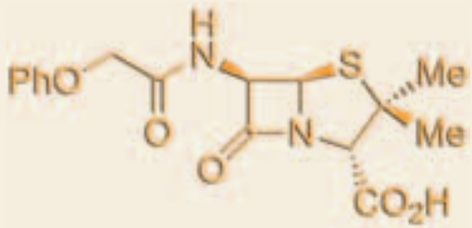
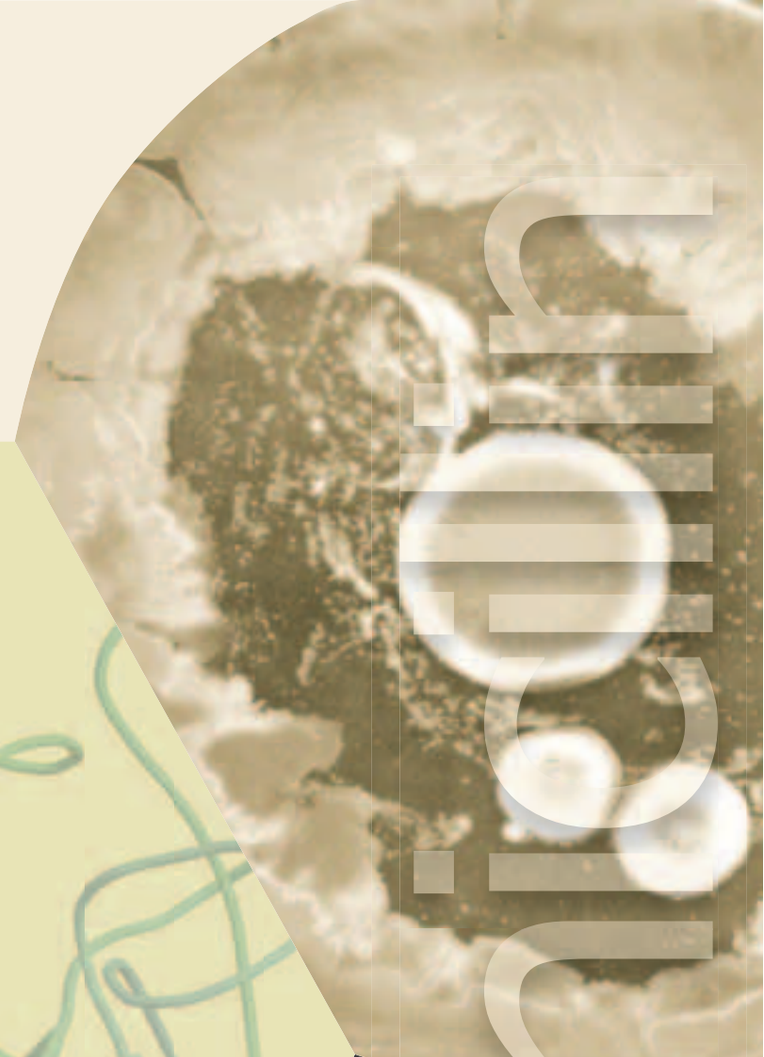
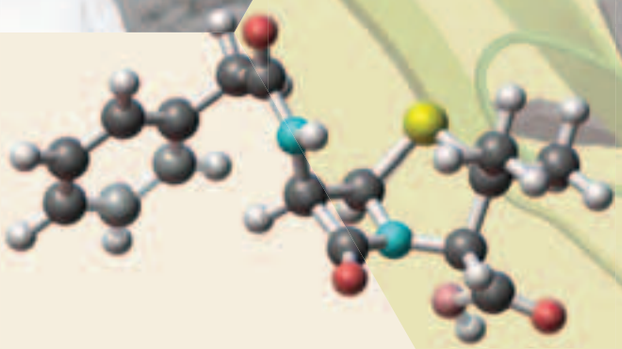




penicillin

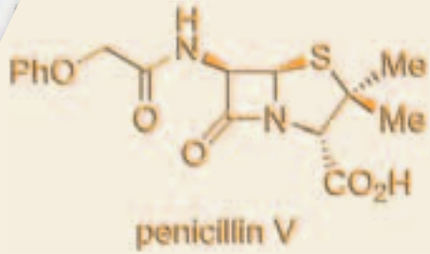


Penicillin

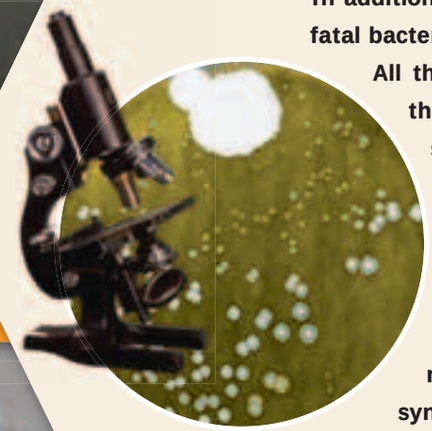
Chapter 13

1957

Alexander Fleming in his laboratory



penicillin V



The narrative surrounding the discovery and development of penicillin is truly remarkable, possessing all the ingredients of a best-selling novel: serendipitous discovery, wartime political intrigue, fierce competition, and an eclectic cast of characters. In addition, the dreadful problem of fatal bacterial infections was solved. All this was a consequence of the extraction of a broad-spectrum antibiotic from an ordinary mold, following an observation made by Alexander Fleming in 1928. Despite its importance and the efforts of many chemists, the total synthesis of penicillin would not be accomplished until 1957.

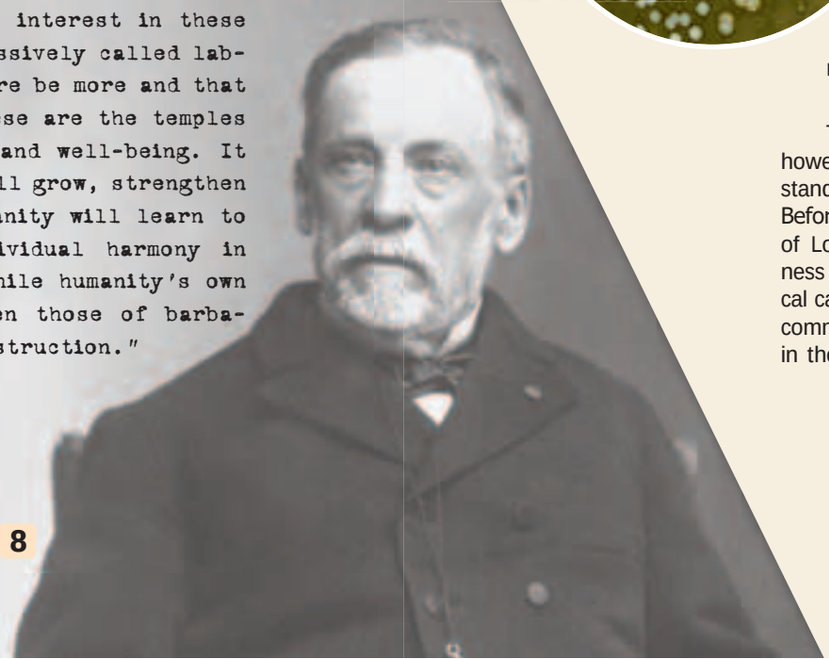
This epic story begins much earlier, however, with a revolution in our understanding of the underlying cause of disease. Before the magnificent accomplishments of Louis Pasteur, disease, death, and illness were frequently ascribed metaphysical causes. In the western world, this idea commonly meant that sickness, especially in the form of epidemics, was deemed to be the wrath of God, punishing man for his sins. Pasteur's seminal works culminated in the 'germ theory of disease,' which asserted that infectious diseases were caused not by God's vengeance, but by microbes.

Louis Pasteur studied chemistry in Paris, and in 1848 he made his first profound contribution to the advancement of science, aged just twenty-six. He examined tartaric acid crystals under a microscope and noticed that they existed in two distinct forms that were mirror images of each other. He was able to separate these forms and discovered that, in solution, they rotated the plane of polarized light in opposite directions. This investigation represents the inception of the pivotal and fundamental field of stereochemistry. The tartaric acid Pasteur studied came from wine sediments, and his interest in the science of fermentation would lead to more important discoveries. In 1856, Pasteur came to the aid of a student's family, who were experiencing production problems at their fermentation plant; sometimes alcohol was produced as expected, but other batches gave lactic acid instead. Pasteur examined the fermentation mixtures by microscopy and noticed that during normal production the yeast cells were plump and budding, but when lactic acid was being produced, the yeast cells were smaller and accompanied by rod-like microbes. He found that briefly heating the liquid medium before fermentation began would kill the undesirable microbes and lead to reproducible fermentations. This sterilization procedure, known as *pasteurization*, is still in common use today, particularly for dairy products. Pasteur was subsequently able to show, using an ingeniously designed swan-necked flask containing a fermentable solution, that microbes such as these were airborne particles. Next, he tackled a serious disease in silk-producing

Box 1 Pasteur's philosophy on nature, research, and humanity

"I beseech you to take interest in these sacred domains so expressively called laboratories. Ask that there be more and that they be adorned for these are the temples of the future, wealth and well-being. It is here that humanity will grow, strengthen and improve. Here, humanity will learn to read progress and individual harmony in the works of Nature, while humanity's own works are all too often those of barbarism, fanaticism and destruction."

Louis Pasteur

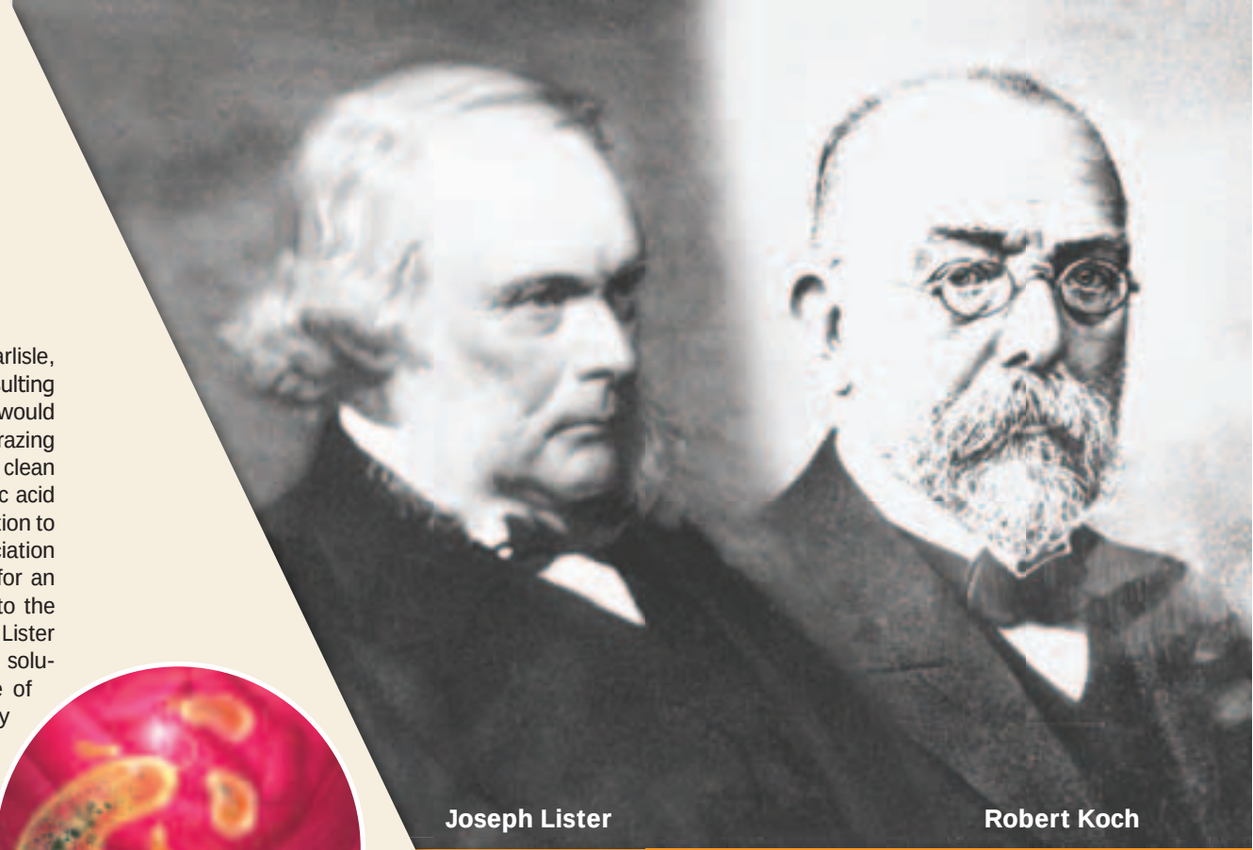


worms whose malaise was having a devastating economic impact on the buoyant European silk trade. He showed that healthy worms could become infected by nesting on leaves previously occupied by diseased specimens. These early influential studies led Pasteur to develop his idea that germs spread contagious diseases, and that these foreign particles were living micro-organisms. Pasteur went on to do more groundbreaking work. Building on Edward Jenner's discovery of a vaccine for smallpox, Pasteur developed vaccination as a prophylactic strategy to outwit other viral contagions, such as rabies, as well as the bacterial infections of anthrax and cholera. Having sensed the widespread recognition of his work, Pasteur died with the knowledge that he had succeeded in bequeathing France a powerful and vibrant establishment for research, The Pasteur Institute, in Paris. He had pursued this project with passion for he strongly believed in applying experimental science to eradicate humanity's plagues and often spoke emphatically on this subject (Box 1). His words ring true and are surprisingly relevant even in today's much altered world.

Meanwhile, in the United Kingdom, a young Quaker surgeon by the name of Joseph Lister was enthralled by Pasteur's work, prompting him to propose a connection between wound sepsis and microbes from the air. Lister's search for the cause of sepsis had begun because he was troubled by the assertion made earlier by the famous chemist Justus von Liebig that sepsis was a kind of combustion occurring when expanding moist body tissue met with oxygen from the air. In Lister's surgical ward at the Glasgow Royal Infirmary, wound sepsis killed fifty percent of his patients. Lister was further inspired to do something about this dreadful situation when he read that carbolic acid (Box 2) was being used to treat sewage in Carlisle, and that fields treated with the resulting slurry were freed of a contagion that would normally lead to infection in cattle grazing on the same site. Lister began to clean wounds and dressings with a carbolic acid solution, and in 1867 he was in a position to announce to the British Medical Association that his ward had been sepsis-free for an astonishing nine month period due to the implementation of this protocol. Lister had pioneered the use of antiseptic solutions and broadcast the importance of hygiene in operating theatres, thereby saving innumerable patients from a painful gangrenous death.

The German doctor Robert Koch, working alone as the District Medical Officer for Wollstein during the Franco-Prussian War in the 1870s, was finally able to show definitively that the anthrax bacillus directly causes disease in test animals. He also developed techniques for culturing bacteria, thereby illustrating the phenomenal resilience of these specific microbes, while also proving that, in general, a host animal was not necessary for these germs to thrive. He photographed cultures, studied the conditions required for bacterial growth, and developed stains to improve their visibility. It was by employing this knowledge that he was able to identify both the *Vibrio cholerae* and the *Mycobacterium tuberculosis* that cause the deadly diseases of cholera and tuberculosis, respectively, work which earned him the 1905 Nobel Prize in Physiology or Medicine.

Vibrio cholerae



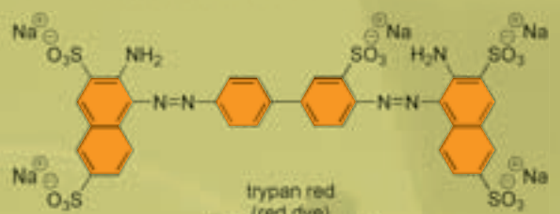
Joseph Lister Robert Koch

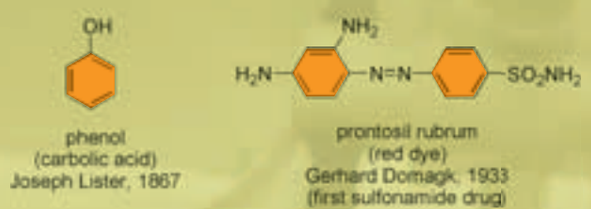
Joseph Lister, pioneer of antisepsis, attending to a patient

Paul Ehrlich

Gerhard Domagk

Box 2 Pre-penicillin antibacterial agents

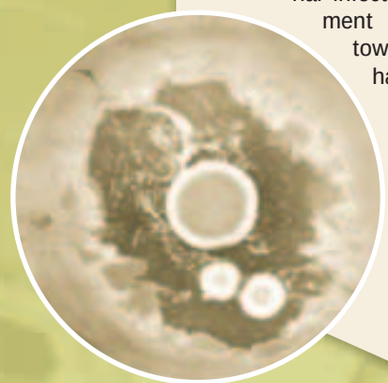




Identifying these disease-causing agents had been a long and arduous battle requiring all the energy of those distinguished scientists whose ingenious work had already revolutionized medical practice, as we have just seen. Each of these men left his own indelible mark on history, immediately contributing to saving lives, yet our story is just in its beginning. At the start of the twentieth century life expectancy in England was just forty-five years, and infant mortality was at a rate of one hundred and fifty per thousand live births. Many of the premature deaths that contributed to these appalling statistics could be attributed to fatal bacterial infections. Bacteria had been identified, routes of transmission had been elucidated, and sterilization and surgical hygiene to prevent infection had been proven, yet still no solution to the problem of successfully treating an already-infected patient was available. People had good reason to dread bacterial infections since they frequently ended with the death of their victim.

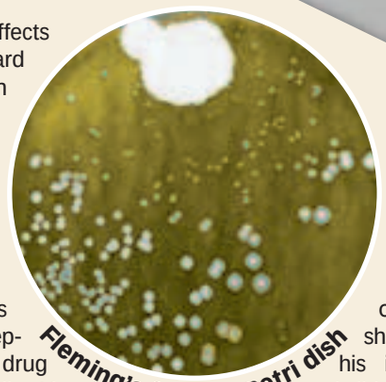
Paul Ehrlich, a friend and one-time colleague of Koch, suggested that bacterial infection might be curable by treatment with a drug that was toxic towards the bacteria, whilst being harmless to the patient, thus introducing the so-called 'magic bullet' concept. This idea had been inspired by the selective uptake of dyes into bacterial cells, a technique that had been developed for enhancing microscopy, and Ehrlich was fascinated by what influenced the disparity between various cell types. The large number of dyes produced by the German dye industry at the time afforded Ehrlich with a vast array of chemical candidates for testing. He and his Japanese colleague, Kiyoshi Shiga, eventually found that trypan red (an azo dye, Box 2) could effectively kill the bacteria *Trypanosoma gambiense*, the causative agent of the fatal sleeping sickness transmitted by the tsetse fly in Africa. However, in tests on humans in Uganda, its use resulted in unacceptable side effects ranging from blindness to death. Ehrlich is also famous for introducing the arsenic salts salvarsan (arsephenamine) and neosalvarsan (neoarsephenamine) as drugs to treat syphilis (caused by *Treponema pallidum* infection). Pain at the injection site, side effects, and frequent relapses rapidly consigned these agents to history. Nevertheless, Ehrlich's studies launched the age of chemotherapy. Ehrlich was also a pioneer in the fields of hematology and immunology, with his contributions being recognized by the awarding of the 1908 Nobel Prize in Physiology or Medicine, which he shared with the Russian Ilya Ilyich Mechnikov, "in recognition of their work on immunity."

In 1927, Gerhard Domagk, while continuing investigations in the same vein at the laboratories of I. G. Farbenindustrie, concentrated mainly on finding agents effective against haemolytic *Streptococci* (the bacteria associated with throat infections). He recommended prontosil rubrum (Box 2), a red azo dye, for clinical trials – a fortunate choice, for this compound contains the critical sulfonamide functionality and proved to be very effective against these bacteria. So it was that the first clinically used sulfonamide antibacterial agent was born, to be followed soon by many other members of this same class. The dis-



Petri dish

covery of the antibacterial effects of prontosil won Gerhard Domagk the Nobel Prize in Physiology or Medicine in 1939. His real triumph, however, was much more personal, as it emerged later that Domagk had, in desperation, used prontosil rubrum successfully to treat his infant daughter, who was dying of *Staphylococcal* septacemia, long before the drug became available to the public. After an initial period of euphoria over the discovery of the sulfonamides the febrile activity calmed and their shortcomings began to become all too apparent. Although still used in rare cases today, these antibacterials are very narrow in spectrum and essentially obsolete.



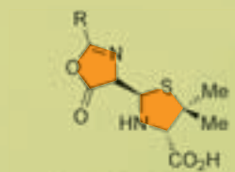
Fleming's famous petri dish

Enter Sir Alexander Fleming, the son of a farmer from rural Scotland, whose entire career could be said to have been based on a series of serendipitous happenings. In 1900, the young Alec, as he was called, joined a Scottish regiment in order to fight in the Boer War with two of his brothers. This experience honed his swimming and shooting skills, but never actually took him as far as the war-torn Transvaal region of South Africa (a province lying between the Vaal and Limpopo rivers). On returning to London, and in need of a profession, Fleming decided to follow his older brother into medicine. He obtained top scores in the qualifying examinations, giving him a free hand over which school he might choose for his studies. He elected to enroll at St. Mary's, one reason being that he had played water polo against them in a previous sporting fixture. In 1905 Fleming found himself specializing in surgery, a career choice which would require him to leave St. Mary's in order to take up a position elsewhere. The captain of St. Mary's rifle club, who relied on Fleming's flawless shooting skills, heard of his impending departure and did his best to prevent it by winning him over to his own discipline, bacteriology, thereby maintaining the integrity of his champion team. Alexander Fleming never left St. Mary's, becoming instead the world's most famous bacteriologist.

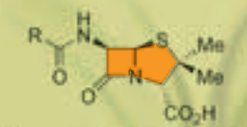
In the 1920s, Fleming identified lysozyme, an enzyme found in tears, which exhibited a natural and mild antibacterial action. This protein was the first antibiotic to be isolated from the human body, but as it was not powerful enough to attack the most prevalent and aggressive infections, Fleming continued his search. One day, so the legend goes, when he was clearing out petri dishes containing bacterial cultures that had begun to accumulate in one of his sinks, he noticed that one of the containers had a mold growing on the nutritional agar. This rather common occurrence was made fascinating by the fact that Fleming's habit of careful observation also revealed that no bacterial colonies were growing around the periphery of the fungus. Fleming went on to show that not only was bacterial growth inhibited, but that healthy bacteria underwent cell lysis and death when exposed to the mold (*Penicillium notatum*). It should be noted at this point that the full detailed tale of the discovery of penicillin has its roots earlier than Fleming's investigations.

Sir Alexander Fleming

Box 3 Highly contested structures of penicillin



oxazolone thiazolidine structure
(incorrect)

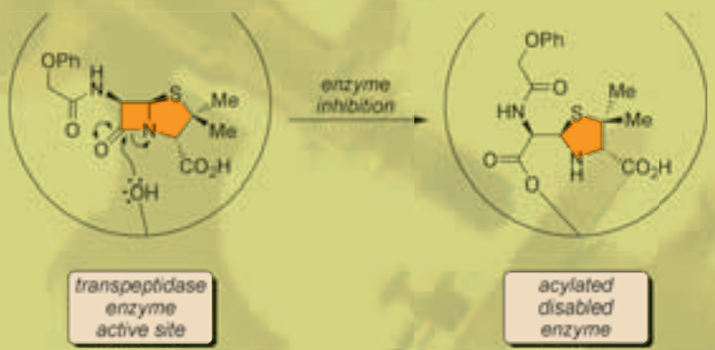


β-lactam thiazolidine structure
(correct)

Bacterial strains

Alexander Fleming and the Oxford penicillin team:
Fleming, Florey, Chain, Sanders, Abraham (left to right)

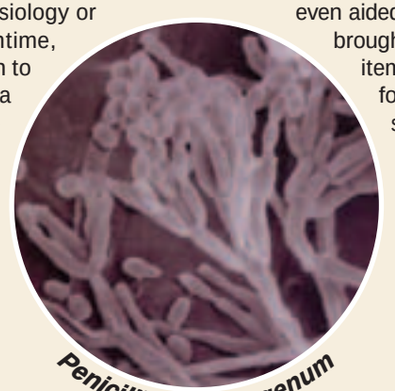
Box 4 The mechanism of action of penicillin



Both Joseph Lister and Ernest Duchesne (a French medical student) independently reported the use of *Penicillium* molds in bandages to treat infected patients at the end of the nineteenth century. These compresses were ignored by the scientific and medical communities due to their low potency until Fleming rekindled interest in them. Furthermore, Chinese medical texts dating back some 3000 years advocated the use of moldy soybean curd to treat skin infections.

At the beginning of the 1930s, frustrated by progress in advancing his discovery to the next stage, Fleming passed on some of his culture to Howard W. Florey and Ernst B. Chain at Oxford University. The Oxford group, which also included Norman G. Heatley and Edward P. Abraham, refined the growth and isolation of the penicillin extracts just enough to facilitate the instigation of clinical trials, which immediately began to deliver very promising results. However, pure penicillin was still in such short supply that it had to be recovered from the urine of patients for reuse. The spectacular success of penicillin as an antibiotic would later earn Fleming, Chain, and Florey the 1945 Nobel Prize in Physiology or Medicine. In the meantime, the elevation of penicillin to its legendary status as a world-changing antibiotic would require the launching and successful execution of one of history's most captivating international scientific adventures, the so-called Penicillin Project.

With the outbreak of World War II, interest in penicillin had intensified. Numerous scientists strove to produce the antibiotic on a large scale in response to an urgent new need for the drug to treat wounded soldiers and civilians who had subsequently contracted infections. The proximity of the battle frontline, the frequent aerial bombardments of the UK, and the need for a rapid solution to the problem led to a huge Anglo-American collaborative project on penicillin. The Rockefeller Foundation in New York arranged for Florey and Heatley to come to America in 1941 to meet with Charles Thom, chief mycologist at the US Department of Agriculture. A two-pronged strategy for the procurement and development of penicillin was immediately formulated. The first approach was directed towards the elucidation of the structure of penicillin, which would make the ultimate goal of its chemical synthesis at least conceivable. Upwards of forty independent laboratories and hundreds of chemists became involved in this labyrinthine task. The second line of attack was directed towards further improving the fermentation process for production of the drug. The latter of these pivotal works was relocated to the US Agriculture Department Laboratories in Peoria, Illinois. Here an intense research program drew on a myriad of sources to find extra momentum; in this eclectic project, progress was even aided by local residents who brought moldy household items to the laboratories for investigation in the search for more productive penicillium strains. It was the second of these two approaches that paid



Penicillium chysogenum

Ripening cantaloupe on a vine

the earliest dividends. Thus, a much-improved yield of penicillin was secured in the 1940s from *Penicillium chrysogenum*, a discovery made courtesy of a moldy cantaloupe melon brought into the laboratory by Mary Hunt, an employee at the Peoria laboratories. Within three years, twenty-nine plants were fermenting this high-yielding fungal strain to produce penicillin using a corn-steep liquid medium (a by-product of the massive Mid-West corn production) in an amazing effort organized by an extraordinary conglomeration of scientific establishments. The pharmaceutical companies of Merck, Pfizer, Squibb, and Abbott were all involved, along with leading British and American academic and governmental institutions.

General Dwight D. Eisenhower began the invasion of Europe from the southern shores of England on D-Day supported by some three million doses of penicillin (300 billion units or approximately 180 tonnes), the product of one of the most exciting and lucrative joint ventures in history. Only the notorious Manhattan Project directed towards the development of the atom bomb exceeded it in magnitude during that period. Thus, in less than four years, scientists had gone from recovering penicillin from patients' urine, due to its short supply, to the phenomenal level of production whereby 1,633 billion units of penicillin were produced in 1944 alone. The tremendous effort extended towards the development of penicillin may be less familiar to us than the Manhattan Project, but it is easy to argue that the former collaboration yielded far greater benefits to mankind. Overall, this venture succeeded admirably in optimizing fermentation and production protocols, allowing for a successful and practical supply of the new miracle drug, ultimately saving countless lives. The process, however, still relied on natural biosynthesis of the intact penicillin molecule, a fact that limited investigations into producing analogs with enhanced activity, especially to combat the new demon of bacterial resistance that was just beginning to rear its ugly head. These imperatives stipulated urgent attention be paid to finding a chemical synthesis.

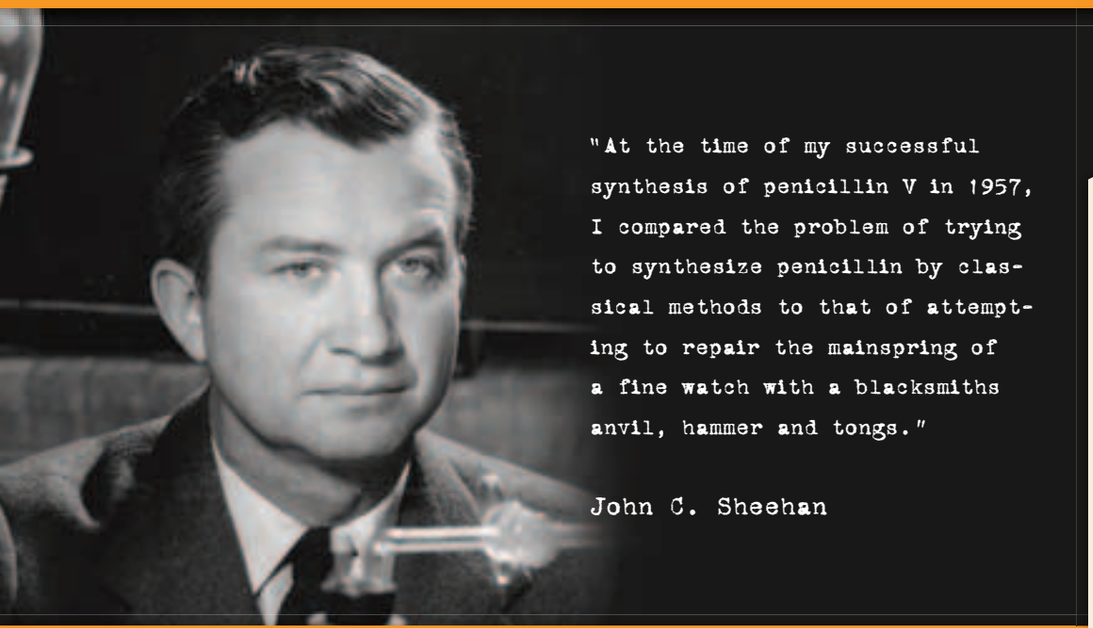
Before a chemical synthesis could be attempted the non-trivial task of deconvoluting the molecular structure of penicillin had to be accomplished. From the ardent debate amongst many of the most renowned chemists of the time, two possible structures emerged in the early 1940s as leading contenders for the honor of representing the magical molecule of penicillin (see Box 3). The so-called oxazolone-thiazolidine formula was proposed by Sir Robert Robinson (Nobel Prize in Chemistry, 1947, see also Chapter 7) and fiercely defended by him, as well as by a number of other notable chemists such as Sir John Cornforth (Nobel Prize in Chemistry, 1975). Its β-lactam rival was advocated by Merck scientists and by the Oxford axis of Abraham and Chain. Despite

X-Ray crystallography

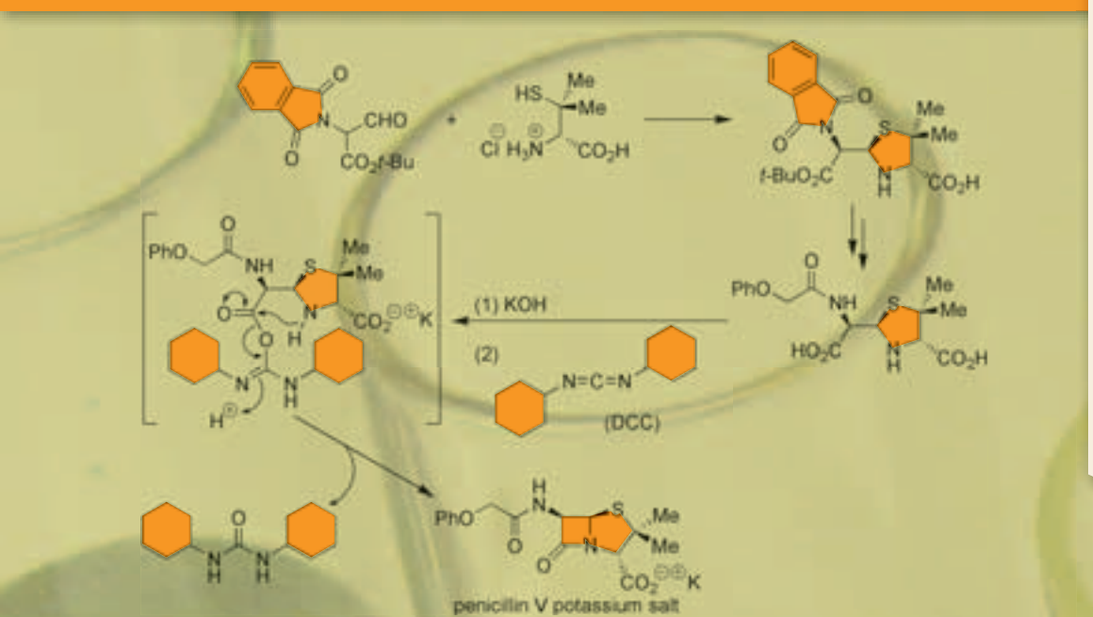
Dorothy Crowfoot Hodgkin and husband

General Dwight D. Eisenhower (left) conferring with General Bernard Montgomery

Box 5 Sheehan's thoughts on the total synthesis of penicillin V



Box 6 Sheehan's total synthesis of penicillin V



experimental evidence for the existence of a β-lactam structural motif provided by the Merck scientists, conventional wisdom could not accept the presence of such a strained and reactive feature within a naturally occurring substance. It was only after the brilliant crystallographic work of Dorothy Crowfoot Hodgkin that the dispute was finally settled in favor of the β-lactam structure in 1945, pleasing its backers and winning fame for the unobtrusive crystallographer from Oxford.

The striking four-membered β-lactam ring of penicillin, which was so decisively revealed by Hodgkin's crystallographic analysis, also turned out to be the motif that was responsible for the lethal action of the drug against bacteria. This activity was found to be related to the conformation adopted by penicillin, wherein the fused 4,5-ring system enforces an orthogonal alignment of the nitrogen lone pair and the carbonyl π-bond such that the resonance stabilization exhibited by traditional amides cannot be attained in this case. This feature, in combination with the intrinsic strain of the four-membered ring, creates a situation where the carbonyl functionality of the β-lactam ring acts as a highly effective acylating agent due to its particularly strong electrophilic reactivity. Thus, it is now known that penicillin irreversibly acylates the bacterial transpeptidase enzyme responsible for the cross-linking reaction which unites the terminal glycine residue of a pentaglycine strand with the D-alanine residue of a neighboring pentapeptide, in an indispensable step during the construction of bacterial cell walls (Box 4). The acylation process deactivates this cross-linking enzyme, thereby compromising the integrity of the bacterial cell wall, resulting in rapid cell death. Unlike bacteria, only a phospholipid membrane surrounds mammalian cells, so transpeptidase inhibition is completely selective for bacterial cells. It has been shown that the penicillin molecule adopts an overall conformation that is very similar to the D-alanine-D-alanine residue of the substrate involved in this chain elongation process, thus it gains ready access to the active site of the enzyme where it reacts to disable its host.

The biosynthesis of penicillin, including the unprecedented β-lactam ring, was elucidated through a series of brilliant chemical and biological studies, many of which were carried out by Sir Jack E. Baldwin and his group at Oxford University. In addition to this seminal work, Baldwin is also known for his contributions to biomimetic synthesis, as well as for a set of rules he devised to predict the outcome of certain ring-closing reactions.

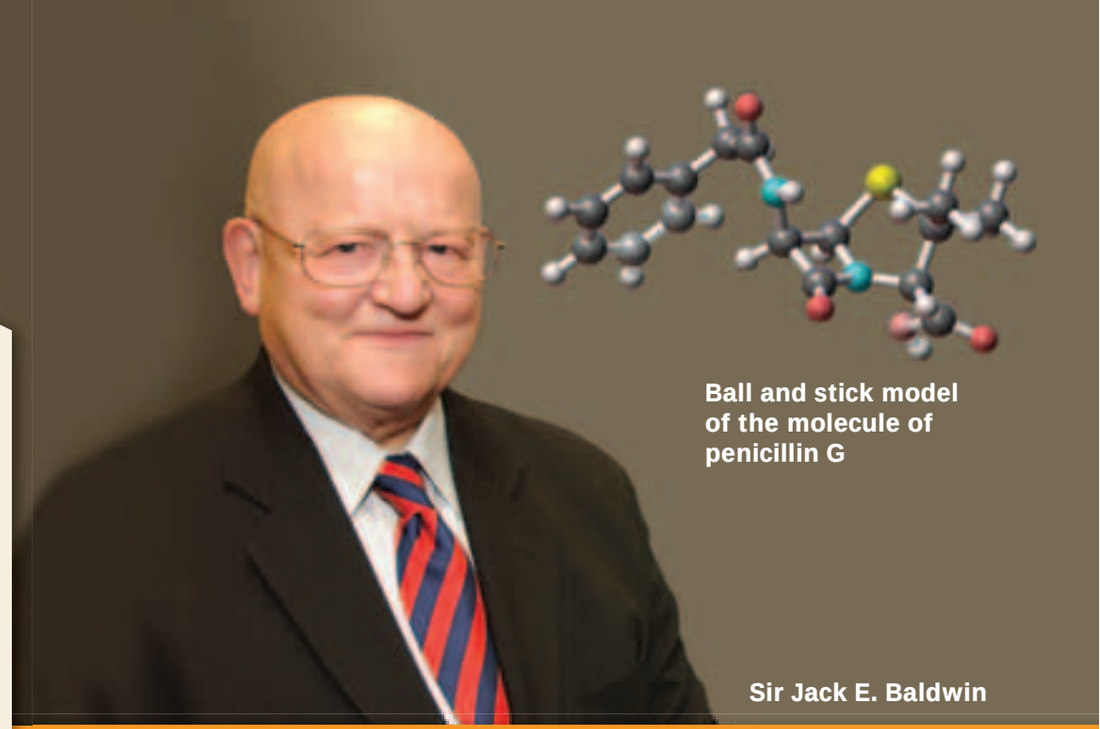
Ironically, while the biological activity of penicillin relies on the characteristic lability of the β-lactam ring, it is this same feature that led most synthetic organic chemists of the wartime period to consider penicillin an impossible target to conquer by chemical synthesis. Indeed, despite a huge effort directed towards its synthesis, both during and after World War II, no success was reported until much later. Indeed, it was 1957 before John C. Sheehan at the Massachusetts Institute of Technology (USA) was able to announce triumphantly the total synthesis of penicillin V, the result of a relentless ten-year campaign (Boxes 5 and 6).

Sheehan's celebrated success was due to his innovative and daring approach. He

had recognized very early on that the main hurdle to be overcome prior to any total synthesis of penicillin was the construction of the highly strained and sensitive β-lactam ring. In addition, it was clear to him that a suitable method for accomplishing this challenging ring formation had to be developed since none of the existing technologies could be expected to rise to the challenge. It is here that Sheehan's insight and brilliance ensured his team's triumph. He conceived of and developed the *N,N′*-dicyclohexylcarbodiimide (DCC)-mediated coupling of carboxylic acids with amines to afford amides. Applied in its intramolecular version (wherein the amine and acid both belong to the same molecule) on an appropriately functionalized precursor, this method would solve one of the most recalcitrant problems in chemical synthesis of the 1940s and 1950s – the construction of the β-lactam ring of penicillin. The DCC coupling reaction, later extended to provide a solution for ester bond formation, remains a powerful synthetic tool in contemporary organic synthesis, and has been the inspiration for many similar reactions. This innovation was not the only one made by Sheehan during the penicillin synthesis; the phthaloyl protecting group for primary amines was another important and enduring contribution emanating from his group.

With these two innovations at their disposal, the Sheehan group was able to complete the synthesis of this previously impossible target through the sequence briefly outlined in Box 6. Thus, coupling of a phthaloyl-protected amino aldehyde with a suitable amino thiol led to the construction of the thiazolidine ring of penicillin. Further elaboration furnished an advanced amino diacid intermediate which served as the precursor for the β-lactam ring of penicillin V. Indeed, exposure of this precursor to Sheehan's DCC coupling conditions provided penicillin V as its potassium salt. So it was that the first total synthesis of penicillin was accomplished, a milestone event in the history of the art of total synthesis. This feat marked not only the beginning of a highly productive era in the synthesis of β-lactam derivatives, many of which were subsequently synthesized, but also represented the addition of a new dimension to total synthesis endeavors, that of seeking to invent new synthetic technologies along the way. The task of developing this paradigm further, taking it to impressive new heights, would be assumed by one of Sheehan's students, the now highly celebrated master of this science E. J. Corey, about whom we will learn much more later in subsequent chapters.

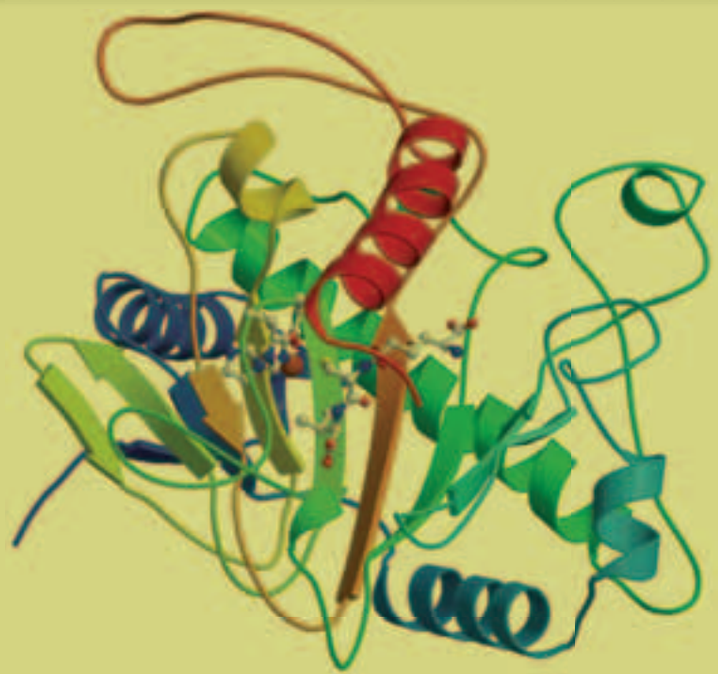
Penicillin ushered in a new epoch in antibiotic research, one that grew by leaps and bounds over the ensuing decades. A series of new naturally occurring β-lactams such as cephalosporin C, clavulanic acid, and thienamycin were delivered in quick succession for use as drugs (Box 7). As soon as these discoveries had been made in microbiology laboratories, synthetic chemists busily focused on synthesizing the newly discovered natural products and modifying their structures in an effort to discover new antibacterial agents with improved pharmacological profiles. Their work led to an equally impressive collection of synthetic or semisynthetic β-lactam antibiotics including ampicillin, amoxycillin, and methicillin (Box 7). During this golden



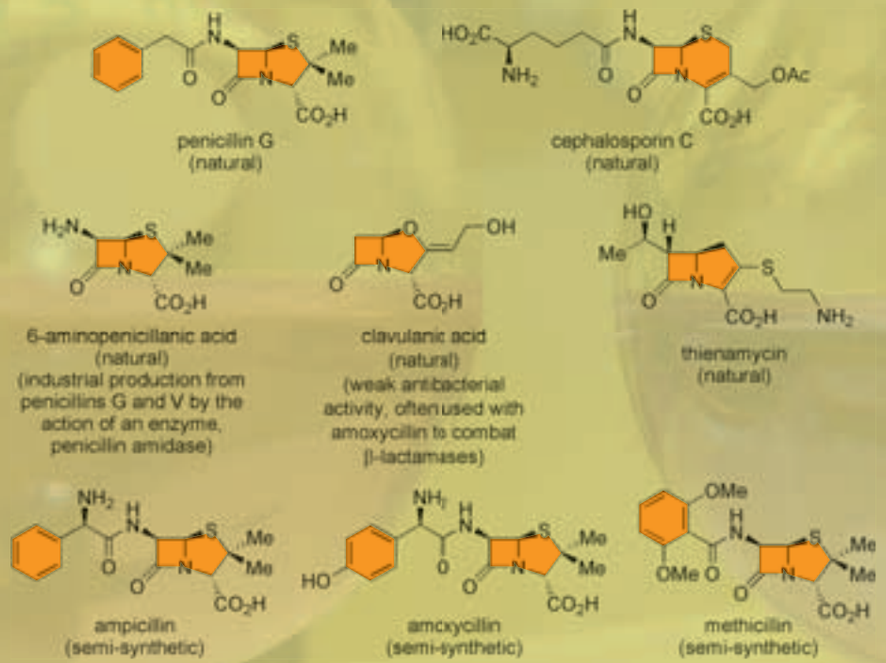
Ball and stick model of the molecule of penicillin G

Sir Jack E. Baldwin

Ribbon model of isopenicillin *N*-synthase



Box 7 Molecular structures of various β-lactam antibiotics



era for antibiotics, however, came some disturbing news. Their widespread use, and sometimes misuse, led to the rapid evolution and spread of antibiotic-resistant bacterial strains. A new menace for humanity was now looming on the horizon!

Bacterial strains have evolved the capability to evade the action of β-lactam antibiotics by producing an enzyme, called β-lactamase, which can cleave the β-lactam ring, thus deactivating the molecules before they reach their site of action. To combat these newly acquired enemies, scientists have since sought out and developed various novel antibacterial agents; some of these powerful new antibiotics came from nature and some emerged from totally manmade designs. The story of drug-resistant bacteria, and of the antibiotics used to defend us against them, will be revisited in the chapter introducing vancomycin (Chapter 31), a natural product that is today the lifesaving drug of last resort in cases of severe infection.

We must conclude this account by underscoring once again the profound significance of the discovery of penicillin. Besides constituting a landmark medical breakthrough that saved lives and alleviated human suffering, this fortunate event also revealed to scientists a new treasure trove of biologically active molecules ripe for exploration. Thus, to the forest, which held the key to the development of Aspirin®, we now add the kingdom of microbes as a rich hunting ground for molecules endowed with healing powers. Indeed, many such molecules have since been isolated from the soil and other habitats where bacteria and fungi hold sway and, from this bountiful harvest, scientists have derived a host of 'magic bullets' and billion dollar drugs, as we shall see in forthcoming chapters.

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Further Reading

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