Antibacterial Warfare: The Production of Natural Penicillin and the Search for Synthetic Penicillin During the Second World War

B Short

Abstract

Begun in 1940 during World War II, the research into natural penicillin later involved British, United States and Australian military forces in carrying out the necessary extensive field testing. Penicillin-G and penicillin-V are the two natural penicillins whose chemical structures were ultimately uncovered in the US and Britain, respectively. The difficulties in determining the beta-lactam ring structure greatly hampered the second wartime project, to synthesise penicillin, a program not completed until 1957. Wartime military organisation and joint scientific collaboration integrated with large dedicated funding grants permitted the realisation by 1944 of adequate allied stockpiles of natural penicillin, a feat not thought possible in the absence of a wartime imperative. Included are brief biographies of the principal three wartime Oxford scientists.

Preamble

The international penicillin program was one of the largest wartime initiatives and among the most significant achievements in science and technology during World War II. Penicillin production went from laboratory microbiological study in 1940 to mass production by 1945. This commentary examines the participation of military medical services in the wartime development of mass-produced natural penicillin by fermentation processes and the establishment of suitable clinical testing forums. Included is a brief professional biography of the three principal Oxford experimenters following the launching of penicillin within clinical medicine.

Historically military associated improvements to the health of twentieth-century populations did not start and end with the discovery of antibiotic therapy, a theme that Roger Cooter canvassed in *Medicine and the Goodness of War.*[1] Equally important was the groundbreaking development of an effective suppressive chemoprophylaxis agent against malaria by schizonticide drugs that proved lethal to the asexual blood stages of *plasmodia,* the cause of *falciparum* and *vivax* malaria. Innovative reconstructive surgical techniques by military surgeons in both World Wars readily spilled into the domain of civilian critical trauma therapy.

The number of lives saved from the effects of bacterial infections associated with conflict by the appropriate exhibition of penicillin therapy during the latter half of World War II is incalculable. During the Great War, between 12% and 15% of the wounded treated in front-line hospitals died of infections, most notably from gas gangrene. The mortality rate from this infection during the subsequent World War was significantly reduced to 3%.[2] Battlefield case fatality rates have slowly declined throughout the twentieth and twenty-first centuries, from 19% in World War II (1939–1945) to 15.8% in the Vietnam War (1955–1975), down to 9.4% during each of the Iraq War (2003–2011) and the War Against Terror fought in Afghanistan (2001–2014).[3] Despite the combination of immediate parenteral antibiotic administration, vigorous intravascular fluid replacement and rapid helicopter evacuation to a combat-surgical facility, infectious complications remain a leading cause of both morbidity and mortality in combat-injured personnel.

Dr Alexander Fleming's penicillin

Penicillin is the 1928 name designated by physician-microbiologist Dr Alexander Fleming FRS, later Sir Alexander, to the filtrate of the mould broth that had been accidentally contaminated by the green mould fungus, *Penicillium notatum.* Eric Lax, in *The Mould in Dr Florey's Coat,* details Fleming's work in the Inoculum Department at St Mary's Hospital, London that he joined in 1906.[4] Lax questions the quaintly serendipitous explanation offered by Fleming, written 16 years after the event in 1944, of how a mould spore entered through the open laboratory window and settled on an uncovered Petri dish containing a culture of *Staphylococcus aureus.* The mould elaborated a lethal toxin in response to bacterial growth as a primitive response to a threat to a food source. When food in the microbiological microcosm is scarce, the mould expresses an intense amount of penicillin. Repeated attempts by Fleming and others to replicate the growth of penicillin under the conditions Fleming described failed.[4] Nonetheless, the mould broth filtrate inhibited the in vitro growth of staphylococci. Fleming documented in a 1929 paper the in vitro antibacterial properties of penicillin but not tests of the filtrate in vivo using experimental animal models.[5] He found that the antibacterial principle in the concentration used, was not toxic either to white cells or rabbits, nor was it a protein.[6] The paper was largely ignored until 1938 when the Australian physician Howard Florey, German biochemist Ernst Chain FRS, later Sir Ernst and English biochemist Norman Heatley commenced work at Oxford investigating the antimicrobial enzyme, lysozyme. Lysozyme is found in tears, saliva, milk, mucus and white cells and was first isolated by Alexander Fleming in 1922. In August 1940, Florey's Oxford team reported the testing of penicillin in vivo using haemolytic streptococcus-infected mice as the experimental model.[7] By early 1941, sufficient penicillin had been produced by surface culture to attempt treatment in six patients suffering severe streptococcal infections. The initial clinical applications were unfortunately flawed due to using inadequate drug dosages consequent to supply constraints.

British-American Research Cooperation

Unable to convince British pharmaceutical interests to assist in the further manufacture of penicillin, Florey and Heatley travelled in July 1941 to the United States. The mission was to seek techniques to improve penicillin production to permit formal clinical drug trials. They met with the US Department of Agriculture's Northern Regional Research Laboratory (NRRL) in Peoria, Illinois. The NRRL had pioneered the use of submerged culture fermentation techniques whereby the so-called 'deep fermentation' process allowed for a marked increase in surface area for mould growth. The culture was grown throughout the medium rather than on the surface, which the British scientists had relied on, thereby enhancing the mould output of penicillin. In addition, the NRRL workers suggested culture medium additives and the isolation of higher-yielding *Penicillium* strains.[8]

In June 1941, President Roosevelt issued an executive order establishing the Office of Scientific Research and Development (OSRD) and its agency, the Committee on Medical Research (CMR), created entirely for military purposes. In addition to penicillin, the wartime output by CMR included antimalarial drugs, insecticides, blood and blood substitutes and novel aviation medicine techniques. An outstanding achievement involved encouraging the widespread use of DDT to control malaria and dengue-carrying mosquitoes and the killing of typhus-bearing body lice. Today US authorities regard the synthetic insecticide DDT as a probable human and animal carcinogen.[9]

A pivotal meeting on 7 August took place with Florey and the new chair of the CMR appointed by President Roosevelt, Professor Alfred Newton Richards, professor of pharmacology at the University of Pennsylvania, who became a strong supporter of Florey's venture.[2] After leaving the CMR in 1946, Richards was elected President of the National Academy of Sciences for the term 1947 to 1950. Focusing primarily on the clinical testing of penicillin, Richards immediately commenced the difficult task of convincing US pharmaceutical interest in the worthwhile investment to manufacture penicillin. The long-term outlook for producing penicillin by the fermentation process was considered doubtful due to rising costs and low yields. This, more than anything else, provoked Richards to launch a new pharmacological project: the synthesis of penicillin. Initial drug firm resistance to natural penicillin production was largely overcome with the release in December 1941 of high-yield drug data from the NRRLs deep fermentation processes. The first pharmaceutical chief to commit to penicillin manufacture was George W Merck, with the heads of Squibb, Pfizer and Abbott later signing to undertake US production. The president of Merck & Co, George W Merck in August the following year was appointed by the Secretary for War, Henry L Stimson, to the innocently titled War Research Service as the inaugural director, a facility for researching biological warfare agents.[10]

The leading research organisation in England tasked to meet the medical needs of Britain's fighting services was the Medical Research Council (MRC), established by the Privy Council in 1920. It became the duty of the two agencies, the ORSD in the US and the MRC in Britain, to organise, promote and subsidise penicillin research as a matter of national defence.[8] Early US penicillin outputs were small, with quantities at the end of 1942 only sufficient to treat 100 patients. An agreement was reached in the US that the entire supply of the antibiotic should be turned over to the CMR, and from the small stock, a select group of accredited investigators were permitted access to the drug.

Early penicillin therapy in clinical practice

In November 1942, one of the most important early penicillin testing took place, treating the survivors of, until then, one of the worst civilian disasters in American history. After the World Trade Centre bombing in September 2001, the second-worst building fire ever recorded in the US took place at the popular Cocoanut Grove Nightclub in Boston on the night of 28 November 1942. Fire enveloped the brick building, housing nearly twice the legal patron capacity, with the loss of 492 lives and 173 survivors with extensive burns. Permission was granted immediately to treat 13 burns cases by prescribing an empirical dose of 5000 units of penicillin administered 4-hourly. The penicillin supply came from an emergency order by the CMR for the Merck Corporation to produce 32 litres of the drug. Sulphadiazine was co-administered with the penicillin, in a dose later determined as inadequate. Unfortunately the low-dose penicillin regimen caused an inability to accurately analyse the efficacy of the therapy.[2]

The first civilian case to receive penicillin therapy within the US was at Yale New Haven Hospital five months earlier, on 14 March 1942. A young female with beta-haemolytic streptococcal sepsis received a course of penicillin provided by Dr Heatley during his stay in the US. The patient rapidly responded and survived to the age of 90.[11] During early 1943, the first opportunity for US military testing of penicillin arose from a request by the Bushnell General Hospital in Brigham, Utah, accommodating over 200 war-related orthopaedic patients. The majority of the cases had compound fractures complicated by osteomyelitis, for which sulphonamide therapy was uniformly ineffective. No clinical trial was undertaken, and the penicillin-testing program was classed as an 'intent to treat'. The drug was administered exclusively to wounded soldiers, given parenterally and locally, in optimum dosages with diligently recorded side effects. Adverse effects were unfortunately common due to the high levels of contaminants within the penicillin solutions containing concentrations of impurities that varied from batch to batch. Phlebitis was a particularly common problem following intravenous administration together with local pain with induration in the wake of the intramuscular route. Filtration of the penicillin solutions immediately prior to injection led to a marked diminution of the unwanted reactions. The results of the Bushnell military penicillin testing were so encouraging that the US Army now regarded penicillin as vital to the war effort. A second test centre commenced in June 1943 at Halloran General Hospital, Staten Island, New York, where a further 200 wounded Army personnel were treated. Excellent drug responses were again recorded, prompting the Army to use both hospitals as penicillin therapy training schools for US Army medical officers.[2] By October 1943, the total US penicillin output had increased from two to nine billion units a week.

The first large supply of penicillin from the US to Britain was delivered in May 1943. However, Britain had conducted tests among wounded Army cases at Cairo since July 1942 during the North African campaign, using penicillin supplied by the Oxford group. The clear message from the early British testing was that administering penicillin weeks after the wounding was far too late. All subsequent British Army investigations were then moved to forward military hospitals in North Africa. From the same theatre of war, sexually transmitted disease records were also collected on the predictable beneficial effects of penicillin in sulphonamide-resistant gonococcal infections.

Synthetic penicillin studies at Oxford

Ernst Chain was joined by another biochemist, Edward Abraham FRS, later Sir Edward, to forge the Oxford scientific inquiry to synthesise penicillin, with tasks to purify natural penicillin and to determine its molecular structure. Three reasons arose for implementing a second major project to discover the synthetic pathway of penicillin side by side with solving the problems of natural penicillin mass production. The first involved the variably high levels of contamination of the penicillin cultures produced by deep fermentation. The second was the perennial problem of low yields coupled with immense production costs. Based on the cost of one gram of crude penicillin material of at least US$100, containing on the average only 5% pure penicillin, compared with the costs as late as April 1944 reported as one pound of pure penicillin costing approximately US$45 000 to manufacture.

Finally, biochemists on both sides of the Atlantic were initially quite optimistic that a synthesis was entirely feasible and achievable within a practicable time frame.[8]

By 1943, coordinated transatlantic research into the biochemistry and synthesis of penicillin was well established. Abraham and Chain, in late 1943, identified the novel beta-lactam four-numbered chemical ring attached to a side-chain as the definitive structure of penicillin. However, workers at the Merck laboratories in the US were unable to confirm this particular chemical structure. Indeed, the penicillin grown by cultures at Oxford and the US was different, suggesting the existence of two separate molecular forms of penicillin. By January 1945, X-ray crystallography at Oxford confirmed the beta-lactam ring model as the structure of penicillin-V. At the beginning of 1945, the US monthly production of natural penicillin was already 40 to 50 times greater than when the program began and represented the combined outputs of three companies, Merck & Co at Rahway, New Jersey, Pfizer at Brooklyn, New York and Squibb at Chicago, Illinois. However, by December 1945, no commercially practical synthetic process for penicillin production had been developed, prompting the US government to withdraw support.[8] The final chapter in the wartime search program for synthetic penicillin would await 1957 when an American organic chemist, Professor John C Sheehan at the Massachusetts Institute of Technology, developed the first complete synthesis of penicillin.[8]

Australian Army experience with natural penicillin

Following early penicillin fieldwork in 1943, the Australian Army Medical Corps first became involved in penicillin testing during March 1944, carried out at the Heidelberg Military Hospital, Melbourne, utilising penicillin produced at the state-owned Commonwealth Serum Laboratory (CSL) at Parkville, Melbourne. The CSL was under the wartime leadership of Army Captain Percival (Val) Bazely, a former CSL technical officer, on appointment by a colleague Colonel E V (Bill) Keogh, then Army Director of Hygiene and Pathology. Bazely and a small staff visited the US pharmaceutical companies during September 1943 and were introduced to the deep fermentation processes. Creditably, the laboratory produced a high grade, safe and reliable penicillin solution that was soon shipped to the New Guinea theatre of operations.[12]

The first Australian trials with military personnel were made using the sodium salt and the crude and refined preparations of the calcium salt administered to 141 patients. Dosage regimens included 200 000 units daily via intramuscular or intravenous routes and topical application into body cavities of up to 20 000 units. Toxic effects were virtually absent and without febrile reactions to penicillin. Initially, due to low stocks and re-supply, penicillin was limited to administration to hospital cases and then only with specific clinical indications. Later, field testing in forward areas within the South-West Pacific Area was undertaken to enable the primary suturing of wounds after cleansing, debridement and the topical application of penicillin powder. It soon became very obvious that penicillin could be employed to make operative procedures safe, or indeed possible, and mechanical fixation of fractures became virtually uncomplicated.[13] In *Medicine and Victory* (2004), Professor Mark Harrison observed that, 'with the sole exception of the official histories, there have been no books on medicine in any of the British armed services'.[14]

Australian physician Allan Walker's *Clinical Problems of War*, written in 1952, documents the medical experiences of the Australian Armed Forces during World War II and is thereby, according to Harrison, an unusual yet highly informative account of this important subject.[13]

German encounters with microbicidals during the Second World War

The antibiotic drug experience of the German armed forces during the Second World War, an important theme that Neushul omitted from his 1998 discourse, is in stark contrast to that of the allies.[2] A leading reason why the Germans were slow to develop penicillin was their long-standing commitment to sulphonamides that had been an original German chemical development. German experimental pathologist Professor Gerhard Domagk, working with the I G Farben companies, patented the sulphonamide azo-dye *Prontosil* in 1932 after demonstrating it possessed significant in vivo antibacterial activity. He published his findings in February 1935 in the pre-eminent German medical journal, the *German Medical Weekly*, entitled 'A Contribution to the Chemotherapy of Bacterial Infections'. The sulphonamide drugs, inhibiting bacterial growth, became the forerunner of the antibiotic class, drugs that killed bacteria. Domagk's work with sulphonamide synthesis won him the Nobel Prize for Physiology or Medicine in 1939, an honour that the German political regime forced him to refuse, but which he graciously accepted after the war.[15] The frequent toxic side effect profile and the rapid development of bacterial resistance exhibited

by sulphonamides proved to be serious limitations to their widespread use. However, incongruously, penicillin was administered several times to the lifelong hypochondriac Adolf Hitler by his treating physician Dr Theodor Morell, a doctor also well known for employing unconventional treatments.[16] Although work on penicillin commenced in Germany as early as 1942 at the Hoechst Dye Works, by the Normandy landings in June 1944, the company could only produce enough penicillin to use as dusting powder for superficial wounds. As late as October 1944, German supplies of injectable penicillins first became available in very limited amounts.[17]

Brief biographies of the principal wartime Oxford experimenters

To mark the one-hundredth anniversary of the birth of Lord Florey OM FRS, Professor Henry Harris delivered the Florey Centenary Lecture at Oxford on 19 September 1998.[16] Harris records that on the recommendation of Florey, a former South Australian Rhodes Scholar and newly appointed professor of pathology without any experience in the study of antibiosis, offered Ernst Chain the first position in the laboratory. An experienced biochemist, Ernst Chain was a Jewish refugee from Hitler's Germany who left in 1933 and joined Florey in 1939, by then head of the prestigious Sir William Dunn School of Pathology at Oxford. Chain completed the ongoing work on lysozyme and undertook a review of other known antibacterial substances. He proposed three candidates for further in-depth study: one was penicillin. On scant evidence, Harris reported that it was Chain who first proposed that penicillin should be further investigated.[6] The first published scientific paper on penicillin appeared in August 1940, documenting inter alia the consequences of two strokes of luck. The first involved Chain and Florey's work with mice injected with crude extracts of penicillin. The scientists' choice of mice was serendipitous since penicillin given to guinea pigs invoked unfavourable reactions. The second good fortune was that the impure penicillin contained no major toxic contaminants.[6 19]

Exerting excellent personnel management skills, the energetic Florey quickly assembled a collaborative team at Oxford. Though not considered by Harris as a profound visionary, Florey had one supreme virtue, he knew exactly what had to be done next, and he got it done. Norman Heatley, a biologist and biochemist, was the next to be invited to join the group, later introducing several important and major innovations. Heatley devised ways of measuring the activity of penicillin in fermentation liquors when present in concentrations far too low for conventional chemical evaluating methods. His cylinder plate diffusion technique provided a much easier, more reliable and sensitive assay for penicillin that was later adopted as the standard assay technique for antibiotic activity. His method for extracting the highly unstable penicillin from very dilute and heavily contaminated solutions by constructing a solvent-to-water transfer cycle, permitted impure but stable penicillin to be prepared from mould culture fluid. Due to heavy wartime restrictions, Heatley had little or no access to purpose-built apparatus, leaving him no option other than to improvise. He designed 400 rectangular ceramic vessels that were stackable and in which the medium could be changed. Finally, it was left to Heatley to monitor the first experiment in which the protective effect of penicillin was assayed in mice infected with streptococci.[19 6 20]

Howard Florey's recognition by the international scientific world was predictable and laudatory. He had been elected a Fellow of the Royal Society as early as 1941, when aged 43, not for his work with penicillin, but for work on the circulation of lymph and the secretion of mucus.[19] He was appointed knight bachelor along with Alexander Fleming in the King's Honours List in June 1944. The surrounding media-linked publicity of this event paradoxically focused almost entirely on Fleming, much to Fleming's chagrin. In 1960 Florey was elected President of the Royal Society and further acknowledged by the monarch on his appointment to the Order of Merit. In 1965 Sir Howard Florey was ennobled Baron Florey of Adelaide and Marston, and during the same year, he was invited to be the Chancellor of the Australian National University. It is said that the chancellorship was for him a deeply satisfying distinction.[20]

The first post-war Nobel Prize for Physiology or Medicine was awarded at the Nobel Institute in Oslo on a winter's afternoon, the 10 December 1945, to three joint laureates, Fleming, Florey and Chain. Nobel's will describes the prizes as awards for a discovery 'conferring the greatest benefit to mankind'. Writer Ronald Bentley commented that one measure of scientific achievement is developing a major school that creates significant and sustained conceptual and/or experimental advances and serves as a training ground for future scholars. In Bentley's opinion, only Florey had the strongest credentials of this kind.[19] The Nobel Committee's prize may be awarded to a maximum of three recipients. In all the narratives on the history of penicillin, Norman Heatley's name seems never to have been mentioned as a likely recipient of prestigious awards. Indeed Heatley's later proposal for a Fellowship of the Royal Society was also declined, adding to the appreciable injustice. The nation finally acknowledged him in

1978 on appointment as an Officer of the Order of the British Empire. More importantly, yet as late as 1990, on the occasion of the fiftieth anniversary of penicillin's development as a therapeutic drug, the 78-year-old Heatley was formally recognised. Within the 800 year history of Oxford University, Norman Heatley was awarded the university's first honorary Doctorate of Medicine. Dr Heatley continued working at the Sir William Dunn School of Pathology through to retirement, dying in 2002 at the age of 92.[20]

Ernst Chain became an expert in the developing applied science of industrial microbiology. Manifesting a flamboyant character, at times a cause of controversies within the laboratory, that ultimately prompted his departure from Oxford in 1948 to take up an academic post in Rome. The same year he married a fellow biochemist, Anne Beloff. His work with penicillin fulfilled the requirements of providing 'contributions to the improvement of natural knowledge'[21], Chain was successfully proposed for Fellowship of the Royal Society in 1949. Later in 1964 he took the Chair of Biochemistry at Imperial College, London and continued to consult with leading pharmaceutical companies. He was knighted in the 1969 honours list and died ten years later in County Mayo, Ireland, aged 73.[4]

Conclusion

Penicillin-G (benzyl-penicillin) and penicillin-V (phenoxymethyl-penicillin) are the two natural penicillins whose chemical structures were uncovered during World War II in the US and Britain, respectively. Unhappily, the difficulties in determining the beta-lactam ring structure significantly hampered the wartime synthetic penicillin production project. Today, high-resolution mass spectrometry and nuclear resonance spectroscopy can identify chemical structures within rapid time frames. Consequently, a raft of semisynthetic and synthetic penicillins appears within international drug compendiums. Wartime military organisation and scientific collaboration integrated with large dedicated funding grants permitted the realisation by 1944 of an adequate stockpile and supply of natural penicillin, sufficient for both national military and civilian needs. The rapid technological developments in the production and supply of natural penicillin between 1940 and late 1945 could not have been possible without a wartime imperative.

To encapsulate the immense contribution made by each of the 1940 Oxford tri-national experiment triumvirates, Professor Henry Harris explained, 'without Fleming, no Chain or Florey; without Chain, no Florey; without Florey, no Heatley; without Heatley, no penicillin'.[6]

The benefits to the health of humankind provided by the evidence-based prescription of an expanded penicillin class of antibiotics remains assured.

Corresponding Author: Air Vice-Marshal Bruce Short AM RFD (Ret'd)shortbandj@bigpond.com
Authors: B Short[1]
Author Affiliations:
1 Independant Researcher

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