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Development of the Miracle Drug Penicillin

Abstract

Since penicillin's discovery in 1928, the medical landscape has changed drastically; countless lives have been saved due to the subsequent proliferation of antibiotics. Today, it is difficult to imagine living in fear of a bacterial infection, but that was not the case two generations ago. The last 50 years are considered a golden age in medicine because of the extended use of antibiotics to treat bacterial pathogens. The story behind the development of what people would eventually dub "the miracle drug" is miraculous; scientists have overcome numerous obstacles, ranging from intra-group conflicts and scale-up limitations, amidst a backdrop of political and economic turmoil caused by World War II. In contrast to the perception that the discovery of penicillin was a strictly scientific process, it was in fact, a triumphant collaboration between science and engineering.

Introduction

Until the 1950's, a mere scrape to the knee or blister could lead to infection and possibly, to death. Without penicillin or some other antibiotic to treat infections, minor topical wounds that seem trivial by today's standards could facilitate the onslaught of a serious infection. In addition, children often died from scarlet fever, measles, or tonsillitis; many survivors of those diseases were later affected by sometimes fatal strep-throat, pneumonia, meningitis, or tuberculosis. Sexually transmitted diseases also posed a serious threat and mothers often died from infections following childbirth.

During World War I (1914-1918), roughly half of the 10 million dead soldiers died not from injury by bombs or bullets, but rather from infections in relatively minor wounds.[1] At that time, wounds were packed with antiseptic-soaked gauze, then replaced as needed. This method had proven useful during the Boer War (1899-1902). However, the fighting took place in South Africa where the battlegrounds were dry and combatants used high-speed bullets; as a result, wounds were clean. In contrast, World War I took place on fields covered with mud and fertilizer. Soil bacteria quickly infected jagged wounds from shrapnel. Soldiers died in droves due to tetanus, blood poisoning, and gangrene, in addition to other now-treatable diseases such as pneumonia.

Because of medical progress, in World War II (1939-1945), deaths from wounds decreased by roughly 15%, and deaths caused by pneumonia dropped from 18% during World War I to less than 1% in World War II. In the early 1940s, posters showing a soldier treating another featured lines such as "thanks to penicillin…he will come home!" The development of penicillin was still underway at this time; production facilities were painfully inadequate, using milk churns for extractions, bathtubs for growing cultures, and trashcans as steam-heated stills. Nonetheless, penicillin had proven its potential as a miracle drug.

Discovery

The common myth surrounding the discovery of penicillin focuses on a serendipitous glance by Alexander Fleming at some culture plates. In July 1928, Fleming, then working at St. Mary's hospital in London, decided to take a month-long vacation from his work on *Staphylococcus aureus*, a pathogenic bacterium. He left the culture plates seeded and studied them upon his return. Finding nothing interesting, Fleming tossed the plates into a tray of Lysol

to kill the bacteria. But, because there were many plates, several stayed above the bacteria-killing fluid. In September, Fleming's assistant dropped by to inquire about recent developments, and Fleming started to hand him plates. According to legend, Fleming then noticed a blue-green mold that was inhibiting bacterial growth. Penicillin research began from that moment.

Several aspects of the story raise suspicion: first, penicillin does not kill bacteria in such a clean fashion. Rather, penicillin causes cell walls to disintegrate during mitosis, thus preventing bacteria from replicating. Second, the mold would have overgrown the Petri dish if unattended for the duration of Fleming's vacation. Third, Fleming's first recorded mention of penicillin in his lab notebook is on October 30, nearly two months after he is said to have shown plates to an inquisitive assistant.

A more probable scenario as written by science historians rests on Fleming's search for antibacterial agents as early as 1920. Fleming was motivated by his World War I experience where he witnessed the deaths of many soldiers from blood poisoning. In 1922, Fleming discovered lysozyme, an enzyme that causes bacteria to burst. Fleming had been testing culture plates for lysozyme activity; having found none, he tossed the plates into a Lysol bath. Contrary to the story that the mold floated in from an open window, unlikely in a bacteriologist's laboratory, scientific historians suspect that a penicillin spore floated up from the workbench of a mycologist—one who studies molds—who worked in the laboratory below that of Fleming. Fleming noticed the mold on one of the plates when showing them to his assistant and then isolated the mold and tested it for lysozyme activity. Finally, at the end of October, Fleming noted that the mold stopped the growth of a strain of bacteria that was immune to lysozymes. He noticed a zone of inhibition around the contaminant mold of the *Penicillium* family and

eventually isolated an extract from it that could singularly inhibit the growth of bacteria. He named the inhibitor ‘Penicillin’.

The idea that mold could serve as an antibiotic was not novel. Folk medicine in the early Greek and Roman societies prescribed fungi and molds from bread for the treatment of wounds and disease. Hippocrates noted that fungi and yeast can cure certain gynecological ailments. The late 1800’s also show records of the therapeutic value of the microorganism *Penicillium*. However, it was Fleming who realized that it was not the mold itself but rather a substance produced by the mold that prevented bacterial growth.

Fleming continued to work on penicillin. In 1929, he delivered a talk, and subsequent paper, regarding his findings. However, because Fleming was such a poor orator and painfully terse writer, his work sent only the faintest of ripples through the scientific community.

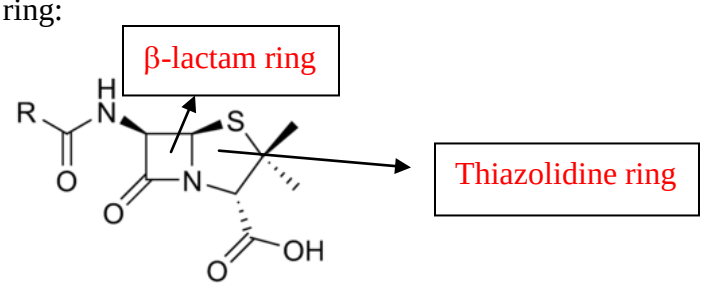
One of Fleming’s students, Ernst Chain, studied penicillin with Howard Florey at Oxford University. Whereas Fleming lacked a large scientific staff and funding, Florey’s lab was well endowed with both. In 1940, with a limited budget and crude equipment, Florey and his team produced penicillin. At this time, England prepared for an anticipated Nazi invasion. The scientists realized that they would have to hide their work to prevent it from reaching enemy hands. Florey and his team devised a plan to rub the spores of *Penicillium notatum* in their lab coats, in the hope that, should their facilities be bombed, someone would be able to continue penicillin research.

Penicillin Production

Penicillin's potential as an antibacterial agent stirred the team at Oxford University. Lack of funding and the war-time climate did not deter the scientists from generating enough penicillin for use on animal subjects. The team collected culture vessels, bedpans, ceramic utensils, and in May of 1940, the first animal trial was conducted wherein 20 ml of penicillin was injected into the tail veins of mice. This was followed by a 1941 publication regarding human clinical trials. Chain soon recognized that "we had a substance that was non-toxic, and not destroyed in the body and therefore was certain to act against bacteria *in vivo*." Penicillin was suddenly no longer just a chemical, but a drug.

Penicillin is the first antibiotic produced for widespread use. Today it remains an ideal therapeutic substance because of its high activity, negligible toxicity, and low cost of production. Penicillin is a metabolite of the fungus *Penicillium*, and it belongs to a class of antibiotics referred to as "β-lactams." The drug interferes with the ability of bacteria to synthesize peptidogylcan cross-links in the bacterial cell wall. As a result, the bacteria lengthen, but cannot divide, and thus the weak cell wall bursts.

Penicillins share a nucleus chemically synthesized by a cyclic fusion of two constituent amino acids valine and cysteine. This nucleus consists of a thiazolidine ring fused to a β-lactam ring:



The side chain (R group) dictates the antibacterial activity. The most commonly used penicillin with the highest degree of antibacterial activity is "Penicillin-G" where R is a benzyl group.

When injected intravenously, Penicillin-G is given in doses of 2-24 million units/day- units denote potency- and the drug is devoid of toxicity in this therapeutic range.

In the early 1940's, many difficulties plagued both small-scale and large-scale production of penicillin. Funding from the British government for new pharmaceutical efforts was scarce during World War II because the government directed most financial resources to the study and production of sulfa drugs. Formally known as sulfonamides, these drugs showed anti-bacterial properties and had proven useful in the early 1930's. Also, because British laboratories were under risk of bombing, the government was reluctant to build large-scale installation of fermentation tanks necessary to the making of penicillin.

In July 1941, Florey secured funding from the Rockefeller Foundation of New York to move his efforts to America. The main problems with the parent culture were two-fold: Florey's production method produced only 1 part penicillin per million parts culture medium, and what penicillin was produced, once given to a patient, would be washed out of the system within 2-3 hours. Over the course of 18 months, only 4 million units of penicillin had been produced, far less than the maximum 24 million unit dosage required for one adult's daily dosage.

The first step towards increasing the production of penicillin required ingenuity for modifying reactor designs. Scientists originally manufactured penicillin by surface culture, a process which involved propagating the mold in large, milk-bottle flasks. However, the active substance in the medium was present only in small amounts and was unstable. Researchers found that they could instead use a large metal tank, aerate the contents and thereby grow mold throughout the entire volume of the 25,000 gallon (approx. 95 m^3) reactor. The Pfizer Company applied its knowledge gained from the deep fermentation of a dietary supplement, gluconic acid, to the production of penicillin. Pfizer engineers developed a reactor wherein sterile air was

introduced into the bottom of the tank from a high pressure nozzle. A central shaft then prevented the mold from gathering on the surface of the liquid medium.

Meanwhile, scientists also studied different molds—collecting samples from moldy jams, bread, and even shoes—in hopes of finding a more potent penicillin strain than the one originally found by Fleming. In 1943, Mary Hunt of Peoria, IL discovered on an overripe cantaloupe, a green mold that came from *Penicillium chrysogeum*. *Penicillium chrysogeum* produced 200 times more penicillin than Fleming's original strain, *Penicillin notatum*. Scientists further attempted to increase production by irradiating *Penicillium chrysogeum* with x-rays to induce mutations. Eventually, a strain was found that produced 5 times more penicillin (leading to a net 1000 fold increase). Yield increased greatly in the span of only 2 years; by 1943, drug companies were mass-producing penicillin for the war effort at 400 million units and $200 per million units. In 1944—just in time for the military priority of D-day—production rose to 100 billion units, and by 1945, the cost was reduced to only $6 per million units.

The synergy of scientists

In addition to the production of an antibacterial agent that has been widely regarded as perhaps the single most beneficial outcome of modern science, Florey and his scientists also were pioneers in their collaborative approach to scientific discovery and development. Florey's lab was assembled to encourage independent scientists to work together as an informal team, an unusual arrangement in British science at that time. Ernst Chain wanted a research laboratory where biochemists, microbiologist, chemical engineers and other specialists could work together. As Gordon T. Stewart noted in his discussion of penicillin discovery, this idea sounded like a "romantic dream to those in authority in post-war Britain; to finance committees, it was like a

hideous nightmare."[2] However, this multitalented research process proved to be successful. The integration of a variety of scientific and engineering disciplines led to large-scale manufacture of penicillin. Since then, biological scientists began to interact with engineers in industry.

Modern development of antibiotics-triumph or tragedy?

The antibiotic era began in 1943. However, 1947 marked the emergence of antibiotic-resistant bacteria. Excessive and improper use of penicillin "educated" bacteria to resist penicillin; the first penicillin-resistant pathogen was *Staphylococcus aureus*. Regrettably, penicillin was prescribed for infections it could not cure and was given in small doses that were ineffective in killing the bacteria, but successful in evolving penicillin-resistant strains of bacteria. The medical community response was to search for other antibiotics. The 1950's added tetracycline, erythromycin and cephalosporins; the 1960's produced aminoglycosides and the 1980's introduced fluoroquinolones such as Ciprofloxacin. Every year, new antibiotics appeared on the market. However, in 2002, no new antibacterial drugs appeared, and in 2003, there were only two. Economic reasons help to explain this downturn. The Oxford scientists developed penicillin for a few thousand dollars while today it takes about $900 million to introduce a new drug into the market. Further, there is little incentive to develop new antibacterial drugs when bacteria resistant to the new drugs appear within a few years of clinical use.

Conclusion

Penicillin more than earned its title as a miracle drug; minor scrapes would no longer portend possible death and such ailments as strep-throat became curable. Thanks to penicillin and its effect against blood-poisoning agents, Mickey Mantle, who was told as a child that his leg

may need to be amputated, instead received penicillin and went on to be inducted into the Baseball Hall of Fame. The discovery of penicillin led to a concerted search for more antibiotics and heralded a new age of medicine. In many ways, as miraculous as the drug is, so is its improbable development; Fleming's poor communication skills nearly left penicillin unnoticed and World War II, with its tumultuous political and economic challenges, could have stopped penicillin development. Several scientists who later were key contributors would have taken positions elsewhere had their intended destinations not been bombed, and human clinical trials of severe cases would have been delayed without the urgency presented by wounded soldiers. Researchers had to beg for economic support and attention from risk-averse governments. Personal and cultural differences strained relations within the Florey group. Outside of the group, scientists and engineers had much difficulty to maintain focus as their family lives buckled from the stress of work and war. While the number of lives saved clearly demonstrates the huge importance of penicillin, manufacturing penicillin contributed strongly to a more effective organization of scientific research. Interdisciplinary teams were established; these encouraged collaboration of academic and industrial researchers.

The best way to celebrate the accomplishments of chemists and engineers who transformed medicine and social life with the first successful antibiotic is to continue their legacy to improve people's lives through the combined power of biology, chemistry and chemical engineering.

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[1] Eric Lax, The Mold in Dr. Florey's Coat, p.14
[2] Gordon T. Stewart, The Penicillin Group of Drugs, p.20

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