THE DEVELOPMENT OF
MICROBIOLOGY

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For the Manchester Diploma in Bacteriology students who persuaded me to write this book

PREFACE

Science is a continuously developing system: a series of models each replacing a previous less satisfactory one. If we are to understand the contemporary corpus of knowledge and critically assess future developments we must learn how we have arrived at the present position.

This book based on lectures given to postgraduate students is an attempt to tell the story of the development of certain ideas in microbiology, relating the views held at different times to the contemporaneous state of knowledge in other fields and showing how successive models grew out of the internal contradictions of their predecessors.

My thanks are due to Mr D. F. Cook and the staff of the Manchester University Medical Library for unstinting help in obtaining references and to my secretary Miss Brenda Gardner for her unfailing patience during the preparation of the typescript for the press.

November 1975 Patrick Collard

CONTENTS

1
Introduction

Microbiology, like all the sciences, is founded upon the twin pillars of craft techniques and philosophical speculation. Without the empirical observations of the first, the subject would be but a mass of meaningless verbiage, and without the organizing hypotheses of the second, would be but a collection of descriptions and receipts.

The crafts of food preservation and fermentation have been highly developed for many thousands of years. Early Egyptian papyri contain detailed instructions for the brewing of wine and beer, and it is clear from these documents that the importance of excluding air from the secondary fermentation was well recognized. The principle of using a starter was standard practice in the making of leavened bread and as the use of the deposit from fermented beer as an agent for the raising of dough is mentioned, it is clear that the Ancient Egyptians were aware of the identity of the agent that produced these two processes, although of course they were quite unaware of its nature.

Other microbiological processes such as the retting of flax are also of great antiquity and had been brought to a high degree of technical excellence three or four thousand years ago.

The use of microbes to produce various milk products such as cheese and the various sour milk drinks, yoghurt and kamous, probably goes back to the time of the neolithic agricultural revolution when men first domesticated grazing animals and began to tend them in herds.

The preservation of foodstuffs by methods such as drying, salting, and dehydration by immersing them in strong sugar solutions, seem to have come in early in the neolithic period, as soon as men had a surplus of foodstuffs sufficient to last them from harvest to harvest.

While the craft techniques have been known for several thousand years, the speculation of the philosophers at first lagged behind. The phenomena of fermentation, putrefaction and infectious diseases were well recognized, but the explanations advanced to account for them were all unsatisfactory because they were grounded in a world view that was prescientific and often magical, and within such a framework the true explanation of these phenomena as by-products of microbial growth was inconceivable.

Formal microbiology may be considered as having passed through four eras. Firstly the era of speculation, lasting from about 5000 BC to 1675. Secondly the era of observation, lasting from 1675 to the mid nineteenth century. Thirdly the era of cultivation, lasting from the mid nineteenth century until the beginning of the twentieth century. Fourthly the era of physiological study, commencing at the turn of the century and continuing to the present.

During the long era of speculation several thinkers advanced the hypothesis that contagious diseases might be due to the growth of minute living organisms, but in the absence of microscopes their theories could not be put to the test and were therefore, in the words of the logical positivist school of philosophers, 'meaningless statements'. In the classical period Cicero discussed the possibility of fevers being caused by the multiplication of minute animals, and some fifteen hundred years later the Renaissance scholar Fracastorius wrote of a '*contagium vivum*', but neither of these hypotheses was fruitful in the circumstances prevailing when they were advanced.

The era of observation dates from the work of the Dutch microscopist Antony van Leeuwenhoek, first published in 1675. He was undoubtedly the first man to see and describe bacteria. His drawings are still extant and so clear are they that one can recognize bacilli, streptococci and other characteristic forms. The era of observation continued for nearly two hundred years and although numerous microscopic forms of life were described, some bacteria, some protozoa and some fungi, and known to their discoverers as 'animalcules', knowledge of the function of these micro-organisms was not advanced at all. They remained scientific curiosities and nothing more. Such progress as was made during this period was the result of a continuing controversy concerning the possibility or otherwise of spontaneous generation. The scholastic philosophers of the Middle Ages were convinced of the reality of spontaneous generation and regarded it as a common phenomenon. As late as the early seventeenth century van Helmont gave a recipe for the spontaneous generation of mice from grain, but during the years of the enlightenment men ceased to believe in the spontaneous generation of whole animals, as a result of the experimental work of men such as Francesco Redi (1626–1697) who showed unequivocally that maggots were not produced by spontaneous generation from rotting meat, as was the general belief at the time, but appeared if, and only if, adult flies laid their eggs in the meat. Redi's experiments, which involved protecting fresh meat from contamination by flies by means of a gauze shield, were elegant, simple and conclusive. William Harvey, the discoverer of the circulation of the blood, summed up the opinion of the 'anti-spontaneous-generationists' in an aphorism '*Omnia ex ovo*'. There were, however, a number of scholars who maintained that spontaneous generation took place, at least in the case of the animalcules of van Leeuwenhoek, and in the effort to decide whether or not this was the case much was learned about the behaviour of microbes, and many of the techniques which were later to be perfected as the basis of cultural bacteriology were first developed.

The most famous of these controversies was that carried on in the mid eighteenth century between Father Joseph Needham and the Italian priest Lazaro Spallanzani, a professor at the University of Pavia. Needham claimed to have shown that microbes were generated in samples of broth that had been boiled and then sealed in the vessels that had been used for the boiling. His work convinced the great French naturalist Buffon, but was challenged by Spallanzani who conducted further and better experiments in which he continued the boiling for much longer periods and carried out the boiling in previously sealed vessels, thus initiating the use of steam under pressure as a sterilizing agent. His work showed that it was possible to preserve broth indefinitely, so long as it had been heated to a temperature above the boiling point of water and was preserved from contamination by the outside air. An interesting by-product of these experiments to refute the doctrine of spontaneous generation was the first observation of anaerobic micro-organisms. The significance of Spallanzani's observations was not appreciated at the time and anaerobiosis had to be rediscovered by Louis Pasteur a hundred years later, but it is quite clear that the first observations ever made were his.

The era of cultivation was initiated by Louis Pasteur's studies on the nature of fermentations. Commencing in 1857 with the 'Mémoire su la fermentation apelée lactique' the work was extended to cover studies on butyric fermentations, fermentations producing ethanol and the production of vinegar. Pasteur's hypothesis that each type of fermentation was caused by the growth and metabolism of a specific micro-organism forced him to develop methods for the cultivation of each organism uncontaminated by any other species. In the early years he used very tedious liquid dilution methods to obtain pure cultures, but later used the methods of isolation on solid media developed by Robert Koch. In order to carry out this work Pasteur had to develop methods for the sterilization of his media and glassware as well as aseptic techniques for carrying out the dilutions and subcultures. The use of the naked flame, the hot air oven and the autoclave all originated in Pasteur's laboratory. Pasteur's work on fermentation started as an attempt to solve a specific industrial problem and this concern with the application of microbiology led him to publish his works on the diseases of wine and the diseases of beer. It is important to remember that the concept of the specific action of microbes was first developed as a result of what we should today call 'trouble shooting' in the fermentation industry. Pasteur was next asked to turn his attention to a disease which threatened to ruin the French silk industry. Silkworms were dying in large numbers and no method for the control of the outbreaks had been evolved. Pasteur's studies on this condition led him to the conclusion that the disease (pébrine) was caused by a protozoan parasite which he was able to demonstrate by microscopy in diseased silkworms but not in healthy ones. A policy of segregation of healthy worms and destruction of colonies which showed signs of infection brought the disaster under control. Pasteur's successes in controlling the diseases of wine and beer and the silkworm disease led to his being asked by the French Government to investigate the problem of anthrax, a condition that was causing great losses to livestock in the country. The work on anthrax and fowl cholera led to the concept of attenuation of bacterial cultures and the development of effective live vaccines for the control of both these infections. The culmination of Pasteur's work was the extension of these principles to the case of rabies, where he was able to produce an effective attenuated vaccine without at any time isolating the virus, and indeed without any idea of the nature of the causative organism.

The great contributions of Robert Koch to cultural bacteriology were the development of solid media and the technique of sorting out mixed cultures and obtaining pure single species cultures by streaking out onto the solid media. This technique revolutionized cultural bacteriology and made possible the great flowering of the subject during the last two decades of the nineteenth century. Between 1882 and 1900 the causal organisms of almost all of the bacterial diseases were isolated and effective preventive measures became possible.

At the same time as the foundations of hygienic bacteriology were being laid by the isolation of the causal organisms of the major epidemic diseases prevalent in Europe, the basis of chemotherapy and immunology was being developed by workers such as Paul Ehrlich, Elie Metchnikoff and Pfeiffer. Ehrlich's earliest work was concerned with the differential staining of leucocytes. The specific way in which certain cells took up different dyes suggested to him the possibility of exploiting this property for the differential destruction of parasites in the body of the host. His preliminary work on the treatment of malaria with methylene blue led ultimately to the synthesis of effective chemotherapeutic compounds such as trypan red and salvarsan.

The search for the basis of immunity followed two lines; the examination of the sera of immune and non-immune animals and the study of the cellular responses to infection. For many years there was a continuing controversy between the followers of Metchnikoff who adhered to the cellular hypothesis and the German scholars who believed in a humoral basis for immunity. Finally in 1908 the work of Wright and Douglas reconciled the two hypotheses and showed them both to be correct.

During the last decade of the nineteenth century great advances were made in our knowledge of the bacteria in soil and water which are responsible for the completion of the nitrogen, sulphur and carbon cycles, and thus for the continued fertility of the land. As a result of the work of Winogradski and Beijerinck a whole new world of bacteria became known, organisms with previously unheard of types of nutrition able to live and grow when supplied only with elementary nitrogen, iron or sulphur and carbon dioxide; the autotrophic bacteria.

The study of bacterial physiology and biochemistry that was the dominant theme of the subject during the twentieth century was stimulated by the isolation of cell-free enzymes and the consequent development of dynamic biochemistry which took place both in Germany and England at the beginning of the century. Perhaps the most famous names in the field of microbial biochemistry are those of Marjorie Stephenson who elucidated many of the energy-yielding pathways in her laboratory in Cambridge and Kluyver of Delft who had the genius to see the underlying unity in the diversity of microbial energy-yielding mechanisms. The detailed study of synthetic pathways came twenty years later and arose out of the application of biochemical genetics, originally worked out using fungi, by Beadle and his school in California.

The study of nutritionally deficient mutants not only disclosed the stepwise pathways by which complex molecules are synthesized in bacteria, but also led to a deeper understanding of the mechanisms of gene expression summed up in the aphorism 'one gene one enzyme'. This stimulated further research in bacterial genetics, at first applied to the problem arising from the emergence of antibiotic-resistant strains of bacteria in clinical practice, but soon extended to more fundamental studies which demonstrated the existence not only of sexuality in bacteria, but of other more bizarre types of genetic exchange, some of which had been reported previously as cases of inexplicable variation but were now for the first time understood. From these studies in bacterial genetics there evolved the new subject of molecular genetics, students of which have solved the genetic code and given us deeper insight into biology than anyone before them.

Pari passu with these advances in fundamental biology a revolution in the treatment of infectious diseases was taking place. In 1935 Domagk working along the lines that had been laid down by Ehrlich, reported the synthesis of prontosil red, a dye which while almost non-toxic to mammals, killed streptococci, neisseria and various other bacteria at very low concentrations. Within the year, French workers had hydrolysed the molecule and shown that the antibacterial activity resided not in the chromophore group but in the colourless sulphanilamide portion of the molecule. As a result of these discoveries many hundreds of sulphonamide drugs have been developed which are extremely effective in the treatment of a number of infectious diseases.

The observation by Fleming in 1928 that the mould *Penicillium notatum* produced a *diffusable* antibacterial substance did not immediately lead to any therapeutic advance, for although it was shown to be a non-toxic substance for animals, it proved impossible to prepare extracts of sufficient purity in sufficient quantity for therapeutic tests to be carried out. The problem of extracting and purifying the antibacterial substance, now known as penicillin, was re-opened in 1939 by a team of brilliant chemists headed by Sir Howard Florey, the Professor of Pathology at Oxford. By then chemical techniques for separating organic molecules were so far advanced that the problem was solved in principle within a year and adequate amounts of sufficiently pure penicillin were made available for first animal, and, when these proved dramatically successful, for human therapeutic trials. Penicillin proved to be virtually non-toxic to man and to be active against Gram-positive organisms and spirochaetes in very low concentrations.

The industrial production of penicillin was followed by a worldwide hunt for other organisms producing antibiotics, which resulted in the discovery first of streptomycin and later of the many broad-spectrum antibiotics. In recent years a new vista has been opened up by the development of semi-synthetic antibiotics. These are made by adding certain substrates to the fermentation which force the antiobiotic-producing organism to modify the nucleus of the compound and thus allow synthetic side groups to be added later.

The study of virology is usually assumed to have commenced with the recognition by Iwanowski in 1892 that tobacco mosaic disease was caused by a filter-passing ultra-microscopic organism, and the demonstration six years later by Loeffler and Frosch that foot-and-mouth disease was caused by a similar organism. Pasteur's work on rabies antedates this, but at that time there was no concept of a class of ultra-microscopic filter-passing infective agents. Just as bacteriology was liberated by Robert Koch's invention of solid media so virology took great steps forward after Goodpasture introduced the fertile hen's egg as a culture medium in the mid nineteen-thirties, and reached its present state of maturity only after Enders introduced the use of tissue cultures with added antibiotics in 1949. Today, virologists are able to grow most viruses, to detect specific antibodies in the serum of patients and to produce effective vaccines against a number of virus diseases.

Microbiology has provided the knowledge that has enabled the industrialized countries of the world to bring all the major infective diseases under control, but it must not be forgotten that they are controlled, not eradicated, and it is only by the continued vigilance of public health bacteriologists that this control can be maintained. Industrial microbiology is a fast-developing field where fresh developments may be expected year by year. Fundamental microbiology is more and more part of molecular biology and in this field we may expect some of the most exciting developments in the next decades.

FURTHER READING

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2
The development of the microscope, staining methods and morphological description

The simple microscope, made up of a single lens of very short focal length, evolved from the magnifying lenses which have been in use since the days of antiquity. The earliest examples known are biconvex lenses made from gem stones and found in the Assyrian excavations of Lugard. Classical authors mention the use of spherical glass vessels filled with water as magnifiers. It was not, however, until the late seventeenth century that lenses of a sufficient quality for the observation of bacteria were produced.

The first man to see bacteria was the Dutch microscopist Antony van Leeuwenhoek (Fig. 2.1). Van Leeuwenhoek was a draper in the quiet town of Delft. He ground his own lenses and during his lifetime he made several dozen simple microscopes with short-focal-length lenses ground so accurately that they gave magnifications of about ×300. Similar apparatus had been described by Descartes earlier in the century, but the quality of the lenses in the earlier models did not permit magnifications great enough to visualize bacteria.

As can be seen in Fig. 2.2, the lens was fixed and the specimen was moved into focus by the use of a series of fine screws. The type of illumination used is not known with certainty; it may have involved the use of a convex mirror, as did the model described by Descartes, or it may have made use of a larger biconvex lens to focus the light onto the specimen. Almost certainly the light source must have been sunlight as no contemporary artificial light would have been able to achieve the intensity of illumination required when working at such high magnification.

van Leeuwenhoek examined rain water, well water, sea water, and water in which peppercorns had been infused. He reported

Fig. 2.1. Antony van Leeuwenhoek (1632–1723). From A. van Leeuwenhoek, *Arcana natura detecta* (1695). By courtesy of 'The Wellcome Trustees'.

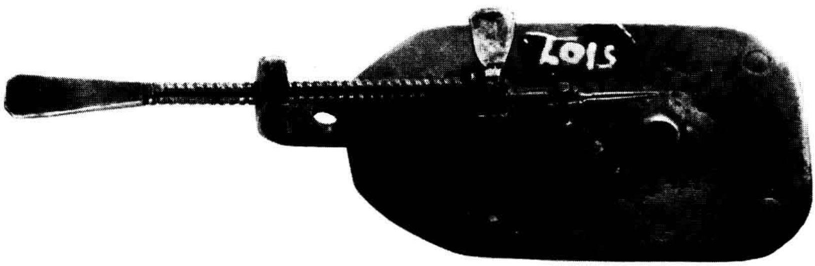

Fig. 2.2. The Leeuwenhoek microscope. A facsimile of the original in the University of Utrecht. By courtesy of 'The Wellcome Trustees'.

his observations to the Royal Society of London in a letter dated 9 October 1676. The letter is published in the *Philosophical Transactions of the Royal Society* for 1677, No. 133, pp. 821–31. A few years later in 1683 he contributed a further letter to the Society containing 'microscopical observations about animals in