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NEW LIGHT ON THE HISTORY OF PENICILLIN

by

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PENICILLIN WAS discovered by Alexander Fleming in September 1928 while he was a member of the staff of the Inoculation Department (now the Wright Fleming Institute) at St. Mary's Hospital, London, and had just been appointed Professor of Bacteriology in the University of London. He had noticed an unusual phenomenon, absence of fully developed colonies of a common microbe, *Staphylococcus aureus*, round a large colony of a common mould, *Penicillium notatum*, on an old culture plate. Research during the following winter showed that this had been due to the production by the mould of a hitherto unknown substance which was unique in that, although harmless to animals, it could kill disease-producing microbes. This naturally suggested its employment for the treatment of the diseases caused by such microbes, but proof of its value for this purpose was not obtained until twelve years later, when a team of workers led by Professor Howard Florey in the Sir William Dunn School of Pathology in the University of Oxford was successful.[1,2,3]

Florey and his colleagues were wise enough to publish a definitive account of their work soon afterwards.[4] Unfortunately, Fleming did no such thing, contenting himself with a few sentences or very short paragraphs in medical journals, most of them with very limited circulation.[5] This, together with what Maurois had to say in his biography of Fleming, whose English translation by Gerard Hopkins was published in 1959, has provided most of what is known about the part played by its discoverer in the development of penicillin as a therapeutic agent.[6]

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[1] A. Fleming, 'On antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*', *Br. J. exper. Path.*, 1929, **10:** 226–236.

[2] E. B. Chain, H. W. Florey, A. D. Gardner, N. G. Heatley, M. A. Jennings, J. Orr-Ewing, and A. G. Sanders, 'Penicillin as a chemotherapeutic agent', *Lancet*, 1940, **ii:** 226–228.

[3] E. P. Abraham, E. Chain, C. M. Fletcher, H. W. Florey, A. D. Gardner, N. G. Heatley, and M. A. Jennings, 'Further observations on penicillin', ibid., 1941, **ii:** 177–189.

[4] H. W. Florey, E. Chain, N. G. Heatley, M. A. Jennings, A. G. Sanders, E. P. Abraham, and M. E. Florey, *Antibiotics*, London, Oxford University Press, 1949.

[5] (a) Fleming, op. cit., note 1 above. (b) A. Fleming, 'Some problems in the use of antiseptics', *Br. dent. J.*, 1931, **52**, 105–117. (c) A. Fleming, 'On specific antibacterial properties of penicillin and potassium tellurite; incorporating a method of demonstrating some bacterial antagonisms', *J. Path. Bact.*, 1932, **35**, 831–842. (d) A. Fleming, 'Penicillin – its discovery, development, and uses in the fields of medicine and surgery. The Harben Lectures, 1944', *J. Roy. Inst. Pub. Hlth Hyg.*, 1945, **8:** 36, 63, 93. (e) A. Fleming, 'Antiseptics old and new', *Proc. Staff Meet. Mayo Clinic*, 1946, **21:** 65–75. (f) A. Fleming, *Nobel lecture on penicillin*, Stockholm, Kungl Boktrichereit, P.A., Norstedt & Soner, 1947.

[6] André Maurois, *The life of Sir Alexander Fleming*, translated by Gerard Hopkins, London, Jonathan Cape, 1959, pp. 124, 137.

Although I had been working in the same department under Dr. John Freeman and Dr. Leonard Colebrook from 1925 to 1930, and was well acquainted with chemotherapeutic thinking at the time and the techniques employed, I myself played no part in the discovery itself or the researches that followed, and I did not discuss them with Fleming. Nor did I have anything to do with the composition of Maurois' biography. However, soon after Fleming's death in 1955, I obtained as much information as possible from Fleming's colleagues. I was unable to proceed further until 1968, when I received photostats of sixteen pages from the laboratory notebook kept by Dr. Stuart Craddock, who had been Fleming's assistant at the time of the discovery. These enabled me to show, in a book published in 1970, that on scientific grounds alone there were discrepancies in the series of events that led to the discovery, the source of the mould, and what Fleming had said about the chemical researches. But I was unable to put forward any very satisfactory reasons for Fleming's failure to demonstrate the therapeutic value of penicillin.[7]

Much of this had been because I had been unable to locate or obtain access to Fleming's laboratory notebooks. But in 1970, soon after the publication of my book, they were deposited in the British Library. They have proved to be much less valuable than had been anticipated. There are long breaks in the dating for which there is no obvious reason, and the complete absence of some researches known to have been carried out. To make matters worse, although the books are available for inspection, they cannot be used or cited without the permission of the owner of the copyright, who refused it for this article.*

Craddock's complete notebook for 1929 has also reached the British Library, and shows that a number of experiments thought to have been carried out by Fleming had actually been done by Craddock. He was also the chronicler of the chemical researches, which were much more extensive than had been assumed. His invaluable notebook has been available for use and quotation.[8]

With this new material it is possible to describe with a greater degree of accuracy than has previously been possible the events that preceded and followed the discovery, and to furnish more acceptable reasons for Fleming's failure to demonstrate its value than he or his biographer had put forward. This is the main purpose of this paper.

THE DISCOVERY

In the paper reporting the discovery, Fleming described the phenomenon and the

[7] R. Hare, *The birth of penicillin*, London, Allen & Unwin, 1970.

* The author and editors wish to thank the British Library, Mr. Peter Levi, and Mr. Michael Bootle for their help in locating and communicating with the copyright-holder, Lady Fleming. A first draft of the article was shown to her, and the editors offered to reconsider any passages to which she objected and to publish a disclaimer dissociating her from the views expressed. However, permission was not granted, even for the reproduction of some drawings, lest it lead to the wrong suspicion that she agreed with the conclusions of the author. The editors consider it unfortunate that the facts about a discovery of such importance, made over half a century ago, cannot now be made available for public discussion except from one particular standpoint. Historians of modern medical science, when dealing with the Fleming Papers, should be warned in advance of this hazard.

[8] S. Craddock, notebook 1929. Now British Library, Add.MSS. 56224. Although Craddock's notebook is still technically copyright, the British Library, in response to our request to quote from it, replied in January 1981 that the holder of the copyright was not known. We have since been unable to trace any members of the Craddock family, with whom the copyright would rest.

processes he thought had brought it about in the following terms:

> 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 (see Fig. 1).[9]

There is no doubt that this explanation was based on the assumption that penicillin acted on microbes in the same manner as lysozyme, which Fleming had discovered six years previously after noticing a very similar phenomenon on an old culture plate.[10] For some years, this explanation went unchallenged, but in 1940, Professor A. D. Gardner of Oxford found that pencillin acted on organisms in a very different fashion from lysozyme in that it could only act on them during the very short phase in their life history when they were actually dividing.[11]

Since virtually all the organisms in fully developed colonies would be dead or dying, doubts about Fleming's explanation for the phenomenon began to arise in Oxford, which led to Dr. Margaret Jennings' attempts to produce dissolution of staphylococcal colonies with high titre penicillin. These failed, the colonies remaining intact (Fig. 2).[12] Using even stronger solutions of penicillin, Dr. W. D. Foster was also unable to produce any visible effect on fully developed colonies.[13]

No alternative procedures that could have produced the phenomenon were described until 1970, when I showed that it was possible to produce the phenomenon in the conditions in which Fleming was operating by postponing growth of the staphylococci until the spores of the mould had grown into a colony large enough to produce penicillin. This could only be achieved if the plate had been contaminated before or at the time it was being seeded with the staphylococci and its temperature kept below 20°C for the next five days, following which a high temperature favoured the development of the phenomenon. Study of the temperatures in London during July, August, and September 1928 showed that there had been only nine days when the weather had been continuously cold enough. Altogether these included two Sundays and one Saturday, Fleming could have discovered penicillin if he seeded the plate with staphylococci and contaminated it on Monday or Tuesday, 30 or 31 July.

It is impossible to confirm this theory because the pages recording the discovery and those concerning the staphylococcal variants have disappeared from Fleming's notebooks. In fact, nothing whatever is known about Fleming's activities from mid-January 1928 until Craddock appeared on the scene at the end of July.

At first sight, these minutiae may appear irrelevant, but if the phenomenon had been produced in the manner Fleming thought it had, it would be a very common occurrence in bacteriological laboratories all over the world, and Fleming could claim credit only for observing something unusual and acting upon it. In doing so, he did

[9] Fleming, op. cit., note 1 above, p. 226.

[10] A. Fleming, 'On a remarkable bacteriolytic element found in tissues and secretions', *Proc. R. Soc. Lond.*, series B, 1921–22, **98**: 306–317.

[11] A. D. Gardner, 'Morphological effects of penicillin on bacteria', *Nature, Lond.*, 1940, **146**: 837–838.

[12] Florey *et al.*, op cit., note 4 above, pp. 634, 1152.

[13] W. D. Foster, 'Sir Alexander Fleming as a bacteriologist', *Makerere med. J.*, 1965, no. 8, 11–15.

himself an injustice, because the stringent requirements in terms of time when the mould reached the plate and the temperature to which it was subsequently subjected render its accidental production almost impossible in countries with tropical or continental climates, and very unusual in those with temperate climates. Fleming was a great deal more fortunate than he ever realized.

The discovery probably occurred on Monday or Tuesday, 3 or 4 September 1928, while Fleming, officially on holiday, 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.

As to what occurred at the time, there are two separate accounts which, at first sight, appear to be describing two separate events. They can, however, be reconciled. The first to be published was that by D. Masters in his *Miracle drug*,[14] and quoted by Ludovici in his unofficial biography of Fleming.[15] It depends on what Dr. E. W. Todd knew of the event. Todd shared Fleming's laboratory, but would have had his back to Fleming's bench so that he could have heard but not seen what had been going on there. This is described by Professor Merlin Pryce in a memorandum, a copy of which he was good enough to give me, and which is summarized in both Maurois' biography and my own book. According to Pryce, Fleming had been looking at plates used for researches on staphylococcal variant colonies and had already discarded many of them. In the ordinary course of events, they would not have been looked at again. At this point, Pryce arrived for a morning gossip, and Fleming started to show him plates from the discard pile. One of them was the penicillin plate, the phenomenon was noticed this time, a culture taken of the mould, and the plate can now be seen in the British Museum.

Having discovered penicillin without looking for it and narrowly escaping failure to do so, Fleming then returned to his country home to resume his interrupted holiday, and did not start work again until the end of September.[16] Even so, it was not until the end of October that an experiment with penicillin was recorded, and late November before serious research can be said to have started.

THE SOURCE OF THE MOULD

The mould was sub-cultured at the time of its discovery and kept alive throughout the following years. It was used by Florey and Chain in the early work in Oxford, and even when large-scale production was required during 1944 and 1945. By that time, it had been found that many strains of *Penicillia* could produce some penicillin, and that Fleming's was among the three best producers of penicillin, out of the hundreds that many American workers had tested.[17] It was, accordingly, a very exceptional strain of this organism. This alone provoked interest in its source, but in view of the strict requirements for the production of the phenomenon that lead to the discovery, its

[14] D. Masters, *Miracle drug*, London, Eyre & Spottiswood, 1946, p. 26.
[15] L. J. Ludovici, *Fleming, discoverer of penicillin*, London, Andrew Dakers, 1952, p. 134.
[16] S. Craddock, 1968, personal communication.
[17] K. B. Roper, 'The development of improved penicillin-producing moulds', *Ann. N.Y. Acad. Med.*, 1946–47, **48**: 41

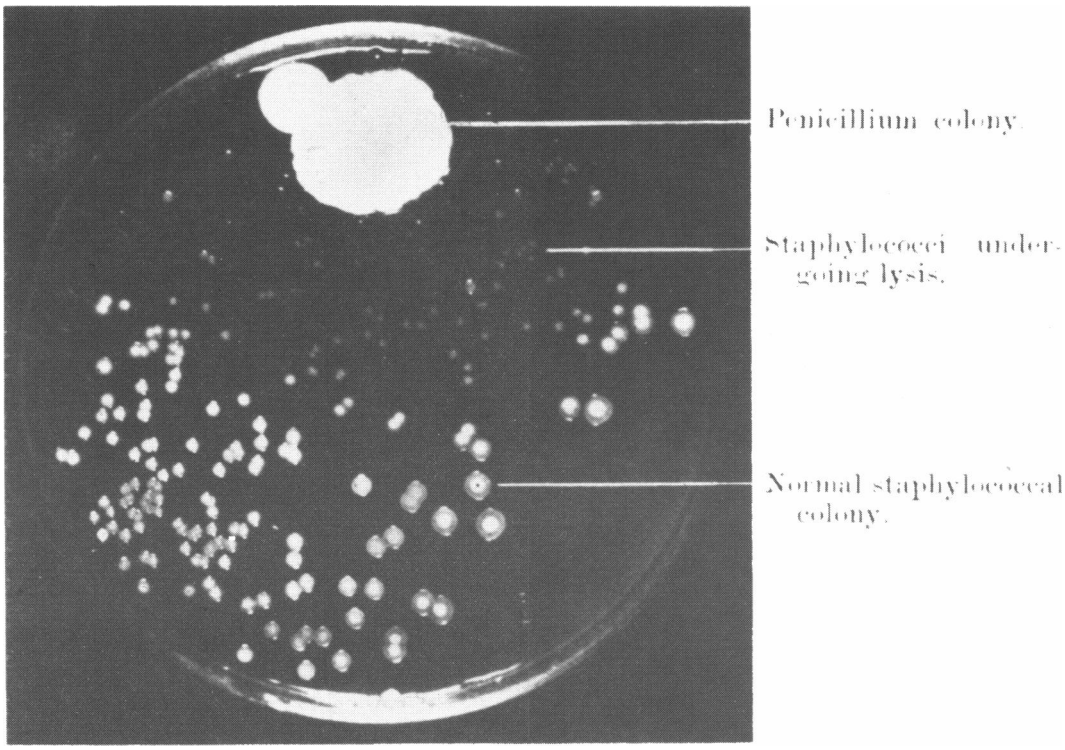
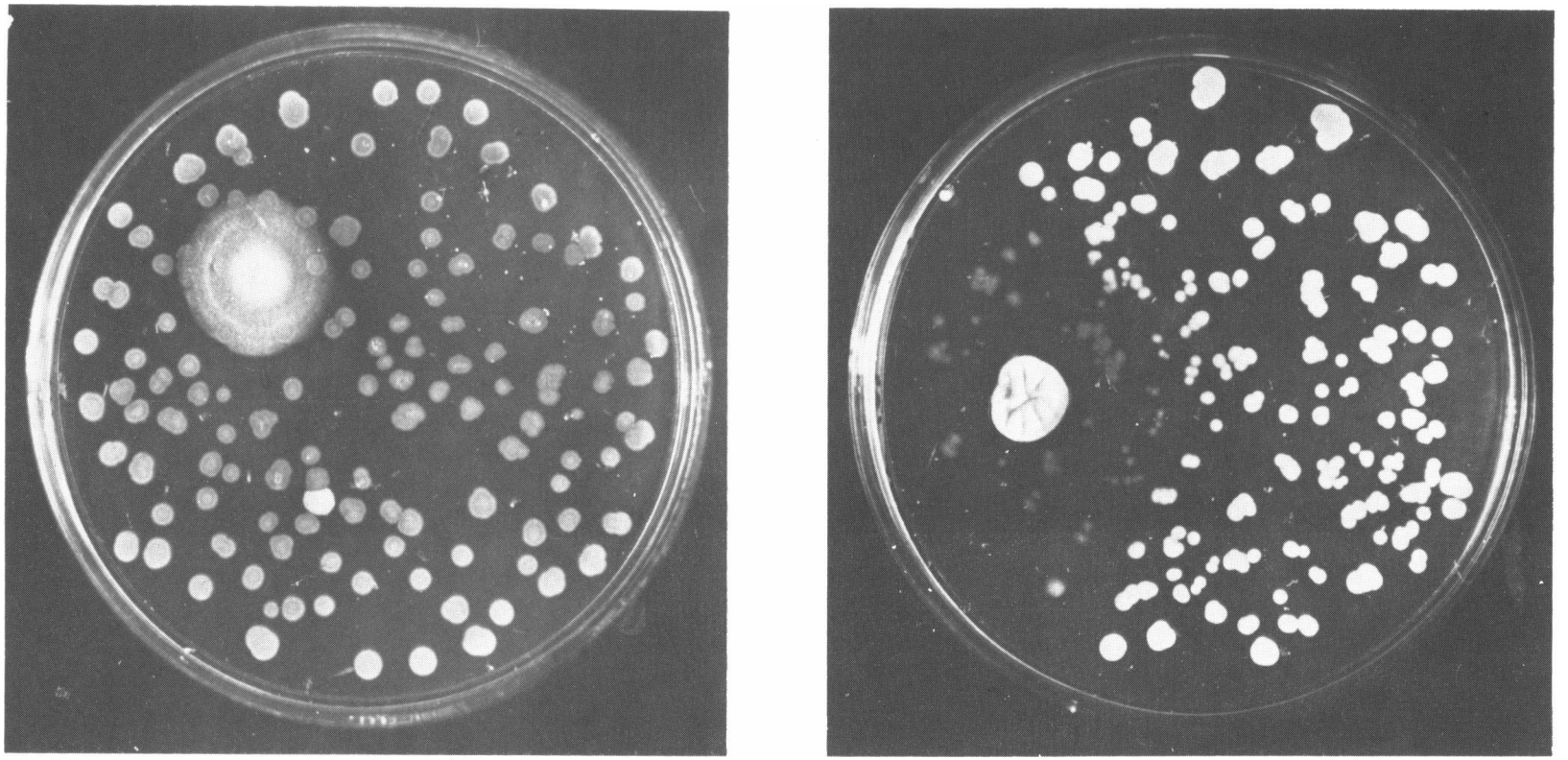


Figure 1. Fleming's photograph of the original culture plate with his captions. (Fleming, op. cit., footnote 1, facing p. 228.)

https://doi.org/10.1017/S0025727300040758 Published online by Cambridge University Press



(a) (b)

Figure 2. The causation of the phenomenon. (a) Contamination by the mould after the staphylococcal colonies had reached maturity and were left at 22–24°C for twelve days. (b) Contamination by the mould at the time the plate was seeded with staphylococci, left at 17–18°C for five days, and then a 22–24°C for twelve days. (Photograph by the author.)

Figure 3. St. Mary's Hospital from Praed Street. The mycology laboratory was on the first floor of the turret and Fleming's on the second. The remainder of the Inoculation Department was behind the balconies on the farther side of the turret. (Photograph by the author.)

https://doi.org/10.1017/S0025727300040758 Published online by Cambridge University Press

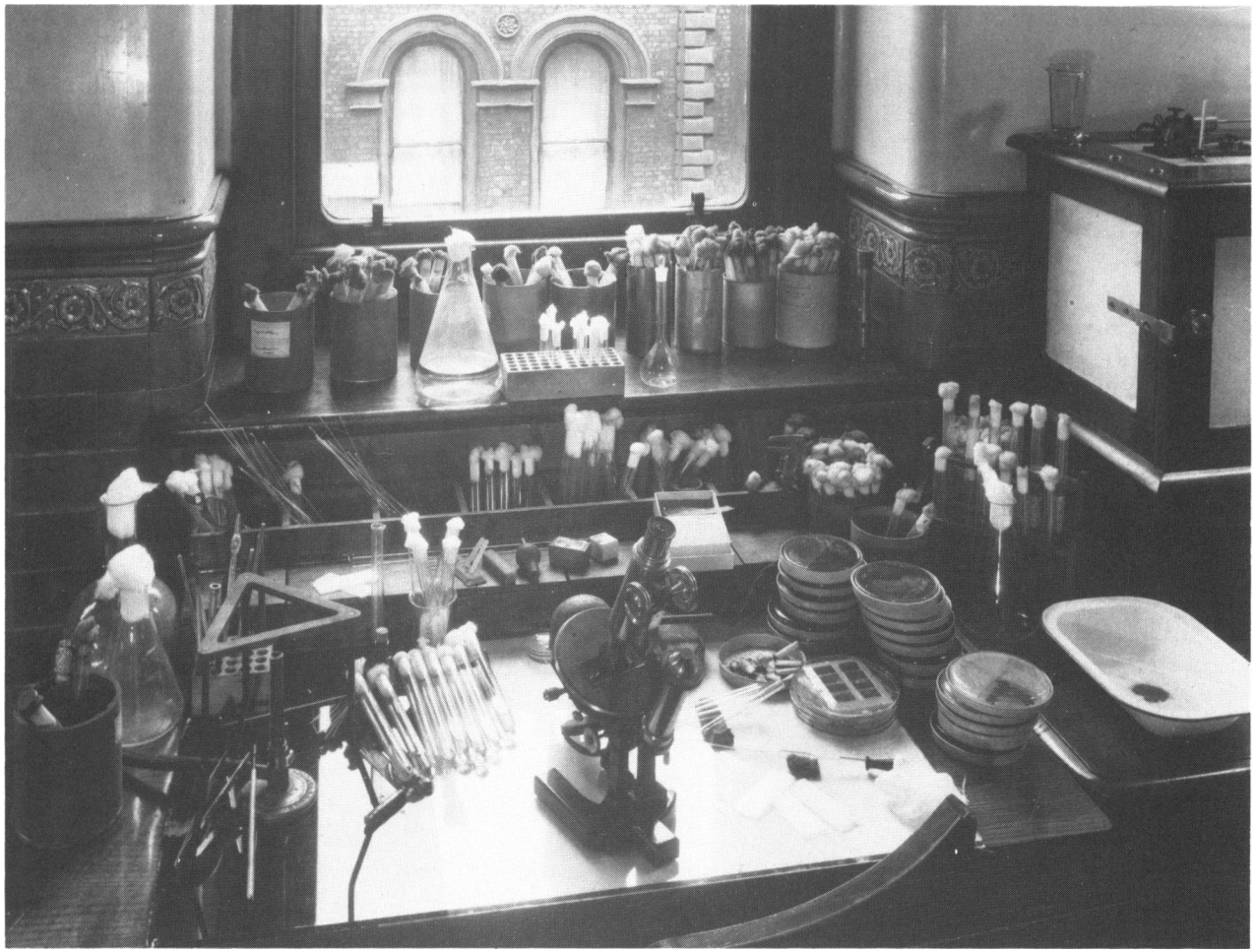


Figure 4. Fleming's laboratory at the time of the discovery showing slide cells on the bench between the microscope and the pile of culture plates. (Photographic Department, St. Mary's Hospital Medical School.)

exceptional ability to produce penicillin may have played a part in this as well. Its source is, therefore, a matter of some importance.

Nothing was said about the probable source of the mould until 1945, when Mr. G. Lacken was preparing a film. He was told by Fleming that it had blown through the window from Praed Street outside his laboratory.[18] Why he said this must remain a mystery, for he had no proof and must have forgotten a good deal that disproves it. Nevertheless, the story obtained wide publicity following its repetition by Maurois in his biography, in which it was even suggested that, almost overnight, Fleming learned enough mycology to isolate and identify ten other moulds.

In 1970, I pointed out[19] 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. I also showed that there had been, at the material time, another source of moulds in the form of a mycological laboratory on the floor below and in the same turret as Fleming's laboratory (Fig. 3). Since the mycologist in charge, Dr. C. J. La Touche, had to contend with very primitive conditions, its atmosphere must have become heavily contaminated by the spores of his moulds, so that they could have reached Fleming's laboratory by way of the stairs and a door that was always open (Fig. 4).

Proof of this was aided by the fact that, soon after the discovery, La Touche gave Fleming ten different moulds to enable him to ascertain how common a property the production of antibiotics was amongst the moulds.[20] These were tested, together with three more obtained from elsewhere; only one produced an antibiotic. This was one of La Touche's moulds, and this, according to Fleming in the original paper, had "exactly the same cultural characters" as the mould on the original plate, and, although he did not say so, ability to produce the same amount of penicillin.

There is, accordingly, good evidence that at the time of the discovery there was present in the mycological laboratory downstairs an exactly similar mould, that it had come upstairs to enter Fleming's laboratory by way of the door, and that Praed Street and an open window played no part in the discovery.

Why Fleming did not tell this story when asked, seventeen years later, whence the mould had come, must remain a mystery. The most probable explanation is that at the time he incriminated Praed Street and an open window as the source, the run-up to the Nobel Prize elections was about to start in Sweden, for which reason it would have been inadvisable to draw attention to the fact that the institute in which Fleming worked could not keep its moulds in order.

PROPERTIES OF THE NEW SUBSTANCE

After preliminary experiments in early December, Fleming was joined by Dr. Stuart Craddock on the twenty-first. Craddock had qualified in medicine in July, and had been awarded a Research Scholarship to work under Fleming's direction. Despite his lack of experience, he was responsible for many of the early experiments with the new substance.

[18] G. Lacken, *The story of penicillin*, London, Pilot Press, 1945.
[19] Hare, op. cit., note 7 above, pp. 82–83.
[20] C. J. La Touche, 1966, personal communication.

It was soon found that penicillin could be produced by growing the mould at room temperature in the laboratory's routine broth, which was made in small batches from a tryptic digest of bullock's heart muscle. A pellicle formed on the surface, the fluid below became bright yellow and was usually free of mould particles. Although they could be removed by filtration without loss of penicillin, this was usually omitted. Such a solution of pencillin was referred to in the laboratory as "mould juice" or, more officially, as "the Inhibitor", until, some time in April when the paper was being written, Fleming gave it its final name.

The penicillin produced could be detected by the now well-known ditch-plate method, in which equal quantities of the culture and melted nutrient agar were introduced into holes or gutters cut in the agar of a culture plate and allowed to set. Cultures of different organisms were streaked over these areas from side to side of the plate. It was then incubated overnight, and the inhibition of growth along each streak gave some indication of the amount of penicillin in the broth.

A more accurate method for estimating the pencillin content consisted in making serial dilutions in fresh broth, to each of which were added a few drops of a staphylococcal suspension. Following incubation, the highest dilution in which no growth of the organisms had occurred was recorded as the *titre*. It was soon found that after growth at room temperature for five to seven days the titre was generally 1/100 to 1/300 and very occasionally 1/600. Thereafter, it began to fall so that all but a trace of pencillin had gone after fourteen days.

It was also found that the new substance appeared to be harmless to man and animals. Its effect on micro-organisms depended on the species, those found in or producing diseases in the alimentary canal being insusceptible, whereas those from the respiratory tract were killed. These properties naturally suggested the employment of penicillin for two purposes: one was the treatment of infections; the second was its incorporation in culture media to facilitate the growth of organisms whose isolation had been difficult. This particularly applied to *H. influenzae*. Strange as it may seen, Fleming spent a great deal more time on this aspect of penicillin than on its therapeutic potentialities, and published several papers on it.[21]

In regard to the treatment of infections by susceptible organisms such as staphylococci, streptococci, and pneumococci, research along two separate lines was required: first, an enquiry into the feasibility of producing a more concentrated and purified solution than the broth cultures of the mould; and second, an attempted proof in the laboratory of its probable therapeutic value. The remainder of this paper deals with these two problems.

ATTEMPTS TO PRODUCE A CONCENTRATED AND PURIFIED SOLUTION OF PENICILLIN

For whatever purpose penicillin might be required, the broth cultures of the mould were generally unsuitable, particularly if treatment of deep-seated infections was

[21](a) Fleming, op. cit., note 1 above; (b) Fleming, op. cit., note 5(b) above; (c) Fleming, op. cit., note 5(c) above; (d) A. Fleming, 'Selective bacteriostasis', *Abstracts, Second International Congress of Microbiology*, 1936, p. 3; (e) A. Fleming and I. H. Maclean, 'On the occurrence of influenza bacilli in the mouths of normal people', *Br. J. exper. Path.*, 1930, **11**: 127–134.

contemplated. In the hope of overcoming this difficulty, Fleming suggested to Mr. Frederick Ridley, who had been studying lysozyme under his supervision, that he explore the possibility of producing a more satisfactory solution. Having had more chemical training while an undergraduate than was usual at that time, Ridley agreed, and started work during January 1929. He was allowed the assistance of Craddock, who also kept the records, copies of which were placed on Fleming's bench every morning. These seem to have disappeared, so that the only information available is that found in Craddock's notebook.

These investigations were never published in the usual sense of the term, but in 1968, amongst photostats of sixteen pages of Craddock's notebook, the only information then available, I found details of three experiments which, together with the recollections of the two men, enabled me to describe the methods employed, the many precautions that had to be taken, and the difficulties they had encountered.[22] Now that Craddock's notebook is fully available, it is possible to describe four more experiments.[23]

With regard to the method employed, suffice it to say that in most of their experiments, quantities of about 100 ml of penicillin broth were evaporated under vacuum at a temperature of about 40°C, the pH being adjusted before and at intervals to 6.9 or below by the addition of sulphuric or hydrochloric acid. The evaporation was stopped when only a few ml of a dark red fluid or a sticky mass was left. An organic solvent was then added, the mixtures centrifuged, and the penicillin titre of the supernatant determined.

In the first experiment dated 8 January, ether (volume not recorded) was added to the mass after evaporation. A great deal of material did not go into solution, but the ether itself became bright yellow and its titre was 1/1,000, considerably higher than any of the many broth cultures of the mould they had tested.

In the second experiment, on 14 February, the culture had a titre of 1/300 and that of the ether added to the mass after evaporation was much higher, 1/6,400.

Since these experiments clearly indicated that penicillin was soluble in ether, advantage was taken of the fact that it does not mix with water to omit evaporation in the next experiment on 20 February, and add it directly to the broth. It became bright yellow and was separated from the culture fluid. There is no record of its titre, but its spectrum of activity on different organisms was the same as that of penicillin. There is, therefore, no doubt that they had found that penicillin, or something behaving in the same manner, was soluble in ether.

Acetone had been employed as a solvent in another experiment, on 12 March, but, although penicillin had gone into solution from a mass after evaporation, most of it had evidently been lost in the process.

The next solvent to be employed was alcohol. Two experiments, on 20 March and 10 April, have already been described elsewhere, in which extracts with titres of 1/500 and 1/3,000 were obtained.[24] Two more can be found in Craddock's notebook.[25] In

[22] Hare, op. cit., note 7 above, pp. 93–96.
[23] Craddock, op. cit., note 8 above.
[24] Hare, op. cit., note 7 above, pp. 95–96.
[25] Craddock, op. cit., note 8 above.

that of 16 March, 200 ml of broth culture with a titre of 1/250 was concentrated under vacuum to 10 ml. This had a titre of 1/3,000. To it was added 90 ml of absolute alcohol, and the mixture centrifuged. The titre of the supernatant was 1/400 and that of the deposit below 1/10. The supernatant was then concentrated under vacuum to 10 ml, presumably to get rid of as much alcohol as possible. Its titre was 1/3,200. Thus, practically all the penicillin had survived two separate evaporations and was in solution in a mixture consisting largely of water.

In the experiment of 4 April, neither the quantity nor the titre of the culture was recorded, but it was evaporated to dryness. To it was added 2 ml of absolute alcohol, and the mixture allowed to stand for two hours. It was then centrifuged, the supernatant removed, and 2 ml of distilled water added to the residue. The titre of the supernatant was 1/8,000, and that of the residue, 1/4,000.

The results of all these experiments are summarized in Table 1. They show that penicillin was not only soluble in three organic solvents but that, depending on the quantity added, very high titres might be obtained, and, where the data permit calculation, with very little loss in the process. A very considerable degree of purification had also been obtained, judging by the amount of residue that had either not gone into solution or been precipitated by the solvent. It must be added that there are no indications in the records that the instability of penicillin had been a serious problem or had caused any experiment to be discontinued.

Table 1. Solubility of penicillin

Date	Solvent	Culture fluid		Extract	
		Volume	Titre	Volume	Titre
Feb. 6	Ether	N.S.	N.S.	N.S.	1/1,000
Feb. 14	Ether	N.S.	1/300	N.S.	1/6,400
Mar. 12	Acetone	N.S.	1/200	N.S.	1/200
Mar. 16	Alcohol	200 ml	1/250	10 ml	1/3,200
Mar. 20	Alcohol	200 ml	1/100	50 ml	1/500
Apr. 4	Alcohol	N.S.	N.S.	2 ml	1/8,000
Apr. 10	Alcohol	1,200 ml	1/300	120 ml	1/3,000

N.S. = not stated.

(*Source:* Craddock, op. cit., footnote 8 above.)

For no very obvious reason, no attempts seem to have been made (or, at any rate, recorded) to carry out what should have been the third step, transfer of the penicillin from the solvent to a watery base suitable for intravenous injection.

Fleming reported these researches in the original paper under the heading of *Solubility* in forty-six words, as follows:

Solubility. It is freely soluble in water and weak saline solutions. My colleague, Mr. Ridley, has found that if penicillin is evaporated at a low temperature to a sticky mass the active principle can be completely extracted by absolute alcohol. It is insoluble in ether or chloroform.[26]

It is all too obvious that Fleming can have known virtually nothing about these researches and had not troubled to find out more about them, because both Ridley and Craddock told me that they had not seen the paragraph before publication. It is therefore not surprising that no mention is made of evaporation having been carried out under a vacuum at a low temperature, that a low pH had been essential, and that concentrations or extracts with very high titres had been obtained. Of much greater importance are the mistakes, it being stated that penicillin was insoluble in ether, in spite of the fact that two extracts had been obtained, one with a titre of 1/6,400, and a third which contained something behaving in the same way as penicillin. Fleming also stated that penicillin was insoluble in chloroform, which Ridley had not employed, and acetone was ignored.

Why Fleming should have known so little about these investigations must be a matter for speculation, but it must be mentioned that he had a limited knowledge of chemistry. Therefore, he had failed to realize that the discovery that penicillin was soluble in three solvents was of fundamental importance, and indicated that its separation and concentration from the other constituents of the broth cultures would not have been such a serious problem as that encountered with proteins, for example. Fleming would have been much more impressed by the clumsy and temperamental nature of the apparatus Ridley had been using and its unsuitability for the processing of any quantity of culture. This, together with the results obtained in other researches to be described later, may have persuaded him that a preparation suitable for intravenous injection was no longer a matter of urgency and was too difficult to come by.

Support for this conclusion comes from the fact that he never referred to these investigations in any of his subsequent papers until the value of such a solution was proved by Florey and his team at Oxford in 1941. Even so, all that Fleming had to say about Ridley's work was that it had been a failure.[27]

Although an alteration in Fleming's opinion of penicillin may have been responsible for the sudden ending of these researches on 10 April, neither Ridley nor Craddock suspected that this had been the reason. According to them, it was mostly because they were getting tired of producing extracts by a method quite unsuitable for the production of any quantity. But one thing is quite certain. There had been no quarrel or other unpleasantness. On 13 May, Ridley went back to his previous researches with experiments on the effect of antiseptics on tears, which were followed by others on 30 May, 4, 14 and 15 June, 18 July, and still more during the autumn and winter.[28] On 17 May, Craddock also branched off, to study the value of penicillin for the isolation of the acne bacillus. This research occupied him for the rest of the year, but was not

[26] Fleming, op. cit., note 1 above, p. 228.

[27] (a) A. Fleming, 'Penicillin; Robert Campbell oration', *Ulster med. J.*, 1944, **13**: 95–108. (b) Fleming, op. cit., notes 5(d), 5(e), and 5(f) above.

[28] F. Ridley, 1968, personal communication.

published until long afterwards.[29] Thus both men were available had they been required.

A year or so later, unasked by Fleming but suggested by Professor W. W. C. Topley at the London School of Hygiene, Raistrick and his colleagues, Clutterbuck and Lovell, started to study penicillin and found that it was soluble in ether. But, unfortunately, Raistrick seems to have been unaware that living things can produce substances that disappear with great rapidity, such as complement and haemolysins, but which by care and nursing can be kept active for quite long periods. When, therefore, he found that penicillin might disappear in a few minutes when an ethereal solution was allowed to evaporate on the bench, he lost his nerve and returned to his study of compounds robust enough to be crystallized.[30]

Two years later, Dr. Lewis B. Holt, a professional chemist, became a member of Wright's department at St. Mary's. Fleming suggested that he attempt the concentration and purification of penicillin and referred him to the paper by Raistrick *et al.*, but said nothing whatever about Ridley's work. This was of small importance, because Holt immediately appreciated the fact that if the new substance was soluble in ether, other organic solvents might be employed as well. He chose amyl acetate and, provided the pH was dropped to between 5 and 6, penicillin would go into solution. But the losses were very heavy when attempts were made to transfer it to a solution of sodium bicarbonate at a pH of 8.0.[31]

Except for the abortive attempt by Reid in America,[32a] nothing further was done until Chain attacked the problem in Oxford. With the assistance of six graduates, an unspecified number of technicians, and a roomful of highly intricate apparatus, he succeeded in producing enough penicillin to prove its value by treating experimentally-infected animals.[32b]

LABORATORY ASSESSMENT OF THE THERAPEUTIC VALUE OF PENICILLIN

Before penicillin could justifiably be employed for the treatment of human beings, a laboratory investigation was an essential preliminary. But it is a striking fact that in all Fleming's accounts of the development of penicillin, no detailed description of what he had done in this connexion was included. What makes this even more remarkable is his having spent a great deal of time and energy during the years preceding the discovery in devising techniques for this very purpose.[33]

Before describing these techniques and the results he obtained, it is essential to emphasize that, at the time penicillin was discovered, very little was known about chemotherapy, and it was not until 1935, when the first of the sulphonamides was introduced, that modern chemotherapy can be said to have been born. Before that,

[29] S. Craddock, 'Use of penicillin in cultivation of the acne bacillus', *Lancet*, 1942, **i**: 558–559.

[30] P. W. Clutterbuck, R. Lovell, and H. Raistrick, 'The formation from glucose by members of the *Penicillium chrysogenum* series of a pigment, an alkali soluble protein and penicillin – the antibacterial substance of Fleming', *Biochem. J.*, 1932, **26**: 1907.

[31] L. Holt, 1965, personal communication.

[32](a) R. Reid, 'Some properties of a bacterial inhibitory substance produced by a mould', *J. Bact.*, 1935, **29**: 215. (b) Chain *et al.*, op. cit., note 2 above.

[33](a) A. Fleming, 'The action of chemical and physiological antiseptics in a septic wound', *Br. J. Surg.*, 1919–20, **7**: 99–129. (b) A. Fleming, 'A comparison of the activities of antiseptics on bacteria and on leucocytes', *Proc. R. Soc. Lond.*, series B, 1924, **96**: 171–180.

only two diseases could be cured by such methods. They were syphilis and relapsing fever, by Ehrlich's Salvarsan and Neosalvarsan. Very little was then known about their mode of action. It was generally assumed that they killed the spirochaetes responsible in the same manner as did the strong but poisonous antiseptics, such as phenol and mercury perchloride; but that the main difference was that their toxicity was sufficiently low to enable them to be injected intravenously without serious effects. Nevertheless, they retained sufficient toxicity to make it necessary to wait a week before giving another dose.

It was naturally assumed that any new compound likely to be of value for the treatment of the more acute and potentially fatal infections by streptococci, staphylococci, and pneumococci would behave in the same manner, would have to be administered intravenously, and would have to be sufficiently less toxic to allow adequate doses to be given more frequently than had been possible with the arsenicals.

Against such a background it would be legitimate to suppose that the discovery of a compound with such basic properties as penicillin possessed would have been followed by its intensive investigation at St. Mary's Hospital, with a view to its clinical employment as soon as possible. But by that time, the Inoculation Department as a whole had become extremely suspicious about the value of compounds with what seemed similar characteristics. These compounds, advocated by commercial houses, had proved to be frauds when tested clinically. Wright and Fleming had, accordingly, taken it upon themselves to expose these frauds, the former in words,[34] and the latter with techniques.[35] When, therefore, Fleming discovered penicillin, his first reaction would have been to look for its defects rather than its merits. How to do this was still a subject for debate, there being two schools of thought.

One based its techniques on those originally employed by Ehrlich, who had assessed his arsenicals by the effect they had on experimentally-infected animals such as mice, rats, and rabbits. Similar procedures were still being used by his successors in German laboratories, such as Hoerlein, Hegler, and Domagk in their search for new therapeutic substances. It must be added that these methods enabled them to discover the sulphonamides and to lay the foundations of modern chemotherapy.

The St. Mary's school, led by Wright, Fleming, and Colebrook, had grave doubts about the value of the German methods. These doubts were never, so far as I know, actually published but they were very much a part of the departmental doctrines. They were based on three facts: first, the experimental infections were considered to be too severe to serve as models of the normal human infections by such organisms; second, the organisms were human pathogens so that any effect the "natural immunity" mechanisms of the animal might have on the action of the substances under test might fail to come into action; and third, the effect of locally applied substances to surface infections could not be assessed for technical reasons given later in this paper.

Instead, the St. Mary's school employed *in vitro* methods, in which, to put it crudely, human blood took the place of animals, the assumption being made that, as it was the principal antibacterial mechanism in the body, any increase or decrease in its

[34] A. E. Wright, 'A discourse on Ehrlich's "chemotherapy",' *Lancet*, 1927, **ii**: 1327–1334.
[35] Fleming, op. cit., notes 33(a) and 33(b) above.

ability to kill organisms when potential therapeutic substances were added to it would give some indications of their value in disease.

Although this method demonstrated the deficiencies of the many compounds on the market which were clinical failures,[36] no-one had yet shown whether it alone could detect a compound likely to be successful. Nevertheless, in spite of this, Fleming had implicit faith in the value of *in vitro* methods. Of several techniques he had invented, one described in 1924 used home-made pieces of apparatus of which he was extremely proud. They were called "slide cells" (Fig. 5) and were made in the following manner:

> The slide cells in this method are made from two microscope slides separated by means of five strips of vaselined paper arranged at intervals conversely to the long axis of the slides. By means of these strips of paper, the space between the two slides is divided into four very thin compartments or cells open at each end and which will contain rather more than 50 cmm of blood.[37]

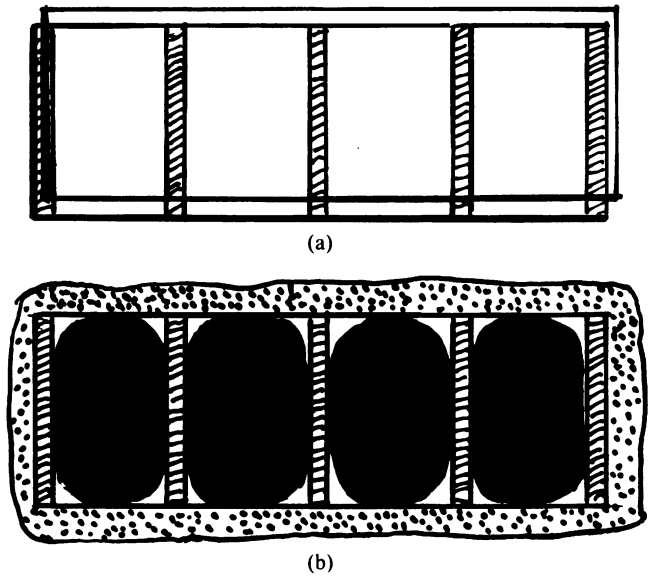


Figure 5. Slide cells (a) before and (b) after filling with mixtures of defibrinated blood, agent under investigation, and microbes and sealed before incubation.

He used nothing more elaborate than thin capillary tubes, rubber teats, minute test-tubes made in the laboratory from glass tubing, and microscope slides covered with

[36] (a) Ibid. (b) R. M. Fry, 'The effect of sanocrysin on *B. tuberculosis*', *Br. J. exper. Path.*, 1926–27, **7**: 174–176. (c) L. Colebrook and R. Hare, 'On the bactericidal power of mecurochrome', ibid., 1927, **8**: 109–114. (d) L. Colebrook, *A study of some organic arsenical compounds with a view to their use in certain streptococcal infections*, (Medical Research Council Special Report Series, No. 110), London, HMSO, 1928.

[37] Fleming, op. cit., note 33(b) above, p. 171.

wax on which to make the mixtures. All the cells contained 5c.mms. of human defibrinated blood to which had been added 5c.mms. of suitable dilutions in saline of overnight cultures of staphylococci or occasionally haemolytic streptococci, and 5c.mms. dilutions in saline of the substance under investigation. When all the cells had been filled, the edges of the slides were sealed with a mixture of paraffin wax and vaseline; they were then incubated for sixteen hours, at which time any surviving organisms would have grown into tiny colonies that could be counted with a hand lens.

In all these experiments a high proportion of the staphylococci or streptococci usually employed failed to survive in the cell containing normal blood without any of the compound under test. They had been ingested by the leucocytes and destroyed inside the cells. When most of the compounds under investigation at that time, which Fleming had tested by this method, were present in high enough concentration, they would usually kill or at least prevent development of all the organisms. But when smaller quantities of the compound were present, they might be in too low concentration to prevent development of the organisms, but at the same time in sufficient concentration to act on the leucocytes and diminish their ability to kill the organisms. Because of this, the position at the infected focus might well be worse than it had been without treatment. This is illustrated in Table 2, showing what occurred when carbolic acid was employed.

Table 2. The effect of carbolic acid on the bactericidal power of human blood on slide cells

Final dilution of carbolic acid	None	1/2,560	1/1,280	1/640	1/320
Number of colonies in each cell	2	7	31	100	0

(*Source:* Fleming, op. cit., footnote 33(b) above.)

Although this method of assessment could condemn a new, untried compound, there was less certainty about its value for the detection and evaluation of what might become a successful compound, largely because no such compound had appeared and been tested during the 1920s. The nearest approaches were the organic arsenicals employed for the treatment of syphilis but which could also kill haemolytic streptococci, for which reason they were being seriously considered for the treatment of infections by that organism. Fleming had also tested one of these compounds, novarsenobillon, and found that it behaved in a manner quite different from most of the compounds he had been testing. The results he obtained are given in Table 3, and show that when present in clinically attainable concentration in blood, it could prevent development of any colonies, and there were no signs that it harmed the leucocytes.

Table 3. The effect of novarsenobillon on the bactericidal power of human blood for haemolytic streptococci

Final dilution of novarsenobillon	None	1/128,000	1/64,000	1/32,000	1/16,000
Number of colonies in each cell	58	32	11	0	0

(*Source:* Fleming, op. cit., footnote 39 below.)

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Having perfected this technique in the early 1920s, Fleming tested almost every substance ever suggested for the treatment of pyogenic infections. They included: eusol, Dakin's solution, urea, alcohol, acetone, glycerine, hydrogen peroxide, chloroform, chloramine T, carbolic acid, emetine, formalin, picric acid, iodine, potassium permanganate, zinc permanganate, sodium salycilate, novarsenobillon, quinine hydrochloride, flavine, brilliant green;[38] to which must be added monsol, samocrysin, and mercurochrome – all introduced after 1924.[39] Then came a lull until 1935, when prontosil and then sulphanilamide appeared, which were duly tested by Fleming soon afterwards;[40] followed by sulphapyridine and sulphathiazole.[41]

It is, therefore, not very surprising that early in December, with nothing better than a crude broth culture of the mould, the only available source of penicillin, Fleming tested its behaviour in slide cells. It is unfortunate that because of copyright restrictions, the results cannot be reproduced here, but they were very much the same as those in another experiment, carried out by Craddock on 19 February, the results of which are given in Table 4.

Table 4. The behaviour of penicillin in slide cells (19 February 1929)

Final dilution of penicillin broth	None	1/12,000	1/1,200	1/120	1/12
Number of staphylococcal colonies in each cell	11	16	15	0	0

(*Source:* Craddock, op. cit., footnote 8 above.)

Up to this point penicillin had behaved in a manner that Fleming would have considered indicative of a successful therapeutic substance that could be injected into the bloodstream or employed for the treatment of surface infections by direct application.

On 7 March, however, the outlook became less propitious as a result of an experiment carried out by Fleming. This, too, cannot be reproduced because of copyright, but Craddock carried out an almost identical experiment on 8 March, which was performed in such a manner that the behaviour of penicillin in a simple bacteriological medium was compared with its behaviour in defibrinated human blood and serum from the same sample of blood. The results are given in Table 5.

They are not so clear-cut as they were in Fleming's own experiment, but they convey the same message, that penicillin was at its best in the bacteriological medium but was only about half as active in blood and barely one-quarter as active in the serum moiety of blood.

Fleming would have concluded from these two experiments that there was something in serum, and therefore in blood, that could in some undetermined manner inactivate penicillin, and that other body fluids such as lymph and the exudate that reaches an open wound, might similarly render penicillin less potent. It accordingly

[38] Ibid.

[39] A. Fleming, 'Discussion on the indications for and the value of the intravenous use of germicides', *Proc. R. Soc. Med.*, 1931, **24**: 808.

[40] A. Fleming, 'Antiseptics and chemotherapy', ibid., 1940, **33**: 127–136.

[41] A. Fleming, 'In-vitro tests of penicillin potency', *Lancet*, 1942, **i**: 732–733.

became imperative to ascertain whether this was likely to occur in the living animal. Craddock therefore carried out the following experiment on 22 March:

> A dose of 20 cc of penicillin broth with a titre of 1/300 was injected intravenously into a rabbit weighing 2,100 gm. Blood samples were taken before, immediately after, and at intervals during the next two hours. Dilutions of the serum were made in broth, staphylococcal suspension was added and incubated overnight.[42]

Table 5. The behaviour of penicillin in slide cells (8 March 1929)

Dilutions of penicillin broth	None	1/320	1/160	1/80	1/40	1/20
Number of colonies in each cell:						
0·05 per cent agar	86	76	61	35	0	0
Defibrinated blood	28	75	34	23	3	0
Serum	88	85	83	70	74	1

(*Source:* Craddock, op. cit., footnote 8 above.)

Table 6. Survival of penicillin in the circulating blood of a rabbit after intravenous injection (22 March 1929)

Dilutions of the serum	Control	1/128	1/64	1/32	1/16	1/8	1/4	1/2
Before injection	+	+	+	+	+	+	+	+
After injection	+	+	+	+	−	−	−	−
30 mins after	+	+	+	+	+	+	+	±
60 mins after	+	+	+	+	+	+	+	+
120 mins after	+	+	+	+	+	+	+	+

+ = Growth − = No growth

(*Source:* Craddock, op. cit., footnote 8 above.)

The results are given in Table 6 and show that the penicillin content of the circulating blood was much as might have been expected immediately after the injection, but its rapid disappearance in less than thirty minutes was probably not. To account for this, Fleming had three alternatives to choose from: (1) rapid excretion by the kidneys; (2) a hastening of the decay in potency responsible for the instability of penicillin when kept on the laboratory bench; (3) its inactivation as a result of its combination with the tissues or the blood, similar to that now known as the protein binding effect.[43]

Although, long afterwards, experience with human beings undergoing treatment by intravenous injection would suggest that excretion by the kidneys had been the

[42] Craddock, op. cit., note 8 above.

[43] J. M. Bond, J. W. Lightbown, M. Barber and P. M. Waterworth, 'A comparison of four phenoxypenicillins', *Br. med. J.*, 1963, **ii**: 956–961.

principal reason for its rapid disappearance, Fleming seems to have favoured the third alternative, its adsorption by the plasma, serum, or tissues, if only because something of this nature had evidently occurred in slide cells. Craddock's comment in his notebook, "This shows that the Inhibitor does not remain free in the serum for very many minutes," is indicative of the opinion at the time.

Further confirmation that inactivation occurred by this method would have come from Fleming's experiments with the chlorine-containing eusol and Carrell Dakin solutions and the yellow dye, flavine. The latter, after intravenous injection, remained free in the plasma for only eight minutes, by which time it had been adsorbed by the tissues as indicated by their colour, while the plasma had lost the bright yellow colour it had been immediately after the injection. Not surprisingly, these compounds had been virtually useless when employed for the treatment of infected wounds during the first world war.[44]

On the other hand, Colebrook, Fleming's colleague, had found that the organic arsenicals, Salvarsan and Neosalvarsan, could still be detected in the human bloodstream six and sometimes more hours after an intravenous injection of a therapeutic dose.[45] And there was no doubt that such compounds could cure syphilis, even when administered only once a week.

Although penicillin seemed to behave more like flavine than the arsenicals and might be equally useless, there was a possibility that if it acted quickly enough, it might deal with the organisms before its inactivation. In this connexion, Craddock had already carried out the necessary experiment on 8 February, which he described as follows:

> To 1 cc volumes of dilutions in broth of penicillin, were added 10 c.mm. volumes of a 1/1,000 dilution of an overnight culture of staphylococci. The tubes were incubated at 37°C and at intervals, 10 c.mm. volumes were plated on nutrient agar and incubated for 24 hours when the number of colonies were counted.[46]

Table 7. Time required to kill staphylococci (8 February 1929). Number of staphylococcal colonies on solid medium

Time	Penicillin dilutions in broth				
	None	1/80	1/40	1/20	1/10
Before incubation	27	27	27	27	27
2 hours after	116	73	51	48	23
4 1/2 after	00	13	1	2	5
8 1/2 after	00	0	0	0	0
12 1/2 after	00	0	0	0	0

00 = Uncountable

(*Source:* Craddock, op. cit., footnote 8 above.)

[44] Fleming, op. cit., notes 33(a) and 39 above.
[45] Colebrook, op. cit., note 36(d) above.
[46] Craddock, op. cit., note 8 above.

The results are given in Table 7. No attempt seems to have been made to ascertain why the new substance allowed the organisms to multiply during the first two hours and required more than another two and a half hours to kill all of them. But it must have seemed obvious that if it survived in the bloodstream no longer than thirty minutes following intravenous injection, it was unlikely to possess much value for the treatment of deep-seated infections such as those of the meninges, lungs, peritoneum, and bones, which, however penicillin was administered, would have required its transportation to the infected area by the circulating blood. But, on the other hand, it might be of value in situations where it could be applied directly without close contact with blood, such as infections of the skin, mucous membranes, ulcers, and open wounds.

Nevertheless, if these were Fleming's conclusions, they were based on nothing more substantial than four experiments in slide cells and one each in a rabbit and test-tubes. But, although nothing further can be found, it is very difficult to believe that during these two months nothing whatever had been done to ascertain the reasons for the strange behaviour of the new substance.

But whatever Fleming did or did not do during these two months, it came to an end on 10 May when the manuscript of the first paper was received by the Editor of the *British Journal of Experimental Pathology*. It provides invaluable information about Fleming's opinion at that time.

In the first place, the slide cell experiments and that showing the rate of disappearance of penicillin from the blood of the rabbit were not described or even referred to. In the second place, all he had to say about the therapeutic value of penicillin was contained in a small paragraph in the Discussion section, as follows:

> Penicillin, in regard to infections with sensitive microbes, appears to have some advantage over the well-known chemical antiseptics. A good sample will completely inhibit staphylococci, *Streptococcus pyogenes* and pneumococcus in a dilution of 1 in 800. It is therefore a more powerful inhibitory agent than is carbolic acid and it can be applied to an infected surface undiluted as it is non-irritant and non-toxic. If applied, therefore, on a dressing, it will still be effective even when diluted 800 times, which is more than can be said of the chemical antiseptics in use. Experiments in connection with its value in the treatment of pyogenic infections are in progress.[47]

By far the most important thing about this paragraph is what is omitted, for although the employment of penicillin for the treatment of surface infections is mentioned, there is no reference to its employment for deep-seated infections or the reasons for its omission. That this was deliberate and not an oversight is proved by the fact that there are similar omissions in papers published two and three years later.[48] In still another paper, in which were described the performance in slide cells of all the many compounds used at that time for deep-seated infections, penicillin was again not mentioned, and no reasons were given for its omission.[49] We must therefore conclude that within eight months of the discovery, Fleming had sufficient doubts about the value of penicillin for the treatment of deep-seated infections to make it inadvisable to say anything about this aspect of its clinical employment. And it is a striking fact, unnoticed by his biographer Maurois, that this opinion never left him. Even when he

[47] Fleming, op. cit., note 1 above, pp. 235–236.
[48] Fleming, op. cit., notes 5(b) and 5(c) above.
[49] Fleming, op. cit., note 39 above.

became a celebrity in 1942, he never claimed that he had wanted to treat the more severe forms of infection for which penicillin was proving so valuable. Those who knew him better than I did have confirmed this for me,[50] and others have reached the same conclusions from his writings.[51]

Fleming's doubts may also have been responsible for the sudden ending of the chemical researches half way through April. Started at a time when the treatment of deep-seated infections by intravenous therapy was not far off, the prospect of using penicillin in this way had faded by that time, and with it the need for a suitable solution or even an accurate description of the researches. But this does not excuse Fleming's telling audiences in several countries many years later that Ridley's and Craddock's work had been a failure.

Fleming's reservations may also account for his not testing penicillin in experimentally infected animals. Quite apart from the departmental objections, this would have required infecting the peritoneum first and then depending on the blood to transport the agent from a subcutaneous, intramuscular, or intravenous injection with, in Fleming's opinion, its inactivation on the way. The omission of these tests would therefore have been deliberate. And it was not until 1935 when the sulphonamides appeared, whose discovery and assessment both depended on animal tests, that their value was finally realized. In spite of this, subsequent commentators have not been slow to censure Fleming for their omission.[52]

On the other hand, Fleming had sufficient confidence in penicillin to suggest its employment for the treatment of surface infections. But unfortunately, no-one had invented an *in vitro* mimic of an infected wound or mucous membrane; only living tissues would suffice. Here, small laboratory animals such as mice, rats, and rabbits were virtually useless, it being impossible to obtain satisfactory infections and even more difficult to treat them with saturated dressings or an irrigation apparatus. Fleming accordingly chose the obvious alternative; treatment of human beings. This is discussed in the next section.

Such would seem to have been the position reached eight months after the discovery, when the first paper went in for publication. The paper itself certainly reflects Fleming's opinion of penicillin as a therapeutic substance, for very little is said about its properties and nothing whatever about what had become his standard method of assessing the value of therapeutic substances; and what he had to say about the chemical researches was inadequate and inaccurate.

But, on the other hand, a large part of the paper and even its title was occupied by his study of the employment of penicillin to facilitate the isolation from mixed cultures of influenza bacilli, which had occupied him for a great deal of the time during the winter months and which were to be resumed in the autumn.

[50] Personal communications: K. B. Rogers (1955); I. H. Maclean (1958); S. Craddock (1968); F. Ridley (1968).

[51] Foster, op. cit., note 13 above. W. C. Noble, *Coli, great healer of men. The biography of Dr. Leonard Colebrook, FRS*, London, Heinemann, 1974, p. 53.

[52] R. Lovell, 1956, personal communication. E. Chain, 'Thirty years of penicillin therapy', *Proc. R. Soc. Lond. [Biol.]*, 1971, **179**: 293–319. G. Macfarlane, *Howard Florey*, Oxford University Press, 1979, p. 188.

THE TREATMENT OF HUMAN INFECTIONS

Fleming's attempts to employ penicillin for the treatment of local surface infections began soon after its discovery. The "patient" was Craddock, whose antrum (a nasal sinus) had been troubling him for some time. It had become infected, and the aperture by which it communicates with the nasal cavity had been enlarged by operation, so that Fleming was able to inject penicillin broth into the antrum on 9 January 1929.

A sample of pus had been plated before treatment began, and had grown a mixed flora of staphylococci and influenza bacilli. After instilling 1 cc of penicillin broth, there was a copious effusion of fluid, and another culture three hours later grew one colony of staphylococci and a few influenza bacilli. Although Craddock irrigated it himself on several occasions during the next few days, the treatment was a failure, probably because the pathogen involved had been what Fleming invariably called Pfeiffer's bacillus (*H. influenzae*), which he found to be unaffected by penicillin.

This is the only "case" I have been able to find in the notebooks. But Craddock's book contains a note dated 26 March recording the filtration of penicillin broth with a titre of 1/600 "for the treatment of patients", but no further details are given. Craddock was also involved some time later with Dr. Claude Dolman in the preparation of several litres of penicillin broth for the oral treatment of a hospital patient whose alimentary canal contained a large number of enterococci thought to be responsible for her rheumatoid arthritis but who, needless to say, did not benefit.[53] But eventually, in 1932, a cure was at last obtained when Dr. Keith Rogers, at that time a medical student, contracted pneumococcal conjunctivitis in an eye required for a shooting match. Fleming treated it with penicillin broth, and St. Mary's fielded its full side.[54]

Some time before this, a more systematic trial was attempted, but virtually nothing is known about it. For such a purpose, Fleming had what would seem to have been quite adequate resources. They consisted in nothing more than the provision of broth cultures containing as much penicillin as possible, which should be ready whenever a suitable patient appeared; either Fleming himself or a deputy was to be available at all times to supervise the treatment. Close co-operation with the clinicians was also essential.

With regard to the penicillin content of the cultures, one of the difficulties was the wide variation in the titres. Although Fleming implied that 1/800 was relatively common, this was a gross exaggeration, 1/100 to 1/300 being more usual.

No attempts seem to have been made to carry out a systematic enquiry to ascertain how the yield might be improved and standardized, and, according to Craddock, they seem to have been content to use the routine broth of the laboratory, no two batches of which can have been the same, for they consisted of only a few litres of a tryptic digest of bullock's heart muscle. Whether or not the Czapek Dox medium was considered is not known, but at that time synthetic media were shunned by Fleming and his colleagues; they were not rich enough. Because of this, the titre of the broth they employed must have been governed by luck more than anything else.

[53] C. Dolman, 1955, personal communication.
[54] K. B. Rogers, 1955, personal communication.

Another problem was the instability of the new substance. Fleming quoted an experiment in the first paper, showing that practically all activity had disappeared after fourteen days at room temperature. But it was soon found that the pH played an important part. Usually as high as 8.5 when the titre was at its peak, it had been found by Craddock that if it was brought down to 6.8 or lower and stored at 0°C, its useful life might be prolonged considerably. In a later paper, it was said to be as much as three months.[55] Somewhat similar findings were reported by Raistrick and his colleagues.[56]

An additional measure designed to provide active penicillin was adopted; it was made a duty of every new recruit of Fleming's department to set up cultures of the mould on a certain day every week (probably Monday or Tuesday) so that potent penicillin would be available during the next week.[57]

Thus, although Fleming frequently complained that the instability of penicillin had hindered him, it is difficult to believe that with suitable organization this could not have been largely prevented. And certainly, the instability of penicillin does not appear to have been a problem when it was employed for selective media, as it continued to be for several years after the discovery.

Material needs in the form of glassware, and equipment for sterilization and filtration were no problem. Nor did he require much assistance, and, in any event, he nearly always had a Research Scholar working for him. Craddock, for example, remained with him until the end of 1929, when his place was taken by Dr. Claude Dolman, who did not leave until August 1931.[58]

Obtaining patients to treat seems to have been Fleming's biggest problem. It is probable that the ophthalmologists were approached first of all, because conjunctivitis was an almost perfect infection for his purpose, and, since Ridley divided his time between their department and Fleming's for several years following the discovery, he would have been a useful link, but they certainly played no part.

Fleming seems to have obtained more co-operation from the general surgeons, but all that can be gleaned about these investigations comes from scraps of information in papers at intervals over a period of thirteen years. The first three[59] show that there had evidently been a delay of one to two years before the trial had started, that what were called "indolent septic wounds" had been treated, that the results obtained had been "superior to dressings containing potent chemicals", and that these were all over by 1931, largely, it would seem, "because of the amount of trouble necessary for its [penicillin's] preparation and the difficulty in maintaining its potency for more than a few weeks". But nowhere was it stated what was meant by "indolent septic wounds", how long the treatment lasted, the organisms involved, and even the number of patients treated.

Following these attempts, Fleming did not refer to the therapeutic value of penicillin in any of his publications during the next nine years, but was heavily

[55] Fleming, op. cit., note 5(c) above.
[56] Clutterbuck *et al.*, op. cit., note 30 above.
[57] Personal communications: K. B. Rogers (1955); C. Dolman (1955).
[58] Personal communications: S. Craddock (1968); C. Dolman (1955).
[59] Fleming, op. cit., notes 1, 39, and 5(c) above.

involved in other matters such as selective media,[60] staphylococcal toxoid and antitoxin (following their discovery),[61] and, when they appeared, the sulphonamides.[62]

LATER DEVELOPMENTS

On 24 August 1940, the first of the Oxford papers was published, demonstrating the value of penicillin for the treatment of experimentally infected animals,[63] and on 2 September, Fleming went to Oxford where he was given a small sample of one of Chain's partially purified preparations. This was tested in slide cells and found to be capable of producing complete inhibition of staphylococci in human blood in dilutions as high as 1/320,000 (Table 8).[64] This may well have vindicated slide cells in Fleming's opinion as a method of assessment. And in any event, any inactivation that may have occurred could be discounted with so powerful a solution.

Table 8. The behaviour of one of Chain's early preparations in slide cells

Final dilutions of penicillin	None	1/640,000	1/320,000	1/160,000	1/80,000	1/40,000
Number of staphylococcal colonies in each cell	28	23	0	0	0	0

(*Source:* Fleming, op. cit., footnote 41 above.)

The results obtained by Florey and his colleagues must have made Fleming realize that he would soon have to find excuses for the long delay in the introduction of penicillin into medical practice, for which he was largely responsible. Nevertheless, he was not very forthcoming in what he had to say. The first sign of this occurred in a paper read to an audience of dentists at the Royal Society of Medicine in April 1941, which contained the following:

> About 1930 it was used as a dressing on a few septic wounds with favourable results but as in peace time, septic wounds are uncommon in hospitals and as the potency of penicillin rapidly disappeared on keeping, the therapeutic aspect of penicillin was dropped.[65]

Only five months later came the second of the Oxford papers, firmly establishing penicillin as a curative agent for human infections and soon afterwards, an annotation in the *British Medical Journal* which stated that "Penicillin does not appear to have been considered as possibly useful from any other point of view than for the isolation of organisms". This galvanized Fleming into publishing a letter in the same journal on 13 September, in which he said,

> Prior to the second article cited (1931)[66] a few tentative observations had been made on the effect of local application of the unconcentrated culture to septic wounds (chiefly carbuncles and sinuses). Although the

[60] Fleming, op. cit., note 21(d) above.

[61] Fleming and Maclean, op. cit., note 21 (e) above. A. Fleming, 'Recent advances in vaccine therapy', *Br. med. J.*, 1939, **ii**: 99–104.

[62] A. Fleming, 'Serum and vaccine therapy in combination with sulphanilamide or M and B 693', *Proc. R. Soc. Med.*, 1939, **32**: 911–920.

[63] Chain *et al.*, op. cit., note 2 above.

[64] (a) A. Fleming, L. Colebrook, E. E. Lewis, and R. Mowlem, 'Chemotherapy and wound infection', *Proc. R. Soc. Med.*, 1941, **34**: 337–350, p. 342. (b) Fleming, op. cit., note 41 above.

[65] Fleming *et al.*, op. cit., note 64 (a) above, p. 342.

[66] Fleming, op. cit., note 5 (b) above.

results were considered favourable, there was no miraculous success.[67]

Although these two quotations seem very much the same, there are important differences. The first suggests to anyone knowing Fleming's previous history that he had wanted to treat the civilian equivalent of the many acutely infected gunshot wounds he had seen in France during the First World War. And nine months after his lecture, Dr. Ethel Florey and Dr. R. E. O. Williams started their study of these civilian equivalents at the Birmingham Accident Hospital in the first controlled trial of the new substance.[68]

But Fleming's letter of 13 September shows, what he had never revealed up to that time, that the infections he had actually treated had been "carbuncles and sinuses" and very different from the "septic wounds" he had wanted to treat. Nor were they suitable, because they would have become chronic and anatomically unfitted for the application of an irrigation apparatus or saturated dressings.

Fleming never gave the reasons why he had treated such unsatisfactory infections until he became a celebrity a year later and could be more candid about the behaviour of the surgeons. Much the same story was told in places as far apart as Belfast, London, America, and Stockholm.[69] The following extract from a speech to an audience of surgeons at the Mayo Clinic is typical of all of them:

> However, penicillin is a very unstable substance, as you know. The culture of *Penicillium notatum* might be good today and in a few days time would have lost its power completely. We tried a little in clinical work, but not much. When we went to the wards and asked the surgeons if they had any septic cases we could try it on they always said, like most surgeons in most places, I think, that they had none. Then perhaps they come along sometime afterwards and say, 'Have you any of that stuff, I have a case I might try it on?'. As likely as not, by that time the potency of the penicillin had faded away. We tried to concentrate the penicillin but we were bacteriologists, not chemists, and we failed.[70]

Of the three excuses put forward, little credence may be given to the lack of a concentrated solution of penicillin, if only because he had never suggested it in any of his papers until the value of such a preparation was demonstrated by the Oxford workers in 1941. Nor is it possible to accept instability of penicillin, in view of the fact that a technique for its preservation for as long as three months had been introduced before 1932. This leaves the third excuse, difficulties with the clinicians. This may have been the real reason for the failure of the trials, because they had reacted in a manner that could have been predicted. Following their promise of co-operation, they had evidently had second thoughts about allowing him access to the acute and potentially dangerous infections he had wanted to treat, when they realized that the new remedy consisted of nothing more than broth in which a mould had grown and which was the fifth in a series of remedies, all based on what seemed, at the time, impeccable scientific evidence which Wright's department had been advocating during the preceding twenty years. These had included vaccine therapy before the First World War, hypertonic saline during the war, and immuno-transfusion after it, and the arsenicals for streptococcal infections, all of which had proved dismal failures in practice.

[67] A. Fleming, [correspondence] 'Penicillin', *Br. med. J.*, 1941, **ii**: 386.
[68] M. E. Florey and R. E. O. Williams, 'Hand infections treated with penicillin', *Lancet*, 1944, **i**: 73–81.
[69] Fleming, op. cit., notes 27(a), 5(d), 5(e), and 5(f) above.
[70] Fleming, op. cit., note 5 (e) above, p. 65.

When, therefore, an acute infection appeared in their wards, Fleming was "forgotten" and he never heard about it. But carbuncles and sinuses were another matter. The acute phase was long past and spread of the infection unlikely to occur. Besides, the patients were occupying beds wanted for much more interesting cases. If Fleming had something that could cure such infections, it was worth trying. With co-operation like this, it is not surprising that he gave up the struggle.

Nevertheless, the surgeons were not entirely to blame, for persuasion of unwilling or sceptical colleagues was not one of Fleming's talents, and he does not seem to have realized that co-operation with clinicians who spent only a few hours a week in their hospitals required more than promises. Dr. Reba Willitts and I had experience of this during a bacteriological investigation in a general hospital that required access to wound infections similar to those wanted by Fleming. Here, the clinicians were willing to co-operate, but we soon found that unless one of us went to their wards every day and cross-examined the sisters, we might never have heard about the infections which were, incidentally, much more common than the surgeons had led us to believe.[71] A few minutes of Fleming's time every day might have given him all he wanted. In the circumstances, it is not very surprising that the trials petered out with nothing very definite to report. Fleming busied himself with other activities, and until his death in 1955, his excuses remained very much the same.

When, however, Maurois' biography was being written, it was impossible to conceal the fact that Fleming had failed to exhibit the fire and energy in his dealings with the surgeons required of someone with a passionate faith in his discovery. Maurois accordingly countered with an entirely new excuse based on doubts about the value or future of antibacterial chemotherapy so frequently expressed by Fleming's chief, Almroth Wright. Certainly, according to Dr. V. D. Allison, Fleming's one-time colleague, Wright had been the reverse of enthusiastic about the curative value of penicillin when the manuscript of the first paper was submitted for publication. So much so, that he had demanded the omission of the short paragraph suggesting its employment for surface infections.[72] Fleming stood his ground, and the paragraph was published without alteration.

That Wright would behave in this manner could have been predicted, because he would have asked how penicillin behaved in slide cells and why all the information on the subject had been omitted, if only because he had played a part in their invention and used them as often as did Fleming. On being told, he would have made the obvious comment that there was insufficient evidence to justify any statement about the therapeutic value of the new substance.

Assuming that Wright's behaviour would have made it difficult for Fleming to ask for special help or facilities, Maurois succeeded so successfully in laying the blame on Wright for Fleming's failure to prove the value of penicillin that this episode has become an important part of the penicillin myth.

[71] R. Hare and R. E. Willitts, 'The source and prevention of septic infection of wounds', *Canad. Med. Assoc. J.*, 1941, **44**: 230–237. R. Hare and R. E. Willitts, 'The bacteriology of recently inflicted wounds with special reference to haemolytic streptococci and staphylococci', ibid., 1942, **46**: 23–30.

[72] V. D. Allison, [correspondence] 'Fifty years of penicillin', *Br. med. J.*, 1979, **i**: 1625.

Such were what might be called the official excuses for the long delay in the development of penicillin that followed its discovery, which were put forward at a time when its therapeutic value had been firmly established and Fleming had become a celebrity. But it is very doubtful whether they were the real reasons for his inactivity that lasted from 1930 until 1940, when others completed the story for him. For, as described in this communication, soon after the discovery what seemed to be good scientific evidence had been found that penicillin was unlikely to be of much value as a therapeutic substance. This may well have generated doubts in his mind about the feasibility of spending a great deal of time and energy on further research with nothing of any value at the end of it. Rather than take this risk, he allowed penicillin to lie fallow while he pursued what seemed more profitable lines of research.

Why the researches that prompted him to do this were never published or referred to, even when he had become a celebrity and a Nobel Laureate, will never be known. But they would have been much better than his laying the blame on the clinicians, the chemists, and even his own assistants for his own failure to follow up his discovery.

SUMMARY

The main objective of this communication is a review of the reasons for Fleming's failure to prove the therapeutic value of penicillin, in the light of information that has become available during the past decade.

The principal reason would seem to have been the behaviour of penicillin in laboratory tests which Fleming had devised five years before the discovery. He had implicit faith in these tests, which he was to employ at intervals during the twelve years that followed the discovery for the assessment of the therapeutic value of any compound put forward as an agent for the treatment of infections by pyogenic organisms. First employed only two months after its discovery, penicillin behaved in a manner indicative of a compound that was likely to be of therapeutic value for the treatment of such infections. But another series of tests three months later suggested that penicillin could become rapidly inactivated by blood, particularly its fluid elements (and possibly those of the tissues as well), to such an extent that it seemed unlikely that penicillin could be of much value for the treatment of any form of infection that necessitated its transportation by the bloodstream (such as meningitis, pneumonia, and peritonitis).

There was, however, a possibility that penicillin might be of value for the treatment of surface infections, such as those of the conjunctiva and mucous membranes, and open wounds, where it could be applied directly with less risk of inactivation.

These limitations in the value of penicillin and the investigations that had led to them were never published or even referred to in any of Fleming's communications. But it is a striking fact that he never advocated the employment of penicillin for deep-seated infections. He not only suggested its employment for surface infections, but attempted to treat them. These failed for a variety of reasons, including the lack of a sufficiently stable and concentrated solution of penicillin, and inadequate co-operation on the part of the clinicians.