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HISTORICAL ARTICLE

Reappraising Fleming's snot and mould

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Alexander Fleming, a Scottish physician at the St. Mary's Hospital, London, made two epoch-making discoveries, lysozyme and penicillin, the bacteria killers, in his own words. But contrary to popular fables, the events were not that serendipitous. He was already an established microbiologist and it took him dogged labours to vindicate his discoveries. He simply had the right mind. Penicillin was especially a hard nut to crack upon which he toiled for half a year with his associates just enough to make a convincing conclusion on the antibacterial property. He in fact utterly failed in understanding what it actually was. As he himself unpretentiously stated: "I did not invent penicillin. Nature did that. I only discovered it by accident." But that did not debar him for sharing the 1945 Nobel Prize in Physiology or Medicine with Howard Florey and Ernst Boris Chain, who isolated the compound and worked out the medicinal applications. Strangely, Fleming's biography has been presented in bits and pieces on the crucial elements of his discoveries, and usually contradictory. This chronicle is trying to mend the gaps and broken pieces in the historical records.

Keywords: Bacteria, drug discovery, lysozyme, penicillin, *Penicillium*, *Staphylococcus aureus*.

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Who was Little Flem?

> It is not the marble halls which make for intellectual grandeur - it is the spirit and brain of the worker
> – Alexander Fleming

A World War I veteran in the British Royal Army Medical Corps, Captain Alexander Fleming, or Little Flem as he was affectionately called in his laboratory, resumed his profession as a bacteriologist in the Inoculation Department of St. Mary's Hospital, London, as the war ended in 1918 (**Figure 1 & 2**). His experience with casualties of war led him to discover two important phenomena in the late 1910s on antiseptics such as carbolic acid, boric acid, mercury salts and hypochlorous acid used at the time. The first was that the right amount of antiseptic applied determined the effectiveness against bacteria. In fact, he found that certain concentrations of the antiseptics could actually enhanced the growth of bacteria in the wounds. Another phenomenon was that antibiotics interfere with white blood cells that eliminate bacteria during immune response. The antiseptics kill the phagocytic blood cells thereby increasing the bacterial infection and exacerbating the wounds.[1,2]

The biographer André Maurois described Fleming as a circumspect young Scot, possessing an inexhaustible gift of silence, and remarked, "In the truest meaning of the word he was an artist."[3]

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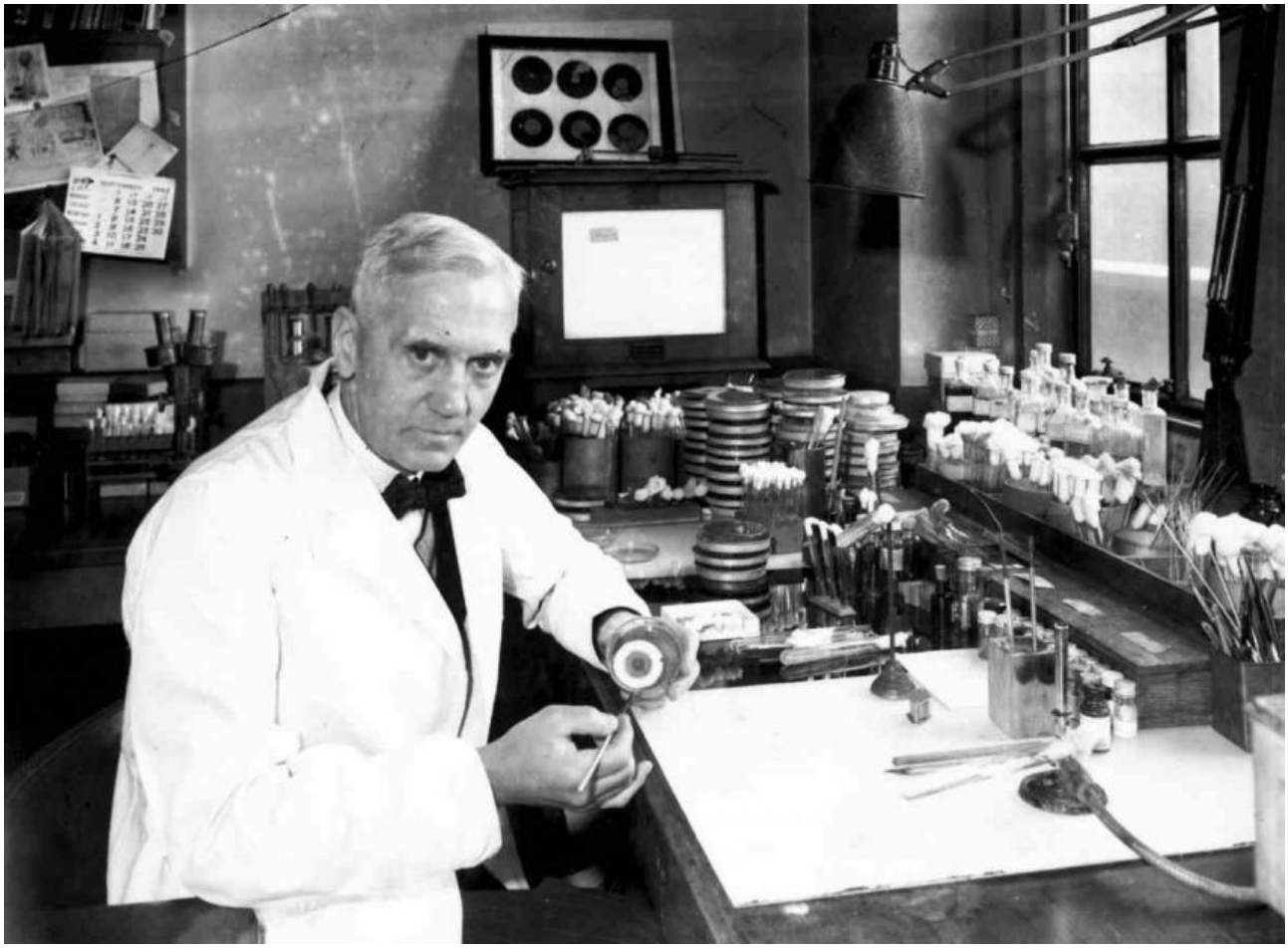

Figure 1 | Alexander Fleming in his lab at St Mary's Hospital, London, displaying bacterial culture. Also note the window on the right overlooking the which once stirred a debate but was quite inaccessible.

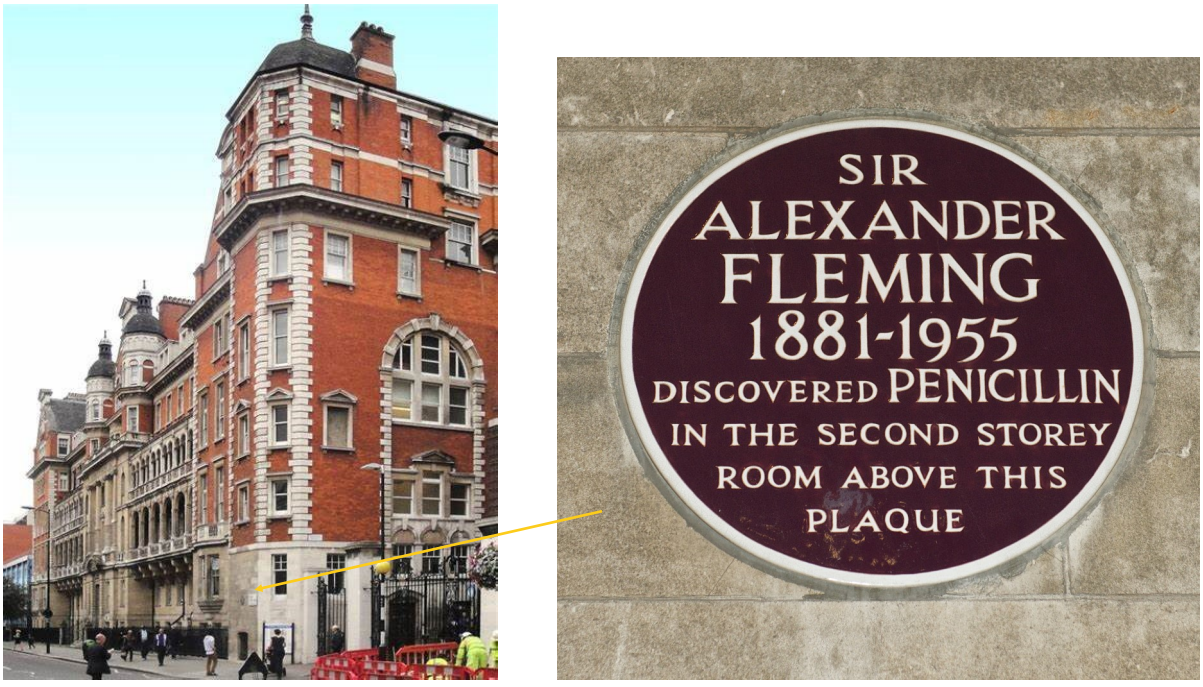

Figure 2 | St Mary's Hospital on the Praed Street (left). A blue plaque under on the wall outside, right below Fleming's lab (right).

Fleming excelled in research but not in conversation. Remembered by his fellow students as the invincible champion in most sport competitions at the medical college. And just like in sports he employed the same methods to his work. As he completed his medical course, he was laden not only with sport accolades but also with flying colours in the whole subjects. He was quite inhibited and modest and often found difficult to make friends quickly, not that he had no friend, as my account will surely repudiate that. To make up his shortcoming in making friends, he was quick to grasp the essentials of a technique, concentrate on them, and so win with ease.

John Freeman, a classmate at St Mary's reminisced about Fleming saying," "[Fleming could] be more eloquently silent than any man I have ever known. He seldom or never gave himself away. In the stress of the moment I sometimes called him a blithering idiot, or used some equally opprobrious epithet. In reply, Fleming would merely look at me with his barely noticeable Gioconda-like smile, and I think he had the best of the exchange."[4]

His sense of humility was well recognised. Fleming considered his becoming a bacteriologist as a chance as it was only the first vacant job he had after completion of his medical studies. But then he also said that he had been interested in antibiotics since his graduation classes. How steadfast and resolute one could be with all kinds of jests and insinuation poked at him by his colleagues, most frequented by his boss Almroth Wright and sometime by his assistant Merlyn Price. He never reacted or retaliated to such provocations. All in all, Fleming had all the dispositions to be a notable man.

Having said that it is manifestly clear that Fleming was a deplorable chronicler, never jotting down critical details of his important moments. Almost all accounts of his scientific contributions came from his family, students and associates, which were often incongruous at specific points. Even his official biography is no exception to untrustworthiness in factual details. This is the underpinning reason behind this article – to set the records straight as far as possible.

The Case of a Snotty Physician

1 May 1922 issue of the *Proceedings of the Royal Society B: Biological Sciences* published a report of an important discovery under the title "On a remarkable bacteriolytic element found in tissues and secretions". The author, Fleming wrote:

> In this communication I wish to draw attention to a substance present in the tissues and secretions of the body, which is capable of rapidly dissolving certain bacteria. As this substance has properties akin to those of ferments I have called it a "Lysozyme," and shall refer to it by this name throughout the communication. The lysozyme was first noticed during some investigations made on a patient suffering from acute coryza.[5]

He was a bit euphemistic as it turns out later that the "patient" was none other than he himself.[2] How do we come know that it was him? Because his notebook dated 21 November 1921 revealed a sketch of the culture plate with a small note saying "Staphyloid coccus from A.F.'s nose."[6] There were not many other people with an initial A.F. at St Mary's at the time; in fact, none.

This was nonetheless a hugely unexpected discovery, a drop of snot on the bacterial culture and the bacteria were dying. But behind the accidental discovery was a meticulous experiment. He carefully isolated the enzyme by treating with salt solution, filtering several times and performed a series of tests on bacteria specifically obtained from (his) nasal mucous, and that was only the first test, which he described as:

> [The] plate was incubated at 37°C. for 24 hours, when it showed a copious growth of the coccus, except in the region where the nasal mucus had been placed. Here there was complete inhibition of growth, and this inhibition extended for a distance of about 1 cm. beyond the limits of the mucus.[5]

It was not only that. He even identified lysozyme from tears, nasal mucus, sputum, cartilage, blood, semen, ovarian cyst fluid, pus, and egg white, and showed that they exhibited similar bactericidal activity. He isolated (at least partially) and identified the bacterium from the nasal mucus as a Gram-positive coccus of unknown identity, and gave the species name *Micrococcus lysodeikticus*, apparently for its susceptibility to lysozyme activity (**Figure 3**).

This remained the standard name until 1972 when taxonomic reevaluation was performed and the bacterium was reassigned to an existing species *Micrococcus luteus* (Schroeter 1872),[7] although the name is still in use to designate Fleming's particular strain. He also tested the lysozyme on 11 human bacteria, of which he found that it was active against *Staphylococcus* and *Streptococcus* species only. It was hardly a purely accidental discovery.

The Most Important Accidental Discovery in Medicine

There were actually three phases in Fleming's discovery of penicillin, which are never properly documented systematically. Of all accounts taken into consideration, what happened in the early and late September of 1928 were never cleared up. This is important because without phase-wise chronological account, the discovery as told by Fleming would be contradictory. For one, in a personal interview, he explicitly mentioned that the discovery was from one culture plate that was accidentally left open,[8] whereas his scientific report

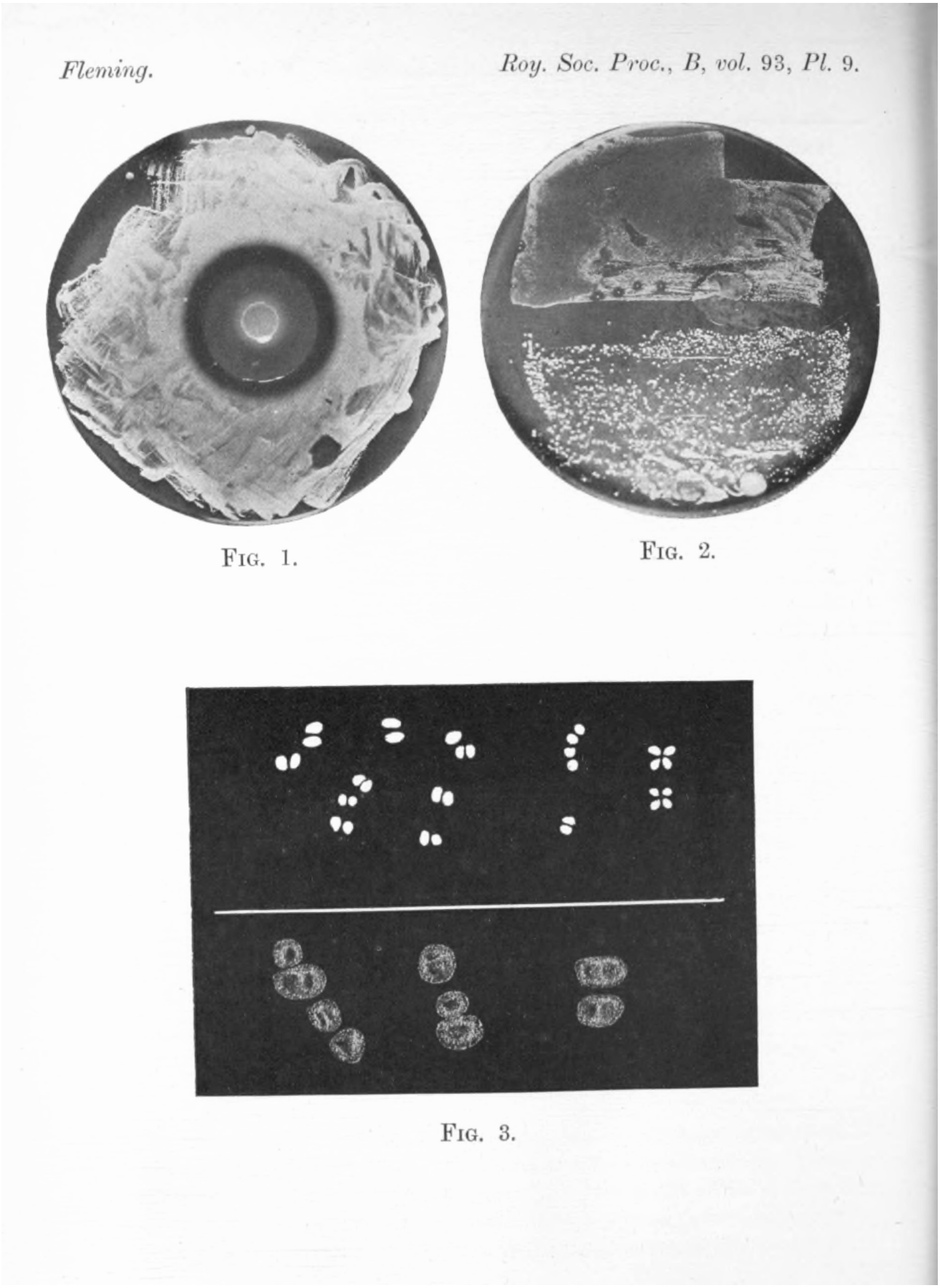

Figure 3 | Fleming's original test of the lysozyme. 1 is agar plate with a smear of tear. 2 is the growth of *Streptococcus* in 1. 3 is *Micrococcus lysodeikticus* before (upper half) and after (lower half) acted upon by tear.[5]

candidly states that several plates were deliberately left open for contamination.[9]

The apparently contradicting statements can be recoconciled by reconstructing the events in these sequential phases. The first phase was an accidental contamination of his bacterial culture, but not that serendipitous. Fleming appropriately preferred the phrase "chance observation".[10] And the second was a scrupulous experimental examination of the "funny" bacterial growth. The third being the identification of the antibacterial source.

The bacteria with many coats of colour

> Had my laboratory been as up to date and as sterile as those that I have visited here, it is possible that I would never have run across penicillin.
>
> – Alexander Fleming

An Irish physician Joseph Warwick Bigger and his two students C.R. Boland and R.A.Q. O'meara at the Trinity College, Dublin, Ireland, published an article "Variant colonies of *Staphylococcus aureus*" in January 1927 in *The Journal of Pathology and Bacteriology*. A Gram-positive bacterium, *S. aureus* is a kind that causes a range of diseases in humans from skin infection, acne, pneumonia, meningitis, sinusitis to food poisoning; in short, dangerous. It was about the bacterium strain of *S. aureus*, which they designated "Y", that they isolated a year before from a pus of axillary abscess from one individual. They made a culture of it and to their utmost surprise, the bacterium grew into a variety of forms (strains). Their description was no less provocative:

> We were surprised and rather disturbed to find, on a number of plates, various types of colonies which differed completely from the typical *aureus* colony. Some of these were quite white; some, either white or of the usual colour were rough on the surface and with crenated margins.[11]

This existence of variation of a single bacterium from a single colony source did not fail to amaze Fleming. Fleming and his research scholar Daniel Merlin Pryce tried the experiment and successfully produced unusual strains of *Staphylococcus*. Dates are not specified for this incidence, but apparently it was in 1927 because in the early 1928 Pryce was transferred to a different laboratory.[12] What Pryce did not (and could not have) realised was that he missed an opportunity of eternal fame as his tenure with Fleming ended and had to leave Fleming, while the latter achieved eternal fame out of it. Pryce remained a good friend and often visited Fleming.

Based on their findings Fleming continued the experiments on his own (until a new scholar Stuart Craddock was assigned to him) and had every intention of writing an article on *Staphylococcus* variation. He even had agreed to contribute his works to *A System of Bacteriology* to be published by the Medical Research Council.

Fleming went for a vacation with his family at his country home, The Dhoon at Barton Mills, Suffolk, England, at the end of July 1928.[13] While on holiday he was appointed Professor of Bacteriology at the St Mary's Hospital Medical School on 1 September 1928.[14] It was time to officially join his new position and head back to London.

It is even said that he was called back to his laboratory as unusual growths were seen in his culture plates,[15] while Ronald Hare assumed that Fleming was "was on a flying visit to London to assist a surgical colleague with the treatment of an abscess from which a haemolytic bacillus had been isolated. It was probably while waiting for his colleague to appear that Fleming took the opportunity to discover penicillin."[16] But these testimonies do not stand to verification. His going to London had apparently nothing to do with his experiment or a visit.

Before he left for vacation, Fleming had inoculated some culture plates with *S. aureus* as part his ongoing research on the variation of the bacterium. Another misconception of this event is that Fleming is often described as leaving the plates carelessly unattended in a mess. The fact is that he was still doing the experiment on *Staphylococcus* growth. Of course, he pushed the pile of plates aside on the corner of the laboratory bench to keep them away from the sunlight as well as to make space for Craddock who would work during his absence.[13]

Fleming returned to his laboratory on 3 September 1928.[14] As a faithful friend Pryce was there to greet him. Fleming was sorting out his culture plates as they were exchanging conversation; but one suddenly struck their eyes. In that particular plate the bacterium grew nicely but not around one corner where there was a large blob of mould. The area just in the vicinity of the blob had no bacterial growth, and at farther region little growth, while at the opposite end was normal colonies of the bacterium (**Figure 4**). Who would have expected the solution to that puzzle would lead to the single most important discovery in medicine, and a Nobel Prize? Pryce obviously did not, and simply commented to Fleming, "That's how you discovered lysozyme," and left without a smidgeon of curiosity.

The cannibal mould

> When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionise all medicine by discovering the world's first antibiotic, or bacteria killer. But I suppose that was exactly what I did.
>
> – Alexander Fleming

Fleming had the right inquisitiveness and immediately worked on the culture plate, carefully measuring the area of bacterial inhibition. Pryce obviously was not impressed by the possible

implication and departed without a trace of inspiration, but to Fleming it was source of illumination, as he said, "My only merit is that I did not neglect the observation and that I pursued the subject as a bacteriologist."[10] Some of the surrounding misinformation (such as Pryce as the original discoverer, it was careless contamination, it was discovered in an instant) be invalidated by Fleming's own words:

> While I was working on some bacterial cultures the cover of one of the dishes in which they were being grown was left off. A few days later I noticed that a spot of mold had formed. A mold spore had fallen and had begun to grow. I noticed another thing also. This was that the bacteria around the spot of mold had apparently disappeared while those some distance from it had continued to increase.[8]

Fleming proudly showed the plate to everyone who came around his lab. But he failed to captivate anyone's interest, including his boss Wright. They all seem to show not a faintest gesture of exhilaration. He remarked to one of his colleagues with a reserved tone, "It may well turn out to be important." The apathetic colleague politely replied with a harsh tone of indifference, "Yes, very interesting."[17] Not disheartened in the least, Fleming carefully took the photograph the plate, which would later be replicated and famously displayed at museums; and who would have known a sample would fetched as much as $14,597 (£11,863) such as at an auction in 2017 (**Figure 5**).[18]

Pryce and colleagues may turn a blind eye, but for Fleming it was time for excitement and a new vista, and even to forget about the *Staphylococcus* project. A saviour of humankind was imminent. But then the mere discovery of a "bacteria killer" or "cannibal mould" (as Charles Hill put it)[19] had no application whatsoever.

According to Craddock's notebook, Fleming went off to resume his vacation and returned for the experiments late in September. It was Fleming who said that the true discovery was on 28 September 1928, when he experimentally verified and reproduced the unusual bacterial growth, or inhibition for that matter.[20] This kicked off the journey of struggle to the discovery of the mysterious antibacterial mould. After planning and scheming, the main experiments were started only a month later. The only surviving note of Fleming indicates the date as 30 October.[21]

The second phase of the discovery was exhausting and painstaking. Fleming collected the mould sample from the original plate and transferred it to culture plates containing agar, a growth medium. After four days he found that the plates developed nice colonies of the mould. Now armed with a stock of the cannibal mould, he inoculated different bacterial cultures. He discovered that the mould was not an omnivorous cannibal but instead a very finicky eater of only specific species of bacteria. For instance, inhibition of growth was very clear on *Staphylococcus*, *Streptococcus*, and diphtheria bacillus (*Corynebacterium diphtheriae*) cultures; whereas there was no effect on typhoid bacterium (Salmonella typhimurium) and influenza bacillus (*Haemophilus influenzae*).[9]

It was not a rocket science for Fleming to deduce that the mould contains antibacterial substance with high but selective activity and that it could have an immense therapeutic value. But then again, a speckle of a mould would hardly do any good. He tried to device a culture method for large-scale production. He used a large receptacle and huge quantity of different broths. A few days after introduction of the mould sample into the receptacle, he noticed that a thick layer of mould developed on the surface while the underlying broth turned into a turbid yellow liquid. He was quick to suspect that the antibacterial substance must be present in that pallid mould juice.

Applying his method of filtration and isolation of lysozyme, Fleming could produce a highly concentrated mould juice. Confirming his assumption, the mould extract was exactly as effective as the original mould. The next step was to test the effectiveness at different concentrations. He diluted the mould extract several hundred timed and found to his astonishment that as long as the yellow colour remained the juice was still effective.

He also experimented with other species of fungi including *Eidamia viridiscens*, *Botrytis cineria*, *Aspergillus fumigatus*, *Sporotrichum*, *Cladosporium*, and eight strains of *Penicillium* to see whether they produce the same antibacterial substance. They did not. Only his original *Penicillium* was an authentic bacteria-killer fungi – it was unique. He further demonstrated that the mould juice was several times more potent than other antibacterial compounds used at the time. But the real extraordinary nature was that it was innocuous to rabbits and mice, and even human blood cells, meaning that it was exceptionally safe to apply. It was the first time a highly non-toxic but potent antibacterial agent (or any drug) was ever found.

The mould from Old Mouldy

It was thus imperative to identify the exact identity of the antibacterial mould. Fleming himself had no knowledge on fungi so he started rummaging through mycology literature. The most he could convince himself was that his mould was most similar to what were known as *Penicillium* fungi belonging to the species *P. chrysogenum*. Fortunately, a young Irish mycologist Charles J. La Touche had settled just below Fleming's laboratory. La Touche was investigating the cause of allergy and was working on fungi, on which he was the only expert at St Mary's Hospital and for which he was fondly known as Old Mouldy. In fact, it was much

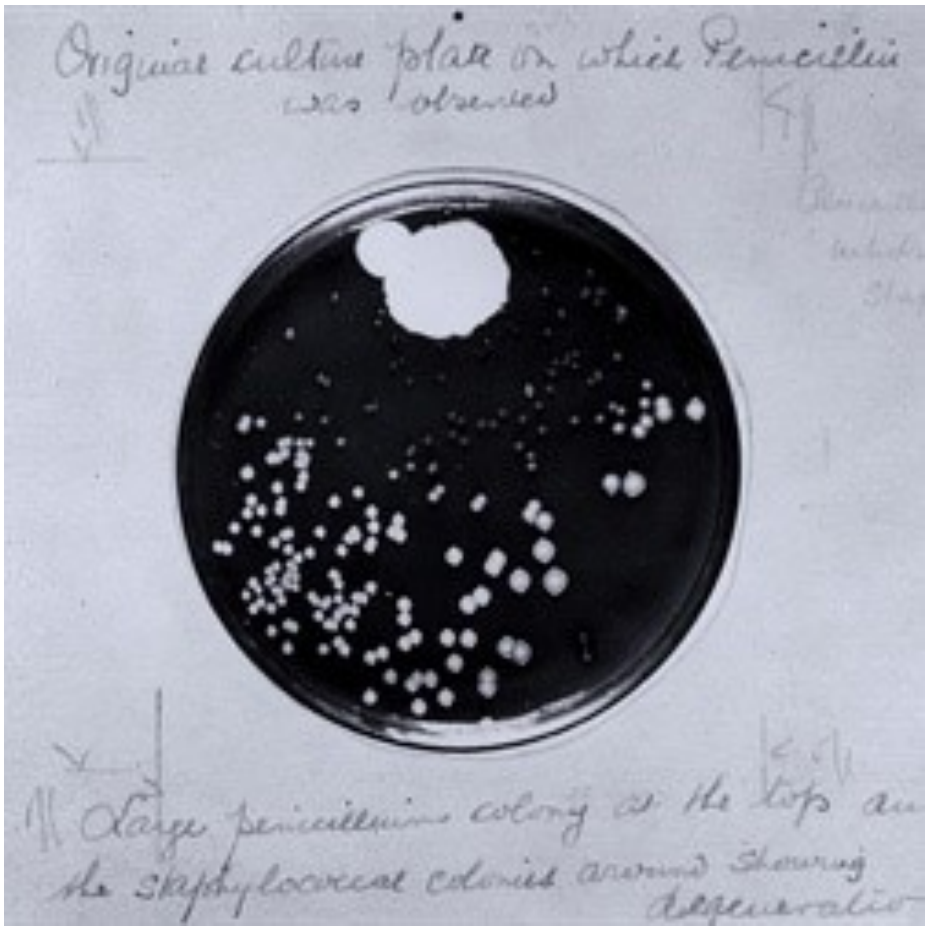

Figure 4 | Fleming's original photo of the antibacterial mould.

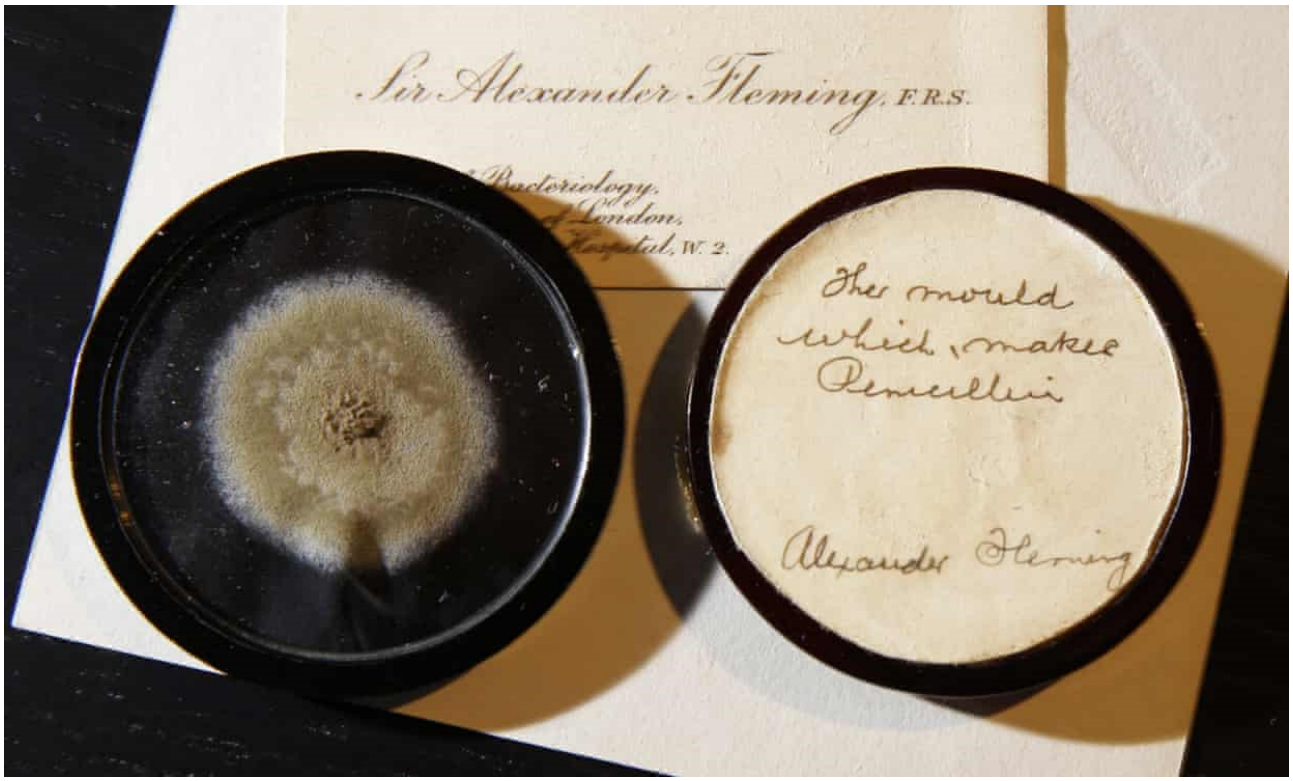

Figure 5 | Fleming's replica of the original penicillin effect auctioned by Bonham in 2017.[18]

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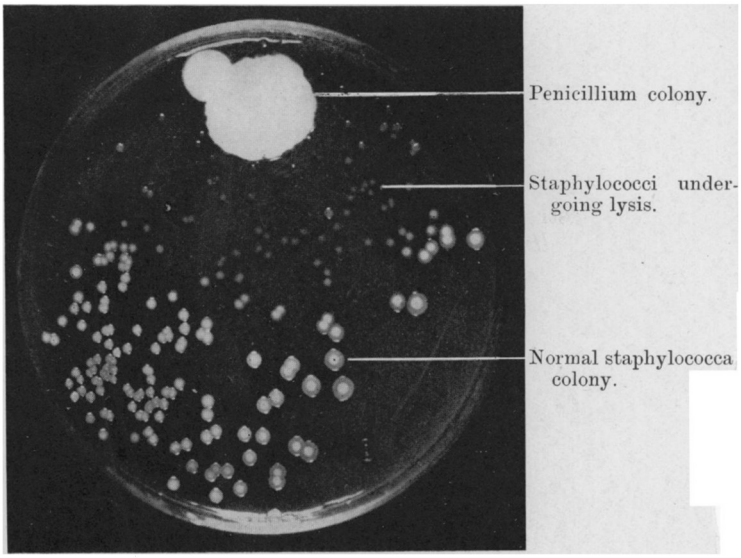

FIG. 1.—Photograph of a culture-plate showing the dissolution of staphylococcal colonies in the neighbourhood of a penicillium colony.

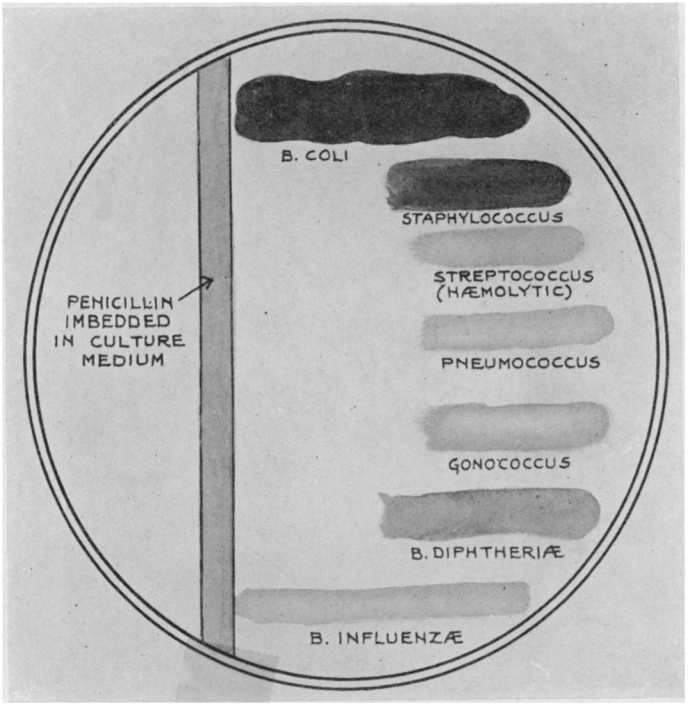

FIG. 2.

Fleming.

Figure 6 | Fleming's original test of penicillin from his original paper.[9]

later established that the original mould of Fleming's penicillin came from La Touche's laboratory with the spores spreading in the air to Fleming's culture plates.[22] The fungi specimens were a major suspect of La Touche as one of the causes of asthma, and that was later proven to be correct, but only after almost a century.[23]

Fleming consulted La Touche and gave him his mould. It was too easy to recognise for La Touche that he confirmed the mould as *Penicillium rubrum*. The name was noted in Fleming's notebook dated February 1929.[24]

The bombshell publication

As the experiment progressed, Fleming described the discovery on 13 February 1929 before the Medical Research Club. His topic "A medium for the isolation of Pfeiffer's bacillus" utterly obscured the nature of the new antibacterial mould, so that no one paid any particular attention to it. Henry Dale, the then Director of National Institute for Medical Research and chair of the meeting, much later reminisced that he did not even sense any striking point of importance in Fleming's speech.[25] After a series of experiments Fleming must have conceded that isolation was the chemical substance was not going to happen. His data was more than enough, he ended the discovery experiments on 10 April 1929. It was time to let the world know.

Fleming reported his discovery under the title "On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae*" to the *British Journal of Experimental Pathology* on 10 May 1929, and was published in the next month issue. Although it did not receive any special attraction at the time, it became one of the most important papers in the history of medicine. The article starts with an unassuming opening statement:

> WHILE working with staphylococcus variants a number of culture-plates were set aside on the laboratory bench and examined from time to time. In the examinations these plates were necessarily exposed to the air and they became contaminated with various micro-organisms. It was noticed that around a large colony of a contaminating mould the staphylococcus colonies became transparent and were obviously undergoing lysis.[9]

The ten commandments Fleming made out of his results were:

1. A penicillium mould produces a powerful antibacterial substance. The antibacterial activity is highest in about seven days at 20°C and diminishes after ten days until it has almost disappeared in four weeks.
2. The best medium for the production of the antibacterial substance has been ordinary nutrient broth.
3. The name "penicillin" has been given to the antibacterial filtrates of broth cultures of the mould.
4. The activity of penicillin decreases after 10 to 14 days at room temperature but can be sustained a bit longer by neutralization.
5. The antibacterial substance is not subdued by boiling briefly but losses its activity after boiling in alkaline solution for 1 hour. It is completely destroyed by autoclaving for 20 minutes at 115° C. It is soluble in alcohol but not in ether or chloroform.
6. It is most effective on the pyogenic cocci and the diphtheria group of bacilli. Other bacteria such as the coli-typhoid group, the influenza-bacillus group, and the enterococcus are not affected (**Figure 6**).
7. Penicillin is harmless to animals even in enormous doses and does not cause irritation. It does not affect leucocytes any more than does ordinary broth.
8. It could have an application as a good antiseptic against infection with penicillin-sensitive microbes.
9. The effects of penicillin on bacteria is unique.
10. It is possible useful in the isolation of *Bacillus influenzae*.

He also explained the rationale of naming penicillin as "to avoid the repetition of the rather cumbersome phrase 'Mould broth filtrate,' the name 'penicillin' will be used." It is interesting to note that by his definition, penicillin never actually meant the substance, but the entire culture filtrate solution. In fact, his team would variously used names such as "mould juice" and "the Inhibitor" to designate the antibacterial principle.[16] He came up with an idea and used "penicillin 5" just to designate the *Penicillium* sample from which he originally discovered and which he allotted fifth in the series of eight strains he tested.[26] Of course, it was insightful of him to choose the simple "penicillin" which he did on 7 March 1929,[14] and with it established the naming convention of antibiotics with the ending of "in" (or sounding like it).

The suffix was in fact inspired by the discovery of digitalin, a cardiac toxin from foxglove (*Digitalis*), a century before, as he explained in his Nobel lecture:

> I have been frequently asked why I invented the name "Penicillin". I simply followed perfectly orthodox lines and coined a word which explained that the substance penicillin was derived from a plant of the genus *Penicillium* just as many years ago the word "Digitalin" was invented for a substance derived from the plant *Digitalis*.[10]

Fleming's Allies and His Dilemma

To start with, Fleming's previous assistant Pryce

was of Welsh extraction and after several years of the discovery of penicillin and later Nobel Prize for it, there was renewed vigour in the Welsh environment to reprise Pryce's contribution. The *Western Mail* (of Wales) had reported on 26 February 1954 that Pryce "played no small part in the epoch-making discovery of penicillin." But the first most provocative was an article in Welsh newspaper *Yr Hogwr* written by D. Vivian Thomas titled "Penisilin: Y Cysylltiad Cymreig" (Penicillin: The Welsh Connection). It expressed that the contribution of Pryce was overlooked, and stated: "Penicillin would never have been discovered if he had not been working with Fleming at the time and that if he [Pryce] had not noticed this particular dish." A systematic appraisal by Emyr Wyn Jones and Gareth Wyn Jones in 2002 made a startling conclusion:

> It is likely that Professor Daniel Merlin Pryce, a somewhat unconventional but gifted son of the Welsh mining valleys played an important, quite possibly a crucial, role in that original observation. However one which, except for a very few occasions, he himself sought to downplay, even virtually to deny.[27]

If so, did Pryce make contribution larger than just meets the eye?

Pryce was a Junior Research Scholar assigned to Fleming in 1927 at St Mary's Hospital. A reticent and dedicated man, he was in all a perfect match for Fleming. However, his scholarship term was for one year and on 19 April 1928 he was appointed Second Assistant Pathologist and moved to the Pathology Department. Short may be the stint Pryce greatly admired Fleming and remained a lifelong friend. As most accounts have a delight in mentioning Fleming's habitual carelessness, or untidiness, even as his official biography would say about "his disorderly habits,"[28] it is obviously far from the truth, as Pryce would later praised Fleming as "one of the *tidiest* of workers and because of this, well able to work efficiently in a small lab even often shared with another."[27] This evidently has a more face value if one bothers to read Fleming's scientific papers – his refined reasoning and meticulousness shone through in every detail.

Pryce was also a modest and authentic man. There was a prevailing hunch that the fungal contamination of Fleming culture plate came through the window – again, often used an indication to ascribe Fleming's untidy behaviour. By the way it was Fleming himself who gave the first suggestion in 1945 that the contaminant could have come from Praed Street through the window. But Ronald Hare, a co-worker in the same department, remembered and reported in 1970 "that the windows were seldom opened because they were too difficult to reach, and because bacterial cultures always present on the window-sills might fall on the heads of passers-by in the street below the opened windows." It was upon Hare's idea that La Touche's samples were examined and experimentally identified as the source of Fleming's mould. It is interesting here to reiterate that La Tocuhe gave 13 samples of different fungi to Fleming for examination and only one had antibacterial activity on *S. aureus*.[16] Pryce also testified much later about this and said that Fleming always kept the window tight shut.[27] The only way for then for the mould to enter Fleming's lab was not like a stealthy burglar through the window, but as a regular visitor through the doors.

On that fateful day of 3 September 1928, Pryce, then from a different office, was at his old lab to welcome Fleming on his return from vacation. There are debates as to who first saw the contaminated plate, Pryce or Fleming. Pryce later on claimed on two occasions that he was the first to see the moulds.[12] In contradiction, Gwyn Macfarlane is more succinct and said that as Fleming was about to discard the plate, but

> [He] suddenly noticed something about the appearance of a plate as he was about to hand to Pryce. 'That's funny,' he said and looked more closely.[29]

Popular accounts such as Pryce's family recollection (for example his sister Hilda Jarman's in 1998) are unimpressive and implausible as they are riddled with factual errors. One story claimed that Pryce was cleaning the lab in preparation for Fleming's return and found the unusual bacterial growth in the culture plate, destroyed the rest and after showing to Fleming, he moved to Pathology Department.[27] This just is not true in three points. Firstly, Fleming's new research scholar Craddock was working in the lab and it would be a great surprise had he required external assistance for cleaning up. Secondly, it would be purposeless if Pryce discarded any culture plate because Fleming left there intentionally for bacterial growths. It would not be hard to imagine the incensed and enraged Fleming had his precious culture plates are destroyed. Thirdly, at the time Pryce was already at least for four months working in another department.

And what of Craddock and Ridley's contributions? Why were not their names in the final publication (although they were properly credited in the acknowledgement)? It is crucial to point out that Fleming toiled for half a year to gather all the necessary experimental data, and that most of the chemical experiments were done by his research assistant Craddock and his once scholar Frederick Ridley, who joined the lab in January 1929 and was entirely entrusted on the chemical isolation part. Fleming specifically sought for a biochemistry-trained Ridley as he once commented: "I am a bacteriologist, not a chemist." The answer to the missing coauthors is a series of personal affairs that occurred before the completion of their target. In

short, Craddock got married and left for a new job at Wellcome Research Laboratories, while Ridley suffered from a severe boil and was compelled to abandon the research, and Fleming knew that they were going nowhere with the chemical identification.

The point is, much as he is attributed as the discoverer of penicillin, Fleming never produced the actual substance or demonstrated the practical application. After a decade of research, the *British Medical Journal* in 1941 went so far as to report, stating "the main facts emerging from a very comprehensive study in which a large team of workers is engaged" was that the penicillin "does not appear to have been considered as possibly useful from any other point of view."[30] The matter of fact was, and still is, that natural compounds are difficult to identify, isolate and produce in workable quantities. Fleming did not have the means or knowledge to do that. In fact, the scientific standard of the 1930s did not permit it. It required the World War II to revolutionise the demand and the technology – but that is another grand story worth telling separately.

A good friend at the Chelsea Arts Club, G.E. Breen once asked Fleming, tapping on his shoulder, "I just wanted you to tell me whether you think it will ever be possible to make practical use of the stuff [penicillin]. For instance, could I use it?" Fleming gazed vacantly for a moment and then replied, "I don't know. It's too unstable. It will have to be purified, and I can't do that by myself."[31]

It was for this hugely uncertain reason that Fleming could only made a diffident assertion (which his boss Wright sharply objected to due to its speculative nature, but which Fleming obstinately included):

> It is suggested that it [penicillin] may be an efficient antiseptic for application to, or injection into, areas infected with penicillin-sensitive microbes.[9]

What's in a Name? The Mould with a Misleading Identity

Although a mycologist of repute, La Touche identified Fleming's mould as *Penicillium rubrum*, it can be construed that Fleming was not entirely convinced. In his paper Fleming asserted that based on La Touche's interpretation, the mould most closely resembled *P. rubrum*. But then went on to cite the French microbiologist Philibert Melchior Joseph Ehi Biourge who made the original description of these fungi and who had mentioned that *P. rubrum* does not exist in nature except only in laboratory contamination. It is worth noting that Fleming initially suspected it to be the species *P. chrysogenum*. After decades of research his was proved to be correct, or was it? The controversy and confusion were beyond expectations.

Fleming's mould was later identified as a rather common mould in indoor environments and are present and spread in dust, indoor air, and damp building materials, including food spoilage. The uniqueness of the fungi is that it produces penicillin. But not so unique as that there are other related species that also produce penicillin.

In the earliest beginning, a Belgian mycologist R.P. Dierckx described three species of *Penicillium* in 1901 such as *P. griseoroseum*, *P. citreoroseum,* and *P. brunneorubrum*. In 1910*,* an American microbiologist Charles Thom at the US Department of Agriculture, Peoria, Illinois, independently described *Penicillium chrysogenum*. It was later realised in the late 1980s that all Dierckx's species were the same species (conspecific) of *P. chrysogenum.*[32]

To add to the confusion, a Swedish chemist Richard Westling had already described *Penicillium notatum* a century earlier in 1811. In 1931, Thom made a peculiar report that "Ad. 35 [Fleming's mould] is *P. notatum* WESTLING. This is a member of the *P. chrysogenum* series with smaller conidia than *P. chrysogenum* itself."[33] It means that Fleming's mould was *P. notatum* which in turn was *P. chrysogenum*. Thom wanted to preserve his own discovery and continued to use and popularise *P. chrysogenum* as Fleming's mould. He was more than harsh on Fleming to put a blame on him for misidentification, as he wrote: "Not being a mycologist, he undertook to identify the mould from the literature and selected the name."[34] He should have at least a decency to stretch his sightedness on Fleming's acknowledgement in the original paper, in which Fleming thanked the "mycologist, Mr. la Touche, for his suggestions as to the identity of the penicillium."[9]

The characteristic features and properties of the species was methodically reevaluated by Kenneth Brian Raper and Charles Thom in 1949. Based on the key diagnostic features, they came to the conclusion that *P. chrysogenum* is in nature a series of four species that included *P. chrysogenum*, *P. notatum*, *P. meleagrinum,* and *P. cyaneofulvum.*[35] *The taxonomic problem* intensified as the number of penicillin-producing species of *Penicillium* was mounting.[36,37]

Taxonomic revision in 1977 by Dutch microbiologists Robert A. Samson, R. Hadlok and Amelia C. Stolk made a decision that *P. notatum*, *P. meleagrinum,* and *P. cyaneofulvum* were just (synonym of) *P. chrysogenum*, so that the scientific name should only be the latter.[38] To ward off the trailing chaos, a collaborative team of expert in the *Penicillium* nomenclature proposed the name *P. chrysogenum* in 1992 to be adopted as the conserved name (*nomen conservandum).*[39] After 13 years, the proposal was formally approved by the Committee for Fungi and Lichens under the International Code of Botanical Nomenclature (Vienna Code) as adopted by the Seventeenth International Botanical Congress in Vienna, Austria, held in July 2005.[40] Fleming was proven right, the

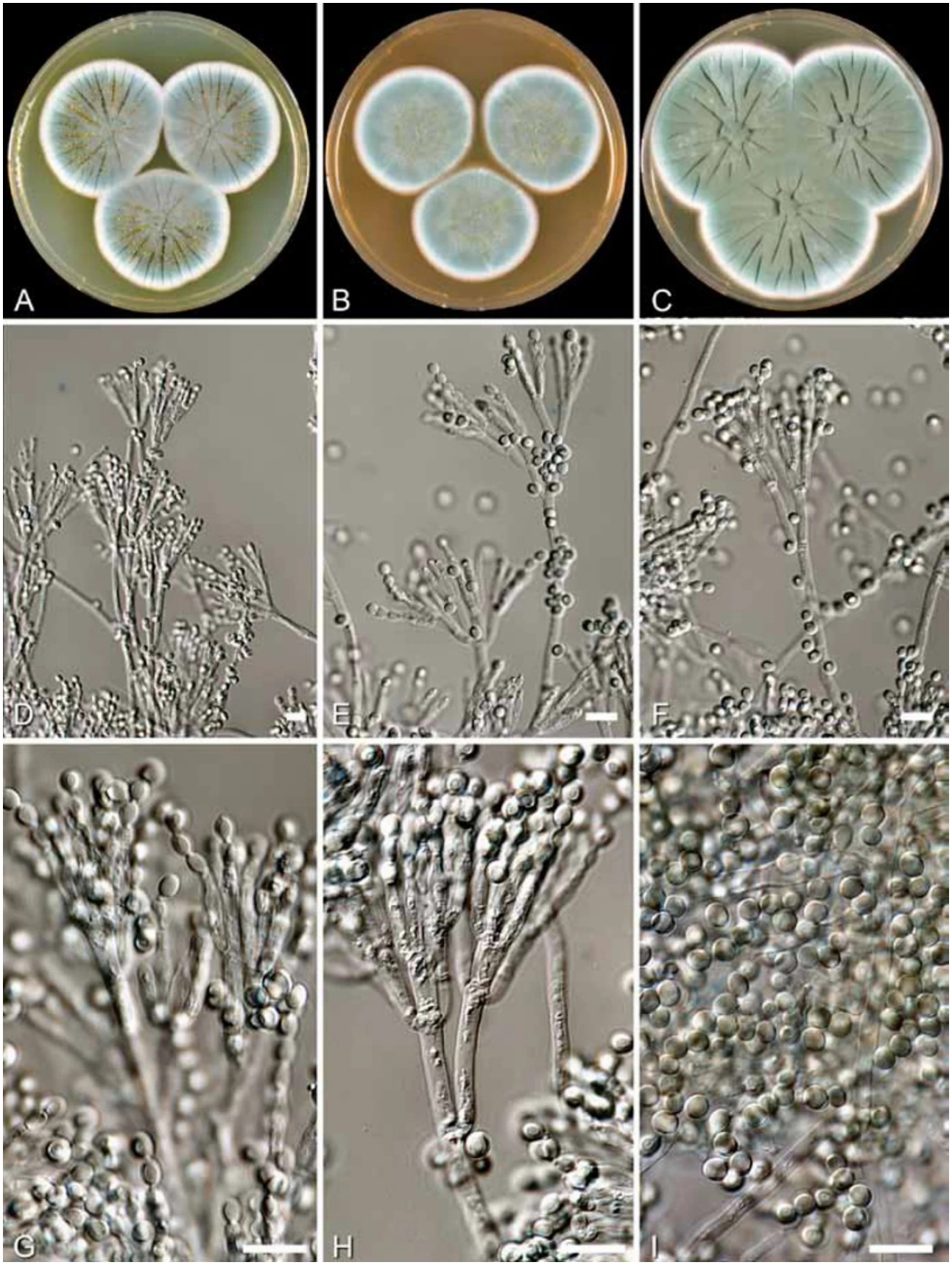

Figure 7 | Fleming's original mould, now identified as *Penicillium rubens*. A. Colonies in Czapek yeast extract agar (CYA). B. Colonies in malt extract agar (MEA). C. Colonies in yeast extract sucrose agar (YES). D–H. Conidiophores. Scale bar = 10 µm.[41]

mould was rechristened *P. chrysogenum* for all purposes. But then had Fleming were alive by then he would have really bemoaned of not clinging to his own identification than wielding to the "expertise" of mycologist.

Yet again Fleming (or Thom and followers) did not have the last laugh, which the future reserved for a French microbiologist Philibert Melchior Joseph Ehi Biourge. With the Vienna Code, the confusion quite settled down, but for molecular-based taxonomy the controversial fire was not a thing to be put out by a splatter of morphological criteria. Jos Houbraken, Jens C. Frisvad and Robert A. Samson reported in 2011 a phylogenetic analysis based on the genome sequence and β-tubulin, calmodulin and RPB2 (RNA polymerase II subunit) datasets and made a startling finding. They could not help but to report that "Fleming's penicillin producing strain is not *Penicillium chrysogenum*", and conclude that: "Fleming's original penicillin producing strain and the full genome sequenced strain of *P. chrysogenum* are re-identified as *P. rubens*"; and also that *P. chrysogenum* is a different species.[41] *P. rubens* was a species discovered by Biourge in 1923.[42] Of the eight strains under *P. chrysogenum* and *P. rubens* compared, the Wisconsin strain (NRRL 1951), Fleming's strain (CBS 205.57 = NRRL 824 = IMI 015378) (**Figure 7**), both designated *P. chrysogenum*, and the strain first used for producing penicillin in submerged conditions (CBS 197.46 = NRRL 832), designated *P. rubens*, were all proved to be *P. rubens*; the rest were *P. chrysogenum*.[41] Thom, who was keenly content with his *P. chrysogenum*, was also proven wrong. It shows that expert taxonomists are not always reliable, and a physician could very well be closer to taxonomic precision.

And thus far, Fleming's mould *P. rubrum* became *P. notatum* became *P. chrysogenum* became *P. rubens*.

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