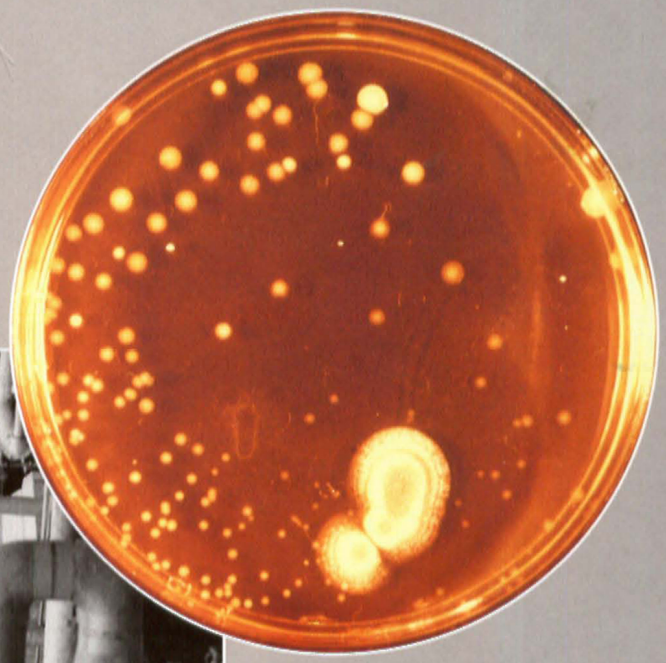
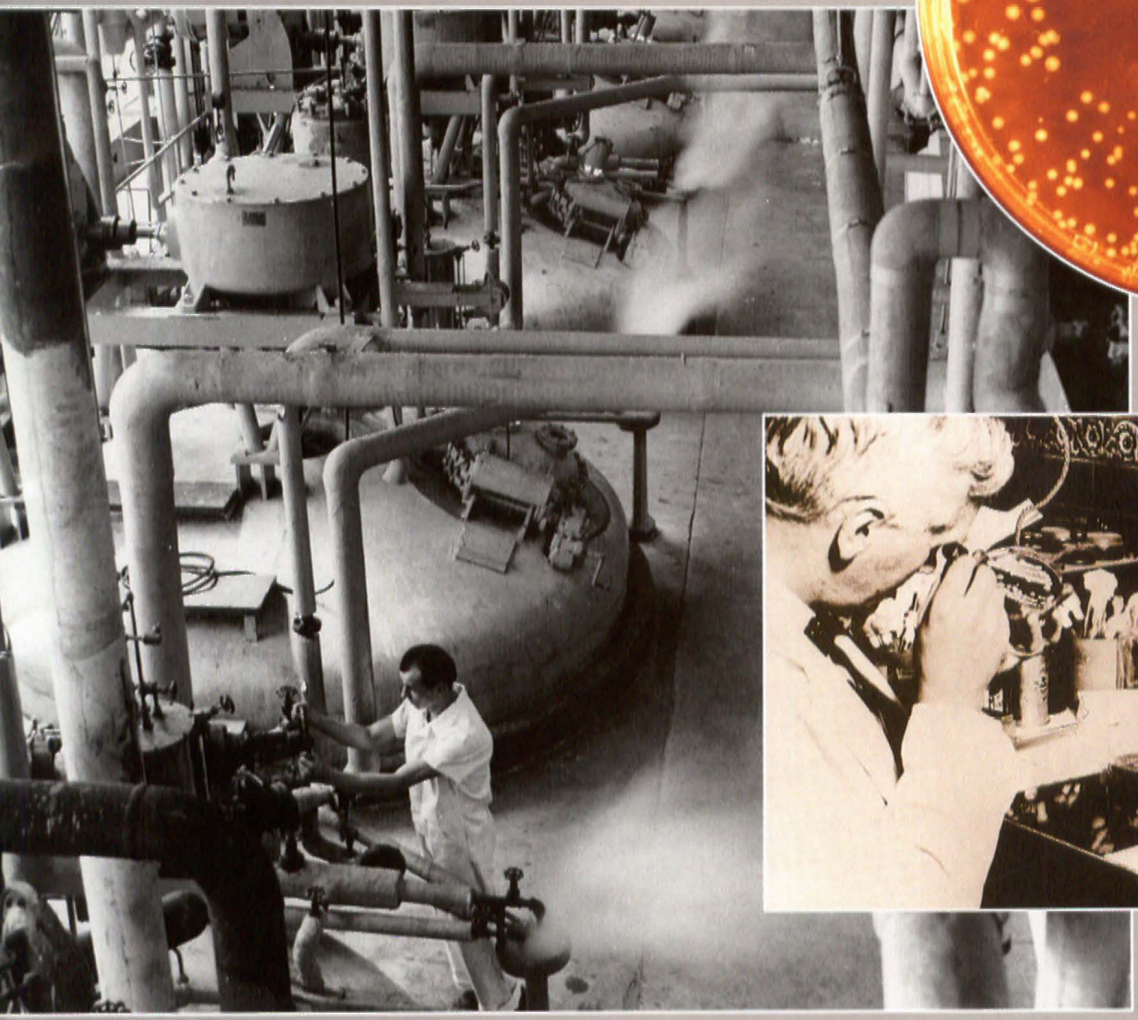
An International Historic Chemical Landmark

The discovery and development of penicillin

1928–1945





The Alexander Fleming Laboratory Museum, London, UK

November 19, 1999

American Chemical Society

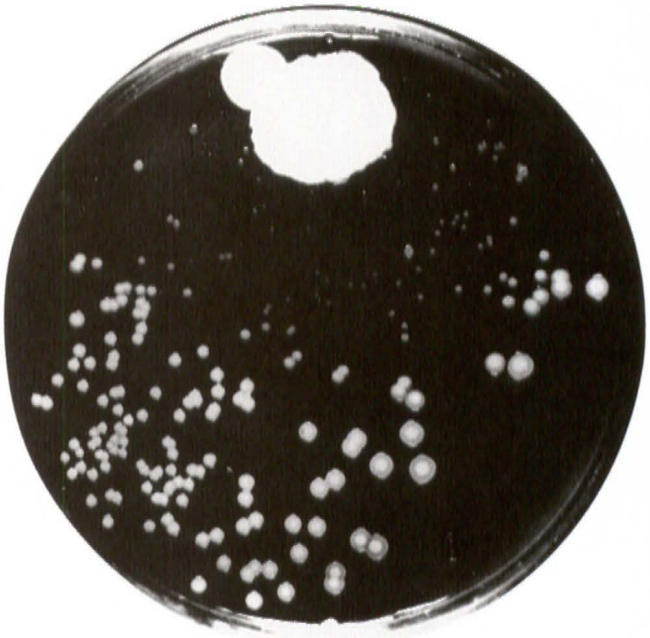
RS•C ROYAL SOCIETY OF CHEMISTRY

This booklet commemorates the designation of the development of penicillin as an International Historic Chemical Landmark. The designation was conferred jointly by the Royal Society of Chemistry and the American Chemical Society, learned societies whose aims are to promote the interests of chemists and chemistry and to serve the public interest.

Left: Clarence Wing, St. Mary's Hospital (courtesy of St. Mary's Hospital)

The commemorative plaque inscription reads:

'In 1928, at St. Mary's Hospital, London, Alexander Fleming discovered penicillin. This discovery led to the introduction of antibiotics that greatly reduced the number of deaths from infection. Howard W. Florey, at the University of Oxford working with Ernst B. Chain, Norman G. Heatley and Edward P. Abraham, successfully took penicillin from the laboratory to the clinic as a medical treatment in 1941. The large-scale development of penicillin was undertaken in the United States of America during the 1939–1945 World War, led by scientists and engineers at the Northern Regional Research Laboratory of the US Department of Agriculture, Abbott Laboratories, Lederle Laboratories, Merck & Co., Inc., Chas. Pfizer & Co. Inc., and E.R. Squibb & Sons. The discovery and development of penicillin was a milestone in twentieth century pharmaceutical chemistry.'



Penicillium Notatum. *Fleming's own photograph of the original mould which produced penicillin (courtesy of St. Mary's Hospital)*

Sir Alexander Fleming in his laboratory, c. 1931 (courtesy of St. Mary's Hospital)

Acknowledgements:

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The booklet was written jointly by Susan Aldridge, UK, John Parascandola, US National Library of Medicine and Jeffrey L. Sturchio, Merck & Co., Inc. the American Chemical Society, National Historic Chemical Landmarks Programme Advisory Committee liaison. It was designed by Stairway Communications, London.

Photographs courtesy of Abbott Laboratories, Merck Inc., Pfizer Inc., Bristol-Myers Squibb, USDA, the Chemical Heritage Foundation, St. Mary's Hospital and the University of Oxford, Sir William Dunn School of Pathology.

The discovery and development of penicillin

The introduction of penicillin in the 1940s, which opened up the era of antibiotics, has been recognised as one of the greatest advances in therapeutics. This dramatic medical breakthrough was a result of combined efforts in the UK and the USA. The discovery of penicillin and the initial recognition of its therapeutic potential occurred in the UK, but, due to World War II, the USA played the major role in developing large-scale production of the drug, thus making a life-saving substance in limited supply into a widely available medicine.

Alexander Fleming Laboratory Museum, St. Mary's Hospital, London, UK (courtesy of St. Mary's Hospital)

Penicillin heralded the dawn of the antibiotic age. Before its introduction there was no effective treatment for infections such as pneumonia, gonorrhoea or rheumatic fever. Hospitals were full of people with blood poisoning contracted from a cut or a scratch and doctors could do little for them but wait and hope.

Antibiotics are compounds produced by bacteria and fungi which are capable of killing, or inhibiting, competing microbial species. This phenomenon has long been known, and may explain why the ancient Eyptians had the practice of poulticing infected wounds with mouldy bread. But it was not until 1928 that penicillin, the first true antibiotic, was discovered by Alexander Fleming, Professor of Bacteriology at St Mary's Hospital in London.

Returning from holiday on 3 September 1928, he began to sort through petri dishes containing colonies of *Staphylococcus*, bacteria that cause boils, sore throats and abscesses, and noticed something unusual on one dish. It was dotted with colonies, save for one area where a blob of mould was growing. The zone immediately around the mould – later identified as a rare strain of *Penicillium notatum* – was clear, as if the mould had secreted something that inhibited bacterial growth.

Fleming found that his 'mould juice' was capable of killing a wide range of harmful bacteria, such as streptococcus, meningococcus, and the diphtheria bacillus. He then set his assistants Stuart Craddock and Frederick Ridley the difficult task of isolating pure penicillin from the 'mould juice'. It proved to be very unstable, and they were only able to prepare solutions of crude material to work with. Fleming published his findings in the *British Journal of Experimental Pathology* in June 1929, with only a passing reference to penicillin's potential therapeutic benefits. At this stage it looked as if its main application would be in isolating penicillin-insensitive bacteria from penicillin-sensitive bacteria in a mixed culture. This at least was of practical benefit to bacteriologists, and kept interest in penicillin going. Others, including Harold Raistrick, Professor of Biochemistry at the London School of Hygiene and Tropical Medicine, tried to purify penicillin but failed.

Penicillin research at Oxford

It was Howard Florey, Ernst Chain, and their colleagues at the Sir William Dunn School of Pathology at Oxford University who turned penicillin from a laboratory curiosity into a life-saving drug. Their work on the purification and chemistry of penicillin began in earnest in 1939, just when wartime conditions were beginning to make research especially difficult. To carry out a programme of animal experiments and clinical trials the team needed to process up to 500 litres a week of mould filtrate. They began growing it in a strange array of culture vessels such as baths, bedpans, milk churns and food tins. Later, a customised fermentation vessel was designed for ease of removing and, to

Florey (front row, centre) with his group at Oxford (courtesy of University of Oxford, Sir William Dunn School of Pathology)

save space, renewing the broth beneath the surface of the mould. A team of 'penicillin girls' was employed, at £2 a week, to inoculate and generally look after the fermentation. In effect, the Oxford lab was being turned into a penicillin factory.

Meanwhile, biochemist Norman Heatley extracted penicillin from huge volumes of filtrate coming off the production line by extracting it into amyl acetate and then back into water, using a countercurrent system. Edward Abraham, another biochemist who was employed to help step up production, then used the newly discovered technique of alumina column chromatography to remove impurities from the penicillin prior to clinical trials.

In 1940, Florey carried out vital experiments, showing that penicillin could protect mice against infection from deadly

Small-scale penicillin production at the Sir William Dunn School of Pathology (courtesy of University of Oxford, Sir William Dunn School of Pathology)

Streptococci. Then, on February 12, 1941, a 43-year old policeman, Albert Alexander, became the first recipient of the Oxford penicillin. He had scratched the side of his mouth while pruning roses, and had developed a life-threatening infection with huge abscesses affecting his eyes, face, and lungs. Penicillin was injected and within days he made a remarkable recovery. But supplies of the drug ran out and he died a few days later. Better results followed with other patients though and soon there were plans to make penicillin available for British troops on the battlefield.

War-time conditions made industrial production of penicillin difficult. A number of British companies, including Glaxo (now GlaxoWellcome) and Kemball Bishop, a London firm later bought by Pfizer, took up the challenge, however.

A move to the USA

Substantial amounts of penicillin would be needed for the extensive clinical trials required to confirm the promise of the early results, and to provide adequate supplies of the drug for therapeutic use if it did live up to its potential. Florey recognised that large-scale production of penicillin was probably out of the question in Britain, where the chemical industry was fully absorbed in the war effort. With the support of the Rockefeller Foundation, Florey and his colleague Norman Heatley travelled to the USA in the summer of 1941 to see if they could interest the American pharmaceutical industry in the effort to produce penicillin on a large scale.

Yale physiologist John Fulton helped to put his British colleagues in touch with individuals who might be able to assist them in their goal. They were referred to Robert Thom of the Department of Agriculture, a foremost mycologist and authority on the Penicillium mould, and eventually to the Department's Northern Regional Research Laboratory (NRRL) in Peoria, Illinois because of the expertise of its Fermentation Division. This contact proved to be crucial to the success of the project, as the NRRL was a key contributor of innovations that made large-scale production of penicillin possible.

Increasing the yield of penicillin

Orville May, Director of the NRRL, agreed to have the Laboratory undertake a vigorous programme to increase penicillin yields under the direction of Robert Coghill, Chief of the Fermentation Division. It was agreed that Heatley would remain in Peoria to share his expertise with his American colleagues. Within a few weeks, Andrew Moyer found

Robert D. Coghill

Dr. Andrew Moyer examines the mould Penicillium chrysogenum *on various nutrient media to develop optimum conditions for production of penicillin*

that he could significantly increase the yield of penicillin by substituting lactose for the sucrose used by the Oxford team in their culture medium. Shortly thereafter, Moyer made the even more important discovery that the addition of corn-steep liquor to the fermentation medium produced a ten-fold increase in yield. Corn-steep liquor was a by-product of the corn wet-milling process, and the NRRL, in an attempt to find a use for it, tried it in essentially all of their fermentation work. Later, the Peoria laboratory increased the yield of penicillin still further by the addition of penicillin precursors, such as phenylacetic acid, to the fermentation medium.

Kenneth B. Raper

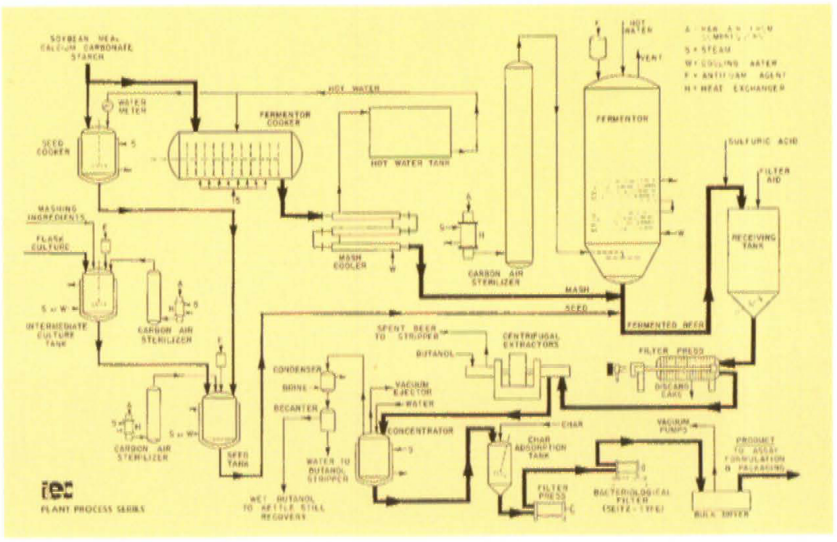
It was recognised that the Oxford group's method of growing the mould on the surface of a nutrient medium was inefficient, and that growth in submerged culture would be a superior process. In submerged culture fermentation, the mould is grown in large tanks in a constantly agitated and aerated mixture, rather than just on the surface of the medium. Florey's Penicillium culture, however, produced only traces of penicillin when grown in submerged culture. Under the direction of Kenneth Raper, staff at the NRRL screened various Penicillium strains and found one that produced acceptable yields of penicillin in submerged culture. Soon a global search was underway for better penicillin-producing strains, with soil samples being sent to the NRRL from around the world. Ironically, the most productive strain came from a mouldy cantaloupe from a Peoria fruit market. A more productive mutant of the so-called cantaloupe strain was produced with the use of X-rays at the Carnegie Institution. When this strain was exposed to ultraviolet radiation at the University of Wisconsin, its productivity was increased still further.

The involvement of pharmaceutical companies

While Heatley remained in Peoria helping the NRRL staff to get the penicillin work started, Florey visited various pharmaceutical companies to try to interest them in the drug. Although Florey was disappointed in the immediate results of his trip, three of the companies (Merck, Squibb, and Lilly) had actually conducted some penicillin research before Florey's arrival, and Pfizer seemed on the verge of investigating the drug as well. At this time, however, the promise of penicillin was still based on only limited clinical trials.

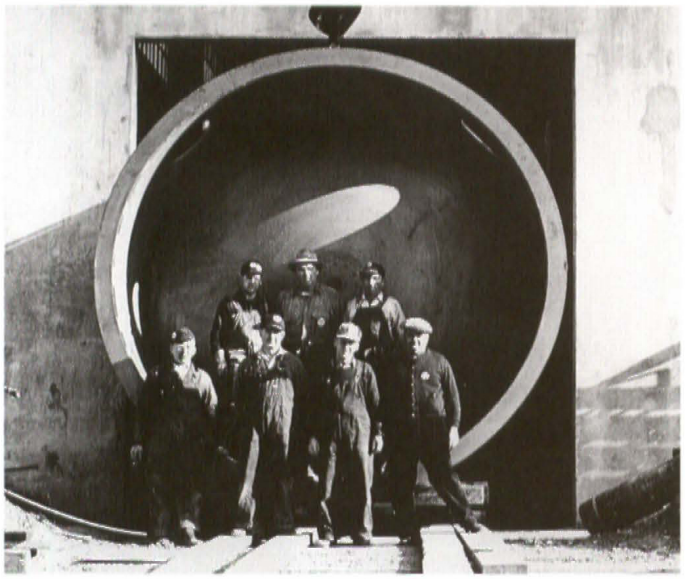
Florey next visited his old friend Alfred Newton Richards, then vice president for medical affairs at the University of Pennsylvania. More importantly, Richards was chair of the Committee on Medical Research (CMR) of the Office of Scientific Research and Development (OSRD). The OSRD had been created in June, 1941 to assure that adequate attention was given to research on scientific and medical problems relating to national defence. Richards had great respect for Florey and trusted his judgment about the potential value of penicillin. He approached the four drug firms that Florey indicated had shown some interest in the drug (Merck, Squibb, Lilly, and Pfizer) and informed them that they would be serving the national interest if they undertook penicillin production and that there might be support from the federal government.

Flowsheet for production of Bacitracin at Squibb & Sons Inc. (courtesy of Bristol-Myers Squibb)



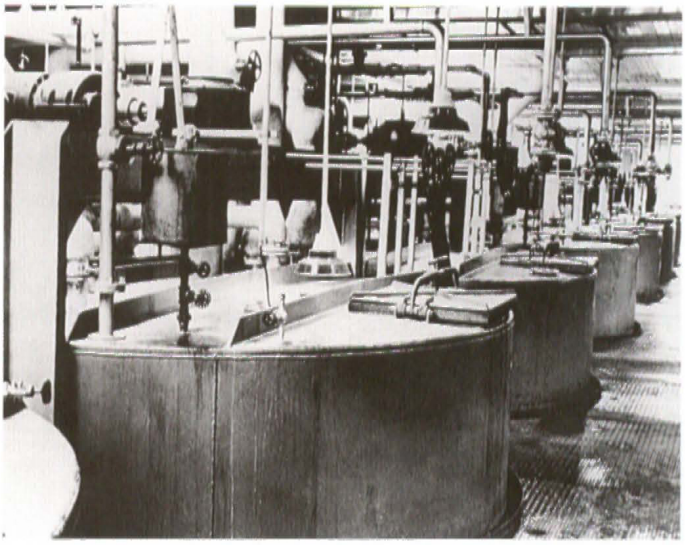
Penicillin fermentation bottles at Squibb & Sons, Inc. (courtesy of Bristol-Myers Squibb)

Richards convened a meeting in Washington, D.C., on October 8, 1941, to exchange information on company and government research and to plan a collaborative research programme to expedite penicillin production. In addition to representatives of the CMR, the National Research Council, and the U. S. Department of Agriculture, participants included research directors Randolph T. Major of Merck & Co., Inc.; George A. Harrop of the Squibb Institute for Medical Research; Jasper Kane of Pfizer; and Y. SubbaRow of Lederle. The next CMR penicillin conference, held in New York in December, ten days after Pearl Harbor and U. S. entry into the Second World War, was more decisive. At this meeting, which was attended by the heads of Merck, Squibb, Pfizer, and Lederle, as well as the company research directors, Coghill's report on the success at the NRRL with corn steep liquor was encouraging to the industry leaders present.



Penicillin plant at Squibb & Sons Inc., New Brunswick, NJ (courtesy of Bristol-Myers Squibb)

As Coghill later recalled, George W. Merck, who had been pessimistic about the possibility of producing adequate quantities of penicillin given the constraints of available fermentation techniques and yields,"... immediately spoke up, saying that if these results could be confirmed in their laboratories, it was possible to produce the kilo of material for Florey, and industry would do it!". It was agreed that although the companies would pursue their research activities independently, they would keep the CMR informed of developments, and the Committee could make the information more widely available (with the permission of the company involved) if that were deemed in the public interest.

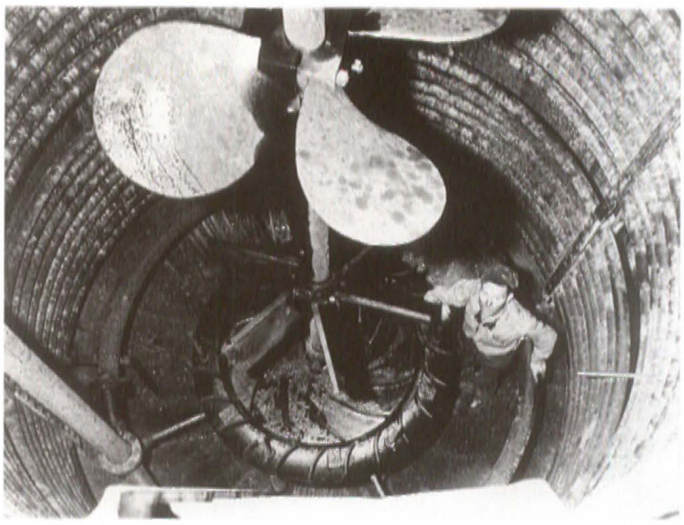


Penicillin deep tanks at Pfizer (courtesy of Pfizer Inc.)

Although there was some concern that investments in fermentation processes might be wasted if a commercially-viable synthesis of penicillin were developed, other companies also began to show an interest in the drug. Some firms worked out collaborative agreements of their own (e.g., Merck and Squibb in February 1942, joined by Pfizer in September). Merck's pilot plant continued to produce several hundred litres of penicillin culture per week using both flasks and tray, and in December Heatley joined the Merck research staff for several months, where he introduced the Oxford cup plate method of penicillin assay, which soon became a standard method industry-wide. By March 1942 enough penicillin had been produced under OSRD auspices to treat the first patient (Mrs. Ann Miller, in New Haven, Connecticut); a further ten cases were treated by June 1942, all with penicillin supplied by Merck & Co., Inc.

The challenge of scale-up

Pharmaceutical and chemical companies played an especially important role in solving the problems inherent in scaling up submerged fermentation from a pilot plant to a manufacturing scale. As the scale of production increased, the scientists at Merck, Pfizer, Squibb, and other companies faced new engineering challenges. Pfizer's John L. Smith captured the complexity and uncertainty facing these companies during the scale-up process:" The mould is as temperamental as an opera singer, the yields are low, the isolation is difficult, the extraction is murder, the purification invites disaster, and the assay is unsatisfactory."Because penicillin needs air to grow, aerating the fermentation mixture in deep tanks presented a problem. When corn steep liquor was used as the culture medium, bubbling sterile air through the mixture caused severe foaming. Squibb solved this problem by introducing glyceryl monoricinolate as an anti-foaming agent. Submerged fermentation also required the design of new cooling systems for the vats and new mixing technology to stir the penicillin mash efficiently. Lilly was particularly successful in making the mould synthesize new types of penicillin by feeding precursors of different structure.Once the fermentation was complete, recovery was also difficult; as much as two-thirds of the penicillin present could be lost during purification because of its instability and heat sensitivity. Extraction was done at low temperatures – Pfizer, responding creatively to wartime shortages, adapted an old ice cream freezer! – and methods of freeze-drying under vacuum eventually gave the best results in purifying the penicillin to a stable, sterile, and usable final form. (Journalists at the time spoke of"yellow magic"but pure penicillin was a white powder.) The steps of fermentation, recovery, and purification and packaging quickly yielded to



Fermentation vat at Merck c.1945 (courtesy of Merck Inc.)

the cooperative efforts of the chemical scientists and engineers working on pilot production of penicillin. On March 1, 1944, Pfizer opened the first commercial plant for large-scale production of penicillin by submerged culture in Brooklyn, New York.

Meanwhile, clinical studies in the military and civilian sectors were confirming the therapeutic promise of penicillin. The drug was shown to be effective in the treatment of a wide variety of infections, including streptococcal, staphylococcal, and gonococcal infections. The United States Army established the value of penicillin in the treatment of surgical and wound infections. Clinical studies also demonstrated its effectiveness against syphilis, and by 1944, it was the primary treatment for this disease in the armed forces of Britain and the USA.

The war effort

The increasingly obvious value of penicillin in the war effort led the War Production Board (WPB) in 1943 to take responsibility for increased production of the drug. The WPB investigated more than 175 companies before selecting 21 to participate in a penicillin programme under the direction of Albert Elder; in addition to Lederle, Merck, Pfizer, and Squibb, Abbott Laboratories (which had also been among the major producers of clinical supplies of penicillin to mid-1943) was one of the first companies to begin large-scale production. These firms received top priority on construction materials and other supplies necessary to meet the production goals. The WPB controlled the disposition of all of the penicillin produced. One of the major goals was to have an adequate supply of the drug on hand for the proposed D-Day invasion of Europe. Feelings of wartime

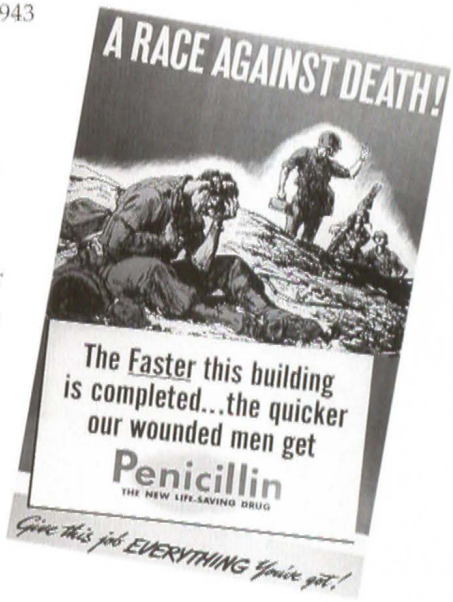
Packaging penicillin at Merck in Rahway, NJ 1943 (courtesy of Merck Inc.)

patriotism greatly stimulated work on penicillin in the UK and the USA. For example, Albert Elder wrote to manufacturers in 1943: "You are urged to impress upon every worker in your plant that penicillin produced today will be saving the life of someone in a few days or curing the disease of someone now incapacitated. Put up slogans in your plant! Place notices in pay envelopes! Create an enthusiasm for the job down to the lowest worker in your plant."

As publicity concerning this new "miracle drug" began to reach the public, the demand for penicillin increased. But supplies at first were limited, and priority was given to military use. Dr. Chester Keefer of Boston, Chairman of the National Research Council's Committee on Chemotherapy, had the unenviable task of rationing supplies of the drug for civilian use. Keefer had to restrict the use of the drug to cases where other methods of treatment had failed. Part of his job was also to collect detailed clinical information about the use of the drug so that a fuller understanding of its potential and limitations could be developed. Not surprisingly, Keefer was besieged with pleas for penicillin. A newspaper account in the *New York Herald Tribune* for October 17, 1943 stated: "Many laymen – husbands, wives, parents, brothers, sisters, friends – beg Dr. Keefer for penicillin. In every case the petitioner is told to arrange that a full dossier on the patient's condition be sent by the doctor in charge. When this is received, the decision is made on a medical, not an emotional basis."

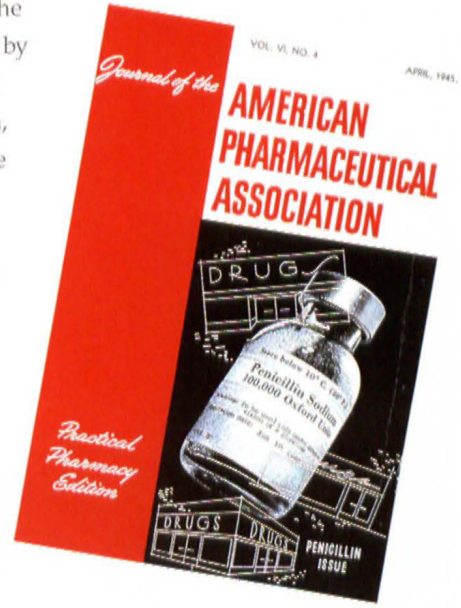
Fortunately, penicillin production began to increase dramatically by early 1944. Production of the drug in the USA jumped from 21 billion units in 1943, to 1,663 billion units in 1944, to more than 6.8 trillion units in 1945, and manufacturing techniques had changed in scale and sophistication from one-litre flasks with less than 1% yield to 10,000-gallon tanks at 80–90% yield. The American government was eventually able to remove all restrictions on its availability, and as of March 15, 1945, penicillin was distributed through the usual channels and was available to the consumer in his or her corner pharmacy.

By 1949, the annual production of penicillin in the USA was 133,229 billion units, and the price had dropped from twenty dollars per 100,000 units in 1943 to less than ten cents.



Most British companies moved over to the deep tank fermentation production of penicillin, pioneered in the USA, after the end of the war to meet civilian needs. In the UK, penicillin first went on sale to the general public, as a prescription only drug, on June 1, 1946.

In the UK, Chain and Abraham continued to work on the structure of the penicillin molecule, aided by the X-ray crystallographic work of Dorothy Hodgkin, also at Oxford. The unique feature of the structure, which was finally established in 1945, is the four-membered highly labile beta-lactam ring, fused to a thiazolidine ring. In the same year Fleming, Florey and Chain were awarded the Nobel Prize for their penicillin research.



The co-operative efforts of American chemists, chemical engineers, microbiologists, mycologists, government agencies, and chemical and pharmaceutical manufacturers were equal to the challenge posed by Howard Florey and Norman Heatley in 1941. As Florey observed in 1949, "too high a tribute cannot be paid to the enterprise and energy with which the American manufacturing firms tackled the large-scale production of the drug. Had it not been for their efforts there would certainly not have been sufficient penicillin by D-Day in Normandy in 1944 to treat all severe casualties, both British and American."

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The Historic Chemical Landmarks Programme

The Historic Chemical Landmarks Programme recognises our scientific and technical heritage and encourages the preservation of historically important achievements and artifacts in chemistry, chemical engineering, and the chemical process industries, It helps to remind chemists, historians, students and teachers of how chemical discoveries are made and developed, and how they are exploited for the benefit of people.

The Programme is applied in a number of different ways. An historic chemical milestone designation marks a landmark step in the evolution of the chemical sciences and technologies. The designation of sites and artifacts notes events or developments of clear historical importance to chemists and chemical engineers. Collections can also receive the designation, thus recognising the contributions of a number of objects with special significance to the historical development of chemistry and chemical engineering.

The American Chemical Society started a National Historic Chemical Landmark Programme in 1992 through its Division of History of Chemistry and the ACS Office of Public Outreach. It has now been extended internationally. The Royal Society of Chemistry has joined the American Chemical Society in designating the development of penicillin as an Historic Chemical Landmark, the second to be designated in the UK under the Programme.

For further information about the Historic Chemical Landmarks Programme contact:

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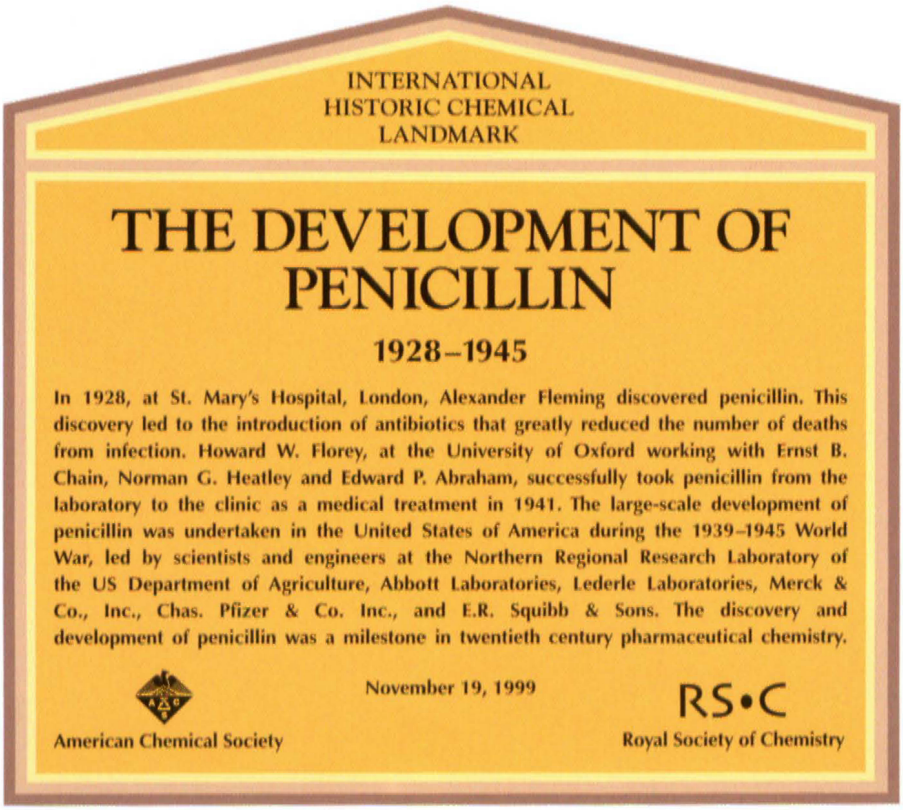

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