THE PRINCIPLES OF BACTERIOLOGY AND IMMUNITY

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THE PRINCIPLES OF BACTERIOLOGY AND IMMUNITY

To

K. T. AND J. W.

PREFACE TO THE SECOND EDITION

THE seven years that have elapsed since the publication of the first edition of this book have witnessed a rapid advance in the science of bacteriology—more rapid, perhaps, than any that has occurred since its early infancy. As a consequence, the task of revision has been peculiarly difficult. Adequate reference to all the new knowledge that has been acquired would have entailed expansion to a size altogether inappropriate to a textbook of this kind; and we have been compelled to push selection very far. We have tried to give an account—full enough to be clear—of all those recent studies that have added significantly to our knowledge, particularly those that have an important bearing on the diagnosis, prevention, or treatment of infective disease. In certain cases, where recent work has greatly altered our conception of a particular bacterial group, or of the diseases caused by it, we have frankly adopted the style of an abbreviated monograph, or review, rather than of a section in a textbook, since the serious student would otherwise be forced to consult a scattered literature to obtain an understanding of important current problems. We have, on the other hand, omitted altogether many observations the significance of which is as yet difficult, or impossible, to assess; and, where recent advances have solved long-standing problems, we have severely curtailed the account of earlier work, at some loss, perhaps, to historical perspective.

As in the first edition, we have dealt with the infective diseases of animals rather more fully than is usual in a textbook intended mainly for medical bacteriologists; but the boundary between medical and veterinary science is becoming very indistinct, and this is as it should be. The importance of animal diseases to the public health is such that both the medical and veterinary bacteriologist will find themselves at a serious disadvantage, unless each has some familiarity with the territory covered by the other.

This has involved extensive alterations. Many of the chapters have been entirely re-written, and additional chapters have been introduced. We have, however, adhered to the original arrangement of the text, except that we have included, in the systematic description of bacteria, a fuller account of their pathogenicity for animals and man, transferring the relevant sections from later chapters.

The two volumes, in which the first edition was published, have been combined into one. This step has been taken in response to requests from many of those who have used the book in its original form; and we hope that the change will be of assistance to readers.

In giving references to the literature we have adopted the abbreviations contained in the World List of Scientific Periodicals.

We should wish to take this opportunity of thanking those friendly critics who have pointed out to us various errors in the first edition. These we have tried

to correct; but we cannot have avoided replacing them by others in the mass of new matter that has been included. We need not say that we shall welcome their detection and removal.

Finally, we should wish again to express our indebtedness to many colleagues and friends, in this School and elsewhere. To most of those who were mentioned by name in the first edition we owe a further debt. Among many others, from whom we have received help and advice in the revision of various parts of the text, we would acknowledge in particular our indebtedness to Dr. W. R. Wooldridge and Dr. B. C. J. G. Knight in connection with certain chemical problems, to Dr. A. B. Hill in relation to the chapter on elementary statistical methods, and to Dr. Leonard Colebrook, Dr. Dora Colebrook, Dr. R. Hare, Dr. F. C. Minett, Dr. A. W. Stableforth, Dr. W. M. Scott, Dr. A. A. Miles, and Dr. I. N. Asheshov who have helped us in a variety of ways.

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July, 1936.

PREFACE TO THE FIRST EDITION

It has become increasingly evident, during the past ten years, that there is a need for the provision of organized teaching in the principles and technique of bacteriology, of a far more detailed and extensive kind than can be included within the four corners of an overcrowded medical curriculum, or in the scarcely less crowded courses of post-graduate study which lead to a Diploma in Public Health, in Tropical Medicine and Hygiene, or in Veterinary State Medicine. There is an increasing demand, at home and abroad, for the services of the trained bacteriologist; and it is no longer possible, or justifiable, to meet this demand by trusting to the emergence of a certain number of bacteriologists, as a by-product of a training designed for those whose work in life will lie in the clinical or administrative field.

The demand for a separate and organized course of training has been met in various ways. We have, for some years past, been concerned in teaching post-graduate students taking a full-time course in bacteriology lasting over one academic year, and leading to a University Diploma. One of the main difficulties we have encountered has been the lack of a textbook covering the ground over which such students have to travel. The many admirable textbooks which are available for the ordinary medical student are designed to meet his needs. At the moment of writing we have no comprehensive handbook of bacteriology in the English language to which the student may turn for reference; though this long-felt want will shortly be supplied under the auspices of the Medical Research Council. Numerous monographs have been published within the last ten years, dealing with such aspects of bacteriology as classification and nomenclature, the chemical and physical behaviour of bacteria, infection and resistance, and a host of allied subjects; but even with these, some parts of the field are left untouched, and it is inconvenient for the student to have to rely on a multiplicity of textbooks reinforced by extensive reading of original papers in different languages. To consult different books, and to read and criticize a selection of the more important contributions to bacteriological literature, is of course an essential part of any sound academic training in this subject; but no student, at this stage in his career, can be expected to make himself familiar at first hand with the mass of controversial literature which forms the raw material on which any conclusions must be based.

We have attempted, on the basis of our personal experience in post-graduate and undergraduate teaching, to provide a textbook which will be of service to those students of medicine and biology who wish to make a serious study of bacteriology and its application to the problems of infection and resistance. The order of presentation is that which is followed in the course of study pursued in this School; and the matter contained in Parts I and II of this book

is, we think, of a kind with which any bacteriologist should be familiar. The book, as a whole, has however a frank bias in the direction of the application of bacteriological principles to the study and control of infective disease; and Parts III and IV deal entirely with general and particular problems of this nature. We have tried to present these problems in their general biological aspect, as instances of variations in the relations of living things to each other, and to their environment, rather than as isolated problems of diagnosis, treatment or prevention, centering round a sick man or animal. Only in this way, we believe, can bacteriology play its full part in the control of infective disease. For the same reason we have included, in Part IV, numerous instances of natural disease in live-stock, and descriptions in some detail of experimental infections in laboratory animals. The liaison between research workers in human and veterinary medicine —the need for which was so strongly stressed by the late Sir Clifford Allbut— is making rapid headway; and in no branch of medical science is the sterilizing effect of the anthropocentric attitude more obvious than in the study of bacterial infection.

We are personally convinced that this order of presentation is sound—that the student should gain some knowledge of bacteria as a distinctive class of living things, and of their systematic relationships and ecology, before considering their reactions with more highly differentiated organisms, the characters of which he has learned while studying zoology, physiology, or clinical medicine. The formal separation of description which this treatment involves is, however, by no means easy; nor can it be followed consistently. So many bacterial species have been studied almost entirely from the point of view of the pathologist that their systematic description is still impossible. It will, therefore, be found that there is considerable overlapping between Parts II and IV of this book, and an arbitrary allotment, based on convenience, of particular sections to one Part or the other. In compiling some of the descriptions contained in Part II, we have had to rely, in certain minor particulars, on our own observation of comparatively small samples of strains. The recorded descriptions of bacterial types or species are often incomplete; and we have attempted, where possible, to fill in some of the more obvious lacunæ from our own experience and that of our colleagues.

To bring our material within a reasonable compass, we have been forced, in most instances, to omit all detailed descriptions of technique. We have done so with no great reluctance; since the convention of mingling practical instructions for laboratory work with the discussion of general principles has obvious disadvantages, and the student must in any case learn this part of his work by practical experience at the bench. Moreover, bacteriological technique is susceptible to so many minor modifications, and the practice of different laboratories may differ so widely in its minutiæ, that the detailed description of a single method for carrying out each routine procedure would be of little service, while the presentation of a host of alternatives would clearly be impossible.

We have found our task peculiarly difficult. Any teacher of bacteriology, at the present time, will realize the impossibility of following the didactic method. Where so much is uncertain, one must be content to present the available evidence, suggest tentative conclusions necessarily affected by personal bias, and encourage the student to form his own opinions. A firm grasp of general scientific principles, and a well-developed critical faculty, are primary essentials in the intellectual equipment of any student in this field. We have, therefore, frankly abandoned

any attempt to simplify the issues by limiting ourselves to well-attested facts, or to undisputed conclusions. To do so would be to neglect some of the most stimulating bacteriological problems. There is a considerable fascination in studying a branch of science which is growing so rapidly that the charting of its territories is in many places sketchy and uncertain. There are disadvantages. Every teacher and student of bacteriology would be happier if there were some stability in bacteriological nomenclature. So many problems are still in dispute that it is necessary to master the basis of an alarming number of conflicting hypotheses. But, at least, the bacteriologist has no lack of problems which invite attack.

In giving references to the literature, we have endeavoured to afford the reader the means of following up for himself any point in which he is interested. The selection we have made has, of necessity, been somewhat arbitrary. We have omitted many papers of the middle bacteriological period, which, though important in their day, have lost much of their significance at the present time. Among the mass of more recent publications, we have certainly omitted much that is important, and have probably included more than a little which future developments will show to be trivial or misleading. In the absence of historical perspective, personal judgments must exert an undue weight.

We would acknowledge our indebtedness to various monographs and reviews, too numerous to mention here. These have been referred to freely in the text. Among our colleagues and friends who have given us assistance in various ways, we should desire particularly to thank Professor Major Greenwood, F.R.S., and Miss Woods for providing us with certain statistical data, Mr. J. E. Barnard, F.R.S., for information on several points in connection with the application of optical methods, Dr. J. F. C. Haslam and Mr. R. L. Sheppard for tracing certain original papers, Professor Delafield and Miss M. Stephenson for their advice on certain chemical questions, and Mrs. Smith and Mr. R. Lovell for their help in many directions. We would also acknowledge our indebtedness to Mr. F. V. Welch and Mr. W. Bale, who have taken the photo-micrographs with which this book is illustrated. Finally, we would express our sense of gratitude to our past and present students, whose questions, answerable and unanswerable, have provided the stimulus for our work.

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and Tropical Medicine.

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PART I

GENERAL BACTERIOLOGY

CHAPTER I

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 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,

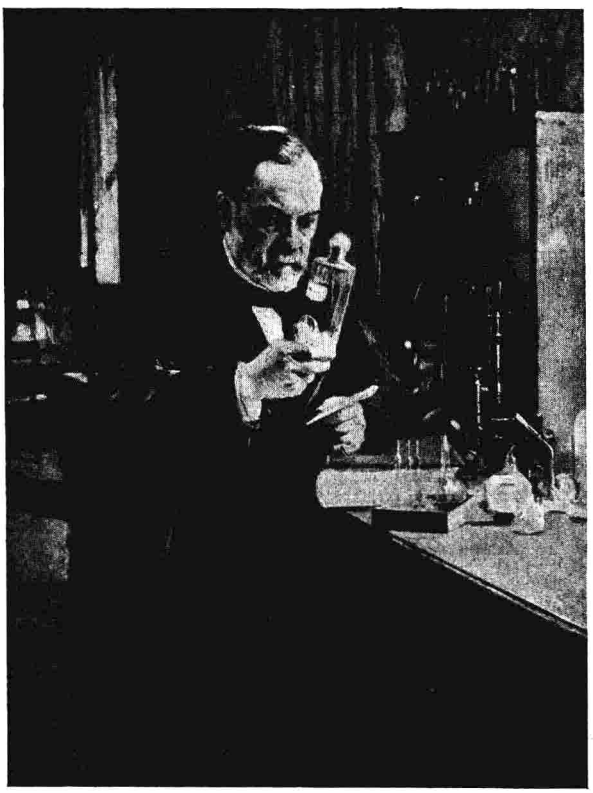

FIG. 1.—LOUIS PASTEUR (1822–1895).

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 Caignard-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, which 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 Caignard-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