BRITISH MEDICAL JOURNAL

LONDON SATURDAY AUGUST 5 1944

PENICILLIN: A SURVEY*

BY

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The chemotherapeutic properties of penicillin were discovered in 1940, but before this there was a long history which for convenience can be divided into stages. (1) The discovery of naturally occurring antibacterial substances—or antibiotics, as they are now beginning to be called—and the early attempts to utilize them in medicine. (2) The discovery of the antibacterial substance penicillin by Alexander Fleming. (3) The discovery of its chemotherapeutic properties at Oxford. (4) The stage of development in which we are at present, which consists of three interrelated lines of research—namely, (i) exploration of methods for mass-producing penicillin by the growth of the mould *Penicillium notatum*; (ii) investigation of the chemical structure of penicillin with the hope that it may eventually be synthesized by chemical means; and (iii) the clinical exploitation of the known properties of penicillin.

Stage 1

We have to go back to 1877 for the first observation of a naturally produced antibacterial substance. In that year Pasteur and Joubert described how when common air bacteria contaminated flasks of broth containing the bacillus of anthrax the growth of the anthrax bacillus was stopped. That phenomenon was probably the first observation that one organism may produce a chemical substance—or antibiotic—which is capable of stopping the growth of another, though Pasteur did not realize its true significance. In the succeeding years many examples were discovered, of which the most interesting was *Bacillus pyocyaneus*. From the medium on which this organism had grown Emmerich and Loew extracted a substance which they called pyocyanase. This was found to be capable of stopping the growth of certain organisms causing disease, notably anthrax and diphtheria. They applied it to the lesions of the skin caused by anthrax with, they claimed, some benefit. Although this product was on sale in Germany as recently as the 1930's its use in medicine never became widespread.

Stage 2

In 1928 Fleming was studying the staphylococcus. One day he examined and then put aside on his bench a plate on which colonies of the staphylococcus were growing. Several days later there was a colony of mould growing on one side. Fleming noticed that in the neighbourhood of the mould the colonies of staphylococci were disappearing. He recognized this as a phenomenon of interest, and subcultured the mould, which was later identified as *Penicillium notatum*. When grown on nutrient broth it was found to produce some substance which passed into the liquid. By experiments in test-tubes Fleming showed that the liquid had the property of stopping the growth of many bacteria. Fleming called the active liquid penicillin. He carried out experiments on the effect of his broth on numerous organisms in test-tubes and showed that many which can cause disease in man were affected, although some disease-producing organisms were quite insensitive. He also injected some of the broth containing penicillin into rabbits, and found that it was no more toxic than ordinary broth. He found, too, that the broth did not harm the white blood cells. Fleming, who had been working on antiseptics, recognized that penicillin had some very desirable properties as an antiseptic, and proposed that it might be useful for local application to infected surfaces. He did, in fact, so apply it in a few cases, with results indicating, as he said, that "it certainly appeared to be superior to dressings containing potent chemicals." About this time an attempt was made by Clutterbuck, Lovell, and Raistrick to extract the penicillin. They succeeded in growing the mould on a purely synthetic medium and found that the active substance could be extracted into ether when the watery medium containing penicillin was acidified. However, when they tried to concentrate the penicillin by evaporating the ether most of the activity was lost, and they concluded that penicillin was "extremely labile."

We may briefly summarize the position at the end of this phase by saying that Fleming had discovered the existence of an antibiotic produced by *Penicillium notatum*. Some test-tube investigations had been made of the antibacterial power of the crude broth and, as a result, it had been suggested that it might be useful as an antiseptic locally applied to infected lesions. But as the result of both Fleming's and Clutterbuck, Lovell, and Raistrick's work the conclusion had been reached that penicillin was an unstable substance and therefore unlikely to have any practical value in medicine.

Stage 3

Stage 3 deals with the work done at Oxford. My own interest in the phenomena of bacterial inhibition began in the 1920's. Since 1929, at first alone and later with collaborators, work had been in progress, but it was not till 1938 that Dr. Chain, a biochemist, and I prepared a plan for the systematic study of some of the naturally produced antibacterial substances. After much discussion the choice was narrowed down to three—*Bacillus pyocyaneus, Penicillium notatum,* and the subtilis-mesentericus group of bacteria. Eventually work was undertaken on the first two. Miss Schoental obtained three antibacterial products from *Bacillus pyocyaneus,* which all proved to be very toxic, but fortunately the results with penicillin turned out rather differently.

Both Fleming and Clutterbuck, Lovell, and Raistrick had noticed that under certain conditions the crude broth might retain its activity for at least several weeks. This indicated that in appropriate conditions the substance might not be so unstable as had been pictured. To work on the metabolic products of moulds from biological as well as chemical aspects needs a team of specialized workers, so that the various fields of investigation may be covered, and it was most fortunate that such a team was available in Oxford at that time. I should like to stress that this work could not have been carried through had it not been for the unremitting labours of the following people: Dr. Chain, Dr. Abraham, Prof. Gardner, Dr. Heatley, Dr. Jennings, Dr. Sanders, Dr. Fletcher, and Lady Florey. Nor

* Abridgment of the Peter Le Neve Foster Lecture delivered before the Royal Society of Arts on June 7, 1944.

could we have got far without the work of our technical assistants, Mr. Glister and his "penicillin girls," Mr. Kent, and, for the chemical work, Mr. Callow and Mr. Burtt.

The body of work done by this team in the next two years produced a single end-result—penicillin as a proved chemotherapeutic drug. The steps cannot be set out chronologically because different aspects of the work were in progress simultaneously, and the accent was first on one thing and then on another until a fairly complete picture was built up. The first step in all work of this type is to grow the mould on a medium into which it will produce the active substance. Here we were able, in the first place, to use the information which had been obtained by Fleming and by Clutterbuck, Lovell, and Raistrick, and we began by growing the mould on the synthetic medium proposed by the latter workers.

In studying an antibiotic its fundamental property of inhibiting bacteria can be made use of as a test method, and we owe to Dr Heatley the elaboration of a test which has proved invaluable for work not only on penicillin but on many other antibiotics as well. By means of his method it was possible to follow the various fractionating processes. The crucial chemical observation was the demonstration that not only did penicillin, when made acid, pass from a watery into an organic solvent such as ether or amyl acetate, but that it could be recovered from the organic solvent when shaken with water and an appropriate amount of alkali. By repetitions of this process purification and concentration were effected and the first stable products containing penicillin produced.

Chemical Properties of Penicillin

The principal chemical properties of penicillin are these: (1) An acid, unstable in the acid form but stable as salts between *p*H 5 and *p*H 7. (2) Ba, Ca, and Na salts highly soluble. (3) Destroyed by acids and alkalis and by heating. (4) Inactivated by oxidizing agents, heavy metals, primary alcohols, and ketonic reagents. (5) Inactivated by enzymes produced by some common bacteria.

This last is a very important observation, because it explains why large-scale production has proved to be more than usually difficult. The ubiquitous air bacteria may produce ferments which destroy the penicillin as fast as the mould makes it, so that although the mould may appear to grow well no penicillin is produced. As a consequence, all penicillin has to be manufactured with the exclusion of all air bacteria, and to do this on a large scale is a very difficult technical feat.

The production of a partly purified extract made possible the biological investigations which formed the next stage. It was found that the extract, even when highly diluted, would stop the growth of many organisms causing disease.

Bacteria Sensitive to Penicillin

Gram-positive.—*Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus viridans, Bacillus anthracis, Corynebacterium diphtheriae, Actinomyces bovis, Clostridium tetani, Cl. welchii, Cl. septique, Cl. oedematiens.*

Gram-negative.—*Neisseria gonorrhoeae, N. meningitidis.*

Bacteria not Sensitive to Penicillin

Relatively insensitive.—Gram-negative: *Salmonella typhi, S. gaertneri, Vibrio El Tor.*

Almost or completely insensitive.—Gram-positive: *Mycobacterium tuberculosis.* Gram-negative: *Pasteurella pestis, Vibrio cholerae, Bacterium coli, Pseudomonas pyocyanea, Proteus, Brucella abortus, Br. melitensis, Bacillus of Friedländer.*

This list is substantially the same as Fleming found with his crude broth containing penicillin, with the important addition among the sensitive organisms of the bacilli of gas gangrene and, unhappily, among the insensitive of the tubercle bacillus. Only those diseases caused by sensitive bacteria are susceptible of cure by penicillin. It was shown that penicillin, except possibly in very strong solution, does not kill the bacteria, but is a bacteriostatic. The penicillin preparations which we had at that time were bacteriostatic for sensitive bacteria at the dilution of 1 in 1 million, but we now know that these were very impure products. Pure penicillin will stop the growth of some kinds of bacteria at the astonishing dilution of 1 in 50 million or more.

It was further shown that the activity of the substance was maintained with scarcely any diminution in serum, in pus, and in the presence of autolysed body tissues. During the process of autolysis many breakdown products are formed, but none of them interfere with the action of the penicillin. These properties differentiate penicillin very sharply from the sulphonamide group of drugs, which are rendered largely ineffective by pus or tissue breakdown products. Another important finding was that penicillin would act almost equally well when large numbers of bacteria were present as when there were few; in either case it would inhibit the growth of the whole lot. This again is in sharp contrast to the sulphonamides, which are rendered less effective if many bacteria are present.

The fact that penicillin is a very powerful antibacterial agent would not by itself differentiate it from a number of other mould products or from some of the familiar chemical antiseptics. But whereas nearly all such substances are quite toxic to body tissues, even concentrated extracts of penicillin had practically no poisonous action on animals. It was further shown that individual body cells, such as the white cells of the blood, were unaffected by concentrations many hundreds of times greater than those necessary to stop the growth of sensitive organisms.

When administered to an infected animal or man in sufficient quantity penicillin stops the growth of the germs, thus giving the white blood cells in particular, and possibly other defence mechanisms, the opportunity effectively to attack and destroy them. It was found, too, that tissue cultures would survive and grow in concentrations very much greater than those necessary to produce bacteriostasis. In animals the active material was rapidly excreted by the kidneys into the urine, and, to a lesser extent, by the liver into the bile. It was readily absorbed after injection under the skin or into the muscles or into the small intestine, but it could not be given by mouth because of the hydrochloric acid in the stomach, which destroys penicillin very rapidly. Neither could it be given by the large bowel, because the bacteria there destroy it.

The position at which we had now arrived was that we had in our hands a substance which combined very low toxicity to animals with a very powerful action against disease-producing bacteria. We knew a good deal about its fundamental behaviour in the animal body. The most important step had now been reached—we had still to learn whether it would cure disease in animals and man.

It is worth while to digress for a moment in order to take up the question of antiseptics, so that the real significance of the experiments about to be described may be understood. Everyone is familiar with antiseptics such as mercuric chloride, acriflavine, dettol, lysol, etc. All these are capable, under appropriate conditions, of killing bacteria—mark the word "killing"—but cannot be used for injecting into the animal body because they have a damaging effect on animal cells as well as on bacteria. All the antiseptics in common use destroy protoplasm quite quickly, and this applies equally to the protoplasm of the bacterium and of the animal. As might be expected, although antiseptics can be used for sterilizing instruments and similar purposes, little success has attended their use in dealing with infected wounds, still less their injection into the body. A chemotherapeutic agent differs from antiseptics in that it selectively attacks the organisms causing the disease, without at the same time doing any serious injury in the body. For this reason it can be given internally or by injection. There are several examples of such chemotherapeutic substances. The one which has been known the longest, and is perhaps the most familiar, is quinine, used to combat malaria. Quinine is swallowed by mouth, passes into the blood stream, and exerts its beneficent action in killing the malaria parasite while being carried round to all parts of the body. Another example is salvarsan, the discovery of Ehrlich, produced after many years' work. It is an arsenic compound which has a very profound effect on the spirochaete of syphilis without being too toxic to be borne by the person suffering from the disease. Other substances were discovered which were effective against various tropical diseases, but only one class of substance, the sulphonamides, had been found of any use in common diseases

such as sepsis. Their use was, for various reasons—some of which have been mentioned earlier—somewhat limited. These are all true chemotherapeutic agents, not antiseptics.

The following experiments demonstrated that penicillin belongs to the class of true chemotherapeutic agents. So far as the use of penicillin in medicine is concerned this was the crucial discovery. Such experiments are carried out in the following way. Mice are injected with bacteria such as streptococci and staphylococci so that they will certainly die from the infection within one or at most two days. To show that a substance suspected of having chemotherapeutic properties is active it is necessary to secure survival of a substantial number of mice which would otherwise certainly die. In the case of penicillin this was accomplished by injecting some penicillin under the skins of the infected animals every three hours for several days. The drug was absorbed from beneath the skin into the blood stream, which carried it to the place where the infecting bacteria had previously been placed. Knowing that penicillin was a soluble substance quickly distributed round the body, that it was not toxic to animal tissues, and that it was just as active in the presence of body tissues as in a test-tube, we were justified in hoping that it would stop the bacteria growing as effectively in the body as it did outside. And this proved to be the case. The groups of treated mice survived almost without exception, while the untreated mice all died. These first experiments indicated without any doubt that penicillin belonged to that rare class of drugs which can be used as chemotherapeutic agents.

From this demonstration it appeared that penicillin was likely to have very great potentialities in the field of human medicine. Penicillin at that time was extremely difficult to produce in substantial quantities, so that some time passed before we were able to show its powers on man. We again have to thank Dr. Heatley and his assistants for unremitting work in producing in the laboratory enough penicillin for the first injections in man. Even after months of work we could treat only six cases of severe infection, but the results were most promising.

The first human patients were treated in the winter and spring of 1940–1, at the time of the worst bombing of England. It seemed improbable that much headway could be made in getting large-scale production started in this country. In these circumstances Dr. Heatley and I went to America, which was not then at war, to ask them whether they could put some of their great resources into the production of penicillin, so that more extensive clinical trials could be carried out. We were extremely fortunate in coming into contact with Dr. Coghill, Director of the Fermentation Division of the Department of Agriculture's excellent research laboratory at Peoria, in Illinois. The work which he and his colleagues have done on the selection of high-yielding strains of *Penicillium notatum*, and on the modification of culture media, has greatly increased the yield which can be obtained from the mould, and has played an important part in the large-scale production of penicillin.

While this work was being initiated in America, enough material was made in Oxford and by Imperial Chemical Industries to enable some eighteen patients with severe infections, most of them caused by the staphylococcus, to be treated. These results were again of such great promise that any effort to produce the drug on a really large scale was clearly worth while. This was more so since certain of the bacteria susceptible to penicillin cause some of the most common and universal infections, including those of war wounds.

Stage 4

From that time the work branched in three directions. First, it was clear that it would be very desirable to make the substance synthetically by chemical procedures without the intervention of the mould. Work is now proceeding along these lines in Oxford, where Dr. Chain and Dr. Abraham are collaborating with Sir Robert Robinson and his colleagues, and elsewhere, both in this country and in America, hundreds of chemists are engaged on this important problem. Progress in this direction cannot be reported as it is now in the secret category, but the fact has already been published that pure penicillin has been obtained. This was done in America and in Oxford at about the same time. Every resource has been mobilized to deal with this chemical question, but whether success will attend the effort to produce penicillin by synthesis it is impossible to forecast.

The second, and more immediately practicable line, has been to increase the manufacture by means of the mould to a really large scale. This has involved a large number of intricate technical problems, which have been tackled along different lines by the various commercial firms, both in this country and in America. As a result of their efforts penicillin can now be issued by the kilogramme, although, of course, the supplies still fall lamentably short of the demand.

The third line has been to explore further the use of penicillin as a curative agent. There are two possible ways of using penicillin. First, it can be injected into the muscles or veins so that it is carried around in the circulation to the parts which are being attacked by the infecting bacteria. This method is obligatory in the more serious and widespread diseases such as pneumonia, diseases of the bones, and septicaemia, where the diseased tissues cannot be reached by any other means. Although in many cases this is a very effective method, it has the disadvantage of requiring relatively large amounts of penicillin, since the drug is rapidly excreted by the kidneys. Secondly, penicillin may be used as a local application to the affected part. This can be undertaken only if every portion of the infected tissues can be reached by the penicillin, and a good deal of the success of local application depends upon surgical ingenuity in ensuring that the penicillin, which is rapidly absorbed from a wound, is kept in contact with all the infected tissues long enough to exert its action. At the present time a great deal of thought and study is being given to the problem of war wounds and how best to utilize penicillin, both locally and generally, for their treatment.

The increasing supplies of penicillin now available permit of extensive explorations of its use in many diseases. Perhaps the most striking recent addition to knowledge is that of the Americans, who have discovered that penicillin is apparently effective in treating syphilis. Another excellent development since larger supplies have become available is that penicillin can now be given as a preventive instead of as a last resort. In battle casualties especially, the effort is being made to prevent serious sepsis from developing by giving penicillin at a very early stage.

TREATMENT OF ACUTE EMPYEMA WITH PENICILLIN

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Treatment of acute empyema has both immediate and ultimate aims—the former to overcome the toxaemia by sterilizing or draining the cavity, the latter to restore the function of the lung and chest wall. This investigation is intended to show how far penicillin can assist in the realization of these aims when it is used in the simplest possible way—repeated emptying of the cavity by aspiration, followed by injection of the drug into it. Penicillin was dissolved in water to give a concentration of 1,000 units per c.cm., and the dosage was adjusted to the size of the cavity. The sensitivity of the infecting organism having been established, the initial injection varied between 10,000 and 40,000 units, 20,000 being usual. As treatment proceeded 10,000 or 5,000 units often sufficed. Tests for the presence of the drug in the pus withdrawn provide essential guidance in this respect. In the cases in which multiple loculi were present each cavity was treated on the same lines. It