The true history of the discovery of penicillin by Alexander Fleming

Frank Diggins trained under Sir Alexander Fleming and Sir Almroth Wright in the 1940s at the Wright-Fleming Institute at St Mary's Hospital. Here, in an article originally published in Imperial College School of Medicine Gazette and reproduced with kind permission, he puts the record straight

On 1 September 1928 Alexander Fleming became Professor of Bacteriology at St Mary's Hospital Medical School in London. He was an acknowledged expert on the staphylococcus and was following up a 1927 report by Bigger *et al.*[1] describing changes in colour, texture and cohesion of *Staphylococcus aureus* colonies over time when left at room temperature. On 3 September 1928 he returned to London from his home in Suffolk, having been on holiday during August with his family. Before leaving for Suffolk, he had stacked all his *S. aureus* culture plates in one corner of his bench, out of the sunlight, so that his new, young research scholar, Stuart Craddock, could work on his bench while he was away.

As Fleming started to examine his culture plates, his former assistant, Dr Merlin Pryce, walked into the laboratory and Fleming picked up the top plate, lifted the cover and said: "That's funny." Near the edge of the culture was a mould about 20 mm in diameter with a smaller satellite attached to it (Figure 1). Around it was a clear area in which organisms apparently had been lysed; further away were degenerate colonies, while still further away were normal colonies of *S. aureus*. Pryce looked and said: "That's how you discovered lysozyme."[2] Pryce left and thought nothing further of it.

In February 1928 Pryce had decided to give up bacteriology and transferred to the Morbid Anatomy Department, leaving Fleming to continue the research by himself. Fortunately, this meant that he was the first person to see the penicillin effect and follow it up.

Mould contamination on culture plates had been seen by Fleming and many others before but he realised that here was something important. He subcultured the mould and kept it going in nutrient broth for further research. He found that the mould grew as a "thick, corrugated, felted mass and after a few

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Sir Alexander Fleming in the Wright-Fleming Institute

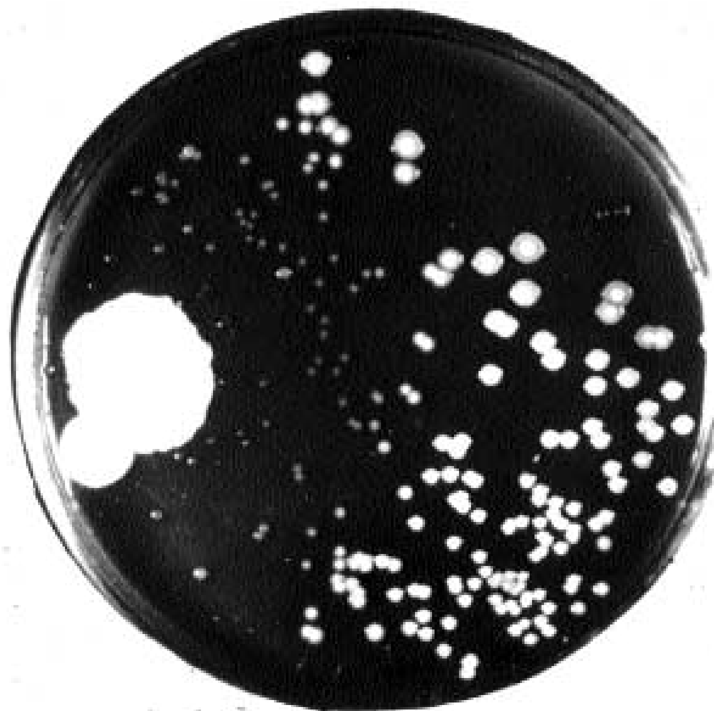

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Fig 1. Photograph of the original culture plate, taken by Fleming in 1928

days an intense yellow colour developed in the underlying clear fluid."[3] He showed that after eight-days' growth at room temperature the culture fluid gave complete inhibition of staphylococci at a dilution of 1 in 500. For some months the culture fluid was known as 'mould juice' but on 7 March 1929 Fleming named the antibiotic 'penicillin'. On 10 May 1929 he submitted his first report on penicillin to the *British Journal of Experimental Pathology*. [4]

Origin of the mould

The first book about penicillin was published by Lacken in 1945.[5] This started the earliest myth – that the spore of penicillium had floated in through the open window of Fleming's laboratory and landed on his open culture plate. No bacteriologist keeps an open window in the laboratory and Fleming could not have reached the window, even if he had wanted to open it (Figure 2). The truth was uncovered by the investigations of Ronald Hare in 1966.[2]

In a room directly beneath Fleming's laboratory was a collection of moulds garnered from the houses in which the allergist John Freeman's asthmatic patients lived. Freeman wanted to know whether or not extracts from moulds could be used to desensitise his patients. A young Irish mycologist, C J La Touche, was appointed to carry out the laboratory work and he had acquired a large collection of moulds to investigate. These were arranged on some tables in the room but it contained no laboratory equipment, such as a fume cupboard, in which to work. Like Fleming, he never closed his door. A spore of *Penicillium notatum* must have drifted up the open lift shaft and stairs surrounding it, into Fleming's laboratory, and, by the most extraordinary coincidence, landed on Fleming's nutrient agar plate while he was spreading a culture of *S. aureus* using a wire loop.

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Fig 2. Interior of Fleming's laboratory where he discovered lysozyme in 1921 and penicillin in1928. Note the crowded bench and window-sill

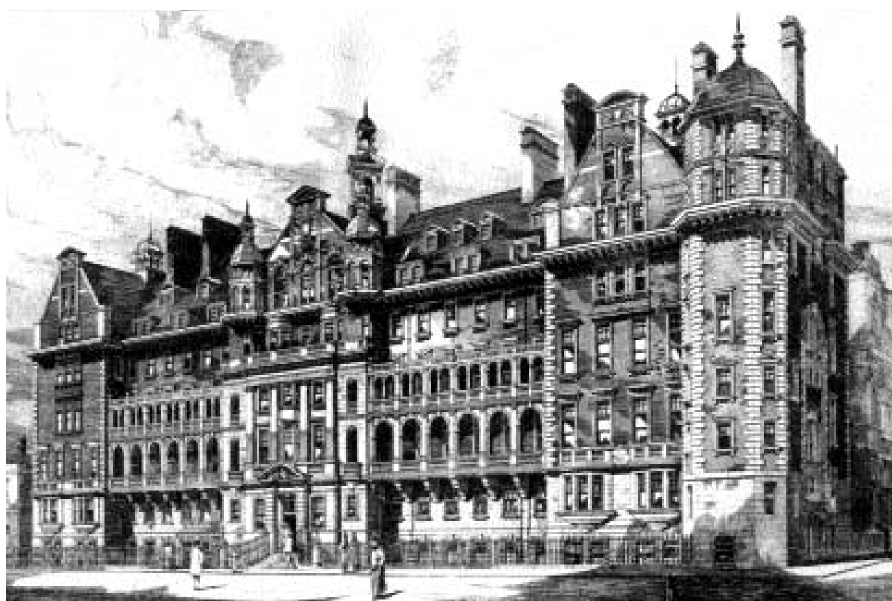

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Fig 3. Drawing of the Clarence Memorial Wing by the architect, W Emerson, 1896. The window of Fleming's laboratory (1909-1933) is on the second floor of the turret on the right, on the corner of Norfolk Place

The essential requirement for the subsequent discovery was that the mould was growing and producing penicillin **before** the staphylococci began to grow, as it only acts on young, growing organisms. The plate was not put in the incubator but left with the others to await Fleming's return from holiday.

The second extraordinary coincidence was that the temperature changes in the laboratory during August were such as to favour the growth of the *P.notatum* **before** the staphylococci began to grow. Fleming's old laboratory was on an exposed corner on the second floor of the turret of the Clarence Memorial Wing in Praed Street (Figure 3). Ronald Hare examined the London temperatures recorded by the Meteorological Office between July and September 1928. During the first nine days in August, the temperature exceeded 20°C on only two days. This favoured the growth of *P. notatum* and the secretion of penicillin. During the remainder of August, the temperatures rose somewhat, reached 25°C on one occasion and then 22.5°C on several days, thus allowing the staphylococci to start growing and produce the phenomenon that Fleming saw. Hare was able to reproduce this on one occasion in 1966, but Fleming was never able to reproduce it in 1928/29 because he was not aware of the conditions required.

Attempts at purification of penicillin

Fleming knew very little chemistry, neither did Stuart Craddock, a medical graduate who had taken the place of Merlin Pryce as a research scholar. However, Frederick Ridley, a young ophthalmologist, had joined Fleming in 1926 and was working on the use of lysozyme in treating eye infections. Ridley had taken the course in biochemistry for BSc students at Birmingham University before moving to St Mary's and was therefore given the task of extracting and purifying the penicillin obtained in bullock's heart digest broth by Craddock. Fleming took no part in this procedure, other than to determine the titre and antibiotic activity

of the mould juice against various microorganisms.

The mould was grown in the broth in 200 mL flat-sided bottles lying on their side in a black incubator at 20°C for five days. The bottles were plugged with non-absorbent cotton wool covered with tin foil. This produced a yellow-coloured liquid that inhibited staphylococci and streptococci at dilutions up to 1 in 600 or 1 in 800. The liquid was filtered through an asbestos pad in a Seitz filter (50 mL capacity), using positive pressure from a bicycle pump. Ridley realised that he must first eliminate as much water as possible and this was done using vacuum distillation at a low temperature.

However, he and Craddock worked together under the most adverse conditions. The only tap with sufficient water pressure to operate their water suction pump was a rising mains tap in a passage outside Sir Almroth Wright's laboratory. It was situated above a large sink and the only place to put the still was on the draining slab. There was no gas tap and therefore a long rubber tube was run from the laboratory to supply gas to the heater for the still. The draughty passage was 11 feet long and four feet wide.

Distillation under partial vacuum was carried out at 40°C and the pH of the liquid had to be kept at under 6.5 to prevent loss of penicillin. This was monitored every hour by drawing off a sample and using pH colour indicators to check the pH. Hydrochloric acid was added as necessary. Later it was found necessary to replace oxygen in the flask with hydrogen from a Kipps' apparatus! Distillation of 200 mL of liquid usually took all day and could never be left alone. By sheer determination, on 20 March 1929, by evaporating to dryness 200 mL of mould juice with a penicillin titre of 1 in 100 and redissolving it in 5 mL of distilled water, they produced a solution with a penicillin titre of 1 in 3000.

The next step was to remove unwanted proteins, assuming that penicillin was not a protein. On 10 April 1929, 1200 mL of mould juice with a titre of 1 in 300 was concentrated to 50 mL, and then 70 mL of 90% ethanol was added. The precipitated proteins were centrifuged out and the supernatant mixture of alcohol and water was removed and found to have a penicillin titre of 1 in 3000. As the concentration was 10 times greater than the original, this showed that all the penicillin had gone into solution in the alcohol and established the fact that penicillin is not a protein and also not a very large molecule.

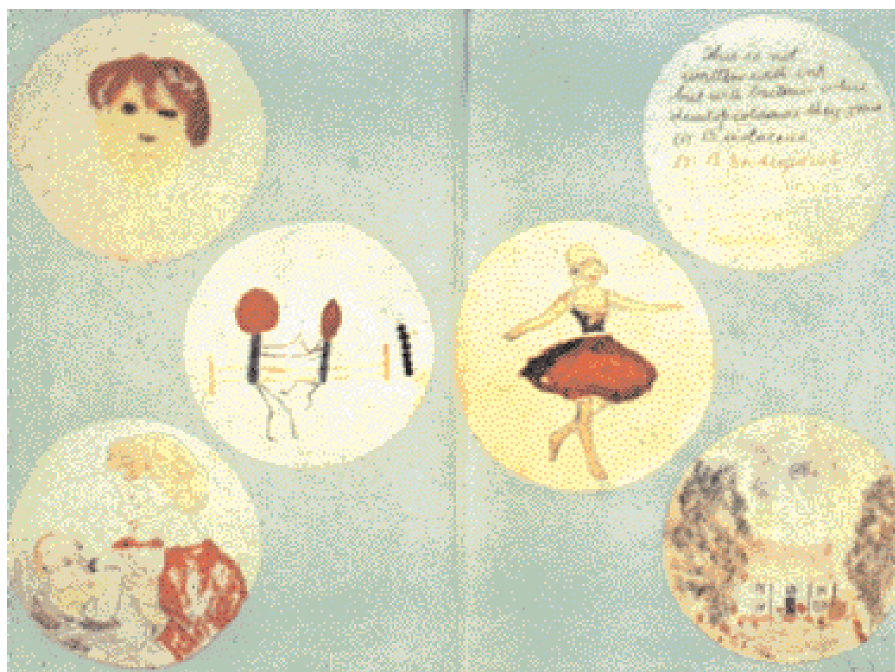
They also assumed penicillin not to be a complicated molecule but this was wrong. The molecule contains a β-lactam ring, which was previously unknown in natural products. The precise molecular configuration of the molecule was determined using X-ray crystallography by Dr Dorothy Hodgkin in Oxford in 1945. The complete synthesis of penicillin was achieved by John C Sheehan in 1957 at MIT in the USA after some nine years' work.[6]

Ridley's alcohol solution of penicillin was useless for biological tests. He removed the alcohol by evaporation under a vacuum and obtained a syrupy residue of about 0.5 mL in volume. This was redissolved in 5 mL of water and had a titre of 1 in 3000 to 1 in 5000. The titre of the concentrates remained high when kept liquid in an ice box for seven to 10 days. In further experiments it was found that ether and chloroform were of no use but acetone had some use. It was a less efficient solvent than alcohol but the addition of small volumes of acetone to the concentrate produced a precipitate that contained very little penicillin but did contain the compound that gave the concentrate its yellow colour. This was named chrysogenin but played no part in penicillin antibiosis.

Ridley and Craddock had produced a full yield of high-potency penicillin. The next step was to infect some mice with a streptococcus, to which they are quickly susceptible, inject half with penicillin and the other half with the solvent only; however, this crucial experiment was never carried out. In fact, Ridley left to join the staff of Moorfield's Eye Hospital, where he became an eminent ophthalmologist. Craddock stayed for about a year and Fleming then helped him obtain a job in the Wellcome Research Laboratories at Beckenham. In 1936 Craddock became a GP in Holsworthy, Devon.[7]

There has been no satisfactory explanation of the failure to carry out the crucial mice experiment but penicillin was used at St Mary's throughout the 1930s to treat eye infections, boils and staphylococcal skin infections. The assertions of Hare and Macfarlane[8] that Fleming abandoned penicillin after 1929 are not true. I have seen Fleming's laboratory notebooks and he was still experimenting with penicillin and different growth media in December 1939.[9,10]

In 1934 Lewis Holt, a chemist, joined the staff of the Inoculation Department. He worked for a while with Sir Almroth Wright on the cause of scurvy, then with Fleming on preparing staphylococcal toxoid. Fleming asked him to try to purify and concentrate penicillin. Unfortunately, he was not told of the earlier work of Ridley and Craddock but he knew that penicillin was soluble in organic solvents. He immediately adopted a chemist's approach by using solvent extraction directly on the mould juice, with amyl acetate as the solvent and the juice adjusted to pH 5–6 with acid. After shaking the mixture in a sepa-



'Germ paintings'. Fleming drew the outline of each figure on blotting paper, then placed the drawing face down on nutrient agar to pick up an initial culture medium. Then the various bacterial cultures were spread on the figure to give the required colour in each section when grown. The blotting paper was then incubated at the appropriate temperature and time to produce the final coloured picture. For example, the red colour was produced by growing Bacillus prodigiosus (now Serratia marcescens), which produces a non-diffusible red pigment – prodigiosin. The figures were finally exposed to methanal vapour to stop growth and preserve them

rating funnel, the amyl acetate layer was 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.

Had Holt known about Ridley's work, and used a lower pH, he could well have come close to the result achieved by the Oxford team in March 1940. Holt did not publish his results and gave up on penicillin. The Oxford team finally adopted ether as the solvent and a final step of freeze-drying to obtain the dry, stable brown powder of penicillin.[11]

The rest, as they say, is history. One final thought, however. Of all the moulds in La Touche's collection, the only one to produce penicillin was *P. notatum*! ■

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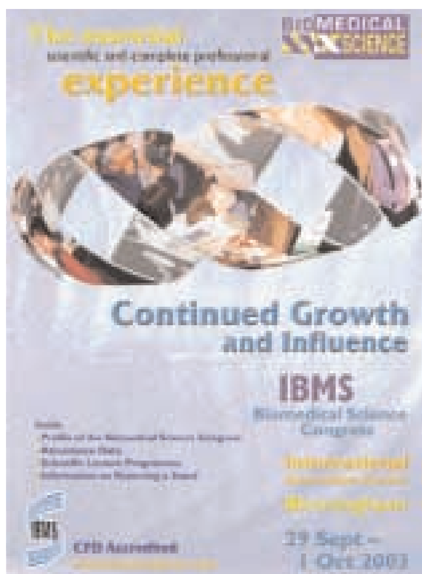

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