Anne Miller had been hospitalized for a month. She was diagnosed with septicemia, a lethal blood poisoning caused by a streptococcal infection. Her condition left her doctors helpless as she slipped in and out of consciousness and suffered from a high fever with her temperature spiking up to 107 degrees. Her condition showed no improvement even after being given sulfa drugs, undergoing blood transfusions, and surgery. On March 14, 1942, as a last desperate attempt, her doctors injected her with an obscure experimental drug. To their surprise, her temperature dropped dramatically overnight and she was no longer delirious the next day. Soon after, she was eating full meals and her doctors breathed a sigh of relief, as they knew the worst was over. Anne’s miraculous recovery marked the first of many lives that were saved by the drug penicillin. Penicillins are a group of antibiotics produced by the Penicillium fungi as a secondary metabolite. These drugs are used to treat bacterial infections often caused by Gram-positive organisms. The discovery and development of penicillin paved the way for advances in the world of medicine that not only saved millions of lives but also opened myriads of possibilities.

The discovery of penicillin and its antibiotic properties is famously credited to Alexander Fleming, a professor of Bacteriology at St. Mary’s Hospital in London. Upon returning from his vacation, Dr. Fleming began to sort through petri dishes in his laboratory containing colonies of Staphylococcus, a genus of bacteria that causes boils, sore throats, and abscesses. One dish in particular caught his attention. It was dotted with colonies of Staphylococcus except for one area where mold was growing. Around the center of growth for this mold radiated a zone of clearing where Staphylococcus colonies were clearly undergoing cytolysis and dying. Dr. Fleming observed that the mold was secreting some substance that inhibited the growth of bacteria. He eventually found out that this mold was *Penicillium notatum* and he named the antibacterial substance it secreted penicillin. Figure 1 features a photograph of Dr. Fleming’s petri dish as presented in his published findings in 1929.

![Figure 1: Alexander Fleming’s petri dish that led to penicillin’s discovery.](image)
Fleming’s research sparked the interest of Oxford University researchers Howard Florey, Ernst Chain, and their colleagues. Their work ultimately paved the road for penicillin’s potential medical use in treating patients with bacterial infections. They went through the arduous task of producing up to 500 liters of Penicillium mold filtrate a week in order to begin animal experiments and clinical trials. Studies in mice that were infected with a deadly strain of streptococcus showed the promise of penicillin’s antibacterial action when half of the mice that had been treated with penicillin survived and recovered while the other half perished. On September 1940, penicillin was used for the first time to treat a human patient, Albert Alexander, an Oxford police officer. He had scratched the side of his mouth while pruning roses and consequently developed a life-threatening infection that spread to his face, eyes, and lungs. A few days after being injected with penicillin, he showed a remarkable recovery. Unfortunately, the Oxford researchers could not produce enough pure penicillin for fully eradicating the infection and Alexander eventually died. Florey and Chain faced the challenge of finding a way to produce penicillin on a large scale so that enough pure penicillin could be provided for treating patients.

The advent of World War II put enormous pressure on researchers to find a way to produce penicillin in large amounts. It was a race against time, as death was just as likely to fall upon soldiers and civilians due to infection as it was from being wounded. Efforts to find a means of mass-producing penicillin were moved to the United States in 1941 because Britain’s resources were focused on the war effort. Daily bombing raids on Britain also made it dangerous to conduct operations in the country. Florey and his colleague Norman Heatley traveled to Peoria, Illinois where the U.S. Department of Agriculture had a research laboratory with expertise on fermentation. The combined work of Florey, Heatley, American scientists and engineers, and the American pharmaceutical industry resulted in the development of a method known as deep tank fermentation. This method of producing penicillin used corn-steep liquid and lactose as a medium for growing Penicillium mold. This increased penicillin yield ten-fold compared to the previous method using sucrose medium. Additionally, *Penicillium chrysogenum*, a new species found on a moldy cantaloupe, was used to replace *P. notatum*. *P. chrysogenum* produced a thousand times as much penicillin as the first batches from *P. notatum* had made, especially when it was subjected to challenging conditions such as exposure to ultraviolet radiation.

Pharmaceutical companies like Merck, Squibb, and Pfizer worked on scaling up penicillin production. As the availability of penicillin rose, priority was given to the military and penicillin was shipped to combat zones where it was used by the U.S. Army to treat surgical and wound infections. By the end of the war in 1945, 6.8 trillion units of penicillin (where 1 unit is equal to 0.6 micrograms) were being produced per month. The wonders of penicillin swept the nation, and it even started appearing on wartime propaganda posters as seen in Figure 2. Penicillin played a crucial role during the war by boosting morale, giving hope to the families of service men and women, and by encouraging more people to participate in the war effort.
It was not until the early 1940s that the chemical structure of penicillin (Figure 3) was determined by Dorothy Hodgkin through x-ray crystallography. Penicillin falls under the category of beta-lactam antibiotics along with cephalosporins, another family of antibiotics derived from the Acremonium fungi. Beta-lactam antibiotics are characterized by three fundamental structural requirements: a fused beta-lactam structure, a free carboxyl acid group, and one or more substituted amino acid side chains. Variations in side chain substitution gave rise to the penicillin derivatives as seen in Figure 3.

<table>
<thead>
<tr>
<th>Penicillin Structure</th>
<th>R Group</th>
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<tbody>
<tr>
<td></td>
<td>-CH₂</td>
<td>penicillin G</td>
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<tr>
<td></td>
<td>CH₂-O</td>
<td>penicillin V</td>
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<td></td>
<td>CH-</td>
<td>ampicillin</td>
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<td></td>
<td>CH-OH</td>
<td>amoxicillin</td>
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<tr>
<td></td>
<td>CH₃O</td>
<td>methicillin</td>
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Penicillin G, also known as benzylpenicillin, was the first form of penicillin to be used on a large scale. It could only be administered parenterally through intramuscular injection or intravenous doses because its instability rendered it vulnerable to inactivation by stomach acid. In 1952, scientists from the Austrian pharmaceutical company Biochemie (now Sandoz) were able to develop the first acid-stable form of penicillin, Penicillin V or phenoxymethylpenicillin, which
allowed oral administration of the drug. Penicillin G and Penicillin V are considered first-generation penicillins, derived naturally from *P. chrysogenum*. There are now many other natural, semisynthetic, and synthetic forms of penicillin available, all with varying spectra of activity and methods of administration.

Antibacterial agents such as sulfa drugs had already been in use before the discovery of penicillin, but penicillin presented a more efficient way of killing bacteria and eradicating infections. It was able to do so through a mechanism of action that targets the peptidoglycan cell wall, a feature unique to bacteria and not found in higher organisms. The bactericidal action of penicillin is similar to that of all other beta-lactam antibiotics. Formation of the rigid and stable peptidoglycan cell wall in bacteria involves the formation of peptide crosslinks that form a two-dimensional network surrounding the cell like a fishing net. This cross-linking is illustrated in Figure 4.

![Figure 4: Peptide crosslinks of the bacterial peptidoglycan wall. (9)](image)

These crosslinks form through a biochemical reaction catalyzed by the enzyme transpeptidase, also known as penicillin-binding protein. The activity of transpeptidase is inhibited by penicillins or other beta-lactams that mimic the structure of the D-Alanyl-D-Alanine end of the peptide that normally binds to transpeptidase. These antibiotics irreversibly inhibit the action of transpeptidase by tightly binding to its active site and reacting with a serine residue in the enzyme, thus blocking the normal physiological reaction and preventing crosslink formation. Failure to form crosslinks at any part of the peptidoglycan wall weakens it and renders it vulnerable, causing the bacterial cell to accumulate water and swell due to high osmotic pressure until it ruptures, thus killing the cell. This process is illustrated in Figure 5.
The accumulation of peptidoglycan precursors also triggers the activation of bacterial cell wall hydrolases and autolysins that further digest the existing peptidoglycan and contribute to cytolysis. All bacteria have a peptidoglycan wall and could potentially be susceptible to penicillin. However, it was found early on that penicillin has no effect on gram-negative organisms but was highly effective against gram-positive bacteria. This can be explained by the differences between gram-positive and gram-negative cell wall structures as seen in Figure 6.

The outermost lipopolysaccharide layer of gram-negative organisms protects them from penicillin by preventing them from entering the cell and binding to transpeptidases. Nevertheless,
the development of penicillin made it possible to combat diseases that were once notorious for their severity and high mortality rates.

Even as early as the 1940s, bacteria had already begun to combat the effectiveness of penicillin by developing defense mechanisms to inactivate it and prevent it from acting on penicillin-binding proteins. Beta-lactamases are enzymes that inactivate penicillin’s function by hydrolyzing the peptide bond in the beta-lactam ring within penicillin’s core structure. They contain a serine in their active site pocket, similar to the serine found in the structure of transpeptidases that penicillin normally binds. This reaction is pictured in Figure 7.

Bacterial defense mechanisms against lethal agents often develop through mutations and natural selection. In this case, beta-lactamases must have developed due to bacterial adaptation to an environment in which a substance, penicillin, inhibits its growth. Since bacteria reproduce and die at a much faster rate, bacterial evolution also happens much more rapidly. Many other antibiotics also become ineffective due to similar situations where enzymes in the bacteria are able to change their chemical structure and inactivate them. The first-generation penicillins, especially Penicillin G and Penicillin V, are known to be susceptible to beta-lactamases, making them ineffective for treating infections caused by bacteria that have beta-lactamases. The development of penicillin-resistant bacteria prompted researchers to look for and develop new derivatives of penicillin that have a broader spectrum of activity, meaning they could be used to treat a wider range of infections.

Penicillin revolutionized the medical field by centralizing the role of antibiotics in the treatment of infectious diseases. Because of penicillin and the discovery of other antibiotics, mortality rates have decreased and deaths from infections have become a rare occurrence. Today, antibiotics are still extensively used in drug therapies. In fact, modern forms of penicillin are the most commonly prescribed antibiotic for treating pediatric patients with ear and throat infections. Anne Miller, the first patient whose life was saved by penicillin, lived up to the ripe old age of 90 and was able to enjoy life and see her three sons, six grandchildren, and seven great-grandchildren grow. Penicillin did this for many people in the twentieth century, and many of us would not even be here today if penicillin had not saved our grandparents’ lives. Penicillin opened the doors for new discoveries and a world of possibilities that have come to shape modern medicine and healthcare today.
Works Cited


