

This section looks back to some ground-breaking contributions to public health, reproducing them in their original form and adding a commentary on their significance from a modern-day perspective. To complement the theme of this month's *Bulletin*, Sir Richard Sykes reviews the 1929 paper by Alexander Fleming on the antibacterial action of cultures of a penicillium. The original paper is reproduced from the *British Journal of Experimental Pathology*.

Penicillin: from discovery to product

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A bacteriology student reading the title of Fleming's landmark paper (1) today in an electronic Current Contents list might well ignore the article. Antibiotic use is commonplace and isolating bacteria is relatively straightforward on modern, selective media. But we need to consider this paper in its 1929 context to appreciate its significance.

Fleming's succinct 10-point summary shows the extent to which he had explored his original finding "that colonies of staphylococci near a mould colony were degenerate". His research covered conditions for optimizing production of the new natural product, penicillin, named following the nomenclature applied to other natural products, e.g. digitalin from *Digitalis*. Fleming explored the spectrum of activity of his novel compound using techniques that he devised to estimate its potency, bactericidal activity, interaction in the presence of leukocytes, efficacy and toxicity in animals. This broad evaluation of penicillin encompasses many of the key areas that would be used today in support of an application to use a new antibiotic for the first time in man. Fleming particularly appreciated the lack of toxicity and irritancy, evidenced by its application topically in the eye and by intravenous injection in mice.

Fleming assessed his own contribution to the discovery of penicillin in his address to the University of Edinburgh in 1952 when he took "Success" as the topic for his inaugural lecture as the newly elected Rector (2). He referred to the phenomenal success of Louis Pasteur, attributing it to hard work, careful observation, clear thinking, enthusiasm, and a spot of luck. He acknowledged the place that chance had had in his own career, beginning with his choice of St Mary's Medical School because he liked swimming and it had an active swimming club. His own assessment of his role in the discovery of penicillin

was that he saw something unusual and appreciated something of its importance and so began to work on it. He also found that the more he worked on it the more interesting it became. Fleming was fortunate in being free to work on his finding, without other pressures, until he had reached the point where he could no longer pursue the necessary chemical steps to exploit his discovery fully. He stressed that it is the lone worker who often makes the first advance in a subject. The details may be worked out later by a team but team members need to work in concert and so may prevent discovery if chance observations cannot be followed up because of other objectives or constraints. He quoted from a friend, Mervyn Gordon: "No research is ever quite complete. It is the glory of a good bit of work that it opens the way for still better and thus rapidly leads to its own eclipse" (3).

Fleming's 1929 paper paved the way to an enormous amount of future work, including an entire industry that now makes antibiotics by the ton around the globe. Fleming began by describing the characteristics of the mould and its growth under various temperature conditions, noting that it was most rapid at 20°C. He checked other moulds to see if they had the same property but realized that his strain was unusual in that the broth filtrate had inhibitory properties. His colleague's original naming of the mould as "most like *P. rubrum*" proved subsequently to be incorrect and *P. notatum* was the designation given by Raistrick and confirmed by Thom in the USA. Fleming noted that penicillin activity diminishes over time, and the labile nature of the new molecule was to prove a problem to others during the chemical scale-up of penicillin.

Fleming is acknowledged by contemporaries as a superb technician, and a glass blower of considerable skill. The methods he used and the principles he applied to testing his new agent are still in use today, so the 1929 paper laid important foundations for the modern science of antibacterial chemotherapy.

To test for antibacterial activity, Fleming used a simple ditch, cut in an agar plate, which he filled with

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filtrate and agar. When the ditch had solidified he cross-streaked it with seven different organisms to see which might be inhibited. The figure in the 1929 paper shows clear inhibition of staphylococcus, pneumococcus, streptococcus, the gonococcus and *Corynebacterium diphtheriae*. *Escherichia coli* and *Haemophilus* strains were not inhibited, both were designated *Bacillus* species at that time. This simple experiment gave an immediate impression of the spectrum of penicillin and is a forerunner of the type of screening that is used today to get an early overview of the extent of activity of a novel antimicrobial compound.

Fleming attempted some quantification of his experiment by measuring the extent of inhibition in mm for each organism and recorded the inhibitory action of doubling dilutions of his filtrates. These methods again were equivalent to today's MIC tests and disk diffusion methods, which have not been superseded because of their simplicity and their ability to convey valuable information quickly and cheaply whilst coping with the relatively high throughput of organisms in busy hospital laboratories. In addition, tests similar to Fleming's bactericidal tests are still conducted today to establish whether new agents are bactericidal or bacteriostatic.

St Mary's laboratory under Sir Almroth Wright had a long tradition of immunological research. Wright had been a friend of Ehrlich, and St Mary's was one of the first places that salvarsan was used in England to treat syphilis. Fleming had published on this topic in 1911 and was technically expert in its administration. This background undoubtedly made him very alert to the downside of toxic substances in interfering with natural host defence processes. His paper therefore showed that intravenous injection of his filtrate into rabbits in very large volume (20 ml) and also in mice had no toxic effects. Fleming was fortunate that he had chosen for his experiments two species that tolerate penicillin well. He was perhaps also fortunate that he chose to inject his filtrate and not to feed it orally: the subsequent finding that penicillin could kill guinea-pigs if administered orally because of effects on caecal flora might have prematurely ended interest in the new penicillin.

Fleming was not constrained by modern regulations on the use of novel agents and clearly experimented with his filtrates on patients or volunteers. He describes irrigation of "large infected surfaces" in man and also of the human conjunctiva every hour for a day without irritant effect. The use on skin surfaces may have been tried because of

Fleming's First World War experience in France, when he was faced with gangrenous infections which he treated with antiseptics. His publications of 1917 and 1919 both relate to the dynamics of wound infection. His experience with lysozyme also showed that lytic effects could be achieved with natural substances. Fleming was acutely aware that antiseptics did not always work beneficially in wounds because they had a damaging effect on leukocytes which he had observed could remove bacteria from pus. He clearly sought agents that did not have a deleterious effect, perhaps partly through the influence of Wright and following Ehrlich's principle of the need for a magic bullet to treat infection.

The last part of Fleming's paper relates to use of penicillin to make a selective medium for isolation of the difficult to grow *Bacillus influenzae*. This methodology again is a forerunner of the many selective media that are available commercially today. Incorporation of antibiotics, dyes, and antiseptic agents is usual in order to select or enrich cultures of strains that otherwise are difficult to isolate.

Fleming continued to work on his finding and to discuss it with others in the years following its publication. Coincidentally, in 1929, Howard Florey began to work on lysozyme and became interested in natural antibacterial substances. Together with Ernst Chain, he used Fleming's and Raistrick's earlier methods of solvent extraction and succeeded in separating penicillin.

The need for medicines to treat infections in the Second World War acted as a spur to unparalleled industrial collaboration on both sides of the Atlantic. Most of the companies involved are major pharmaceutical houses today, employing thousands of people and contributing significantly to health care everywhere. In the following 60 years thousands of novel antibacterials have been described, some of which have been developed to the status of medicines for human or animal use.

Penicillin itself founded a family of related molecules that have contributed significantly to a massive reduction in infections in the last half of the 20th century. Fleming always acknowledged that others might have seen the same phenomenon before him. Indeed when he heard that *P. notatum* had first been found on the herb, hyssop, he recollected the fifty-first Psalm: "Purge me with hyssop and I shall be clean". Fleming's initial paper was certainly a "good bit of work" that opened up enormous opportunities because he chanced to observe carefully and act methodically upon his observation. ■

References

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