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New Light on the Discovery of Penicillin

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Abstract

Although penicillin was discovered by Alexander Fleming at St Marys Hospital, London in the autumn of 1928 it was not widely available for medical use until the late 1940s. Here, emphasis will be placed on the discovery and development of penicillin in England during this period, particularly on the so-called "penicillin-interregnum", i.e. the period between Fleming's discovery and its purification by the Oxford group, led by Howard Flory and Ernst Chain. Emphasis will be placed on some lesser-known aspects of the story, including the role played by Cecil George Paine, the first person to achieve documented cures using unpurified penicillin filtrates. Attempts will also be made to correct a number of common misunderstandings about the discovery, including the myth that Fleming stopped working on penicillin soon after its discovery.

Keywords: *Antibiotic Resistance; Disease; Germs, Pathogens*

1. Introduction

The discovery and early development of penicillin took place in England although the principal scientists involved were born in Scotland, Australia, Germany, as well as England. The crucial work was done in three cities, namely London, Oxford and Sheffield. This work will be detailed here, with special attention being given to what might be called the "penicillin interregnum", that is the period between penicillin's discovery by Alexander Fleming and its development for medicine, by Florey.

Pre-Fleming discoveries of penicillin

The simple, incontrovertible fact is that penicillin was discovered by Alexander Fleming while working at St Mary's hospital (in the Inoculation Department) in London during the autumn of 1928 (Wainwright,1988, Wainwright,1991, Wainwright, 1993, Wainwright,1994, Wainwright,1997, Wainwright, 2002). A number of attempts have however, been made to champion so-called "pre-Fleming discoverers of penicillin", with particular emphasis being placed on the work of the French military doctor, Ernst Duchesne (Diggins, 1999). Such pretenders to Fleming's crown can be readily dismissed because their work is based on the use of anti-bacterial fungal extracts which inhibit the growth of *Escherichia coli*. As Fleming pointed out in his first penicillin paper (Fleming,1929), filtrates from the penicillin producing moulds do not inhibit the growth of this pathogen. As a result, Duchesne and the other claimed pre-Fleming penicillin discoverers were clearly working with antibacterial agents other than penicillin, almost certainly patulin. Patulin is an antibacterial agent which is produced by a number of species of *Penicillium*, and which inhibits *E. coli* and other pathogenic bacteria, but is too toxic to be used in medicine. While there is evidence then that a number of scientists from around the world, from the late Victorian period onwards, demonstrated the antibacterial effects of "mould juices" (Wainwright,1998), there is no convincing evidence that they discovered penicillin before Fleming (Diggins,1999). Such antagonistic effects of fungi on bacteria were well known to late Victorian scientists, with observations being made by, amongst others, the British scientists William Roberts and John Tyndall. There is also evidence that moulds have been used in folk medicine since antiquity, and anecdotal evidence shows that they have been applied, apparently with some success in more recent times. (Wainwright,1989).

Fleming-the Discovery of Penicillin

Fleming's discovery of penicillin appears at first sight to be extremely straightforward. He was called back to St Marys from his summer cottage in Suffolk in order to do some routine pathological work when he entered his laboratory, sat down and plied through a pile of glass petri dishes, which he had been discarded in Lysol, the famous plate having avoiding its sterilizing effects. When he saw the famous penicillin-plate he is said to have exclaimed, "That's funny!" or more probably "Here's a rum go!" (Wainwright, 2004, Wainwright, 2005) Fleming's attention was drawn to a green mould growing on the medium in the plate which was inhibiting the growth of colonies of the pathogenic bacterium, *Staphylococcus aureus*. The mould was producing an antibacterial effect on an important pathogen, the cause of septicaemia, which in the pre-antibiotic age (and increasingly so today, because of antibiotic resistance) was often fatal. More importantly in relation to Fleming's observation was the fact that the mould was dissolving (or lysing) the bacterial colonies. It was this lysis that probably caught Fleming's eye because it suggested to him the novel possibility that that the mould was producing a fungus-lysozyme. Lysozyme is an enzyme which produced in a number of body fluids (such as tears) in order to act as a first line defence against bacterial pathogens. Fleming had discovered lysozyme some years earlier and had spent considerable time studying its properties; it was this discovery that had initially made his name and lead him to being elected a Fellow of the Royal Society (Hobby,1985, MacFarlane,1984).

The observation that a mould might produce of a novel source of lysozyme, which could inhibit *Staphylococcus*, excited Fleming. He therefore transferred the mould to a fresh growth medium and began work on the moulds' antibacterial properties when he returned full-time to the laboratory.

Although it is generally accepted that Fleming arrived in the laboratory on returning from Suffolk and immediately observed the penicillin-phenomenon, it has recently been suggested that the first observation of the penicillin effect was, in fact, made by Fleming's assistant, Merlyn Pryce (Wyn Jones and Wyn Jones, 2002). It is claimed that Pryce observed what was happening on the famous plate and drew the attention of Fleming to the petri dish, noting that no bacteria surrounded the mould. According to this account, Pryce was the first to see the impact of the mould on the bacteria and without his intervention, Fleming might have missed the discovery. Apparently Pryce, from the very beginning, played down his part in the affair and insisted that credit for the discovery should rest solely with Fleming. He did however, claim priority of the discovery in front of none other than Lady Fleming. During an interview with André Maurois, Fleming's first biographer. Fleming's wife is claimed to have said to Pryce, "Anybody would think you discovered the mould." Pryce's response was a simple statement, "But I did.".

Early attempts at penicillin purification

Since Fleming knew little about practical chemistry, especially in relation to chemical extraction and separation techniques he needed to seek help to purify penicillin. Stuart Craddock, who had replaced Merlin Pryce as research Fellow, was similarly restricted by his lack of knowledge, but Frederick Ridley (a young ophthalmologist, who was working on the use of lysozyme to treat eye infections), had concluded an undergraduate course in biochemistry and so was better placed to help Fleming. Craddock grew the mould in Bullock's heart digest broth at 20 degrees centigrade for five days and Fleming determined the titre of any resulting penicillin. (Diggins,1999) A yellow coloured liquid resulted which was capable of inhibiting *Staphylococci* and *Streptococci* at dilutions up to 1 in 600 or 1 in 800. The liquid was then filtered through an asbestos pad in a Seitz filter (50 mL capacity), using positive pressure from a bicycle pump and as much water as possible was removed by vacuum distillation at low temperature. Distillation was carried out at 40°C and the pH of the liquid had to be kept at under 6.5. It is important to note that both Craddock and Ridley were working in an exceedingly primitive laboratory, even by the standards of the time. After a period of frustrating effort, by the 20th of March 1929, 200 ml of mould juice was evaporated to dryness, having a penicillin titre of 1 in 100, which when with re-dissolved in 5 ml of distilled water gave a solution with a penicillin titre of 1 in 3000. This was then treated ethanol and centrifugation to remove any protein present. The whole procedure produced a solution that was ten times greater than the original and showed that penicillin was a small molecule and not a protein. This alcohol solution of penicillin was useless for biological tests until the alcohol had been removed evaporation under vacuum. This gave a syrupy residue of about 0.5 mL which when dissolved in 5ml of water gave a titre of 3000 to 1 in 5000, and which remained stable in ice for ten days. The next logical step was to infect some mice and treat them with penicillin, but this was never done at St Marys. One final attempt by Fleming's colleagues to purify penicillin was made in 1934, when Lewis Holt, a chemist, joined the staff of the Inoculation Department (Bud, 2007). He immediately attempted direct solvent extraction of the mould

juice, with amyl acetate as the solvent after the juice had been adjusted to pH 5–6 with acid. The amyl acetate layer was then removed and shaken with a weak solution of sodium bicarbonate at about pH 8. Some of the penicillin went into solution in the bicarbonate but most was lost because the bicarbonate was too alkaline. Unfortunately, Holt was not told of Ridley's work. Had he used a lower pH, he might have achieved what the by the Oxford team in did in March 1940. Holt failed to publish his results and gave up on penicillin. The Oxford team finally adopted ether as the solvent and a final step of freeze-drying (which was not available to Flemings workers) to obtain the dry, stable brown powder of penicillin.

One final attempt was made in Britain to isolate penicillin prior to the involvement of Florey's team at Oxford. This was achieved by Clutterbuck, Lovell and Raistrick at the London School of Hygiene and Tropical Medicine (MacFarlane,1984) Again, this group came remarkably close to producing an efficient mean of isolating the active ingredient from the mould juice. Raistrick was one of the world's leading fungus-biochemists and the fact that he failed to develop a fully efficient isolation technique must have convinced Fleming that there was nothing could achieve in this direction. A paper written in 1932 on Raistrick's lab their work on metabolites produced by Fleming's Mould" concludes with the statement:

Studies on the isolation and chemical nature of penicillin is being continued. (Clutterbuck *et.al*,1932). Unfortunately, no such studies were ever reported and as a result, attempts to purify Fleming's mould juice continued to languish.

Identification of Fleming's mould

Having isolated the penicillin-producing mould, Fleming naturally enough wanted a name for it and turned to the mycologist, C. J. La Touche who occupied a downstairs laboratory. La Touche identified the isolate as Penicillium rubrum (the red Penicillium) because it produced an occasional red pigment. The mould was however, subsequently identified as P. notatum and later P. chrysogenum. LaTouche, subsequently received much criticism for this apparent miss-identification and apologised to Fleming for his error. However, recent molecular–based identification studies have vindicated La Touche because the mould is now recognised as being P. rubrens, i.e., essentially P. rubrum (based on the classification key used by La Touche) (Houbraken et al., 2011). La Touche was working at St Mary's on moulds which cause asthma and it is assumed that spores of Fleming's mould wafted upstairs to contaminate Fleming's plate, rather than the more generally accepted belief that Fleming's opened laboratory window was the point of contamination (in fact, this was apparently sealed shut) (McFarlane,1984).

In his famous paper, Fleming refers to the use of penicillin filtrates to isolate Bacillus influenzae, an application which is emphasised in its title, which to a modern reader appears to be a boring, or at best, a prosaic way to announce one of medicine's most important -discoveries. Until the mid-1930s however, this species of Bacillus was thought to be the causal agent of influenza. In his paper, Fleming was suggesting that it be added to bacterial growth medium in order to isolate a patient's own flu-bacillus, so as to produce a vaccine which might affect a cure and prevent a reoccurrence. When Fleming wrote his famous paper such a use would have been extremely apposite because of the influenza epidemics which had ravaged the Globe after the Great War. Had Bacillus influenzae really caused flu, then Fleming's suggested use of penicillin might have saved millions of lives, and he might have been awarded two Nobel Prizes-one penicillin the life- saving antibiotic and another for penicillin the life-saving flu vaccine. Fleming's use of penicillin in this way was mentioned in an annotation in the Lancet of 1930. Unfortunately, a mistake in this report would likely have led to considerable confusion, as it refers to Fleming's product as aspergillin, an error which might have led others to prepare ineffective mould juice using a species of Aspergillus rather than Penicillium. Could this help to explain why little interest was shown in penicillin during the 1930s (Anon.1930).

The penicillin interregnum

As we have seen, Fleming was keen to get anyone who had the slightest knowledge of chemistry interested on purifying penicillin and had no proprietorial regard to his discovery, as a result he sent the penicillin- producing mould to anyone who requested a culture.

As has already been mentioned, Fleming emphasizes the use of penicillin in selective isolation medium and in 1934 there appeared a published reference to its use in the isolation of haemolytic bacteria by a certain L. Hoyle (Hoyle,1934); so it least one bacteriologist was reporting the use of Fleming's mould juice during the mid-nineteen thirties. Fleming was keen to talk about penicillin to anyone who would listen, presumably in the hope they had the knowledge and facilities to achieve its purification. The following quote exemplifies Fleming's persistence:

It was at one of these lunches that I first met Professor Alexander Fleming, who rather plaintively said that he had something better than Prontosil, but the he could get no one to take any interest in it…He even suggested that I tried the effect of the mould for vaginal infections, which I did with no result as the mould was rapidly destroyed. (MacLoed,1958).

It is often said that Fleming lost interest in penicillin after the early 1930s, but a search of his notebooks shows that he continued working with penicillin right up until 19389, when Florey and Chain took an interest. This explains why when Fleming met Florey at Oxford he refers to "his penicillin"-Fleming clearly never lost interest and hope in his antibacterial mould juice. His 1930s notebooks also show that Fleming was actively researching other antibacterial agents, including naturally occurring ones like Besredka's so-called antivirus (Wainwright, 1990), bacteriophage, and the potential of fungi other than Penicillium species to produce antibacterial substances; as a result; Fleming shows himself to be one of the first pioneers in the search for antibiotics in general (Wainwright,1991).

Finally, one of Fleming's former students, Cecil George Paine, used penicillin filtrates in Sheffield (around 1929-30) in Sheffield to cure eye infections in newly born infants. Unfortunately, he soon gave up on studies and did not develop penicillin further (Wainwright and Swan, 1986, 1987).

The Oxford penicillin work

Although the main aim of this essay is to discuss the discovery of penicillin, mention must, of course, be made of the subsequent development of penicillin in England. Howard Florey knew of the curative properties of penicillin after Cecil George Paine informed him of his work in Sheffield. Florey was also on the editorial committee of the Orange Journal in which Fleming's famous paper was published and, because of his research-interests, almost certainly refereed it. The idea that Florey was somehow surprised when Chain came across Flemings paper in his search of the antibacterial literature is therefore nonsense and was obvious that Florey would concentrate on penicillin. The forgotten hero of the penicillin story is of course Norman Heatley, who did most of the early work on scaling-up penicillin production, and it was he who, unlike Chain, quickly concluded that it was a small molecule and not a protein and he also suggested the extraction method which was finally employed in the production. The work done at Oxford was truly magnificent and turned penicillin from a substance of passing therapeutic relevance into a miraculous life-saving drug. onwards and to make its cost less than the packaging it was delivered in. The UK pharmaceutical companies, working under the effects of terror bombing did an excellent job of producing penicillin in large-area shallow fermentation, but it was, of course, the deep fermentation methods developed by the Americans at Peoria which enabled sufficient penicillin to be available to the Allies from D Day onwards.

Conclusion

As was mentioned in the introduction to this essay, although the penicillin story has been extensively written, gaps still remain gaps in our knowledge and misconceptions continue to be written about in popular accounts of the discovery. For example, it is routinely stated that Florey came to penicillin "blind" and that Chains reading of Fleming's famous paper which instigated the Oxford work. In fact, as we have seen, Florey knew of penicillin from the beginning an almost certainly reviewed the famous paper before it appeared in the "Orange Journal". In addition, Paine told him about the curative properties of penicillin filtrates in 1932, yet he did nothing with penicillin until Chain came across Fleming's paper much later on. The seminal contribution of Norman Healy to the development of penicillin is also becoming increasingly recognized and it could be argued that his contrition exceeded that of Chain. In the end however, the simple fact is that by the efforts of talented people working in London, Oxford and Sheffield, as well as in other parts of the UK, the first and most important antibiotic and arguably the most important breakthrough in medicine came into being.

Finally, with the increasing problem of antibiotic resistance it is noteworthy that, early on in his studies, Fleming noticed that some bacteria, which had previously been highly susceptible, soon became resistant to his mould juice. In his Noble Prize speech in 1945, Fleming made the following, early, insightful comment:

The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant. Here is a hypothetical illustration. Mr. X. has a sore throat. He buys some penicillin and gives himself, not enough to kill the streptococci but enough to educate them to resist penicillin. He then infects his wife. Mrs. X gets pneumonia and is treated with penicillin. As the streptococci are now resistant to penicillin the treatment fails. Mrs. X dies. Who is

primarily responsible for Mrs. X's death? Why Mr. X whose negligent use of penicillin changed the nature of the microbe. Moral: If you use penicillin, use enough.

It is indeed tragic that Fleming's words were not heeded and that we are potentially at the end of the golden age of antibiotics and we may once again return to a time, before the advent of penicillin when because of the widespread occurrence of septicaemia, trainee doctors would go through the right of passage of entering Septic Wards to get used to the "smell of death".

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Conflict of Interest
No Conflict of Interest

References

Anon (1930). Annotation. *Lancet* 1930, February 1, pp. 247-8.
Bud, R. (2007). *Penicillin: Triumph and Tragedy Oxford*, Oxford University Press.
Clutterbuck, P.W., Lovell, R. and Raistrick, H. (1932). Studies on the biochemistry of microorganisms. *Biochemical Journal*, **26**,1907-1918.
Fleming A. (1929) On the antibacterial action of cultures of a *Penicillium* with special reference to their use in the isolation of *B. influenzae*. *British Journal of Experimental Pathology* 10, 226-236.
Harvard, Harvard University Press.
Hobby, G.L. (1985). *Penicillin Meeting the Challenge*, New Haven, Yale University Press.
Houbraken, J., Frisvad, J.C. and Samson, R.A. (2011). Fleming's penicillin producing strain is not *Penicillium chrysogenum*, but *P. rubens. IMA Fungus* **2**,87-95.
Hoyle, L. (1934). The haemolytic bacteria of the upper respiratory tract. *Journal of Hygiene*, **34**,195-202.
MacFarlane, G. (1984). *Alexander Fleming, the Man and the Myth*.
Paine, C.G. (1931). The source of infection in a minor outbreak of puerperal fever. *British Medical Journal* February 9th, 243-246.
plate. *Journal of Medical Biography* **2**,61-62.
Wainwright M. (1987). The history of the therapeutic use of crude penicillin. *Medical History*, **31**, 41-50.
Wainwright M. and Swan, H.T. (1987). The Sheffield penicillin story. *Mycologist* **21**, 28-31.
Wainwright, M. (1988). Fleming did discover penicillin. *Society for General Microbiology Quarterly* **15**, 30-31.
Wainwright, M. (1989). Moulds in folk medicine. *Journal of the Folklore Society* **100**, 162-6.
Wainwright, M. (1990). Besredka's antivirus in relation to Fleming's initial view of the nature of penicillin. *Medical History* **34**, 79-85.
Wainwright, M. (1991). Fleming's early search for antibiotics other than penicillin. *Society for General Microbiology Quarterly* **18**,46-47.
Wainwright, M. (1993). The mystery on the plate. Fleming's discovery and early contribution to the development of penicillin. *Journal of Medical Biography* **1**,59-65.
Wainwright, M. (1994). More mysteries from Fleming's
Wainwright, M. (1997). A response to some criticisms of Fleming's role in the discovery of penicillin, *St Mary's Hospital Gazette* **103**, 31- 34.
Wainwright, M. (2002). Fleming's unfinished. *Perspectives in Biology and Medicine* **45**, 529-538.
Wainwright, M. (2004). Fleming's unfinished. *Perspectives in Biology and Medicine* **45**, 529-538.
Wainwright, M. (2005). Who witnessed the discovery of penicillin? *The Biologist* **52**, 234-237.
Wainwright, M. and Swan, H.T. (1986). C.G. Paine and the earliest surviving clinical record of penicillin therapy. *Medical History* **30**, 42-56.
Williams, T. I. (1984). *Howard Florey*, Oxford, Oxford University Press.
Wynn Jones and Wynn Jones (2002). Merlin Pryce (1902-1976) and penicillin: an abiding mystery. *Vesalius* **8**,6-25.