TOPLEY AND WILSON'S PRINCIPLES OF BACTERIOLOGY AND IMMUNITY

THIRD EDITION

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IN TWO VOLUMES

VOLUME I

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PREFACE TO THE THIRD EDITION

APART from the war, this edition has been prepared under the shadow of a double misfortune. Early in 1941, Professor Topley accepted the post of Secretary of the Agricultural Research Council, and thereby took a step that rendered his further participation in this book impossible. To replace him, I was fortunate in enlisting the co-operation of our former colleague, Professor Miles. Together we began the arduous task of revision. Our work, however, had not progressed far before the second blow fell. Quite suddenly in January, 1944, Topley died. The effect of this second misfortune was almost as serious as the first. Although Topley could have made no direct contribution to the text, his criticism and advice would have been constantly available, and he would have helped us to maintain that uniformity of presentation for which he and I had always striven. On Miles, in particular, the burden weighed heavily, since, in taking over those parts of the book for which Topley had previously been responsible, he was deprived of counsel that would doubtless have proved invaluable to him.

There is no call to write a long preface. We have endeavoured not merely to bring the book up to date, but to present the new additions to our knowledge in a manner worthy of their importance. One chapter—that on Soil Microbiology—has been deleted, but two new chapters, on Chemotherapy and on the Bacteriology of Air, have been added. For the sake of convenience we have divided the previous *Bacterium* chapter into three, giving separate recognition to the genera *Shigella* and *Salmonella*. We have also removed the psittacosis-lymphogranuloma group of diseases from the other filtrable virus diseases with which they were associated and awarded them a chapter of their own. Except for these alterations, the form of the book remains unchanged. In the first two editions we tried to ensure that scientific literature from all parts of the world was fairly represented, but in the present edition we have suffered under a handicap imposed by the war, which has seriously curtailed the inflow of journals from many parts of Europe as well as, of course, from Japan. This gap we shall look forward to filling in the future. Partly because of the necessarily one-sided picture we have been obliged to paint, we have thought it wise to present our evidence in greater detail than might otherwise have been necessary, and to be perhaps unduly cautious in drawing our conclusions. The bibliography has been much expanded, so as to cater for the needs of those who use the book more as a work of reference than as a textbook. For the increased length of the new edition we tender our apologies. The war has not been conducive to careful leisurely recapitulation, and our plea must be the paradoxical excuse that we have not had time to be more concise.

To those who have assisted us in various ways, we would express our thanks. We are particularly grateful to Dr. N. W. Pirie for his help with some chapters in Part I, to Professor A. Bradford Hill for his advice on Chapter 43, and to Professor S. P. Bedson, Lt.-Col. R. F. Bridges, Dr. A. Q. Wells, Dr. Joan Taylor, Dr. A. W. Stableforth, Dr. R. Lovell, Miss Nancy Hayward, and many of our former helpers for information on particular problems. To Dr. Stuart Mudd and his American colleagues, to Professor A. D. Gardner, Dr. C. F. Robinow, Dr. S. T. Cowan, Dr. N. G. Heatley, Dr. A. Pijper and the publishers of " Endeavour " we are indebted for a number of electron micrographs and photographs ; to Professor J. R. Marrack for Fig. 32 ; to H.M. Stationery Office for permission to reproduce Figures 32, 34, 77, 79, 80 and 81 ; and to Miss Margaret Rees for the preparation of some new diagrams. We should also like to pay our tribute to the library staffs of the London School of Hygiene and Tropical Medicine, the Bureau of Hygiene, University College Hospital Medical School, and the Radcliffe Science Library, Oxford, for their unfailing courtesy and help in the tracing of numerous references.

G. S. W.

June, 1945.

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PART 1

GENERAL BACTERIOLOGY

CHAPTER 1

HISTORICAL OUTLINE

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. It is almost true to say that the clue to the present position of bacteriology is the curious fact that there have been no bacteriologists. From Pasteur onwards, the great majority of investigators have been 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, have presented problems which pressed for solution; and, as a result, we have witnessed a reversal of the normal process. 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. The essential interlocking of pure and applied science has, of course, been in evidence here as elsewhere. The necessity for being able to recognize a bacterium, which has been shown to be of importance in some province of human affairs, or of determining the way in which its harmful or beneficial action is brought about, has led to an intensive study of many aspects of bacterial morphology and physiology; but, in general, it may be said that the study of bacteria themselves has been carried out *en passant*, that amount of knowledge being acquired, or searched for, which would afford adequate data for the solution of some problem in applied bacteriology. Gradually the general structure of our knowledge has been added to, and gaps have been filled. Many of those who have started from some particular application have been led far afield by that desire for knowledge, altogether apart from its technical application, which is the essence of science itself. But this mode of construction has given to the general body of existing bacteriological knowledge a curious patchiness and indefiniteness which are puzzling to the student, and which must be realized and allowed for in any attempt to present the subject as a whole. There can be no question of any future reconstruction *ab initio*. The history of a science is largely a history of technique, and the foundations of bacteriological technique, which presents many peculiar difficulties, have been well and truly laid by those who have worked in this field since the middle of the nineteenth century. The pure bacteriologist of the future will owe a lasting debt to those who have worked on the applied side, and his investigations will necessarily be based upon the knowledge gained by the medical or agricultural bacteriologist. The study of immunology, for instance, has supplied a body of facts, and an armoury of technical methods, which no bacteriologist can neglect, and which will inevitably give to future bacteriological research certain peculiarities of outlook and special methods of attack.

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), concerning a *contagium vivum* as the possible 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 to any considerable extent by arguments, however ingenious, which are propounded without appeal to experiment, or to wide and detailed observation ; and the absence of all real progress until the middle of last century is sufficient evidence that the views of Fracastorius, von Plenciz and others 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, and the reported observation by Kircher (1659) of minute worms in the blood of plague patients forms, perhaps, the earliest attempt at direct microscopical observation in this field. It is, however, more than doubtful whether Kircher could have seen plague bacilli, or indeed any bacterial forms, with the apparatus which he had at his disposal. 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 developed an apparatus and technique (Cohen 1937) which enabled him to observe and describe various microbial forms with an accuracy and care which still serve as a model for all workers in this field. He observed, drew, and measured with considerable approximation to truth large numbers of minute living organisms, including bacterial and protozoal forms. It is, perhaps, somewhat surprising that this marked 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.

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 the phenomena of fermentation. His early studies on the structure of the tartrates, and 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, as contrasted with substances synthesized in the laboratory. It was known that small amounts of an optically active substance, amyl alcohol, were formed during the fermentation of sugar, especially in association with the lactic fermentation. 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 was led to the conclusion that the optically active molecule of the sugar was first broken down to relatively simple substances, which experience had shown to be without optical activity,

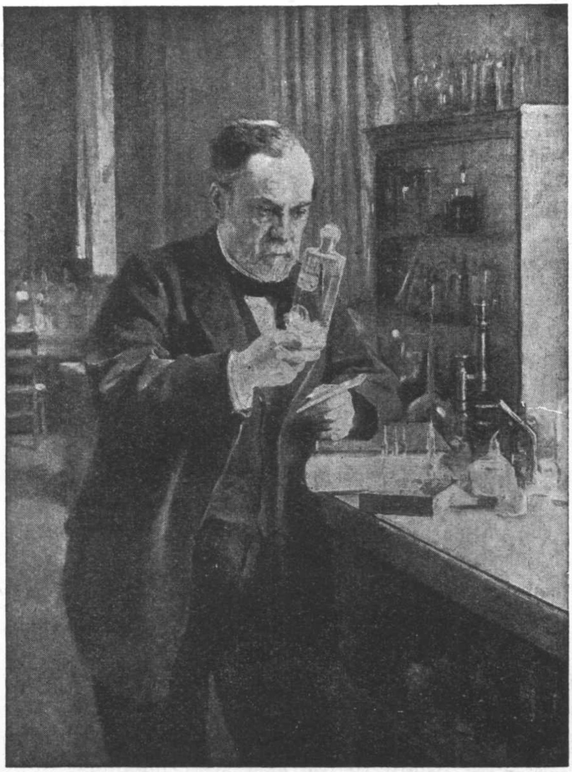

FIG. 1.—LOUIS PASTEUR (1822–1895).

and that from such inactive substances the optically active amyl alcohol was synthesized. For Pasteur this was evidence of the presence and activity 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 fermentations, and which had been described by van Leeuwenhoek in 1680.

Since, however, it was in the lactic fermentation that the production of amyl alcohol had especially been noted, it was this reaction which Pasteur first selected for experimental study, though he had already made numerous observations on material from the vats of the breweries of Lille. He was probably influenced by the fact that the observations of van Helmholtz (1843) had already indicated that the alcoholic fermentation was due to the yeast itself or to some other organized material. Helmholtz had shown that the substance, whatever it might be, which was responsible for initiating alcoholic fermentation, would not pass through membranes that allowed the passage 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, rather than in one kind of fermentation in particular. 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. Pasteur's first memoir was published in 1857, and in it 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 which must be fulfilled for the cultivation of bacteria.

These studies on fermentation occupied Pasteur almost continuously from 1855 to 1860, and he returned to them again at intervals during later years. He was able to show that the fermentation of various organic fluids was always associated with the presence of living cells, and that different types of fermentation were associated with the presence of microscopic organisms which could be differentiated from one another by their morphology and by their cultural requirements. Thus, at this early stage, the idea of specificity entered into bacteriology.

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 their concepts from the great naturalists of antiquity, believed in the spontaneous generation of living things from dead, and especially from decomposing organic matter. It is of little interest to remember the vague terms in which such conceptions were clothed; but one tendency may be noted, which did not escape the astute mind of Pasteur. 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. In the beginning, the supporters of spontaneous generation were prepared to attribute this mode of origin to relatively large animals. Van Helmont, in the sixteenth century, offered a prescription for making mice. It needed the experiments of Redi (1688) to substitute, for the idea that worms were spontaneously generated in decomposing meat, the truth that these worms were the larvæ of flies, and that their appearance could be very simply prevented by protecting the meat with gauze, through which the flies could not pass to deposit their eggs. 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 leaned towards the opposing school of those who believed that spontaneous generation was a myth, 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 marked 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 has made possible the cultivation and study of bacteria.

Neglecting for the moment the vaguer conceptions of the pre-experimental era, the position in 1859 was as follows. Needham, an Irish priest, had published in 1745 a memoir describing the spontaneous generation of microbial organisms in closed flasks of putrescible fluids, which had been heated to destroy pre-existing life. These views were strongly supported by the celebrated naturalist Buffon in 1749. An Italian abbot, Spallanzani, countered in 1769 with the publication of a series of admirable experiments in which he criticized Needham's results, and showed that, with longer heating, the fluid in such flasks remained clear and sterile. This controversy narrowed into a dispute as to the nature of the principle which survived short periods of heating, but was destroyed by long heating in flasks hermetically sealed. For Spallanzani the principle was a living germ, for Needham it was a " vegetative force," resident in the air, or perhaps in the putrescible fluid. In any case such argument was sterile, and although it was generally admitted that the honours remained with Spallanzani, no final judgment was pronounced.

At this time oxygen was regarded as an element of quite peculiar power and significance, and the experiments of Appert (1810) on the preservation of food-stuffs, 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. Schwann (1837) showed that the air in a flask containing a putrescible fluid, which had been sterilized by boiling, could be renewed by drawing in air which had passed through a glass tube immersed in a bath of fusible alloy kept at high temperature, and by this means he demonstrated that the presence of oxygen alone would not cause the appearance of micro-organisms in the fluid. Unfortunately, in the same memoir, Schwann reported other experiments, in which he introduced heated and unheated air into flasks, containing a sterilized solution of sugar in a watery extract of yeast, by inverting the flasks over a mercury bath and admitting the air through the mercury seal. Here his results, as regards the occurrence of fermentation, were altogether uncertain, and his conclusions lost much of their force. Helmholtz (1844) confirmed certain of Schwann's observations. Schultze (1836) had already obtained similar results by admitting to his flasks air which had been drawn through strong potash solutions or through concentrated sulphuric acid. Schroeder and Dusch (1854) showed that the active principle could be removed from the air by drawing it through cotton-wool. This last method was a real advance, since the incoming air had not been subjected to high temperatures, nor to strong chemical reagents. Unfortunately another element of doubt was introduced. Schroeder and Dusch relied, for their preliminary sterilization, on a short period of heating to the boiling-point. They experimented with four kinds of material—water containing meat, malt of beer, milk, and meat without the addition of water. With the first two materials their results were quite uniform: the fluids remained unaltered. With the last two materials fermentation usually occurred. They concluded that there were two kinds of decomposition, associated with the presence of living organisms, the one spontaneous, needing only the presence of oxygen, the other requiring some additional principle, which could be removed from the air by filtration through cotton-wool.

This, then, was the position when Pasteur began his investigations in 1859. In a series of admirable memoirs, 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, or through similar filters, contained organized particles which were neither crystals nor starch granules, but which were similar in appearance to the spores of moulds. By introducing these particles into flasks of sterilized organic material, he demonstrated that they were capable of giving rise to the growth of numerous kinds of living organisms. Using other methods, he showed that the air in different situations differed in its content of these germs; that they 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 Schwann's failures were due to his use of mercury, from the surface of which his fluid had acquired the germs, which had settled on it from the air. He showed that the failures of Schroeder and Dusch were due to the inadequate sterilization of their material.

He also showed 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.

The controversy with Pouchet, Joly and Musset, which continued from 1860 to 1864, did not lead to the collection of many new facts, except those with regard to the unequal distribution of micro-organisms in the atmosphere; but a later dispute with Bastian, who became a veteran in the dwindling army of the supporters of spontaneous generation, was more fertile, because it caused Pasteur to reconsider some of his ideas, and to elaborate the technical methods which he had partially developed during his re-investigation of the results obtained by Schroeder and

Dusch. 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° C., 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° C., and that organisms which had become deposited on the surface of glass-ware 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° C. under pressure for the purpose of sterilization, thus introducing the autoclave into the laboratory, and the practice of sterilizing glass-ware by dry heat at 170° C. In this connection a very 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 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 between them. This method is still known as Tyndallization. It was first described in a letter to Huxley in 1877 (see Bulloch 1930).

While investigating the phenomenon of fermentation, and the problem of spontaneous generation, Pasteur had studied the behaviour of very various kinds of natural organic fluids and solutions, and had succeeded in growing micro-organisms on simple synthetic media. As a result he had become assured of the fact that a medium, which is eminently suitable for the growth of one bacterium or mould, may be ill-adapted for the growth of another, and that one of the primary necessities for the successful cultivation of any species of micro-organism is the discovery of a suitable medium for its growth. Quick to grasp the general significance of isolated observations, he pointed out the decisive effect which must be exercised by the selective action of various environmental factors in determining the constitution of any naturally occurring bacterial flora ; and he later developed these ideas in connection with the problem of infection.

As the result of these studies Pasteur had collected a mass of data, which enabled him to deal successfully with bacteriological problems that could not previously have been attacked. He had learned the need for the scrupulous sterilization of everything that came into contact with material which was to be submitted to bacteriological examination. He had learned the necessary methods of sterilization, in the steamer, in the autoclave, in the hot-air oven, or by direct flaming, which enabled these conditions to be fulfilled. 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 with enthusiastic care the troubles of the local vintners or brewers, or vinegar-makers, and many of his memoirs are devoted to the diseases of wines or of beers, and the methods of preventing them. It was in connection with these studies that Pasteur faced a new problem of fundamental importance. He had shown that ferments were living organisms, that they were specific, that they were reproduced from parent forms and not by spontaneous generation. He was now faced with the problem as to 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.

Anyone who reads for himself the original memoirs on fermentation and spontaneous generation (see Vallery-Radot, P., 1922–1933) will realize that the possibility of applying this new knowledge to the elucidation of infective disease was already in Pasteur's mind. It needed only the spur of a request from Dumas to investigate the disease, which was then ruining the silkworm industry in the South of France, to turn his steps 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 will be referred to in later chapters. We must, however, note certain contributions which Pasteur and his colleagues made to the fundamental data of bacterial infections. 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 as regards many points in the demonstration of the nature and action of the anthrax bacillus. It was Pasteur who introduced into bacteriology the conception of virulence and of attenuation, and who demonstrated the fact 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 a perfectly efficient 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. The correct procedure for preparing good wine, good beer, good vinegar, and the methods of preserving them, the control of pébrine, of anthrax, of chicken cholera, of hydrophobia, these were the problems which occupied the last thirty years of his life, and 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.

He had learned how to isolate and cultivate bacteria, and how to study their effect on animals; but with the minutiæ of their morphology or physiology, apart from any significance these might have for the problem in hand, he was not greatly concerned. Duclaux relates that a clever and positive microscopist, who told Pasteur in very cautious language that a certain organism which he had taken for a coccus was in reality a very small bacillus, was much astonished to hear him reply: "If you only knew how little difference that makes to me!"

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

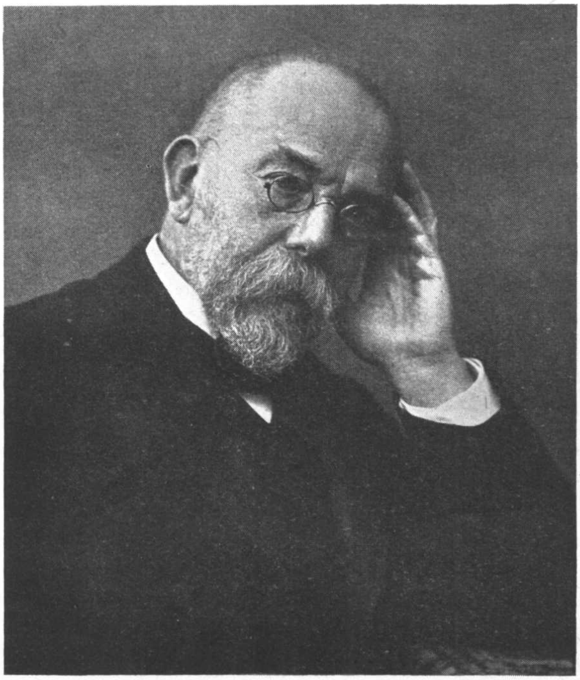

FIG. 2.—ROBERT KOCH (1843–1910).

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 cases in which it was possible to employ 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.

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, and described his methods of photographing such preparations. In 1878 he published his memoir on traumatic infective 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 1930). 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 Infective 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 great 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.

The fruits of this revolution appeared with surprising rapidity. During the last quarter of the nineteenth century a succession of discoveries was reported, bearing on the relation of bacteria to human and animal disease, which opened a new era in medicine.

In 1874 Hansen described the bacillus of leprosy, and Neisser, in 1879, the gonococcus. In 1880 Pasteur recorded the isolation of the bacillus of fowl cholera, and Eberth observed the bacillus of typhoid fever. In 1881 Ogston published an adequate description of the staphylococcus. In 1882 Koch discovered the tubercle bacillus, and Loeffler and Schütz the bacillus of glanders. In 1883 Koch discovered the cholera vibrio, Fehleisen isolated the streptococcus of erysipelas, and Klebs described, but did not isolate, the bacillus of diphtheria. In 1884 Loeffler isolated, and subjected to thorough study, the bacillus which Klebs had briefly described in the previous year, and Gaffky isolated and studied the typhoid bacillus, which