THE PENICILLIN GROUP OF DRUGS

BY

GORDON T. STEWART

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PREFACE

This book was begun in England and completed in America. It is, therefore, my pleasure to acknowledge the help and encouragement which I have received from colleagues on both sides of the Atlantic. In England, my interest in penicillin dates from 1944 when, as one of the first (and most ignorant) "Penicillin officers" in the Royal Navy, I was initiated into problems of production, assay and usage at the R. N. Medical School, Clevedon, by Surgeon Commander C. A. Green, R.N.V.R., now Professor of Bacteriology at the University of Durham. Subsequently, I had the privilege while working at the Wright–Fleming Institute of Microbiology in London of contact with Sir Alexander Fleming who did not often talk about penicillin but laid more emphasis than anyone I have known upon personal experimentation and observation. He was a man whose extreme reticence concealed a great depth of kindliness, sincerity and sagacity but few of the younger people seemed to appreciate this. Among those few, I must mention Dr. Amelia Voureka (Lady Fleming), herself an able bacteriologist, whose understanding of her husband's qualities contributed largely to the biography written so ably by M. André Maurois. For information about earlier events, I have to thank Professors R. Hare, W. D. Newcomb, M. Pryce and Dr. W. H. Hughes.

The contribution of the Sir William Dunn School of Pathology at Oxford to the development of penicillins and cephalosporins is incalculable. I hope that, in these pages, I have indicated the significance of the continuing interest of this School in fundamental aspects of antibiotic activity. I am personally indebted to Professor Ernst Chain for his interest, as well as to other present and past members of the Dunn School, notably Professor E. P. Abraham, Dr. N. G. Heatley and Dr. G. G. F. Newton for helpful discussions of past events and present trends, as well as for samples of cephalosporins when supplies were scarce.

The greater part of the book is concerned with the newer penicillins which arose out of a programme planned by Chain and the Beecham Research

Laboratories Ltd. Having enjoyed for six years close collaboration with the Beecham research team, I find it difficult to confine my thanks to a few individuals but in thanking the late Dr. John Farquharson, Mr. F. P. Doyle, Mr. F. R. Batchelor, Dr. E. T. Knudsen, Dr. J. H. C. Nayler and Dr. G. N. Rolinson, I am trying to thank the whole management and staff and, at the same time, paying tribute to the immense benefits arising from this kind of research by the pharmaceutical industry. It is especially pleasing to note the recent recognition of Beecham's achievement by the award of the Gold Medal of the Most Sacred Society of Apothecaries to Mr. Doyle and Dr. Rolinson. Among other organisations, I must thank the National Research Development Corporation and Glaxo Research Ltd. for supplying me with cephalosporins and the Connaught Research Laboratories, Messrs. Pfizer, Boots and others for supplying me with various derivatives of 6-APA.

My own recent work in the antibiotic field has been carried out mainly at Carshalton and I am glad of this opportunity to register my gratitude to many colleagues there, in Queen Mary's Hospital for Children and in the Medical Research Council's Laboratories. Again it is difficult to confine my thanks to a few names, but I owe special acknowledgement firstly to my chief assistant Mr. R. J. Holt, whose technical collaboration has been invaluable; also to Dr. J. M. Barnes, Dr. H. M. T. Coles, Dr. S. Duckett, Miss Patricia Harrison, Dr. R. L. Newman and Mr. H. H. Nixon; and my secretary there, Mrs. Eileen Hardy. I am indebted also to Dr. M. T. Parker, Dr. Patricia Jevons and Professor R. E. O. Williams for their collaborative work with staphylococci extending over several years in the Central Public Health Laboratories at Colindale.

In America, I have encountered many colleagues who have stimulated my interest in less familiar aspects of antibiotic research. In the first place, I must thank the National Science Foundation for bringing me here as a Visiting Foreign Scientist. Numerous new colleagues have opened doorways for me at the University of North Carolina to widen my interest in the control of infection. The Squibb and Bristol pharmaceutical companies, among others, have furnished information about past and present research on penicillins. I have also met some of the pioneers of antibiotic research in America, all of whom have provided useful information, often unique. The manuscript for the later chapters was typed by Mrs. Natalie Harbin and Mrs. Carolyn Owen who assisted also in revision, along with my daughter Linda.

Elsewhere in the world, I find myself indebted also. Dr. W. R. Lane of the Commonwealth Serum Laboratories in Australia generously exchanged information with me about the toxicity to tissue cultures of some of the

newer penicillins. Colleagues in Canada, Denmark, Germany, Norway and Poland have sent me strains of bacteria with unusual forms of penicillin resistance which have extended my experience of this problem.

In writing this book, I have become increasingly aware of the beautiful—if accidental—continuity of research and achievement in the subject. The point has now been reached where the benefits to preventive and therapeutic medicine stemming from the β-lactam antibiotics are matched by a scientific understanding which cannot fail to contribute immensely to genetics, pharmacology, microbiology and epidemiology. My own comprehension of these subjects is limited, but I have enjoyed pulling some of the strings together for I find that science is more satisfying when I glimpse its unity. The story of these antibiotics illustrates a continuing interaction of intellectual and practical endeavour, and this is what the book is about.

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ERRATUM

p. 203, first column:

7-Aminopenicillanic acid, *see* 7-ACA

should read:

7-Aminocephalosporanic acid, *see* 7-ACA

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Chapter 1

DISCOVERY IN 1929

Discovery is seeing what everybody has seen, and thinking what nobody has thought.

CLAUDE BERNARD

Great thoughts and great deeds can occur at any time and any place, but great discoveries are dependent on people and places. Paddington is a bustling, nondescript place in West London, best known for its railway terminus and, to a much lesser extent, for the hospital standing almost next door. But it was in this hospital, at the turn of the century, that an Irishman in his early forties named Almroth Wright, who had been educated in Europe, established a small department of research which was to become one of the first nurseries for the infant science of bacteriology in England. The seed of discovery was planted in this nursery by Wright, best remembered as a forceful eccentric who believed that something could and should be done about communicable disease.

The hospital, St. Mary's, was and is unlike the traditional teaching hospitals of London. Many of its students were of middle class origin, often from Wales; the staff were, likewise, heterogeneous, and newcomers were usually welcomed. In this setting, Wright was able to create something much greater than a department of inoculation and research, unusual as that was at the time: he formed a school of thought. It was this school which fostered the philosophy originated by Metchnikoff of direct observation of the mechanism of natural defence against infection, and of the practicability of prevention and cure. It was to this school that several thoughtful men were attracted in their formative years, among them Alexander Fleming, who was as a naturalist as well as a doctor. It was in this school that his mind was prepared for the observation that led to the discovery of penicillin.

The characters of Wright and those who worked and argued with him in London were essential elements in the discovery and preliminary examination of penicillin. Like all great men, Wright had many strings in his bow. From his own writings[1], from contemporary literature, and from subsequent character studies by Colebrook[2], Maurois[3] and Hare[4], as well as from the vivid recollections of those who knew him, there emerges a vigorous and varied personality who could display humour and gentleness along with

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ferocity and impatience; he worked hard himself, at the bench and in his consulting room, for he believed that doctors in laboratories should also see patients, and encouraged his assistants to do so. His intellectual interests and gifts were wide—from poetry to bacteriology—but his outlook was largely pragmatic and his research was applied with constancy to the prevention and cure of infection by methods utilising natural substances. His belief, to which he clung obstinately, was that prevention of infection could be achieved by immunisation and cure by stimulating the various components of the body's natural defences, or by administering these components; his own achievements—demonstrating the natural bactericidal power of the blood and the presence of opsonins—together with his own eloquence and determination, convinced many of his associates that his approach was rational and likely to be fruitful. Events to date have proved him wrong; pioneer as he was in immunology, Wright did not himself discover any therapeutic substance of lasting value.

Nevertheless, today, the Research Department at St. Mary's is renamed the "Wright–Fleming Institute of Microbiology" in recognition of these two key figures in a long chapter which culminated and ended with the early description of penicillin. Wright's importance—in this discovery and in the broader subject of microbiology—was as the founder of a productive and provocative school of thought; he saw the ravages of communicable disease as a challenge to his humanity as well as to his scientific curiosity; and he imbued others, inside and outside his department, with the credulity of his belief. It is no discredit to the other members of the research department, several of whom became distinguished by their own efforts and a few of whom are alive today, to say that no one there except Wright could have given such impetus to these particular researches.

A book on penicillins would be incomplete without recounting this background. Research is now a commonplace, almost a routine, but discovery is still a rarity which can only be understood fully in terms of people and places. It is often said that research workers need isolation and tranquillity; this may be true of some subjects, but the school which unearthed the leading clue to antibiotic therapy was crowded, controversial and as much a part of the motley borough of Paddington as the stale air which yielded the penicillium mould, not inappropriately, on a plate.

Alexander Fleming was born in 1881, the seventh son of a working farmer, at Lochfield, Ayrshire, Scotland. He attended first the village elementary school at Lochfield, then a larger school at Darvel, to which he journeyed on foot, four miles each way each day. At the age of twelve he was sent to

Kilmarnock Academy, returning home at week-ends. When he was fourteen, like many other Scottish country boys, he left school and went to London, joining three of his brothers there, to earn his living. One of his brothers, Tom, who had studied medicine at Glasgow University, was now in practice in London, and found room for Alec, two other brothers and a sister, in his house.

After a brief spell of study at the Polytechnic School, Alec took a post as junior clerk in a shipping company, the American Line, in Leadenhall Street. When the Boer War broke out in 1900, he joined the London Scottish Regiment as a private, along with two of his brothers. At the age of twenty he inherited a legacy of £250 from an uncle and, with encouragement from his doctor brother, Tom, decided to study medicine. He sat the entrance examination of the College of Preceptors and, in 1901, entered St. Mary's Hospital Medical School. As a student he had a distinguished record; when qualified, he prepared for a surgical career by acquiring the Fellowship of the Royal College of Surgeons. He was, however, persuaded by Dr. John Freeman to apply for a post in the Inoculation Department. Wright in 1908 accepted him as a trainee in bacteriology and there he was to remain for the rest of his life. Shortly before this, he had declared his interest in communicable disease by writing an essay on "Acute bacterial infections" which won him the Cheadle gold medal offered by the School.

For the next four years, Fleming studied and applied Wright's immunological techniques but, unlike Wright, he accepted chemotherapy rather than immunotherapy as a means of treating infection. He was one of the first men in England to use Ehrlich's newly-discovered arsphenamine ("salvarsan") in the treatment of syphilis[5], possibly because his hands were safer than those of his colleagues in delivering this highly-irritant drug intravenously. In 1914 he became a medical officer in the Royal Army Medical Corps and served, under Wright, in a laboratory at Boulogne-sur-Mer, established for the purpose of devising methods to control wound infection. His practical experience of this appalling problem, and his interest, deepened beyond words. He drew attention to the role of necrotic tissue in facilitating wound infection[6] and devised methods for the rational use of irrigation, antiseptics and transfusion; he was particularly impressed by the failure of all available antiseptics to sterilise wounds without damaging tissue or killing leucocytes[7].

After the war Fleming, now married, returned to the Research Department at St. Mary's Hospital. In 1922 he described the bacteriolytic substance[8] (christened "lysozyme" by Wright) in mucus, tears and other secre-

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tions as well as in egg albumin, plants and skin. With Allison and Ridley he attempted to extract and purify the active principle but without success[9], though he maintained his interest in lysozyme and said in later years that it could be "more important than penicillin". He showed also that lysozyme was present in phagocytes and that staphylococci could acquire resistance to it on exposure[10].

The story of Fleming's chance observation that a contaminant mould of the *Penicillium* family produced a substance inhibitory to staphylococci is now a classic. His frame of mind at the time and his subsequent activities are less well recognised, though no less important than the chance observation. Maurois[3] gives an excellent description of what happened, based on the recollections of eye-witnesses. It is clear that, in Fleming's well-prepared mind, the importance of the observation was soon registered: not only did he take the essential steps of testing and preserving the mould; he also gave up many other activities so that he could concentrate his attention on the phenomenon which he had encountered. It is fortunate for mankind that he had the intelligence and the opportunity to do so, though he has been criticised—by those who knew and loved him best, as well as by others—for not mustering more energy in his research. He knew that chemical knowledge was the answer to his problem; but he never attempted to learn any more chemistry himself, leaving this part of the problem uncritically to others.

This was in 1928, and the phenomenon known as antibiosis had already been described by many scientists, including Lister[11] (1871), Roberts[12] (1874), Tyndall[13] (1876), Pasteur and Joubert[14] (1877) and several others. *Penicillium* had in fact already been used by Gossio[15] in 1896 to produce an antibacterial substance. The therapeutic possibilities of antibiosis—the word seems first to have been used in France—appealed to French scientists, who have always been attracted by medicinal substances of natural origin. At the end of the 19th century, research was proceeding on those lines, but nothing useful was produced. An occurrence such as that which Fleming witnessed must have been observed and even deliberately produced many times; the importance lay less in Fleming's observation than in his action. Being at heart a good Presbyterian, he felt in an entirely unpretentious way that Destiny entered his laboratory that day; not least among his endearing qualities was his candid admission, years later, that "the fates were wonderfully kind to me"[16].

Fleming then proceeded to test other fungi, including eight strains of *Penicillium*, for antibiotic production. The only species which produced a bactericidal substance was a strain of *Penicillium* identical in appearance and

properties to the original mould which was identified at that time as *P. rubrum*, though in subsequent studies it was reclassified by Thom as *P. notatum*. He characterised the antibiotic, accurately, as being soluble in water and ethanol, insoluble in chloroform and ether, stable at pH 6.8, and moderately stable to heat. In his first paper on the subject[17] published in the following year in the *British Journal of Experimental Pathology*, he described in detail the rate and extent of bactericidal activity and noted that the action of penicillin was directed essentially against gram-positive and gram-negative pyogenic cocci, there being little or no action on gram-negative bacilli. For this reason, he was impressed by the immediate practicability of incorporating penicillin to make blood agar selective for the isolation of *Haemophilus* in sputum. This was featured in the title of his paper "On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae". The title might have been chosen better, for it gave rise to the impression, then and later, that Fleming was not aware of the therapeutic potential of his discovery. The text of the paper belies this, for he mentions the lack of toxicity of the antibacterial substance, which he named penicillin, in the rabbit, mouse, human eye, human leucocytes and wound surfaces. It is also clear, in the paper and in notes made soon after, that he tested the therapeutic properties of the crude brew of penicillin in wounds infected with staphylococci. Attempts to purify it by evaporation *in vacuo*, performed by his colleagues Stuart Craddock and Frederick Ridley, were unsuccessful. A report presented to the Medical Research Club evoked no questions or interest from anyone in the audience.

Fleming then approached other chemists, including Harold King, head of the department of chemistry at the Medical Research Council's Laboratories at Hampstead. Independently, the Professor of Biochemistry at the London School of Hygiene and Tropical Medicine, Harold Raistrick, studied penicillin with the help of a bacteriologist, R. Lovell, and another chemist, P. W. Clutterbuck. They succeeded in isolating the inert pigment from the mould, but not the antibacterial substance. Their work[18] came to a halt with Clutterbuck's untimely death.

Meanwhile Fleming had published another paper dealing with the use of penicillin as a selective agent in media for the isolation of *Haemophilus*[19]. In 1932, when it was clear that attempts at chemical extraction and purification were fruitless, he published an account of his use of the crude brew as a topical agent in the treatment of infected wounds[20]. He gave a paper and a demonstration about penicillin to the 2nd International Congress of Microbiology[21] in 1936, and continued to speak to other scientists about the

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importance of purifying it by chemical means, but again failed to arouse any obvious interest.

To anyone interested in the evolution of medical science, these happenings in 1929–32 are most revealing. Many medical men saw no possibility—or refused to see any possibility—of treating any major bacterial infection by a drug, though the practicability of systemic chemotherapy had already been demonstrated in syphilis and, with empirical remedies, in malaria and amoebic dysentery. And yet, in every general hospital, and especially in children's wards, bacterial infection was by far the greatest challenge to the therapeutic impotence of the day. Since the days of Lister, antiseptics had come and gone. None was fit to swallow or inject, though Browning, following Ehrlich's approach, had found the flavine acridines to be less toxic than most antiseptics. Hindsight is an easy road to wisdom, but it is nevertheless astonishing to reflect that no one at the time, except Raistrick and an American bacteriologist, R. D. Reid[22] of Baltimore, exhibited active interest in any of Fleming's reports, though to any bacteriologist in those days an impure but non-toxic filtrate killing staphylococci at a dilution of 1:600 must have offered the prospect of a strange new experience. Academic research in medicine is often too fundamental to be concerned with the prevention and cure of disease but, even in sympathetic circles, Fleming's work aroused no interest, nor was there any evidence of exploratory action on the part of the pharmaceutical industry. There are fashions in medical science no less than in costumes; Fleming's observations, reported at a time when therapeutic nihilism was the vogue, were completely ignored. Even Wright, to whom research on infection was a religion, was oblivious to the therapeutic potential of Fleming's work, though perhaps appreciative of its technical accuracy.

Some personal factors also have to be considered. Though shrewd in his assessment of men and matters, Fleming was abrupt in manner and devoid of guile; he was also habitually taciturn and an indifferent speaker. Within himself he was wise and surprisingly far-seeing, but in address he seldom pressed a point and was not persuasive. At St. Mary's he had long been respected, even popular, for his sincerity, even temper and athletic ability, but he failed to convince anyone there that research on penicillin should be effectively supported or even repeated. The despotism of Wright may have been an adverse factor here, for Fleming often acknowledged the co-operation of some of his clinical colleagues who allowed him to treat their patients[22]. But this co-operation almost certainly was given to Fleming the doctor rather than to penicillin the drug. Nevertheless, some useful cases came his way[23,24] and a controlled trial at that time might have disclosed

more objectively the therapeutic activity of penicillin; even in the crude brew applied topically, there was enough antibacterial potency to eliminate sensitive organisms from burns, wounds, ocular and other localised infections; but Fleming, like Wright, was distrustful of trends and statistics. Seeing was believing; had they thought otherwise, penicillin might never have been seen.

It must be remembered that biochemistry, wherein lay the solution of Fleming's technical difficulties, was a younger science even than bacteriology. Techniques which are familiar now to undergraduate students were then undeveloped. In these circumstances, wittingly or unwittingly, Fleming probably did the best thing he could do in describing, simply and factually, his basic findings; he set the stage admirably for a subsequent performance by a more expert team who read the literature wisely.

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Chapter 2

TEN YEARS LATER: 1939 AND AFTER

The clever men at Oxford
Know all there is to be knowed.

SONG (K. GRAHAME)

If the first key figure in the discovery of penicillin was a scientific naturalist, the second was a natural scientist, a pathologist with the outlook of a physiologist. Howard Florey, who was born and educated in Australia, became interested in natural antibacterial mechanisms while working in the University Department of Pathology at Sheffield in the early thirties[1]. He confirmed and extended Fleming's work on lysozyme and, when he moved to the Chair of Pathology at Oxford, planned a programme of research on natural antibacterial substances. Among those who joined his staff in Oxford was Ernst Chain, born in Berlin in 1906. With other members of Florey's department, Chain continued the study of lysozyme which was purified and crystallised[2,3] by 1938. Various other antibacterial substances were also investigated, including pyocyanase (whose bactericidal properties had first been described by Emmerich in 1902), actinomycetin and penicillin. It was obvious from Fleming's original paper that penicillin was one of the most promising antibacterial agents ever described among about forty references read in a preliminary survey of the literature. Chain believed that its instability, which had baffled Raistrick[4], was not an insuperable difficulty. Fleming's experiments were quickly repeated and confirmed, and preparations made for growing the mould on a larger scale to fathom the biochemical depth of this marker of bacterial antagonism.

Meanwhile, the climate of opinion had become more favourable toward chemotherapy. Domagk's description of the bacteriostatic properties of prontosil[5] in the Bayer laboratories in Germany, and the subsequent isolation of the active principle *p*-aminobenzene sulphonamide by workers at the Pasteur Institut in Paris[6] established the fact that pyogenic bacteria could be suppressed *in vivo*, while clinical studies conducted in France, Germany, England, and elsewhere by various workers[5-8], demonstrated in a few months that complete cure of severe streptococcal infections was practicable. Within a year the efficacy of sulphonamide in gonorrhoea, bacterial pneumonia, meningitis and other conditions caused a revolution which al-

tered therapeutic outlook no less than procedure, while the synthesis of new derivatives proved that pharmacological drawbacks in the primary sulphonamide could be overcome. To anyone studying medicine at that time, the somersault in therapeutic thinking was quite spectacular. In research circles, however, this channelled thinking in the direction of synthesis, not antibiosis. In selecting their subject, the Oxford workers followed the new fashion but, in their approach to it, they swam against the tide, as they were soon to find.

In their early work on the cultivation of *P. notatum* in 1939, the Oxford workers used simple Czapek–Dox medium, still a favourite for this purpose. They accelerated growth by adding a boiled extract of yeast and they increased the yield of penicillin by harvesting and replacing the medium beneath the surface mat of fungus, though this procedure was eventually abandoned because of the liability to contamination. To extract the penicillin, they used a solvent-transfer process based on the technique of Clutterbuck, Lovell and Raistrick and produced early in 1940 small quantities of a brown powder which inhibited the growth of *Staphylococcus aureus* at dilutions of 1 in 2 million. This powder probably contained less than 2% of penicillin but, even so, it was remarkably non-toxic in animals. When John Barnes, now a well-known toxicologist, gave the first intravenous injection to a mouse, he remarked to Chain that the new substance was at least non-toxic, if less colourful, than pyocyanin, which was still an object of departmental interest; but further toxicology and protection tests in mice infected with β-haemolytic streptococci left no one in any doubt over the therapeutic potential of the crude penicillin which thereafter became the dominant subject of study[9].

The next problem was large-scale production and extraction. The descriptions of Heatley[10] and others[11,12] hint at some of the difficulties experienced in overcoming this problem in a small University department in war-time, but modestly understate the ingenuity and determination of the team which amassed culture vessels, bedpans, ceramic slipware and other utensils for the purpose, and devised a counter-current solvent extraction which yielded, early in 1941, enough penicillin for a preliminary study in human subjects. The cup-plate assay technique developed by Heatley led to the definition of the Oxford unit of activity (0.6 μg of pure penicillin G), still in use today.

The first clinical trial with the crude penicillin powder was conducted on 12th February, 1941. The patient was an Oxford policeman, dying of staphylococcal osteomyelitis and pyaemia in the Radcliffe Infirmary, Oxford. Pen-

References p. 15/16