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The Lesser Known Story of the Development of Chemotherapy and Its Circuitous Path

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Abstract

Although the discovery of penicillin was attributed to Alexander Fleming, there were other talented scientists who participated in bringing about a favorable outcome. However, these individuals were unable to pursue the project to a successful completion for a variety of reasons. These missed opportunities were important lessons and merit attention, since they delayed the search for other antibiotics which would provide the foundation for developing chemotherapy as an extraordinarily effective drug regimen.

Keywords: Penicillin; Penicillium notatum; Alexander Fleming; Paul Ehrlich; Fungus; Ernest Duchesne; Andre Gratia; Cecil Paine; Harold Raistrick; Roger Reid

Introduction

The discovery of penicillin by Alexander Fleming is a familiar story in the annals of biomedical science; but it is also the subject of much debate [1]. While studying the properties of *Staphylococcus aureus,* Fleming observed that this microorganism became contaminated with a mold that inhibited bacterial growth. Which he named after the fungus *Penicillium*. Although he determined that the fungus was non-toxic to laboratory animals, Fleming was unable to extract the compound from the mold broth or reveal most of its basic properties.

Fleming worked on penicillin from 1928-1931 and published only one paper on the subject [2]. Lacking the skills to continue his research with penicillin, Fleming believed that success of the project would require the expertise of a chemist. Fortunately, Fleming's discovery was carried forward by Howard Florey and Ernest Chain, who made penicillin available as a new wonder drug. In 1945 Fleming, Florey, and Chain were awarded the Nobel Prize.

Although the discovery by Fleming has been described by some as accidental, several others did conspire to attempt a favorable outcome. This article provides additional information about those lesser known individuals who played a role in its discovery. Their stories will document that this extraordinary advance in chemotherapy was not a simple chain of causation.

Early Studies

As early as 1500 B**.C.,** there were records describing the use of molds in the treatment of diseases. However, such treatments were carried out without an understanding of the nature of the cellular and biochemical processes of the human body. The Germ Theory of Disease was postulated by Louis Pasteur in the late 19th century, which prompted the search for compounds to cure infectious diseases. During this period, Joseph Lister introduced antiseptics to treat infections and surgeons adopted the concept of antiseptic surgery [3]. In 1871, Lister also observed that the inhibition of bacterial growth in urine samples was prevented by contamination with mold. These rudimentary studies provided the groundwork for the investigations that followed.

Paul Ehrlich's Magic Bullet

In 1897 Paul Ehrlich was appointed Director of the Royal Institute of Experimental Therapy at Frankfurt-am-Main in Germany. There, he devoted his work to chemotherapy [4]. Ehrlich's interest in drug therapy was spawned by his observation that synthetic dyes were able to stain pathogens, but not their host cells. He thus predicted that an infection produced by a microorganism could be treated if the drug was selectively taken up by the microorganism. Coincidentally, at this time the spirochete that caused syphilis had been identified and Ehrlich decided to search for a drug that would be effective against this organism.

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Figure 1: Ernest Duchesne. Taken from Wikipedia, Nov. 2011.

After Ehrlich and his assistant Sahachiro Hata succeeded in infecting rabbits with syphilis, they began testing a large number of compounds in animals; number 606 (salvarsan or arsphenamine) was found to be effective. Salvarsan was a compound that had been previously tested and discarded as being ineffective. Subsequently, Ehrlich and Hata found that the 914th compound called neoarsphenamine possessed less of a curative effect, but greater solubility was more easily administered.

After *Farbwerke Industrie,* a giant conglomerate, distributed large quantities of the drug throughout the world, salvarsan became the most effective treatment for syphilis until the advent of penicillin in the 1940's. Paul Ehrlich was awarded the Nobel Prize in 1908 for his discovery of the "magic bullet" and establishing the foundation of modern chemotherapy. What follows is the slow progression of the development of antibacterial therapy, as well as the factors that inhibited its progression.

Ernest Duchesne (1874-1912)

During the late 19th century, the idea of a systemically administered drug to treat infections was a revolutionary concept. At this time, Ernest Duchesne was working on his doctoral thesis on microbial antagonism at the Military Health Service School at Lyon France (Figure 1). Duchesne completed his dissertation in 1897 by studying the interaction between *E. coli* and *Penicillium glaucum*. His observations included the lysis of bacteria by the fungus and the prevention of an animal from contracting typhoid fever by the mold [5].

Although Duchesne demonstrated that *Penicillium glaucum* was effective against typhoid fever, he failed to establish a link between the fungus and a substance that possessed antibacterial properties [6]. In addition, he was not viewed as the discoverer of penicillin because he employed *enicillium glaucum*, which proved to be a much weaker antibiotic than *Penicillium notatum*. Nevertheless, this work was a key contribution to the antagonism between molds and microbes because Duchesne, not Fleming, was the first to demonstrate that a mold was able to cause a bacterial infection.

Because Duchesne was young and inexperienced, his work was left unnoticed at the time. Moreover, the famed Pasteur Institute did not even acknowledge the receipt of Duchesne's dissertation; and so his work remained unknown until the experiments of Alexander Fleming. Although he urged that further studies be conducted on this subject,

Figure 2: Andre Gratia. (Taken from Wainwright M. *Op. Cit.* [9]; pg. 39).

Duchesne went into the army and died from tuberculosis before reaching forty years of age. In 1949 Duchesne was posthumously honored for his work by the French *Academie nationale de medicine.*

Andre Gratia (1893-1950)

Andre Gratia was mentored by the Belgian Nobelist Jules Bordet (Figure 2). In the 1920's, Gratia and his coworker Sara Dath were examining many molds and fungi at the Pasteur Institute in Paris when they observed that the growth of a culture of *Staphylococcus aureus* was inhibited by a contaminating mold. Instead of culturing the mold, they simply observed its lytic effect. They later identified this fungus as a species of *Penicillium* and presented their findings in a paper published in 1924 [7]. The next year, they observed an anthrax culture that was lysed by a mold which was a variety of *Penicillium glaucum* [8]. Gratia also identified a mold of a type of *penicillium* that he had used to treat furuncles. This lytic agent was able to inhibit several different microorganisms, but was ineffective against *E. coli*. However, Gratia like Duchesne also failed to continue this line of research. Once the dramatic effect of penicillin was revealed some time later, Gratia never forgave himself for his monumental oversight [9].

Therefore, Gratia, not Fleming, was the first to observe that *Penicillium notatum* possessed anti-bacterial actions. In fact, Fleming happened to know Gratia quite well, and Fleming's original paper referenced the article published by Gratia and Dath. But the lack of interest shown by colleagues in his work, together with the failure to understand what he had achieved, consigned Gratia to an obscure role in the annals of science. However, his earlier discoveries set the stage for the advances made by Alexander Fleming and beyond.

Figure 3: Cecil Paine. (Taken from Wainwright M. and Swan HT. *Op. Cit.* [13]).

Cecil Paine (1905-1994)

Cecil George Paine was the first person to effectively treat surface wounds with penicillin (Figure 3). Paine had qualified as a doctor at St. Mary's Hospital Medical School in London where Fleming worked. At the time he carried out his work with penicillin in 1929, Paine had assumed a joint position at the Sheffield Royal Infirmary and the University Medical School. Although Paine had known about the penicillin work at St. Mary's and had read Fleming's recent paper on the subject, he never consulted with Fleming. However, Paine did obtain a culture of an isolate from *Penicillium notatum* from Fleming and grew it in meat broth in his laboratory [10].

His first attempt at employing penicillin involved a patient with a skin infection of the hair follicle with *Staphylococcus* [11]. The application of a crude penicillin extract was unsuccessful. However, undaunted, he employed the crude filtrate to successfully treat patients with eye infections. He subsequently successfully treated a miner with a lacerated eye caused by *Pneumococcus,* which normally would have required the removal of the eye. Another case at the Sheffield Royal Infirmary involved a baby who had contracted a gonorrheal eye infection from his mother; once again the baby's eye was spared after treatment with the penicillin extract.

Although Paine was the first to obtain a documented cure using crude penicillin filtrates, he abandoned his promising experiments when he moved to Sheffield's Jessop Hospital, where he focused his interest on puerperal fever. Because he had used a crude extract to treat patients, Paine never wanted to publish his 1931 experiments or present a paper on his findings. In addition, lacking the skills of a chemist, he failed to pursue this project any further. The lack of availability of a method to preserve the active component of the unstable filtrate also probably played a major role in the loss of interest exhibited by Paine in the project [12].

However, in 1932, while at Sheffield, Paine did discuss his work with Howard Florey, who had recently taken over the Chair of Pathology. Although Paine viewed penicillin only as a possible antiseptic agent, his work ultimately helped to convince Florey of penicillin's medical potential. But another ten years were to elapse before Chain and Florey were able to purify penicillin and achieve the remarkable cures that this miracle drug produced.

Paine's work was largely overlooked until the 1980's when he was interviewed by Dr. Milton Wainwright and Harold Swan, who had found the case notes of his work [13]. These notes provided the first documented evidence for the use of penicillin as a therapeutic agent. Paine was very contrite about not reporting his findings and described himself "as a poor fool who did not see the obvious when placed in front of me" [14]. Paine was eventually awarded an Honorary Degree from Sheffield in 1987. In addition, Florey graciously acknowledged Paine's contribution in his second volume of *Antibiotics* published in 1949 [15].

Harold Raistrick (1890-1971)

The next step in the story of penicillin involved Harold Raistrick, the Director of Biochemistry at the University of London, who identified many fungal metabolites and their chemical composition (Figure 4). Raistrick's accomplishments as a distinguished microbiologist earned him many awards and honors, including membership in the prestigious Royal Society [16]. During the 1940's, Raistrick, acting as Scientific Advisor to the Ministry of Supply and

Figure 4: Harold Raistrick. (Adapted from Birkinshaw J.H. *Op. Cit.* [15]; pg. 489).

a member of their General *Penicillin* Committee, visited the United States and Germany to exchange information with pharmaceutical companies on *penicillin* production. Thus it seems fair to conclude that Harold Raistrick was eminently qualified to make a major impact on the development of chemotherapy [17].

Harold Raistrick and his colleagues decided to investigate the bacterial inhibitory properties of *Penicillium notatum.* Although Raistrick was unaware of previous attempts to extract penicillin, he was provided with a supply of the *penicillium* mold by Fleming. In 1932 an investigation of the production of the mold revealed that the substance was produced on a synthetic culture medium and that it was extractable with ether from an aqueous medium [18]. Raistrick later confirmed that the extract was related to *Penicillium notatum.*

Raistrick obtained another species of *Penicillium* strain from the Lister Institute and concluded that Fleming's mold was *Penicillium notatum* and not *rubrum,* as Fleming had reported [19]. Raistrick's group also found that other strains obtained from Norway failed to inhibit bacterial growth; only Fleming's strain was active. This selective inhibition of growth was a phenomenon that Raistrick had never encountered. In addition, because the substance disappeared following evaporation, Raistrick summarily concluded that the production of this unstable substance would be an insurmountable task. However, if Raistick had performed a simple back extraction from organic solvent into a buffer solution, *penicillin* would have become available ten years earlier [20]. The fact that an expert chemist such as Harold Raistrick abandoned the project was probably a major factor in the lack of interest of the scientific community in pursuing penicillin as a therapeutic agent.

Roger Reid (1905-1979)

There was a significant contribution to the penicillin story by an American microbiologist named Roger Reid (Figure 5). He obtained his doctoral degree from Pennsylvania State University (now known as Penn State University) in 1935. He taught at Penn State from 1931-1936, and then joined the faculty at Johns Hopkins after a short period of working in industry. He ended his career as Professor at the University of West Florida [21].

As a graduate student, Reid's thesis included a study of the properties of *penicillin*. Reid had read the 1932 paper by Harold Raistrick and his colleagues and, in consultation with his mentor, decided to study the bacterial inhibitory properties of *Penicillium notatum.* Reid obtained a sample from Fleming, which he referred to

Figure 5: Roger Reid. (Adapted from Wainwright M. *Op. Cit.* [20]; pg. 14).

as *Penicillium rubrum***.** Charles Thom, an American mycologist, later identified it as *Penicillium notatum*. Using a synthetic medium, Reid found that its antibacterial activity was highly variable, confirming both Fleming's findings and Raistrick's conclusions that the active substance was unstable. Despite testing more than twenty strains of mold, Reid was unable to identify an active agent [22]. However, he was the first to publish details of a systematic search for antibacterial agents. He authored three papers on the subject, the first one in 1933 on the antibacterial activity of *Penicillium notatum* [23]; a second one in 1934 on molds closely related to *Penicillium notatum* [24]; and a third one in 1935 on the properties of the mold [25].

Although Reid was unsuccessful in extracting the active substance [26], he did consider possible medical uses of penicillin in light of the fact that he injected broth filtrates intraperitoneally into guinea pigs without producing any ill effects. Reid also found that most *Gram negative* bacteria were not inhibited by penicillin and that it could inhibit, but not lyse, *Staphylococci*.

Reid's work was directly related to the potential use of penicillin as a therapeutic agent and the factors that affected its production. In his later publications, he did not restrict his tests to *Staphylococci,* but examined a large number of microorganisms to protect against experimental infection in animals. He continued working with *penicillin* even after his extensive, but futile, search for *penicillin*-producing fungi. Although realizing that penicillin would have to be purified before it could be employed as a therapeutic agent, Reid was unable to accomplish this task [27]. Thus, it seems fair to state that Roger Reid's work had little influence on the subsequent development of *penicillin*. Although Reid failed in his quest, it is of interest to note that he continued this general area of research into the 1940's, when he published papers on antibacterial substances from molds and on metabolic products of *Penicillium*.

For the next several years, studies concerned with penicillin were for the most part suspended, although both Eli Lilly and Squibb Pharmaceuticals showed some interest in developing a research program that involved *penicillin*. But the entry of Howard Florey and Ernst Chain into the picture, plus the impetus for *penicillin* production spurred by World War II, all contributed to the development of this miracle drug. One might have predicted that this advance would prompt a search for other systemically effective antibiotics. Indeed, about this time, a class of drugs alternative to antibiotics emerged at *I.G. Farben Industrie* in Germany under the supervision of Gerhard Domagk, which became known as the sulfa drugs. The landscape was now set for the emergence of the age of chemotherapy.

Epilogue

This article has attempted to present a picture of the missed opportunities that arose on the path to the development of antibiotics. The early pioneers of research in developing penicillin were talented scientists who were willing to venture into unchartered territory, but were unable to pursue the project to a successful completion. These missed opportunities were consequential, in that they delayed the search for other antibiotics which would provide the foundation for developing more effective drug regimens and combating drug resistance.

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