

HUMANITIES

Mould juice: A brief history of penicillin

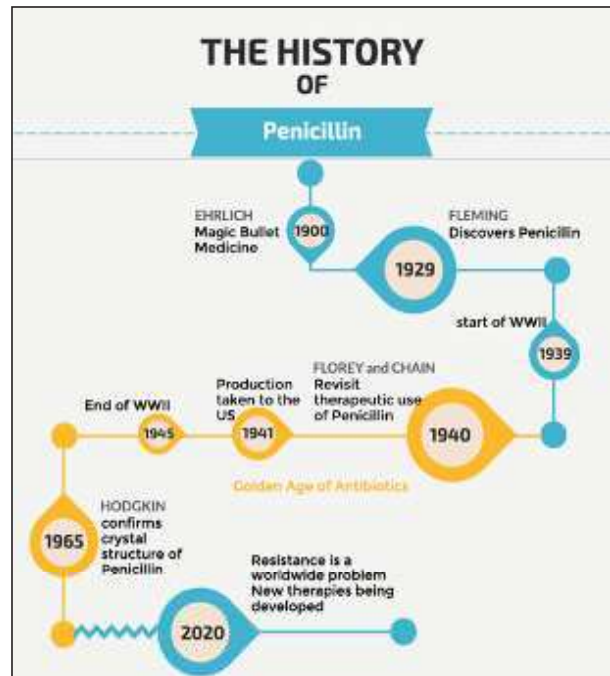
Bridgette Chan

In 1900, Paul Ehrlich developed the concept of magic bullet medicine¹. He theorized that, because different cells could be targeted with different stains, cells could also be targeted with medicines – much like a bullet targets a victim. This simple theory led to the development of antibiotics that are able to kill bacterial cells, but not human cells. Ehrlich dedicated his life to discovering these so-called magic bullet cures and in 1909, he discovered the antibiotic Salvarsan, an arsenic-containing compound that was the first known cure for syphilis. Ehrlich used the term chemotherapy, defined as “treatment of diseases by chemical substances”, to describe his drug². As discussed later, the term antibiotics did not take on its current meaning until the mid-20th century. Ehrlich’s discovery was proof of principle and paved the way for a century of “magic bullet” discoveries.

Unlike Ehrlich’s tireless work to discover Salvarsan, the discovery of penicillin came after a vacation. To briefly retell the famous story, Alexander Fleming returned from a vacation in Scotland to discover that one of his *Staphylococcus petri* dishes had been contaminated with mould and that the mould lysed the *Staphylococcus* (Figure 1)³. In 1929, he published his findings that the “mould broth infiltrate” of *Penicillium notatum* had bactericidal and bacteriolytic properties³. In this paper, he first used the term penicillin to describe the active substance since he considered “mould broth infiltrate” too cumbersome a term.

Although the quest for antibacterial agents had been set in motion years earlier by Ehrlich, Fleming and his team were unable to generate sufficient quantities of *Penicillium notatum* to test the substance and research halted. Fleming predicted that growth inhibition by penicillin may be a helpful characteristic in the classification of bacteria, like urease and catalase, and he and his research team moved on to other topics³.

About a decade later, Howard Florey and Ernst Chain, who investigated how bacteria and mould naturally kill each other, found Fleming’s paper and replicated the experiments. Soon Florey ran into the same problem as Fleming: *Penicillium notatum* was difficult to grow. The mould had to be mass produced because up to 2/3rds of the product could be lost during purification and extraction^{4,5}. Norman Heatley, a biochemist at Oxford, was the first in a long line of chemists to address this challenge. Production was eventually outsourced to the US, but for Florey’s



initial experiments, *Penicillium notatum* was grown in broth in open containers. Heatley repurposed every container available at the Radcliffe infirmary, including bottles and bedpans, to grow penicillin⁶.

Animal testing was conducted by Florey and involved infecting mice with Group A streptococcus and giving half of the mice penicillin^{7,8}. At this point, WWII had begun and scientists knew the impact a compound like penicillin would have on the war. Within a couple of months, they began testing on humans. The first toxicity study was in a woman with terminal cancer who volunteered as a subject⁹. She developed severe tremors and fever, which the scientists realized were due to the impurities in their compound. The first patient to be treated with penicillin was a policeman who had a facial infection after being scratched with a rose thorn^{9,5}. Unfortunately, he died 5 days after initiating therapy because the “penicillin supply was exhausted”^{9,10}. This may seem inconceivable given the current large-scale production of antibiotics, but to achieve the recommended dose for streptococcal pneumonia, Florey and Heatley would have had to produce thousands of litres of broth daily^{5,11}. The scientists observed a noticeable improvement in the policeman after receiving penicillin⁵, so they continued to give their product to patients. They often collected the patients’ urine; roughly 60% of penicillin is excreted

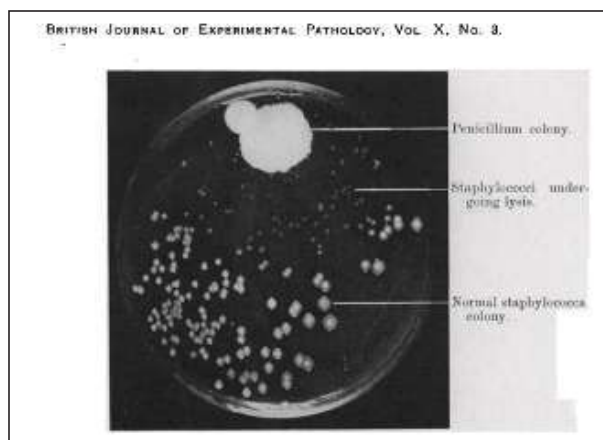


Figure 1. A photo of penicillin-lysed Staphylococci colonies from Alexander Fleming's original paper "On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of *B. influenzae*" (1929).

in the urine unchanged and they could recover about 30% of their initial dose through re-extraction^{5,12}.

Florey and Chain recognized that, with the war, Britain would be unable to produce enough penicillin to supply the troops. In 1941, they took their discovery to the US where the Northern Regional Research Laboratory (NRRL) in Illinois improved the fungus strain and production technique. The NRRL scientists successfully induced mutations in a high-yield strain isolated from a piece of cantaloupe⁴. They also increased yield by substituting lactose instead of sucrose, adding a corn-steeped liquor, and adding penicillin precursors to the broth. Once the NRRL had a viable Penicillium strain and technique, they offered the patent to American pharmaceutical companies, one of which was Pfizer. Pfizer agreed to make the compound and later developed the deep-tank fermentation method, the critical production technique that allowed the supply of the antibiotic to meet wartime demand⁴. In 1944, Pfizer opened a large-scale penicillin manufacturing plant in the Bronx. At this point, the value of penicillin was undeniable, and companies and workers were constantly reminded of the importance of the compound (Figure 2). Eventually, production reached adequate levels and George Stone, a Pfizer employee, wrote an internal report stating that in 1943 they could produce in one day what they did in the entire year of 1942⁴.

Following the discovery of penicillin, we entered the Golden Age of Antibiotics. Within a short span of time, numerous other compounds with antibacterial properties were discovered including streptomycin, bacitracin, nitrofurans, chloramphenicol and the first cephalosporin. Between 1940 and 1960, about 20 antibiotics were discovered. In fact, 50% of antibiotics we still use today were discovered during this time. This is also when the term chemotherapy was exchanged for the more precise term: antibiotic.

Antibiotic, which comes from the Greek word for "against life", was first used in 1860 but took on its current meaning in 1941 when it was used by the soil microbiologist Selman Waksman¹³. In addition to giving antibiotic its modern meaning, Waksman also discovered streptomycin (the first aminoglycoside). Four years later, the first cephalosporin was isolated from *Cephalosporin acremonium*. Giuseppe Brotzu, an Italian pharmacologist and politician, wondered how the people of Cagliari did not get typhoid fever while swimming in water contaminated with *Salmonella*. Upon culturing the water, Brotzu, like Fleming, discovered a fungus with antibacterial properties.

Throughout this Golden Age, numerous scientists received Nobel prizes for their work related to antibiotics. Waksman was awarded the Nobel Prize in Physiology and Medicine in 1952. Years earlier, in 1945, Fleming, Florey, and Chain received the Nobel prize for the discovery of penicillin. During Fleming's acceptance speech, he cautioned listeners about the dangers of resistance in the famous quote:

It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body. The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant¹⁴.

It was unlikely at this time, however, that Fleming grasped the full consequences resistance would have. In part, this was because syphilis – one of the main diseases treated with penicillin – did not develop resistance to penicillin despite repeated treatments. This proved to be the exception rather than the rule.

After the war, Australia was the first country to make penicillin available to civilians and it soon became a panacea worldwide. As resistance developed, penicillin drug development struggled to keep up. Dorothy Mary Crowfoot Hodgkin, the inventor of x-ray crystallography, confirmed the structure of penicillin in 1945. In 1964, she too was awarded the Nobel Prize in Chemistry for her achievements. Other developments included the invention of the first major penicillin derivative, ampicillin. Ampicillin was developed as a broader spectrum alternative. Following ampicillin, the β lactam resistant penicillins (eg. methicillin) were created. Despite multiple iterations and improvements, the inappropriate and widespread use of antibiotics has led to worldwide resistance and a large resistome – a collection of antibiotic-resistant genes in bacteria. In

addition to inappropriate use in humans, an estimated 82% of antibiotic use in Canada and the US is in livestock, which likely contributes to the inappropriate overuse of antibiotics^{15,16}. Encouragingly, the dangerous use of antibiotics in animals in Western countries is decreasing, and in 2006, the European Union banned the use of antibiotics for growth promotion in animals. However, the worldwide use of antibiotics is increasing overall and one estimate expects antimicrobial use to rise by 67% by 2030, largely due to rising consumption in growing and developing countries¹⁷.

Currently, penicillin is indicated as monotherapy for β hemolytic streptococcus infections and syphilis, and it can be used in combination to treat a wider variety of infections. Penicillium species can also be found on mouldy fruit and cheeses such as Brie and Roquefort. While penicillin is not obsolete, antibiotics no longer have the curative power they used to. In 2018, 26% of infections were resistant to first-line antimicrobials¹⁸. The invention and development of penicillin has resulted in numerous Nobel prizes which is indicative of the significance of its advent, but the Golden Age of Antibiotics is behind us and we need to adapt. This includes promoting drug research of novel solutions such as bacteriophage therapy, nanoparticles, and antimicrobial polymers, and while these new magic bullets are being invented, physicians – who prescribe 65% of outpatient antibiotics – must continue to be stewards¹⁹.

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