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The Penicillin saga: a different tale

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Abstract

The Sumerians, but also the ancient Egyptians, as well as Greeks and Indians, used extracts of some plants and fungi for the treatment of infections [1]. Henryk Adam Aleksander Pius Sienkiewicz (1846 - 1916), the author of *Quo Vadis*, Nobel Prize for Literature in 1905, in his novel "*With iron and with fire"* reports that during the 17th-century in Poland, wet bread was mixed with cobwebs (which often contained fungal spores) to heal wounds. In the same period, in England, in the book entitled *Pharmacopoeia Londinensis*, the apothecary and botanist John Parkinson (1567 - 1650) recommended the use of molds as a medical treatment for many infectious diseases that affect humans. These treatments often work since many organisms, including many species of mold, naturally produce antibiotic substances. However, ancient practitioners could not accurately identify or isolate the active components of these organisms.

Similar experiences and evidence have been found in many other countries, including Italy. For example, Bartolomeo Bizio (1791 - 1862), found in 1821, that the red color assumed by "polenta" (a corn meal dish) was due to a bacterium that he named *Serratia marcescens* and that its development was inhibited by the presence of mold.

After 1850, thanks to the progress of chemistry, the chemotherapy concept for the treatment of infectious diseases began to assert itself. Consequently, some effective chemical compounds were synthesized (for example sodium arsenylate by Antoine Béchamp in 1859 and used, at that time, against sleeping sickness and other trypanosomiasis. It was later abandoned due to its remarkable toxicity). However, thanks to Louis Pasteur (1822 - 1895), in the seventies and eighties of the nineteenth century, the interest in substances of natural origin returned. Pasteur in fact highlighted both the inhibiting action of molds on the development of some bacteria and the antagonism between different bacteria. These observations were taken up by Arnaldo Cantani (1837 - 1893), who tried, with poor results, to apply Pasteur's concept, developing bacteriotherapy (fight against pathogenic bacteria with other harmless bacteria) to treat tuberculosis.

Keywords: Penicillin

First evidences

Towards the end of the 19th century, it became evident that the mold-bacteria antagonism gave the best results when using molds produced by the genus *Penicillium*. According to the Dictionary of the Fungi (10th edition, 2008), the genus *Penicillium* is very widespread and includes over 300 species that are of great importance in the natural environment, in spoilage and in the production of cheeses such as Gorgonzola, Camembert, Brie, Roquefort, Stilton, etc. and drugs (*e.g.*, penicillins and mycophenolic acid). In the early stages of research on penicillin, most species of *Penicillium* were generally referred to as *Penicillium glaucum*, making it impossible to identify the strains actually used. Therefore, it is difficult to establish whether penicillin actually prevented bacterial growth in the following situations [2].

Bartolomeo Gosio (1863 - 1944) was a physician, microbiologist and biochemist Italian who worked in the *Scientific laboratories of the health* in Rome, of which he later became director. In 1893, he found in a mold of *Penicillum glaucum*, a metabolite with antibiotic properties and purified it. This line of research was originally aimed at identifying the causes of pellagra, a widespread endemic disease in many regions of northern Italy, in particular Lombardy and Veneto, starting from the end of the 18th century [3]. Gosio supported the hypothesis, originally proposed by Cesare Lombroso (1835 - 1909), that pellagra was caused by a poisoning produced by altered corn, in particular due to the action of some molds [4]. During this research, that obviously was not be able to highlight the toxic origin of pellagra, Gosio purified and crystallized an innominate substance produced by *Penicillium glaucum*, which presented phenolic characteristics. The results describing the toxic and antibacterial properties of the compound were the subject of numerous publications [5, 6]. The

antibacterial substance was identified in 1945, and confirmed in 1968, as mycophenolic acid [7], while the fungus that produces it turned out to be *Penicillium brevicompactum* [8]. Recently, it was shown that other types of *Penicillium* (*stoloniferum* and *echinulatum)* are able to produce the mycophenolic acid [9]. As explained above, the fact that it was produced by *Penicillum* misled several authors, who believed that the mycophenolic acid was an antibiotic belonging to the class of penicillins. Currently the mycophenolic acid (DCI) or mycophenolate is a immunosuppressant drug used to prevent the rejection reaction to transplanted organs and in the treatment of lupus erythematosus. Furthermore, the wording "arsine penicillary", which appears on a bottle still present at the Museum of the History of Medicine in Rome, cannot refer to the hypothetical "first sample of penicillin", as claimed by Borra (1988) and Donelli and Di Carlo (2002), but it is certainly related to the mercuric compound of trimethylarsin mentioned by Gosio. This confusion does not detract from the importance of Gosio's discovery, which was recognized by Florey *et al*. (1946): according to which mycophenolic acid “has the honor of being the first antibiotic, derived from a fungus, to be crystallized" [10]. Unfortunately, this recognition took place two years after Bartolomeo Grosio’s death.

The discovery of penicillin

The first to notice that certain molds could have been attributed antibacterial properties was the Molise physician Vincenzo Tiberio (1869 - 1915). Tiberio had observed that when the walls of the well of the house in Arzano, near Naples, where he was a guest when he was studying medicine around 1892/93, were cleaned, among the users of the well, enteric episodes occurred contrary to what happened in the presence of molds that spontaneously formed there. On the basis of these observations, he experimented the bactericidal action of the aqueous extracts both *in vivo* (on guinea pigs and rabbits), and in culture on *staphylococcus, typhus bacterium*, *carbuncle* and *cholera*.

Tiberio's studies were published in 1895 in an Italian magazine (*Annals of di experimental hygiene - Annali di igiene sperimentale)* [11] which, even in Italy, was scarcely known. Therefore, these researches were soon forgotten. The same Tiberio was subsequently unable to follow up on these studies.

In 1946 appeared on Minerva Medica, an Italian scientific journal, an article by Prof Pietro Benigno (1918 - 2007), at that time Pharmacologist of the University of Padua, entitled "*A pioneer of research on antibiotics*". In this article the author stated: “The Tiberio’s researches are conducted with such accuracy of investigation, to deserve a fundamental place in the research of antibiotic factors" [12].

During the stay of one of the authors of this article in Sicily (December 1 - 10, 1990), Prof Benigno, at that time Professor of Pharmacology at the University of Palermo, told the backstory of his discovery. In the late spring of 1945 just after the Second World War, the laboratories of the Institute of Pharmacology of the University of Padua were rearranged. From some containers placed in the basement of the Institute emerged some laboratory notebooks from the end of the 19th century. In particular, Prof Benigno's attention was drawn to one of Prof Vincenzo Chirone's (1847 - 1908) laboratory notebooks, dated 1894-96, which on the cover read "Vincenzo Chirone - Researches commissioned by Ch.mo Prof Mariano Semmola". It described the experiments carried out on some molds of different origins: Paduan, Neapolitan and collected near 15 national sources of sulphide-alkaline waters. The solutions of the cellular products, obtained from these ifomycetes were investigated, through experiments conducted *in vitro* and *in vivo*; the ability of these solutions to induce bactericidal activity on some bacteria was thus highlighted (*typhus bacillus, cholera vibrio and staphylococci, aureus, album,* and *citreus.* At that time, penicillin-resistant *staphylococci* were not yet present). The results obtained, especially when using extracts of *Penicillium glaucum, Mucor mucedo* and *Aspergillus flavescens* molds, were more than satisfactory. Furthermore, the activity of these extracts was particularly evident for the products that came from Naples. Periodically, as Prof Benigno was able to ascertain, Prof Chirone sent his Master reports, however very brief, on the results obtained and, when he went to Naples, obviously he proceeded to discuss the results with him. On at least two occasions, Prof Étienne Jules Marey (1830 - 1904), who at that time spent a lot of time in Naples also participated in these meetings.

It was, however, from the examination of these laboratory notebooks that Prof Benigno became aware of the studies of Dr Tiberio and subsequently succeeded in identifying the publication of the latter entitled: *On the extracts of some molds* (*Annals of experimental hygiene, vol V, 1895)* [11]. Curiously, during conversations with Prof Benigno it was asked why these researches were not carried out in the Institute of Prof Semmola (Istituto di Clinica Terapeutica, University of Naples), but were commissioned to his pupil Prof Chirone. Prof Benigno explained that he had no definite proof, but that Prof Semmola, as Dean of the Faculty of Medicine, had some doubts about the researches of Dr Tiberio, conducted in the Institute of Hygiene directed by Prof Vincenzo De Giaxa, and that it was obviously less risky to carry out these checks in a location far from Naples.

Unfortunately, Prof Semmola died in Naples on April 5, 1896 and Prof Chirone was named to the Chair of Materia Medica and Experimental Pharmacology at the University of Naples. The research on molds continued slowly and then definitively ceased at the end of 1896 by order of Prof Aristide Stefani, Professor of Physiology.

In time, the Paduan researches fully confirmed the results obtained by Dr Tiberio, and that only some types of molds were able to manifest bactericidal activity. In particular, among all the tested molds, the Neapolitan ones of *Penicillium glaucum, Mucor mucedo,* and *Aspergillus flavescens* were the most effective.

In 1897, a young French student, Ernest Duchesne (1874 - 1912) noticed, during his stay at the Lyons Military Health Service School, that Arab grooms used mold taken from wet saddles to heal themselves the wounds. Having identified that mold as *penicillium glaucum*, Duchesne had tested it on some guinea pigs to treat typhus. The research, which would later also become his doctoral dissertation, entitled "*Contribution à l'étude de la concurrence vital chez les micro-organismes: antagonisme entre les moisissures et les microbes*" (Contribution to the study of vital competition in microorganisms: antagonism between mold and microbes), suggested that, like some bacteria, molds, such as *penicillium glaucum*, could release toxins. He proposed that to treat diseases of bacterial origin, it was appropriate to use broth in which bacteria or molds were cultivated [13]. The results reported in the dissertation were, unlike those of Dr Tiberio, incomplete, contradictory and of limited numbers and, probably, for this reason Dr Duschesne's application for financing the project was not considered by the Pasteur Institute [14]. However, Duchesne's studies, unlike those of Tiberio, are widely cited in articles dealing with the history of penicillins. We do not know whether the decision of the Pasteur Institute to refuse funding to Dr Duschesne was influenced by Prof Marey. Etienne Jules Marey was Professor at the Collège de France from 1869 to 1904, Full Professor of *Histoire Naturelle des corps organisés* (Natural history of organized bodies), as well as a Member of the French Academy of Sciences and the Pasteur Institute. His acquaintance and friendship with Prof Chirone stemmed from the fact that the latter had carried out research in the prestigious Parisian laboratory of Prof Marey, from 1874-76. As mentioned above, at least on two occasions, Prof Marey attended meetings with Profs Semmola and Chirone. In the second meeting, Marey handed Chirone samples of two molds taken in Paris. Subsequent investigations, conducted in Padua, undoubtedly demonstrated that Parisian molds had little or no bactericidal activity on staphylococci. This was confirmed in 2016 by Gilbert Shama: he in fact demonstrated that penicillium glaucum itself is not capable of producing penicillin [2].

In August 1928 bacteriologist Alexander Fleming, who worked in Sir Almroth Wright's Inoculation Department at St Mary's Hospital in London, discovered that a culture dish on which he was growing staphylococcal colonies had accidentally been contaminated with a mold, which he mistakenly identified as Penicillium rubrum (in reality it was Penicillium notatum, due to the shape of the sporophores which resemble a brush). To his astonishment Fleming observed that staphylococci were

unable to grow in the vicinity of the mold. This singular behavior prompted Fleming to investigate that mold more carefully. The mold broth filtrate was called penicillin and was able to inhibit the in vitro growth of some Gram-positive bacteria, such as staphylococci, streptococci, pneumococci and gonococci, in dilutions from 1 to 800; no effect was found on Gram-negative organisms. In addition, Fleming demonstrated that penicillin had, in experimental animals, little or no toxic effects and when applied topically to the human skin did not cause toxic reactions. However, from what can be gleaned from a reading of his publications, he did not foresee any chemotherapy application to penicillin. He thought it was another member in the group of slow acting antiseptics. Therefore, although Fleming recognized penicillin's antibacterial properties, he never tested the antibacterial activity of its filtrate *in vivo.*

In 1938 Fleming came into contact with Howard Florey, Ernst Chain and others who worked at the Sir William Dunn School of Pathology in Oxford. A good friend of this research group was also Prof Egidio Meneghetti (1892 - 1961), one of the most important Italian Pharmacologists of that period, Director of the Institute of Pharmacology of the University of Padua since 1932. Under his school was formed Dr Nicolò Ercoli, a Jewish researcher of Hungarian origin (original surname Herskovits) fled from Nazi Germany and welcomed by Meneghetti in the Institute he directed. Shortly before the racial laws were promulgated in Italy, anticipating those already foretold in Germany, Prof Meneghetti provided Dr Hercules a scholarship that allowed him to reach Florey and Chain's group. They had long been interested in antibacterial substances and part of their work included the study of lysozyme, an antibacterial enzyme that Fleming had discovered in 1922. Precisely the *in vivo* pharmacology of this substance was to be the subject of Ercoli’s investigation. They were unaware of Fleming's research, but they became interested in the ability of penicillin to inhibit the growth of staphylococci and immediately began studying this substance, stopping all other lines of research. In May 1940, this study group collected enough penicillin to test its therapeutic effects on animals. Two groups of animals with particularly virulent hemolytic streptococci were infected and in the group that received penicillin survival was at least three times longer than that treated with physiological solution. Subsequently, they showed that penicillin inhibited the growth of certain strains of *staph-*, *strep-*, and *gono-coccus* in dilutions of 1 to 1,000,000 or higher. In early 1941, the Oxford laboratory produced enough penicillin to treat six patients, all of whom were carriers of s*taphylococcal* or *streptococcal* infections. Some patients died, but their deaths were caused by complications unrelated to their infections or were simply due to the fact that not enough medication was available to complete the treatment. So, these early clinical trials demonstrated the efficacy of penicillin in severe infections.

This was all the more important as sulfonamides show very limited efficacy in *staphylococcus* infections.

Soon enough, Florey and Chain realized that in England it would be impossible to produce penicillin on a large scale. For this reason, in 1941, Florey and Heatley (another member of the group) went to the US to convince pharmaceutical companies to finance massive penicillin production. Pharmaceutical companies proved very skeptical initially, but things changed dramatically when the Oxford Group was able to convince the US and British governments of the importance of their programs. The first research took place at the Northern Regional Research Laboratory (NRRL) of the Department of Agriculture in Peoria, Illinois. This laboratory was chosen because it was already familiar with *Penicillium* strains and fermentation processes. In the next two years, methods for the industrial production of penicillin were developed at the NRRL and high-performance strains of *Penicillum* were isolated [15]. In December 1942, the survivors of a fire in Boston were the first patients with burns to be successfully treated with penicillin [16]. Pfizer Pharmaceuticals was one of the first private companies to enter the program for the development of the antibiotic. In 1943, Jasper H. Kane and other Pfizer scientists in Brooklyn developed a practical method of "fermentation in deep tank" for the production of large quantities of penicillin with suitable characteristics for administration to patients [17].

There were problems, however. For example, contamination of cultures was a common problem, such that, until 1943 penicillin production was slow and was not able to fill the needs of the civilian and the military population in the US and England. Furthermore, the cost of the antibiotic was still exorbitant, a gram of raw material containing, on average, five percent of pure penicillin, cost, until April 1944, at least the equivalent of the current € 100 (today it costs a few cents). These were some of the reasons that convinced American authorities to undertake a research program to obtain penicillin synthetically. Thirty-nine laboratories representing pharmaceutical companies (Abbott, Cyanamide, Lilly, Merck, Parke-Davis, Pfizer, Hoffmann-La Roche, Squibb, Upjohn, Winthrop), universities (Michigan, Illinois and Cornell University Medical College), government agencies (Department of Agriculture) and private foundations in the United States and Great Britain, joined the project. Completion of the project involved several hundred chemists, biologists and pharmacologists. One of the latter was Dr Ercoli who, already at the end of 1941, by virtue of a research agreement between the Foley group and Hoffmann-La Roche, moved from Oxford to the latter's laboratories in Nutley, New Jersey, which was involved exclusively in the development of synthetic penicillin. Merck Pharmaceuticals was the first company that succeeded in the enterprise (1944). However, the costs were significantly higher than the penicillin obtained by fermentation,

which in the meantime had made significant progress. Consequently, on November 1, 1945, the program was discontinued [18-20].

The penicillin chemical synthesis program, which took place during the Second World War, has often been called a failure. But, as can be seen from the publications of Prof Ercoli "it is not entirely right to express such a judgment because the results must be considered as a whole" [21]. Undoubtedly, a commercially useful synthesis could not be developed, but numerous and valuable contributions were made to the chemistry and pharmacology of beta-lactams, thiazolidines, penicilloic acids and beta-lactam-thiazolidines. These results opened the way to Sheehan's general synthesis of penicillins in the 1950s [22] with the consequent development of semisynthetic penicillins, which are still today of inestimable value from the therapeutic point of view.

Declarations

Conflict of Interest

The Author declares that there is no conflict of interest.

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