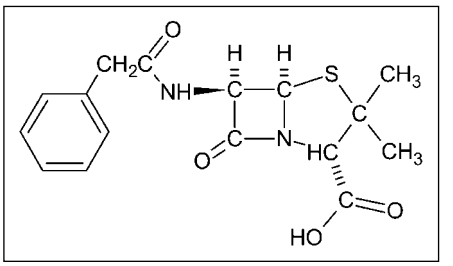
69. Penicillin

CHEMICAL NAME = 2***S,***5***R,***6***R***)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
CAS NUMBER = 61–33–6
MOLECULAR FORMULA = $C_6H_{18}N_2O_4S$
MOLAR MASS = 334.4 g/mol
COMPOSITION = C(57.5%) H(5.4%) N(8.4%) O(19.1%) S(9.6%)
MELTING POINT = 209–212°C (for Penicillin G sodium)
BOILING POINT = decomposes
DENSITY = 1.4 g/cm^3



Penicillin was the first natural antibiotic used to treat bacterial infections and continues to be one of the most important antibiotics. The name comes from the fungus genus *Penicillium* from which it was isolated. *Penicillus* is Latin for "brush" and refers to the brushlike appearance of filamentous *Penicillium* species. Species of this genus are quite common and appear as the bluish-green mold that appears on aged bread, fruit, and cheese. The term *penicillin* is a generic term that refers to a number of antibiotic compounds with the same basic structure. Therefore it is more appropriate to speak of penicillins than of penicillin. The general penicillin structure consists of a β-lactam ring and thiazolidine ring fused together with a peptide bonded to a variable R group (Figure 69.1). Penicillin belongs to a group of compounds called β-lactam antibiotics. Different forms of penicillin depend on what R group is bonded to this basic structure. Penicillin affects the cell walls of bacteria. The β-lactam rings in penicillins open in the presence of bacteria enzymes that are essential for cell wall formation. Penicillin reacts with the enzymes and in the process deactivates them. This in turn inhibits the formation of peptidoglycan cross-links in bacteria cell walls. Peptidoglycans consist of a network of protein-carbohydrate chains that form a strong outer skeleton of the cell. Thus penicillin weakens the cell wall and causes it to collapse. Humans and other animal cells have membranes

rather than walls. Because animal cells do not have corresponding cell wall enzymes that are present in bacteria, penicillins do not harm human cells.

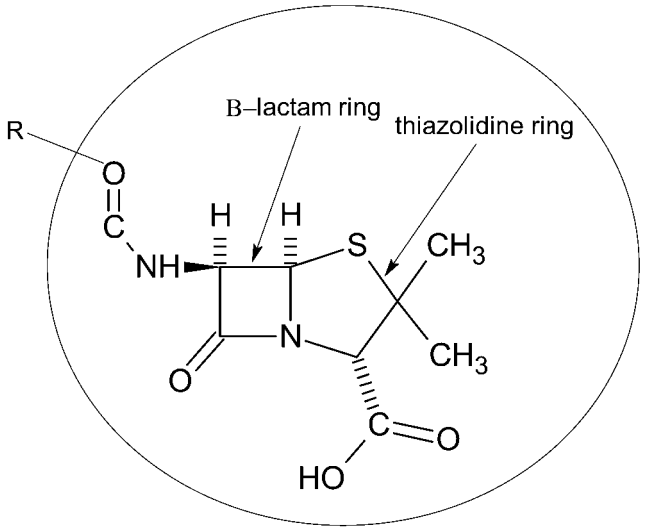


Figure 69.1 General structure of penicillin.

The discovery of penicillin is generally credited to Alexander Fleming (1881–1955) in 1928, but the development of penicillin as an antibiotic took place sporadically over the last decades of the 19th century and first half of the 20th century. Joseph Lister (1827–1912) noted in 1871 that bacteria growth was inhibited by the presence of *Penicillium* molds. Lister used *Penicillium* to treat patients but never published his results. Other researchers published articles that implied that molds could be used to kill bacteria. In 1896, Ernest Duchesne (1874–1912) discovered the antibiotic properties of penicillin while working on his dissertation. He noted that *Penicillium glaucum* prevented the growth of *Escherichia coli* when incubated in the same culture dishes. Other researchers reported before Fleming that extracts from *Penicillium* species inhibited the growth of bacteria, but the difficulty in working with *Penicillium* and mixed results led early scientists to abandon the research.

Alexander Fleming was a medical doctor working in the Inoculation Department at St. Mary's Hospital in London. Fleming studied agents to combat bacteria. In 1922, he discovered the enzyme lysozyme using mucus from his own nose. Lysozyme destroys bacterial cell walls. Fleming's work involved culturing numerous plates, which consisted of Petri dishes, with bacteria and isolating subcultures for study. His discovery of penicillin occurred when Fleming was working on staphylococci for a reference manual on bacteria. During his work at the end of the summer in 1928, Fleming took a month-long vacation. Rather than discard his Petri dishes, he piled them in the dark corner of his small laboratory. When Fleming returned in September to continue his staphylococci work, he retrieved his stored Petri dishes to make observations and to create subcultures of interesting variants. Soon after his return, he was visited by a former co-worker. Fleming had a pile of Petri dishes near a small vessel of Lysol. Lysol

was used for rinsing and disinfecting the dishes. Fleming and his former co-worker observed that one Petri dish was contaminated with mold and that staphylococci growth was absent in the vicinity of the mold. Fleming cultured the mold in a tube of broth and in succeeding weeks showed his plate to scientists who visited his laboratory. Fleming's plate did not raise much interest. He worked on penicillin for several months after his discovery in 1928 and published an article in 1929 reporting his 1928 observation. He then worked sporadically on penicillin over the next several years and published a second article in 1932, but the difficulties that plagued earlier researchers on *Penicillium* also prevented Fleming from making significant progress with the mold.

The commercial development of penicillin can be traced to 1938, when Ernest Boris Chain (1906–1979), a German biochemist who fled Germany for England in 1933, and Howard Walter Florey (1898–1968) expanded their work on lysozyme to search for other antibacterial agents. Chain was part of Florey's research team at the Dunn School of Pathology at Oxford University. He reviewed the literature and Fleming's 1929 article stimulated his interest on penicillin because of its similarity to lysozyme. Chain convinced Florey that penicillin had potential as an antibacterial agent, and laboratory work on *Penicillium notatum* (currently called *Penicillium chrysogenum*) began in 1938. Florey assembled a research team to work on penicillin, but lack of research funds from England and Oxford University initially hampered his efforts. England's entrance into World War II also made difficult the task of acquiring funds for a project with unclear potential.

The first challenge of the research teams was purifying penicillin by extracting the compound from the mold. Norman Heatley (1911–2004) devised extraction and purification methods to obtain quantities of penicillin sufficient for study. It was determined that penicillin was nontoxic when injected in animal subjects. In May 1940, a significant experiment took place in which eight mice injected with streptococci bacteria and half of these were treated with penicillin. The mice injected with penicillin survived and the untreated mice died after a day. Similar results were obtained with subsequent experiments as the researchers refined the amount and frequency of penicillin injections needed to combat infections in different animal subjects.

The next step in penicillin's use for humans involved clinical trials on humans. The large problem that Florey's team faced was producing enough penicillin to treat human subjects rather than small animal subjects such as mice. The penicillin initially used in Florey's Oxford laboratory was produced by cultivating *Penicillium* in hundreds of clay containers modeled after bedpans. The labor-intensive process produced limited quantities of penicillin. Heatley devised a continuous process for extracting penicillin from mold filtrate, and this increased the quantity and purity of penicillin so that limited trials could be performed on several human subjects. The amount of penicillin for human trials was so limited that researchers recycled penicillin by extracting it from subjects' urine. The initial human trials were performed on five subjects and were successful to the extent that penicillin cured infections without harmful side effects, but one subject died when the supply of penicillin ran out and another died of complications unrelated to the treatment. Florey's team published and presented their results in 1941, and Florey was convinced that the evidence for penicillin's efficacy would enable him to obtain financial backing to conduct the human trials necessary to commercialize penicillin. Florey was unsuccessful in convincing government health officials or pharmaceutical companies in Britain to provide financial backing to scale up production, so he arranged a trip to

the United States to seek backing. Florey used several close professional colleagues and friends in the United States to arrange a tour of pharmaceutical companies, foundations, and agencies; Florey and Heatley arrived in the United States in July 1941, carrying with them a small quantity of *Penicillium* cultures. During their tour, they were able to arrange a pilot study to increase mold production with brewing techniques used in fermentation at the Bureau of Agricultural Chemistry Lab in Peoria, Illinois. At the Peoria laboratory Heatley worked with Andrew J. Moyer (1899–1959) to develop methods to increase production. These methods led to using a broth of corn steep liquor and lactose rather than glucose for culturing *Penicillium* and deep-culture fermentation, in which the mold was grown throughout the volume of a vat rather than on the surface. While Heatley collaborated with Moyer on production techniques, Florey continued to promote penicillin to pharmaceutical companies. Several of these companies were already working on penicillin and Florey was able to increase their efforts.

Florey returned to Oxford in late September, and Heatley remained in the United States to continue work on increasing production, first in Peoria and then at Merck's factory in New Jersey. Florey succeeded in increasing interest on penicillin research in the United States, but he was unsuccessful in obtaining more penicillin for his own clinical trials. Florey's team continued to produce penicillin using laboratory techniques and to perform limited human trials. Meanwhile, U.S. pharmaceutical firms, stimulated by the country's sudden entry into World War II on December 7, 1941, made commercial penicillin production a priority. Mass production of penicillin by U.S. firms started in 1943, and it was used immediately to treat wounded soldiers. Penicillin reduced suffering, prevented amputations, cured pneumonia, and saved thousands of lives during the war and was hailed as a "miracle drug." The United States increased production throughout the war years and after the war widespread civilian use commenced. U.S. firms took out patents on the production methods. Chain had urged Florey to patent production processes, but Florey and his supporters thought that this was unethical. Ironically, British firms had to pay American firms royalties for methods that originated in their country. There was also disagreement over credit for the work on penicillin between Florey and Fleming. Although Fleming had written the article that motivated Florey to examine penicillin as an antibiotic, Florey and his team's work led to its commercialization. Fleming, Florey, and Chain shared the Nobel Prize in physiology or medicine in 1945 for their work on penicillin.

Three years after mass production of penicillin began, the first evidence of resistance appeared. Because bacteria exist as large populations that rapidly reproduce, the widespread use of an antibiotic inevitably leads to biological resistance. Bacteria have evolved to produce a number of resistance mechanisms to antibiotics. One is the production of β-lactamase enzymes, which breaks down the β-lactam ring, rendering it ineffective. Another mechanism is modification of the penicillin-binding proteins. These proteins are essential in building the peptidoglycan cross-links of the cell wall.

Because of the ability of bacteria to develop resistance to penicillin, pharmaceutical companies must continually develop different penicillin compounds for continued use as an antibiotic. Different forms are also used depending on the type of infection, delivery method, and individual. The form discovered by Fleming and used by Florey was benzylpenicillin or Penicillin G. Today there are numerous compounds that are classified as penicillins that are marketed under various trade names. Early penicillins were biosynthetic compounds obtained from molds. Modern penicillins are semisynthetic in which penicillin obtained from natural sources is further synthesized to impart specific properties to the compound.

From 1940 to 1945, researchers sought the structure of penicillin. Several forms of penicillin were discovered. In 1945, Dorothy Mary Crowfoot Hodgkin (1910–1994) used x-ray crystallography to determine the structure of penicillin. John C. Sheehan (1915–1992) synthesized a penicillin called penicillin V in 1957 at Massachusetts Institute of Technology. Although Sheehan's work showed that penicillin could be synthesized, the method was not practical on a commercial level. Currently, about 45,000 tons of penicillins are produced annually worldwide.