Penicillin: legend, history, and chance events

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Abstract

The author, based upon the reading of two biographies of Howard Florey, writes about the events that led to the discovery of penicillin. He tries to give a realistic account, putting away any legendary happenings and chances that allowed this discovery to take place.

Key words: penicillin, Fleming, Florey, Chain.

"The discovery begins with the awareness of anomaly" – Thomas Kuhn

"When the facts become legends, the legends imprint and not the facts" – John Ford

The discovery of penicillin was one of the most important events in the history of Medicine. But more than that, it was also an event that had the rare felicity of being told and retold, not only by some of those who were directly involved in it, like Alexander Fleming and Ernst Chain, but also by others, like André Maurois and Ronald Hare, who leave us with detailed reports of the facts and the people involved. Of all these written testimonies, and everything that was said, a legend emerged that was nothing more than a reduced, distorted version of what actually happened. This legend tells the story of Fleming, an obscure bacteriologist of St. Mary's Hospital; a *Penicillium notatum* that entered through the window of his office, and grew in a Petri dish; the inhibition of staphylococcus colonies, which were inhibited from growing around the area where the fungus developed; Fleming's enthusiastic reaction to this phenomenon, in contrast to the indifference of his colleagues and the director of the laboratory; the ten long years in which *Penicillium notatum* was relegated to the pages of the *British Journal of Experimental Pathology;* and the taking up of investigations once again, which culminated in the discovery of penicillin, and led to fame, glory and the Nobel Prize.

Some years ago, during a visit to London, I discovered in a book store two biographies of Howard Florey. Reading them made me realize that the story of penicillin, as I knew it and had sometimes recounted it, was only part of the story; it was full of inaccuracies, and like all narratives that have fallen into the realm of myth, it created a hero – Fleming – but omitted much of the important detail that went before and after. I also discovered some fascinating aspects that contain precious teachings for all those interested in the history of medicine and the genesis of major scientific discoveries. The version that follows, based on my reading of those two biographies, is neither original nor definitive: It seeks only to contribute to a better understanding of what really happened.

We shall begin, therefore, at the beginning. In 1881, Alexander Fleming was born in Nochfield, and in 1906, he became a practicing physician at St. Mary's Hospital. He was a man who was short in stature, introverted, with a great capacity for work. He was also an enthusiastic sportsman, who practiced: shooting, football, boxing and water polo. It was precisely this love of sports that ended up being the deciding factor in his career. In fact, somebody had suggested to Almroth Wright, director of the laboratory, to hire him as a researcher in order to ensure that he remained on the shooting team of St. Mary's, of which he was one of its best marksmen.

Fleming, who by this time had already decided on a career as a surgeon, ended up accepting, perhaps because from his time as a student, he had nurtured a great admiration for Almroth Wright. And so it was that in 1908, he joined the recently-created "Inoculation Department" of St. Mary's Hospital.

Almroth Wright and Fleming were completely different characters. Wright had a brilliant personality and a vast general culture, and was a versatile conversationalist, capable of conversing on any subject

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with rare eloquence. Fleming on the other hand, was precisely the opposite; he was introverted and self-effacing, with a confused and somewhat unappealing way of speaking. Another difference, this time of a scientific nature, would gradually come to separate the two men. Wright believed that the solution for the treatment of diseases was by stimulating the body's natural defenses. Fleming, on the other hand, began to ally himself with those who, in the tradition of Erlich, believed in the hypothesis that a "magic ball" would be discovered, capable of attacking the bacteria without harming the cells of the organisms. It is worth analyzing how these ideas took shape in his mind.

One day in winter of 1922, Fleming, who was suffering from a terrible "cold", decided to cultivate a drop of his own nasal secretion in a Petri dish. The result was as he had expected: various colonies formed around a mixed bacterial flora. But Fleming noted a strange detail: some of the colonies appeared to dissolve in the culture medium. So he isolated these colonies, made new cultures in liquid medium, and submitted them to the action of a drop of nasal secretion. The result was the same: the suspension, which was cloudy, became transparent because the bacteria underwent lysis. Fleming then observed that the phenomenon also occurred with other bodily fluids, such as tears, saliva and bronchial mucus. He concluded that all these fluids contained a substance, probably an enzyme that was targeting the walls of certain microorganisms, and gave it the name of *lysozyme*. The bacteria observed by Fleming were not previously known, and Wright baptized it with a strange neologism: *Micrococcus lysodeikticus*. Unfortunately, the pathogenic bacteria were not affected by this enzyme, which for Fleming, was a great disappointment. After publishing four articles in the *Proceedings of the Royal Society* and in the *British Journal of Experimental Pathology*, the last one being published in 1927, Fleming was unable to make any further progress, and abandoned the lysozyme project.

In 1928, it is known that he was interested in the study of staphylococcus strains, and attempted to link their virulence with the color of the colonies. At the end of July of that year, he was preparing for the start of his vacation, tidying up his laboratory, he stacked the Petri dishes containing staphylococcus colonies at one end of his work bench, to clear the bench for another colleague. When he returned from his vacation, he took the dishes and observed them again, together with a long-standing colleague who was visiting him at the time, and with whom he had discussed his projects. He then observed that in some of the dishes, fungi and yeasts had developed, which was not an uncommon occurrence, particularly in dishes which, like those, had lain untouched for several weeks. But Fleming, with his innate ability to see value in seemingly unimportant phenomena, noticed that around the area where one of the fungi was growing, there was a halo in which the staphylococci had not developed. It is said that he uttered the words "That's funny!", and that day, showed his discovery to various colleagues in the laboratory, in particular Almroth Wright. All of them, including Fleming himself, thought it was another lysozyme, and it appears that someone even said something like: "Here comes that boring Fleming with his lysozymes".

Nevertheless, Fleming decided to investigate further. He photographed the dishes and sub-cultivated the fungus. In the days that followed, the fungus grew, forming a fine layer over which a liquid accumulated. Then he used this liquid to fill a hole in the surface of an agar dish, into which he implanted various bacteria. After incubation, he observed that some of the bacteria (streptococcus, staphylococcus, pneumococcus, meningococcus) had not developed around the hole. The conclusion seemed evident: this liquid, segregated by the fungus (mould juice), contained a substance that inhibited the growth of certain bacteria, by good fortune, all of them pathogenic. A mycologist who worked on the floor below him identified the fungus as *Penicillium rubrum* which, as would later be verified, was incorrect - it was in fact *Penicillium notatum*.

Over the following weeks, Fleming continued to investigate, coming to several conclusions: The liquid, which he called *penicillin*, continued to prevent the growth of bacteria even when diluted 1/800; and it did not affect the activity of the leukocytes; neither did it reveal toxicity when injected into the peritoneum of rats.

Experiments carried out on humans were very limited at that time, and in a certain form, anecdotal. Craddock, who worked in the Department and had an acute case of sinusitis, dropped some of the liquid into his nostrils, without any benefit, but without suffering any side effects either; Rogers, who was part of the shooting team and contracted pneumococcal conjunctivitis on the eve of a competition, improved

quickly when he put a few drops of the liquid into his eyes. But it seems to have stopped there. Fleming thought the penicillin would probably be effective when applied topically to infected wounds, and laboratory man, he placed a high value on its use in the *in vitro* selection of cultures of non-sensitive bacteria, such as *Haemophilus influenza*. But it never entered his head that penicillin could bear any similarity to the "perfect antiseptic" that Lister had dreamed of. If that idea had occurred to him, he would have carried out the decisive experiment, which had already been performed various times by Erlich and his disciples: he would have injected the liquid produced by the *penicillium* into rats, together with lethal doses of streptococcus or pneumococcus. But Fleming did not take this step, and "his penicillin", as it would later come to be known, would wait a further ten years, until a series of strange and fortuitous events would once again bring it to center stage.

In summer of 1929, Fleming published his observations on *Penicillium* in the *British Journal of Experimental Pathology* then apparently, lost interest in the subject, as demonstrated by two facts: in 1931, in a publication of the *Royal Society of Medicine* entitled *"The indication for, and value of the intravenous injection of germicides"*, he made no reference whatsoever to penicillin, and in a conference in 1932, in the Pathology Section of the same Society, he selected the lysozyme his theme, and made no mention of the word "penicillin" at all.

However, some tentative attempts were made by other researchers to isolate penicillin. Soon after Fleming's initial observations, two men from his laboratory, Craddock and Ridley, managed to verify that under certain Ph conditions, and after extraction by alcohol or acetone, followed by concentration in vacuum, it was possible to remove much of the inactive material. After these experiments, it was possible to conclude that penicillin was a small, non-protein molecule which, in the conditions used, was very unstable. However, after several months, these observations were suspended without being published.

In 1932, another group of researchers, comprised of professional biochemists, led by Harold Raistrick, also attempted to purify penicillin. But their interest was far from being focused on the search for a substance with antibacterial action; what they were trying to study was the chemical composition of fungi. Raistrick had isolated sixteen new organic compounds produced by fungi, and asked Fleming for a culture of *Penicillium notatum*. He handed over this project to a colleague, P. W. Cluterbuck, and a bacteriologist, R. Lovell who, in the initial phase, carried out similar stages to those of Craddock and Ridley, whose works they were unaware of. But beyond that, they took a decisive step when they observed that after acidification of the medium, penicillin dissolved in ether, releasing a large portion of the impurities. However, once dissolved, penicillin lost all its biological activity, and attempts to separate it from the ether without destroying it proved futile. This difficulty, which was not resolved at the time, would be a crucial problem, and it was not until seven years later that its resolution enabled a rapid advance in the production of penicillin, and its therapeutic use.

In this first phase of the story of penicillin, there were two more frustrated attempts to isolate the active substance. One, of little importance, was that of R. D. Reid, a microbiologist from Pennsylvania, who did not manage to get much further than the others, but who observed that of the numerous varieties of *Penicillium*, none showed the activity of the original strain observed by Fleming, highlighting, once again, that it was indeed a fortuitous and truly exceptional stroke of good fortune. The other, more important attempt was that of Lewis Hold, in 1934, who worked in Fleming's laboratory, but who appears to have received no support from Fleming. After various experiments, he made two important discoveries, namely: that in acid medium, penicillin was soluble in amyl acetate, and that it could be separated from this solvent after alkalinisation. Now, it was precisely this latter operation that would prove to be of decisive importance later on. But Holt, unable to stabilize the activity of the isolated substances, stopped his research after a few weeks, without publishing his findings.

In the four years that followed, i.e. from 1934 to 1938, there is no record of anybody taking up Fleming's discovery as a subject of research, and penicillin appeared destined to be relegated to the pages of history as a mere curiosity, without any practical application. It is possible that this abandonment of penicillin was further prompted by another scientific discovery that took place in Germany, in the I. G. Farbenindustrie Laboratories of Bayer, in Elberfeld. Domack, who was researching the antibacterial activity of various dyes, following one of the key ideas of Erlich, ended up discovering *protonsil rubrum,* whose

efficacy in the treatment of pneumococcal infections in rats seemed undoubted. The entire scientific community was excited by this event, and Almroth Wright himself traveled to Elberfeld to see the research first hand. Nevertheless, it is interesting to note that when he returned to England, he expressed some contempt for the methods used by Erlich's school, which consisted of blindly experimenting on lists of chemical compounds in the hope that one of them would reveal, for some unknown reason, antibacterial activity. Wright took the view that all scientific investigation should develop out of an original theoretical idea.

At the end of 1938, a strange convergence of events took place that would lead to the rediscovery of penicillin. In fact, somewhat by chance, a group of researchers would stumble upon Fleming's work, and reopen the investigations. At the heart of these events, right from the beginning, was the Australian Howard Florey. Born in 1898 in the city of Adelaide, he studied Medicine there, completing his course in 1920. His vocation for scientific research, and the high scores achieved on his course led to his being granted a scholarship, which brought him to England. In 1922, he obtained a place in the Physiology Department, in Oxford, where he remained until 1924. His career then took him to various places: Philadelphia (1925), London Hospital (1926), Cambridge (1927-1932) and Sheffield (1932-1935). By the time he returned to Oxford, in May 1935, having been nominated professor of Pathology of Dunn School, he had built up an enviable Curriculum, and enjoyed great prestige in the British medical world. During the ten preceding years, he had developed, with considerable success, research projects covering areas as diverse as the behavior of the omentum in infections of the peritoneum, the therapy for tetanus, methods of contraception, lymphatic circulation, cerebral circulation, the protective action of the intestinal mucus, etc. Now he would have an opportunity, for the first time, to set up a department of his own, which would enable him to continue researching and dedicating himself to a subject in which he had had an ongoing and almost obsessive interest since 1929: the lysozyme.

Having begun, some years before, to study the mucus and its protective action and penetration of bacteria in the intestinal lumen, he had learned about the works of Fleming on the lysozyme, and admitted that besides its mechanical effect, the bactericide action of a similar substance, present in the mucus, was also involved in this process of protection of the mucosa. In order to identify the chemical structure of the lysozyme and the substract of the bacterial wall on which it acted, he needed the collaboration of an experienced biochemist. After various attempts, Florey decided to hire Ernst Chain, who had been recommended by Gowland Hopkins, professor of Biochemistry in Cambridge and Nobel Prize winner for Medicine in 1929.

Chain was a German Jew who, in 1936, had fled Hitler's regime and settled in England. Besides being an excellent biochemist he was also a talented musician, and could even have been a pianist of international renown. But in the new department of the Dunn School, directed by Florey, he found conditions of stability and security that would enable him to develop his qualities as a biochemist.

Despite the fact that the department was experiencing some financial difficulties, and the various other research projects that were taking place simultaneously, and were attracting great interest at the time – like the lymphatic project", led Sanders, Medawar and Jean Taylor – Florey's interest in developing his research on the lysozyme continued. His efforts would end up bearing positive results: in 1937, Chain managed to purify the lysozyme, and between 1938 and 1940, he identified its chemical structure – a polysaccharide – and the composition of the substract of the bacterial wall on which it acted – an N-acetylglucosamide. Until the end of his life, Chain never stopped emphasizing the importance of this work, which opened a new chapter in the knowledge of the biochemistry of bacteria.

It was precisely between 1937 and 1938, when he was dedicating himself to researching the lysozyme that Chain decided to carry out an exhaustive literature review on the bacterial lysis provoked by natural substances. This phenomenon, which Vuillemin, in 1889, called "antibiosis", had been the object of a review carried out in 1928, by Papacotas and Gaté. Besides various cases of antibiosis among bacteria, described prior to that time, some were also known in which fungi were involved: Lister, in 1871, made the first unpublished observation of a *Penicillium* with antibacterial action; Gosio, in 1896, described a crystalline substance produced by a *Penicillium* that inhibited the growth of *Bacillus anthracis*, and Duchesne, in 1877, reported on the protective action of *Penicillium glaucum* in animals infected with virulent

microorganisms.

Thus, Chain managed to gather about two hundred bibliographic references, and it was precisely at this time that he discovered, by mere chance, Fleming's article on penicillin. This was a decisive moment, therefore it deserves to be told in some detail. At the beginning of 1938, Chain went, one day, to the library of the Dunn School, searching for articles on the lysozyme published in the *British Journal of Experimental Pathology*. Two of these, written by Fleming, were in volumes 3 and 8; another two, written by Florey, in volume 11. Leafing through the various volumes of the journal, as he was no doubt in the habit of doing, he stumbled upon Fleming's article on penicillin, in volume 10. It is curious that what most attracted his interest about this article was that he wrongly thought Fleming had only found another lysozyme.

Florey was not particularly enthusiastic when Chain told him about his discovery. Knowing that he was a habitual reader of the *British Journal of Experimental Pathology,* for which he had, for some time, been on the board of editors, it would seem that that he already knew about Fleming's article, and had probably not attributed much importance to it. Even so, he gave his agreement to Chain's idea to include penicillin, together with actinomycin and the pyocyanic bacilli, in an investigation on antibacterial substances. Both knew that the search for antibacterial agents with possible therapeutic application could attract sponsors and financing, which the Department greatly needed to carry out its projects. But, as they later affirmed, it never entered their heads at that time that it could help "lessen the suffering of humanity": their objectives were, in fact, purely scientific.

Chain immediately began to investigate penicillin, but the initial results were discouraging, as they were unable to reproduce even Fleming's initial observations. The reason for this, which we now know, was at that time an enigma. As is known today, penicillin, unlike the lysozyme, does not cause bacterial lysis: its action is exerted only in a phase of cell multiplication, inhibiting the synthesis of the wall, a fact which subsequently leads to autolysis. In the presence of already formed colonies, penicillin is, therefore, totally ineffective. Thus, it would have been difficult to see what would have occurred place in the Petri dish with staphylococcus colonies in which Fleming had observed, for the first time, the phenomenon of antibiosis caused by *Penicillium*. Despite the various explanations that were proposed, the mystery continued.

So Chain launched himself into the biochemical study of penicillin, repeating the stages carried out six years earlier by Cluterbuck and Lovell in an attempt to arrive at the same conclusions; it was a small molecule that, certainly, was not an enzyme and that showed great biological instability. But, like the other researchers, he came up against the same obstacle: once dissolved in ether, it did not seem possible to separate it in a way that kept its biological activity intact.

One aspect was, at that time, shrouded in mystery: the exact moment, and the reasons that led Florey to decide to investigate penicillin as a priority project of his department. Everything leads us to believe that the decision took place in the fall of 1938, but the circumstances in which it was taken are steeped in legend. It is seen, for example, that the two accounts of what was one of the most decisive moments in the history of penicillin tell that Florey found himself, at that time, under a tree. But in one of the accounts, it was a chestnut tree, and in the other it was an elm tree. Regardless, some months would pass before Florey became completely involved in the project, probably because the studies with lysozyme and lymphocyte were still in progress, and there were not enough financial resources available. Finally, on 6 September 1939, three days after war was declared between England and Germany, he gave a demonstration to Edward Mellanby, secretary of the Medical Research Council, in a bid to win funds for his research into penicillin. In this demonstration, he began by referring to lysozyme and its action on some non-pathogenic bacteria: he mentioned other microorganisms that were active against staphylococcus, pneumococcus and streptococcus, among which were certain strains of *Penicillium, Actinomices* and some soil bacteria; next, he refers in particular to the penicillin "discovered by Fleming" and its efficacy against staphylococcus; finally, he ends up making some statements of doubtful scientific rigor, which was contrary to his habit, probably pressured by the enthusiasm and impatience of Chain. Specifically, he states that penicillin could be purified and produced in large quantities with relative ease, and that it did not show any toxicity in laboratory animals. The truth is that all these facts were still awaiting confirmation, and would only be verified later on.

The Medical Research Council agreed to assign

funds that were clearly insufficient (one hundred pounds a year for three years), but Florey, probably already convinced of the importance of the project, appealed to the Rockefeller Foundation, where he had some friends. At the beginning of 1940, he was given substantial funds (1670 pounds a year for five years), and Florey, for the first time in his career, had enough financing to carry out a project in which he had decided to take a gamble.

Meanwhile, he decided to invite the young N. G. Heatley to the team, who left his internship in Denmark, faced with the worsening situation on the European continent. Heatley was a specialist in laboratory engineering, and was also highly skilled in the areas of optics, mechanics, carpentry and electricity. He was, therefore, the right man for a phase of the project in which everything depended on the practical problems that emerged to the production of penicillin. He accepted the invitation with the condition that he would work under the direct orders of Florey, without interference from Chain.

At the beginning of 1940, the four problems encountered by this team were: 1 – finding a way of accelerating the growth of fungi that would provide sufficient quantities of penicillin; 2 – achieving rigorous methods of antibacterial evaluation; 3 – studying the biological effects of penicillin on the cells and living organisms; 4 – developing the necessary biochemistry for the purification of penicillin.

The first important contribution came from Heatley, who came to use segments of small glass tubes, containing penicillin, implanted on the surface of the Agar (cylinder plate). The diameter of the circle around the tubes, in which the microorganisms did not develop, came to constitute the physical unit for evaluating the activity of penicillin, which would later be expressed in units.

The major biochemical breakthrough occurred in May 1940, during one of the meetings that Florey frequently held, with all the team members. At the meeting, the same problem as always was being discussed, that Chain was unable to resolve: how to separate penicillin that had been dissolved in ether. Although it was not a subject within his area of specialization, Heatley tentatively suggested a solution that seemed obvious to him: if to dissolve penicillin in ether, it was necessary to acidify the medium, why not do the opposite, i.e. alkalinize it? Florey found the proposal interesting, and thought it should be confirmed by Heatley himself. Chain thought the idea was nonsense, and resented this interference in his area of specialization. But the experiment worked, and from then on, the relations between the members of the team would be seriously affected.

The increased production of raw penicillin was achieved after a visit from Paul Fildes, a personal friend of Florey, who suggested adding beer yeast extract to the medium. Furthermore, it was observed that if the liquid that accumulated on the fungus were aspirated when the concentration of penicillin was at its maximum, it would be possible to obtain successive harvests, without the production of each one decreasing. Finally, with lyophilization, invented in the United States of America in 1935, it became possible to maintain the stability of the organic substances which, like penicillin, proved very unstable. The end result of all this was a brown powder with a much higher biological activity than all the other extracts achieved thus far.

In March 1940, Chain had 100 milligrams of this powder at his disposal, and began to become impatient. He believed that it was time to test the toxicity of penicillin in animals, but Florey, who was in charge of this area, remained oblivious and indifferent to his insistence. So Chain decided to continue alone. He diluted part of the powder that he had available (between 40 and 80 milligrams, the exact amount is not known) in 2 c.c. of water, and asked a biologist, J. M. Barnes, to inject 1 c. c. of the solution into each peritoneal cavity of two rats. The result of this experiment was clear: the animals did not show any secondary reaction whatsoever. Chain, who did not know that Fleming had carried out a similar experiment, communicated the fact to Florey who, as was to be expected, reacted badly. This time, it was he who felt it was an invasion of the area for which he was responsible – the biological assays – and his relations with Chain, which were not very good in the first place, deteriorated even more after this incident. But after that, Florey was unwilling to lose control of the situation again, and in the months that ensued, supported by Margaret Jennings, he repeated the animal experiments, testing various administration routes and gathering information on absorption, excretion, and possible toxic effects. Since that time, it has been discovered that penicillin is destroyed in the stomach that it is active when injected by any route, and that it is excreted unaltered in the urine.

At that time, Florey added two bacteriologists to the team – Gardner and Jean Orr-Ewing – who were responsible for studying the sensitivity of the various microorganisms to penicillin. It was they who, on observing the growth of the sensitive bacteria in the presence of penicillin, came to the conclusion that it did not act like an antiseptic or an enzyme, but rather, as a blocker of the normal process of cell division. Furthermore, Gardner, through detailed experiments, determined the minimum dose of virulent streptococci, which is 100% lethal for rats of standard weight.

Thus, the steps were taken that placed Florey and his team at the threshold of the decisive step: to demonstrate the effectiveness of penicillin in experimentally infected animals. It was Florey himself who prepared the experimental protocol – a true model of economy of means to obtain maximum information, which was so essential due to the lack of active substance. At 11 o'clock on the morning of Saturday, 25th May 1940 – at the same time as the English army was being pushed back to Dunkirk – in Oxford, eight rats were receiving intraperitoneal doses of 100 million streptococci. Four of them did not receive any subsequent treatment, and served as the control group; the other four were divided into two pairs: pair "A" were injected with 10 mg of penicillin by the subcutaneous route; pair "B" received an initial dose of 5 mg, repeated five times over the subsequent ten hours. During the afternoon of that day, everything appeared normal. At 10 o'clock in the night, Florey left a written note for Heatley, informing him of the situation: the rats treated with penicillin were in perfect health, except for one, in group "B", who appeared to be less active; the rats in the control group appeared to be very sick (at 3.30 in the morning, Heatley would verify that all the rats in this group were dead). On the Sunday morning, the 26th, Florey, Chain and Heatley returned to the laboratory, where they confirmed the death of all the rats in the control group, and saw that in the treated group, three rats were in perfect health, while one of them appeared to be sick. At that moment, they realized immediately that they were witnessing a truly rare event, and Florey, usually so circumspect and cautious, telephoned Dr. Jennings and exclaimed: "It looks like a miracle!". Without wasting any time, Florey repeated the experiment on the Monday, with ten rats, and on the Tuesday with sixteen rats, and the results were always the same.

But at that time, the research was seriously limited by the inability to increase production, and the difficulty in obtaining sufficiently purified material. Suffice to say that in 1940, they managed an activity of 5 units per milligram, which increased to ten units per milligram in 1940. When, years later, penicillin was finally purified, the activity increased to 1800 units per milligram. Despite these difficulties, the experiments continued during the months of June and July 1940, with a much higher number of animals (50 and 75) and using variable doses of pathogenic bacteria and active substance. Once the efficacy of the penicillin appeared undisputed, the problem was to define the doses, the interval between the various administrations, and the duration of the treatment.

However, this was the time of the Battle of Britain, and the threat of invasion by Hitler's troops. The professors in Oxford organized an evacuation plan for their families, to Canada, which included Florey's children – Charles and Paquita. Furthermore, faced with the danger that the Germans would one day occupy Oxford, and take control of a discovery as valuable as penicillin, the entire team was prepared to destroy all their records and equipment. But to ensure that the fungus would survive undetected, Florey and some of his collaborators decided to spread *Penicillium notatum* spores in the lining of their clothes, in the hopes that one day they would be able to begin again.

Florey then decided that it was time to publish the results of the experiments. It was in the *Lancet* of 24 August that the article *"Penicillin as a chemotherapeutic agent"* was published, with all the members of the team listed as authors, cited in alphabetic order: Chain, Florey, Gardner, Heatley, Jennings, Orr-Ewing and Sanders. It was a short communication, occupying two pages, and it concluded thus: *"The results are clear-cut and show that penicillin is active in vivo against at least three of the organisms inhibited in vitro. It would be a reasonable hope that all the organisms inhibited in high dilution in vitro will also be found to be dealt with in vitro"*.

After that, Florey waited for reactions from the scientific community, convinced that he would be able to attract financing for the project. He was wrong. The only consequence of the article in the *Lancet* was the unexpected visit of Alexander Fleming, who appeared at the Dunn School on 2 September. It is curious to note the amazement of some members of the team, particularly Chain, who was convinced Fleming had

died some years previously. Fleming was informed about all that had happened, and returned to London without making any comments.

For Florey, it had become clear that the time had come to urgently begin experiments on humans. He knew that to extrapolate for the human organism the results obtained in rats was risky, as there are biological differences between mammals that could involve the enzymatic inactivation of penicillin, or the appearance of unexpected toxic effects. But to start this new stage, it was essential to double the production of the active substance, to 500 liters a week, as a dose of 10 mg, effective on rats, was equivalent to 30 gr. In humans - approximately 3000 times higher. Heatley tried to resolve the problem by designing rectangular ceramic recipients with similar dimensions to those of a bedpan, produced especially for this purpose. In the company of Florey, they spent the Christmas eve and Christmas day of 1940 washing and sterilizing 100 of these recipients which, after being implanted with the spores of *Penicillium notatum*, were placed in an incubator for ten days. Thus began the production of penicillin – albeit in a small-scale way – in sufficient amounts to for use in the first experiments on humans.

The first objective was the study of toxicity, and for this, it was necessary to select a patient for the first experiment. This task was assigned to a young clinical doctor from Oxford. Charles Fletcher, who, on 17 January 1941, endovenously injected 100 mg of penicillin into a patient with a neoplasia in the terminal phase. Several hours later, a reaction of fever and hot flushes was observed, to which Florey did not attribute great importance. It was, without doubt, a case of pyrogenic substances, which reinforced the need to purify the penicillin. This was achieved shortly afterwards, with the introduction of chromatography.

The first administration of penicillin with therapeutic objectives was in a policeman aged 43 years, who had been hospitalized with sepsis staphylococcus, developing from a boil of the labial commissure. It was an extremely severe situation, with invasion of the subcutaneous cellular tissue of the face, affecting the eye sockets, and requiring enucleation of the left eye socket. On 12 February, 200 mg of penicillin were intravenous injected, followed by doses of 100 mg at 3-hour intervals. In the days that followed, a spectacular improvement was observed, with normalization of temperature, decrease in suppuration of the drained abscesses, and return of the patient's appetite. However, the reserve of available penicillin was depleted, and it had to be recovered from the patient's own urine, and on the 5th day, the treatment had to be suspended. At the beginning of March, the clinical condition worsened and the patient died on the 15th of that month.

This case, though not a success, provided undeniable indications as to the efficacy of penicillin. Therefore, as soon as more active substance could be obtained, the trials continued. A further five patients were treated, all with sepsis by streptococcus or staphylococcus. All made a full recovery, except for one who, already apyretic, died as a result of a rupture of a mycotic aneurysm secondary to the sepsis.

These results were published in the *Lancet* of August 1941, but were not sufficiently conclusive from a statistical point of view. The irrefutable proof of the efficacy of penicillin would only come two years later, with the publication, in the *Lancet*, on 27 March 1943, of 187 cases of sepsis treated by Florey, in collaboration with his wife, Ethel.

As is known, the history of penicillin did not end there. But this marked the end of the initial episodes of a fascinating adventure, taken by a small group of researchers who were the protagonists in one of the most fantastic discoveries in the history of Medicine. Thanks to them, the "magic ball" idealized by Erlich had finally been discovered: penicillin, when injected, was capable of curing severe infections caused by a wide range of bacteria, without causing any damage whatsoever to the normal cells of the organism.

This whole story, therefore, is filled with chances and fortuitous happenings that cannot help but cause us enormous perplexity. And chance, in the words of Pasteur, *"only favors the prepared mind"*. But in spite of everything, they were chance events that appeared more given than a capricious destiny, placing in right hands, at the right time, the key to resolving the enigmas that Man had long sought to resolve.

At a time when we find ourselves facing problems such as cancer and AIDS, which has resisted enormous research programs carried out throughout the world, the history of penicillin, and the steps that led to its discovery deserve, once again, to be recorded. It is felt that decisive scientific progresses will come soon, that will enable us to cure the diseases that threaten Humanity. But could it be that, once again,

we are at the mercy of one or more chances offered to men, in the right place and time, by unknown gods? Only in the future will we be able to answer this question. ■

References

Macfarlane, Gwin. Howard Florey. The Making of a Great Scientist. Oxford University Press, Oxford. 1979

Williams, Trevor. Howard Florey. Penicillin and After. Oxford University Press. Oxford.