Penicillin[1]

(A Lecture)

By H. W. FLOREY, Oxford

An enormous literature has now accumulated on penicillin both of a scientific and of a popular nature, though much of the latter is completely misleading if not untrue. On the scientific side Switzerland has been fortunate in having had excellent reviews published in the "Schweiz. med. Wochenschrift" and "Experientia" (WETTSTEIN[2] 1944, WETTSTEIN and ADAMS[3] 1945, v. HALLAUER[4] 1944, v. RIEBEN[5] 1944, LÖFFLER and HEGGLIN[6], PLATTNER[7] 1945) and a symposium in the "Revue médicale de la Suisse romande" with articles by BICKEL[8] (1945) and several others. The reviews necessarily touched only briefly on many points, but so comprehensive were they from the medical point of view that I greatly fear I can add little to what you already know.

It seems probable that moulds have been used in folk medicine by many different peoples, for example, in Central Europe, in the Ukraine, in Central America and very probably in other places, for the treatment of septic lesions, but the first scientific observations on "microbial antagonism" were made by PASTEUR and JOUBERT[9] in 1877. They noticed that if a culture of anthrax bacilli in urine was contaminated by organisms from the air the anthrax bacilli were destroyed. They also found that by introducing one of these common bacteria into the animal body at the same time as the anthrax bacillus the death of the animal from anthrax could sometimes be prevented. They remarked: "Tous ces faits autorisent peut-être les plus grandes espérances au point de vue thérapeutique." PASTEUR himself was mainly interested in immunity and did not pursue the matter, but in 1885 CANTANI[10] proposed to replace the tubercle bacillus in the tissues of the lungs with an ill-defined organism called *Bacterium termo*. This idea of the replacement of one pathogenic organism either by another less pathogenic or, if possible, by an innocuous organism occurs frequently in subsequent literature, but we have no time now to pursue this interesting side-line.

In 1885 methods for showing the inhibitory action of one organism against another were developed by BABÈS[1] who worked in Paris. He correctly deduced from his experiments that the inhibitions of bacterial growth which he observed were brought about by a definite chemical substance produced by the antagonistic organism. Of particular interest to you will be the paper published by the Swiss, GARRÉ[2], in 1887. The methods introduced by him to show bacterial antagonism are in all essential points the same as those commonly used at the present time. His paper can be read with interest and profit by anyone at the present day. Though his paper was not illustrated a picture was published by FROST[3] in 1904 to show one of his methods. Between radiating streaks of *Pseudomonas fluorescens* are planted streaks of *Salmonella typhi* and one sees that the growth of the typhoid organism is inhibited near the *Pseudomonas fluorescens*.

According to LEWEK[4] (1889) GARRÉ's paper gave a great stimulus to the subject at the time it appeared. In 1889 the first photographic record of the phenomenon was published at Kiel in a thesis by DOEHLE[5]. This illustration—the photograph for which was actually taken by HOPPE-SEYLER himself—shows the anthrax bacillus which has been sown in a solid medium being inhibited in the neighbourhood of an organism—some kind of streptococcus called *micrococcus anthraxotoxicus*—which has been planted in the form of a square (Fig. 1).

It was about this time that the word "antibiosis" was introduced into scientific literature by VUILLEMIN[6] (1889). It was used to express the idea of the unfavourable action of one organism on the growth of another. Relatively recently WAKSMAN and his colleagues have proposed that naturally occurring antibacterial substances should be called "antibiotics," and the word is finding increasing use.

Thus, by 1889 the phenomenon of microbial antagonism was well known and had been illustrated. Not only PASTEUR but nearly all the bacteriologists who worked on the subject at that time had in mind the use of the phenomenon for therapeutic purposes.

But it was the observations of BOUCHARD[7] in 1889 which started one of the most serious efforts which had been made to use a natural antibacterial substance in medicine. BOUCHARD observed that a culture of *Bacillus pyocyaneus* could confer some degree of

[1] We regret that many of the interesting figures accompanying this lecture could not be reproduced here for lack of space. The editors.

[2] A. WETTSTEIN, Schweiz. med. Wschr. *74*, 617 (1944).

[3] A. Wettstein and C. ADAMS, Schweiz. med. Wschr. *75*, 613 (1945).

[4] C. HALLAUER, Schweiz. med. Wschr. *74*, 611 (1944).

[5] G. RIEBEN, Schweiz. med. Wschr. *74*, 625 (1944).

[6] W. LÖFFLER and R. HEGGLIN, Schweiz. med. Wschr. *75*, 425 (1945).

[7] PL. A. PLATTNER, Exper. *1*, 167 (1945).

[8] G. BICKEL, Rev. méd. Suisse romande *65*, 657, 670 (1945).

[9] L. PASTEUR and JOUBERT, C. r. Acad. Sci. *85*, 101 (1877).

[10] A. CANTANI, Zbl. med. Wiss. Nr. 29, 513, and G. int. Sci. med., n. s. *7*, 493 (1885).

[1] V. BABÈS, J. Connaiss. Méd. prat. *7*, 321 (1885).

[2] C. GARRÉ (a) Dtsch. med. Wschr., p. 597, and (b) Correspondenz-Blatt für Schweizer Ärzte *17*, 385 (1887).

[3] W. D. FROST, J. infec. Dis. *1*, 599 (1904).

[4] T. LEWEK, Beitr. path. Anat. *6*, 277 (1889).

[5] DOEHLE, Beobachtungen über einen Antagonisten des Milzbrandes. Thesis presented at Kiel (1889).

[6] P. VUILLEMIN, Assoc. franc. Av. Sci. (2nd pt.), p. 525 (1889).

[7] CH. BOUCHARD, C. r. Acad. Sci. *108*, 713 (1889).

protection on animals infected at the same time with anthrax. The next step was to use the metabolic products of the bacteria instead of whole cultures, and I think the first trials on man of such a product were made by HONL and BUKOVSKY[1] in 1899. They described

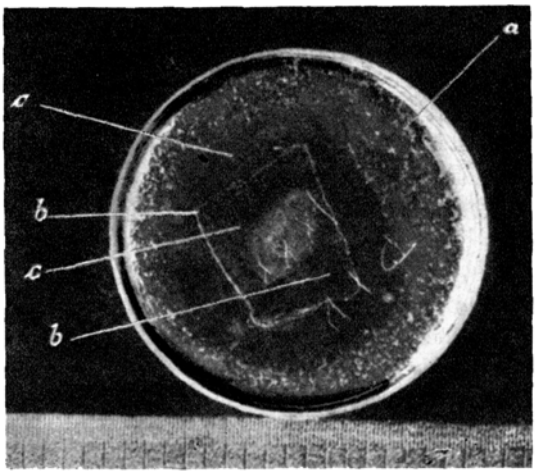

Fig. 1. First photographic record of antibiosis (DOEHLE; *B. anthracis* inhibited by a micrococcus).

the treatment with *B. pyocyaneus* culture fluid of 100 patients with ulcer of the leg, some of them cases in which amputation had been considered. At the same time, quite independently, EMMERICH and LÖW[2] were working with the same bacterium, and in 1899 prepared an extract which they called "pyocyanase." This extract not only killed such organisms as *B. anthracis* and *B. diphtheriae* but also dissolved them. It was thought at first that it would be possible to treat generalized infections by injecting pyocyanase into the body but these attempts came to nothing and the subject became confused with untenable ideas on immunity. As a local application, however, pyocyanase was much employed in the clinics of Germany and Italy and a large number of papers were written on the clinical use of this material. It was particularly employed in the treatment of diphtheria, both in the acute stage and for carriers, and for this purpose usually was insufflated. It was also used locally for treating inflammatory conditions of the eye, for the treatment of meningitis and of carriers of the meningococcus, and it was even injected into the cerebro-spinal fluid. It was also used for gonorrhoea. Nearly all the papers published at this time speak of good results from local application, but nevertheless from about 1908, which seems to have been the peak-year, fewer and fewer publications about pyocyanase appeared and it eventually passed almost out of use, though it remained on sale as a commercial product in Germany at least until 1936.

[1] J. HONL and J. BUKOVSKY, Zbl. Bakt., 1. Abt., *26*, 305 (1899).
[2] R. EMMERICH and O. LÖW, Z. Hyg. Infekt. *31*, 1 (1899).

In 1903 an interesting paper by LODE[1] appeared in which he described a coccus which dropped accidentally on to a plate of *Micrococcus tetragenus*. Round the cocci there were wide zones in which the *M. tetragenus* had not grown (Fig. 2). Experiments showed that the coccus produced a diffusible substance which was heat-labile and that oxygen was needed for its formation, but he did not succeed in extracting it, though he noted that it could be dried and was soluble in alcohol but not in ether. He did some experiments to see if the metabolic product could be used for treating experimental disease in animals, and though he considered his results disappointing he clearly had the right idea.

So far we have only considered examples of the products of bacteria, but fungi or moulds may also elaborate antibacterial substances, indeed one of the first of such substances to be crystallized came from a mould. This was done by GOSIO[2] in 1896 from a *Penicillium* which is now known as *Penicillium brevi compactum*. The substance, mycophenolic acid, was obtained only in very small quantities, and this he said unfortunately prevented him from doing animal experiments, but he ascertained that it would stop the growth of the anthrax bacillus. That, I think, is the first example in history of the preparation of an antibiotic from a mould.

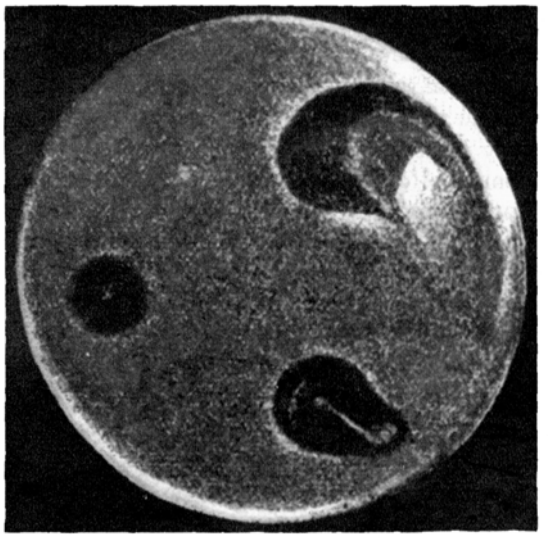

Fig. 2. Inhibition of *Micrococcus tetragenus* by a diffusible substance, produced by a coccus (LODE).

The first use in man of a mould, as opposed to a bacterial product, was apparently made by VAUDREMER[3] and reported in 1913. He stated that he had injected the culture liquid on which *Aspergillus fumigatus* had grown into more than 200 people suffering from tuberculosis. *In vitro* he had found that *A. fumi-*

[1] A. LODE, Zbl. Bakt., 1. Abt., Orig., *33*, 196 (1903).
[2] B. GOSIO, Rivista d'Igiene e Sanità pubblica *7*, 825 (1896).
[3] A. VAUDREMER, C. r. Soc. Biol. *74*, 752 (1913).

gatus was capable of destroying the tubercle bacillus although this apparently took some considerable time. The observation is one of considerable interest because we now know that *A. fumigatus* produces not one but

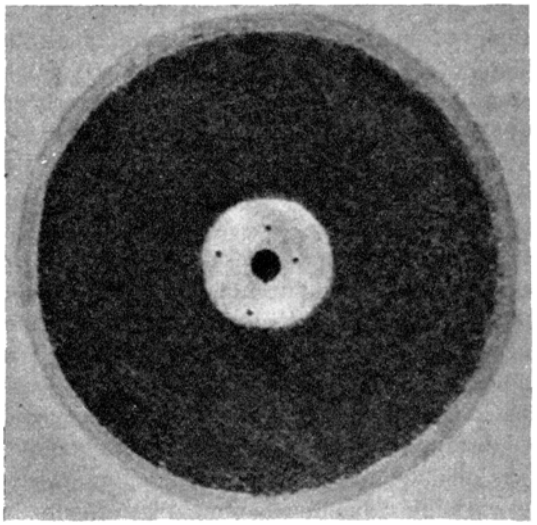

Fig. 3. Inhibitory action of one type of *Bacillus coli*, acting against another (GRATIA).

4 powerful antibacterial substances, all of which have been identified and crystallized. VAUDREMER's[1] experiments were unfortunately not backed up by any animal or pharmacological investigations, but I think this is the first instance that can be recorded of an attempt to use a mould product in man.

I can do no more than choose some examples from the very large number of papers in which bacterial antagonism of one sort or another has been recorded. So COLEBROOK[2] showed in 1915 the inhibition of meningococci by a growth of pneumococci, and further that this inhibition is influenced by the number of bacteria present. In case the meningococci were planted more thickly they were not inhibited.

In 1925 ALIVISATOS[3] showed the inhibition of staphylococci, also by pneumococci.

Fig. 3, from one of GRATIA's[4,5] publications (1925 and 1934), is of interest in that it shows one type of *Bacillus coli* acting against another. This inhibitory action was apparently very specific.

Some important work was done in the 1920's on the antagonistic effect of actinomyces, which was first noticed by LIESKE[6] in 1921. We owe most of the knowledge gained at this time to GRATIA and DATH[7,8] (1925, 1926) who deliberately set out to find antagonistic organisms by planting out such sources as tap-water, pond-water, etc., on plates containing various pathogenic organisms. They were particularly struck by the effects of a *Streptothrix* which caused the dissolution of the staphylococci with which the plate was heavily sown (GRATIA[1] 1934) (Fig. 4). They developed a method of preparing vaccines by dissolving the organism by the *Streptothrix* instead of destroying it by heat. These special vaccines were called "mycolysates" and it was claimed that they produced very good immunity in animals and man. In the same category was the work of MUCH[2] (1925) who used a strain of *B. mycoides—B. cytolyticus* Much—for the production of lytic substances. His preparation was on sale for use in medicine under the name of "Sentocym."

I should like to call the attention of a medical audience to the part that our colleagues the botanists have played, who over the course of years have made many first-class observations on this subject. For example, REINHARDT[3] in 1892 gave a clear description of the inhibitions between *Penicillia* and *Aspergilli* and described most beautifully the inhibition of *Pezizia trifoliorum* by a very small bacterial colony. In 1911 HARDER[4] showed the inhibition of *Stereum purpureum* by *Penicillium luteum* and, in 1924, PORTER[5] the inhibition of *Pestalozzia* by *Penicillium* colonies.

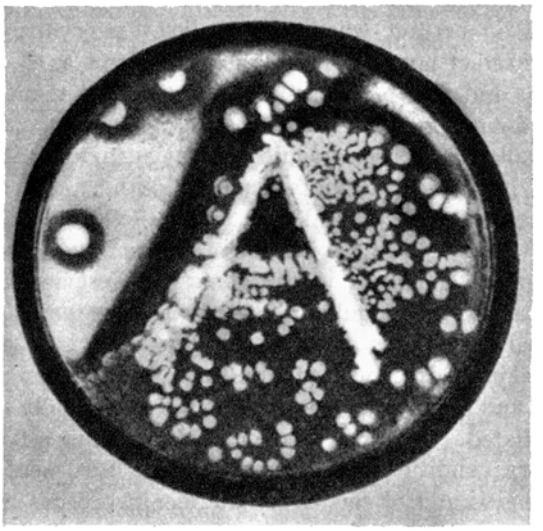

Fig. 4. Lysis of staphylococci caused by a *Streptothrix* (GRATIA).

I think I have said enough to convey to you the idea that much has been known about bacterial and fungal antagonisms for at least 50 years and that many attempts have been made to use the antagonisms in

[1] A. VAUDREMER, C. r. Soc. Biol. *74*, 752 (1913).
[2] L. COLEBROOK, Lancet *2*, 1136 (1915).
[3] G. P. ALIVISATOS, Zbl. Bakt., 1. Abt., Orig. *94*, 66 (1925).
[4] A. GRATIA, C. r. Soc. Biol. *93*, 1040 (1925).
[5] A. GRATIA, Bull. Acad. roy. Méd. Belg. *14*, 285 (1934).
[6] R. LIESKE, Morphologie und Biologie der Strahlenpilze. Bornträger, Leipzig (1921).
[7] A. GRATIA and S. DATH, C. r. Soc. Biol. *92*, 461 and 1125 (1925).
[8] A. GRATIA and S. DATH, C. r. Soc. Biol. *94*, 1267 (1926).

[1] A. GRATIA, Bull. Acad. roy. Méd. Belg. *14*, 285 (1934).
[2] H. MUCH, Münch. med. Wschr. *72*, 374 (1925).
[3] M. O. REINHARDT, Jb. Wiss. Bot. *23*, 479 (1892).
[4] R. HARDER, Naturw. Z. Forst- u. Landw. *9*, 129 (1911).
[5] C. L. PORTER, Amer. J. Bot. *11*, 168 (1924).

medicine. You now have some background against which to view the development of penicillin.

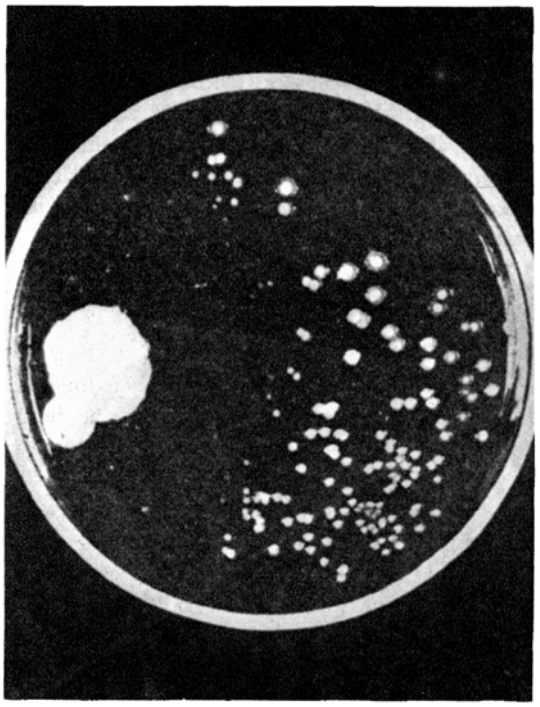

Fig. 5. Lysis of staphylococci by penicillin, secreted by a *Penicillium* (at left) (Original plate of FLEMING).

In 1929 FLEMING[1] made an observation on the inhibition of bacterial growth by a mould of the genus *Penicillium*. He was working on variation in colonies of staphylococci, work which entailed examining his identified as *Penicillium notatum*) in broth and found that it secreted something into the broth which had the power of stopping the growth and eventually of slowly killing many pathogenic organisms. He called the broth in which this antibacterial substance had been secreted "penicillin." He observed that the broth acted on many important pathogenic organisms such as the streptococcus and staphylococcus, while others such as the influenza bacillus and the salmonellas remained unaffected. Fig. 6 shows bacteria planted across a plate with a central gutter in which penicillin is incorporated. The penicillin has diffused from the gutter and some organisms are inhibited while others are unaffected.

FLEMING also found that he could inject 20 cc. of the penicillin broth into rabbits with no more effect than that produced by plain broth, and the same applied to its effect on leucocytes. These observations are frequently quoted as showing the lack of toxicity of penicillin, but it must be remembered that the 20 cc. of fluid injected into a rabbit can have contained at the most 400 units of penicillin (or about 0·25 mg. of the pure substance). It was not until the material had been extracted and purified at Oxford that it was found that very large amounts of the active substance could be injected without toxic symptoms. The experiments of FLEMING did show that a solution of penicillin was considerably more toxic to bacteria than to tissue-cells. Unfortunately this lead was not followed up at that time.

As a result of his work FLEMING[1] wrote in 1932: — "In penicillin (this refers to the broth), we have a perfectly innocuous fluid which is capable of inhibiting

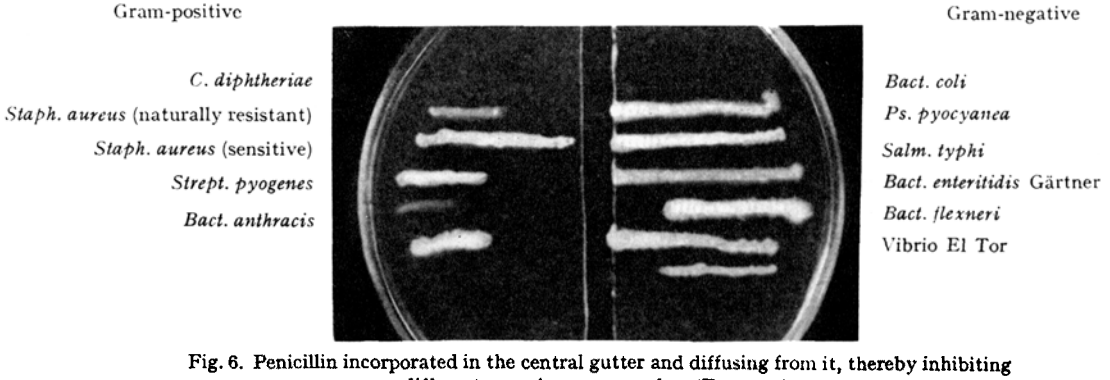

Fig. 6. Penicillin incorporated in the central gutter and diffusing from it, thereby inhibiting different organisms more or less (FLEMING).

plates with the lid lifted at frequent intervals. On one of these plates (Fig. 5) which had been standing for about a week at room temperature, a mould appeared which he noted to be remarkable in that it was dissolving the colonies of staphylococci in its neighbourhood.

He subcultured this mould (which was subsequently the growth of the pyogenic cocci in dilutions up to 1 in 800. It has been used on a number of indolent septic wounds and has certainly appeared to be superior to dressings containing potent chemicals. It is unlikely that it acts by killing the bacteria directly... The practical difficulty in the use of penicillin for dressings of septic wounds is the amount of trouble necessary for

[1] A. FLEMING, Brit. J. exp. Path. *10*, 226 (1929).

[1] A. FLEMING, J. Path. Bact. *35*, 831 (1932).

its preparation and the difficulty of maintaining its potency for more than a few weeks." It is clear from this that his idea was to use penicillin broth in much the same way as pyocyanase had been used before, that is, as a local application.

Nothing more was done towards its introduction into medicine between the early 1930's and the beginning of the next decade. In articles written in 1940 and 1941[1,2] he explained in these words why he gave up working on penicillin: —

"We have been using it in the laboratory for over 10 years as a method of differential culture. It was used in a few cases as a local antiseptic, but although it gave reasonably good results the trouble of making it seemed not worth while." And — "... a few tentative observations had been made on the effect of the local application of the unconcentrated culture to septic wounds (chiefly carbuncles and sinuses). Although the results were considered favourable there was no miraculous success."

The work at Oxford which I am going to describe has often been considered merely as a development of FLEMING's work, but it is essential for a clear understanding of the matter to realize why it is only since the Oxford results became known that there has been such an enormous flood of literature on all aspects of antibiotics. Briefly, the reason is that we were able to show that penicillin when extracted from the medium in which it was grown belonged to that very rare class of drug the chemotherapeutic agents, that is, a drug which can be administered so as to circulate in the blood-stream in sufficient quantity to cause infecting organisms to die or at least to cease multiplication, while at the same time the body-tissues are not harmed. It was this discovery which made worth while that which FLEMING had not thought worth the trouble. The steps leading to this realization were as follows:—

In 1932 CLUTTERBUCK, LOVELL and RAISTRICK[3] had published a paper in which they showed that penicillin could be produced by the mould on a synthetic medium. They also showed that the substance could be extracted from water into ether if the water was made acid, but that large losses occurred on the evaporation of the ether. They recognized that penicillin was most stable around neutrality but they stated that it was a very labile substance, and did not carry the work further.

In 1939 at the beginning of World War II, serious work was begun at Oxford, but I should like to emphasize that our investigation of penicillin had in the beginning no relation to the war but was undertaken as a purely academic study, part of a comprehensive research which had been planned by Dr. CHAIN[1] and myself. After some preliminary work it became clear that a team of workers with special knowledge in various branches of pathology, biochemistry and medicine would be needed for rapid progress, and finally at the Oxford School of Pathology were assembled for the work, Dr. CHAIN, Professor GARDNER, Dr. ABRAHAM[2], Dr. HEATLEY, Dr. JENNINGS, Dr. SANDERS, Dr. FLETCHER, Dr. M. E. FLOREY and myself. I should like to emphasize that it was the combined efforts of these colleagues which made a success of the work.

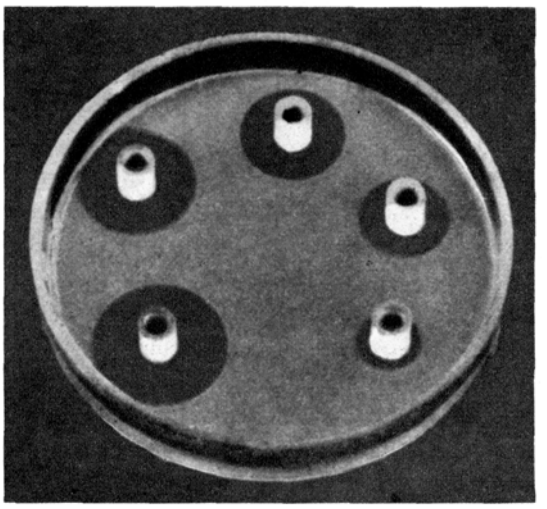

Fig. 7. Cylinder test for penicillin and other antibiotics (HEATLEY).

One of the first essentials in undertaking the extraction and purification of a biologically active substance contained in a complex mixture such as culture fluid is to have a quick biological test which will enable one to follow the substance through the various chemical manipulations (HEATLEY[3] 1944).

The surface of an agar plate is first sown with a broth culture of *Staphylococcus aureus* or other suitable organism. The excess broth is drained away and the plate dried in the incubator at 37° C. Small cylinders of glass or porcelain are then placed on the surface and filled with the solutions under test. Substances in solution in the cylinders diffuse out into the agar. On incubation at 37° C the staphylococci grow up into a confluent layer on the plate, but if penicillin is present in the fluid no growth occurs round the cylinders, where it has diffused out. The diameter of the zones of inhibition bear a relationship to the amount of penicillin in the fluid (Fig. 7). We have found this test easier and

[1] A. FLEMING, Pharmaceutical J. *145* (4th ser. *91*), 172 (1940).

[2] A. FLEMING, Brit. med. J. *2*, 386 (1941).

[3] P. W. CLUTTERBUCK, R. LOVELL and H. RAISTRICK, Biochem. J. *26*, 1907 (1932).

[1] E. CHAIN, H. W. FLOREY, A. D. GARDNER, N. G. HEATLEY, M. A. JENNINGS, J. ORR-EWING and A. G. SANDERS, Lancet *2*, 226 (1940).

[2] E. P. ABRAHAM, E. CHAIN, C. M. FLETCHER, H. W. FLOREY, A. D. GARDNER, N. G. HEATLEY and M. A. JENNINGS, Lancet *2*, 177 (1941).

[3] N. G. HEATLEY, Biochem. J. *38*, 61 (1944).

quicker than serial dilution methods and in our hands more accurate for assay work. It has been of inestimable value not only for penicillin, but for other antibiotics also.

At first culture was undertaken in Erlenmeyer flasks but early in the work it became apparent that larger vessels would be required. It may amuse you to know about the evolution of our first large vessels. At the time we wished to have them the Battle of Britain had been won but the country was being subjected to heavy bombing. It was difficult to get supplies. It was found that the old style bed-pan with a spout and lid was an ideal culture vessel but unfortunately when I tried to procure 600 of them they were not available as they had been replaced by a more modern stream-lined structure without a lid. However, HEATLEY designed a suitable vessel which was made in porcelain and to indicate some of our difficulties, he had to borrow a van and petrol and drive 200 miles to fetch them. He returned in the van with the first load of vessels on Christmas Day 1940. Vessels of this kind are shown in Fig. 8 in place in the incubator.

Clearly one of the first steps in the work was to learn to extract the penicillin from the complex mixture of culture and metabolites. The first key to its successful extraction is the knowledge that in the acid form it is more soluble in organic solvents than it is in water. This had been described by CLUTTERBUCK, LOVELL and RAISTRICK[1] (1932). It is true that in the acid form it is extremely unstable in water but losses can be considerably diminished by cold. It is thus possible to extract penicillin in the cold from watery solution into

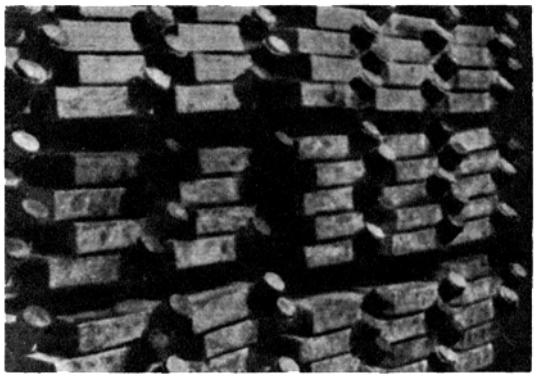

Fig. 8. Porcelain culture vessels for *Penicillium* culture (HEATLEY).

an organic solvent such as ether by first acidifying the water. The second key was, we found, that to get the penicillin into water again it was only necessary to shake the ether extract with about 1/5th of its volume of phosphate buffer at a p_H of about 7. Among other organic solvents which can be used to effect this purification one of the best is amyl acetate. It soon became evident that large volumes of fluid would have to be

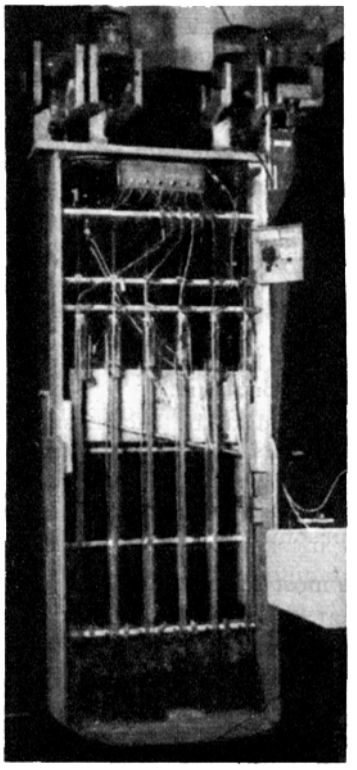

Fig. 9. Counter current apparatus for the extraction of penicillin (HEATLEY).

handled, so HEATLEY devised an extraction apparatus (Fig. 9) based on the counter current principle. Acidified metabolism liquor was allowed to fall in fine droplets through a column of amyl acetate which slowly ascended. During this the penicillin was transferred to the amyl acetate. This apparatus was somewhat temperamental but was of the greatest service. Later it was replaced by another built by Dr. SANDERS, the essential feature of which was the breaking of a thick emulsion formed by mixing amyl acetate and acidified brew by means of a Sharples centrifuge. Many commercial extraction plants were later built on the same general principles. In passing, one may note that the apparatus was set up in an autopsy room—a real case of substituting life for death.

Some of the essential chemical properties of penicillin relating to its stability are:—

Salts most stable between p_H 5 and 7.
Destroyed by acids and alkalis
boiling
oxidizing agents
heavy metal ions, e. g. Cu and Pb
primary alcohols and amines
enzymes produced by bacteria.

[1] P. W. CLUTTERBUCK, R. LOVELL and H. RAISTRICK, Biochem. J. *26*, 1907 (1932).

I would particularly like to call your attention to this last item, namely the influence of a bacterial enzyme on penicillin. This enzyme, which was first studied by ABRAHAM and CHAIN[1] (1940), is found in a large number of non-pathogenic bacteria, and is capable of destroying the antibacterial action of penicillin in an extremely short time. You can imagine the really great skill required in handling thousands of litres of culture fluid with complete sterility. If it were not for this enzyme, penicillin would probably be as easy to make as beer.

Some of the bacteriological properties which were elucidated were:

Penicillin activity was little affected by the number of bacteria.

Its efficiency was little impaired by pus, tissue autolysates, blood or serum.

It was considered to be bacteriostatic (but found later by others to be bactericidal to dividual organisms).

It was found that bacteria can develop resistance to the substance.

One of the most important bacteriological observations was that penicillin retained its activity in the presence of blood, serum, and, most important of all, pus. This activity in the presence of pus sharply differentiated penicillin from any of the sulphonamides known at that time. In addition, the action of penicillin was little affected by the number of bacteria present, again in contrast to the sulphonamides.

In addition to bacteria which may be naturally resistant to the action of penicillin it is possible, as GARDNER first showed, for bacteria to develop resistance *in vitro* by serial subculture in broth containing increasing amounts of penicillin. The resistance of a staphylococcus, which appears to be the organism most amenable to this treatment, has been raised as much as 5,000 times by serial passage. Such induced resistance is apparently not associated with the production of penicillinase. Its cause is unknown but clearly it may be of importance in the clinical use of the drug though few reports of resistance induced *in vivo* have appeared.

The most important biological observations we made were:

Penicillin was of low toxicity when given intravenously to mice and other animals; mice of 20 g. tolerating 10 mg. without symptoms.

It was innocuous on local application.

Leucocytes and tissue cultures were unaffected by solutions hundreds of times stronger than were required for bacterial inhibition.

It was excreted in the urine rapidly and in the bile.

It was absorbed from muscle, subcutaneous tissue and small intestine, but could not be given by stomach (because of acid) or by rectum (because of bacteria).

We can count it as an extremely fortunate circumstance that the many impurities which pass through the extraction processes are themselves of low toxicity, so that relatively impure material can be used for clinical purposes. Not only was toxicity low after a single intravenous injection but repeated subcutaneous injections over several days were without significant effect on the animal. It was shown also that quite strong solutions could be applied to the central nervous system.

It was further shown that substantial doses of penicillin had no effect on the blood-pressure or respiration in cats.

You will have followed how, as the result of this work, we knew that we had an extract which was stable under certain well defined conditions, was remarkably innocuous to animals, not only to the intact animal but to the various tissues of which it is composed, and that the animal could withstand a dose of the material thousands of times greater than that necessary to produce bacterial inhibition throughout the body. We had, in the early stage of these co-ordinated investigations, done a very small scale protection experiment on mice, using the streptococcus as the infecting organism. These tests had been quite promising so that we were not greatly surprised, when we came to do a full scale set of mouse protection experiments, that almost complete protection could be given against fatal infections with the streptococcus and, more important, the staphylococcus, by the subcutaneous injection at suitable intervals even of our crude preparations. In addition, it was shown that a fatal infection of mice with *Clostridium septique* could be controlled (CHAIN *et al.*[1], 1940). These were the crucial experiments from which we knew that penicillin belonged to a rare class of drug and was a true chemotherapeutic substance; and now you will understand why what did not seem worth while to FLEMING in 1930 became so much worth while in 1940 that the greatest efforts seemed called for to produce the drug. The first step was to make, by the methods we then had in use, sufficient penicillin to try on disease in man. This was accomplished and in the course of some ten months or so of arduous work we eventually produced enough in the laboratory to treat the first few cases and to show that our animal and other experiments had not misled us as to what might be expected in man

[1] E. P. ABRAHAM and E. CHAIN, Nature *146*, 837 (1940).

[1] E. CHAIN, H. W. FLOREY, A. D. GARDNER, N. G. HEATLEY, M. A. JENNINGS, J. ORR-EWING and A. G. SANDERS, Lancet *2*, 226 (1940).

(ABRAHAM[1] *et al.*, 1941). It is important to remember that the application to disease in man followed directly on the laboratory investigations, for it is still as true as ever it was that the really successful use of penicillin in the clinic demands a thorough knowledge of the chemical and biological properties ascertained in the laboratory.

The first patient was injected intravenously with 100 mg. of our first preparation. Relatively soon after this injection the patient had a rigor. This observation was repeated and it was clear that the material was pyrogenic. Fortunately it was found possible to separate the pyrogenic substance or substances from the penicillin by means of an alumina chromatographic column. In all material used in the clinic now this pyrogenic factor is removed.

Five of the first 6 patients treated had infections with staphylococcus or streptococcus which were considered hopeless because they had not been controlled by surgical and sulphonamide therapy. From this series of cases, it was clear that substantial doses of penicillin were not toxic to man, and there were very good indications that the most severe infections could be controlled by penicillin as long as the organism was sensitive.

While trying to interest the commercial firms in the new drug we continued to manufacture penicillin in the laboratory at Oxford and, with some material supplied by the Imperial Chemical Industries Ltd., and Kemball, Bishop & Co., we treated 15 more cases of serious illness—10 infected with staphylococci, 1 with a sulphonamide-resistant streptococcus, 3 with actinomyces and 1 with *Streptococcus viridans* (FLOREY and FLOREY[2], 1943). No case of severe staphylococcal infection died. In addition 172 infections of the eye, the mastoid process, chronic wound sinuses and miscellaneous local septic conditions were treated by applying penicillin locally to the infected part, again with most promising results (FLOREY and FLOREY[2], 1943).

In May 1943 we were able with the help of a number of Army surgeons and bacteriologists to carry out the first extensive trials on various types of war wounds in North Africa (FLOREY and CAIRNS[3], 1943). From this work it was soon clear that we had a most powerful weapon for controlling sepsis in war wounds, and this gave a great stimulus to large scale production.

By the middle of 1941 it had become clear that it would be extremely difficult to produce enough penicillin in England to be of more than scientific interest, so with the aid of the Rockefeller Foundation, HEATLEY and I visited the States. I will not chronicle our adventures there but I can say shortly some of the outstanding contributions which America has made since our visit. Firstly, at the Northern Regional Laboratory of the Bureau of Agriculture a very great improvement in the yield of penicillin per litre of culture fluid was obtained by COGHILL and his colleagues by utilizing a medium containing lactose and corn steep liquor—a product of maize. In addition it was at this Laboratory that excellent penicillin-producing strains were isolated and propagated and they were also responsible for initiating the deep culture method for producing penicillin—a method which it appears certain will displace all others. To these three major contributions can be ascribed the relative abundance of penicillin for it is not often remembered that when HEATLEY and I first visited the States it needed perhaps as much as 2,000 litres of metabolism fluid to furnish enough penicillin to treat 1 case by parenteral administration.

American clinicians and bacteriologists were soon able to confirm our findings at Oxford and with relatively abundant supplies of penicillin appearing first in America, then in England, there is now a vast accumulation of literature on its clinical application.

I can only touch now on certain aspects of clinical application.

Firstly, it is useless to expect good clinical results if the disease is caused by an insensitive organism. Here is the latest list of sensitive and insensitive organisms:

1. *Highly sensitive*

	Minimum and maximum inhibition reported: units per cc.
N. gonorrhoea	0·0018 — 0·176
β-haemolytic streptococcus	0·004 — 0·2
Str. pneumoniae	0·005 — 0·14
α-haemolytic streptococcus	0·005 upwards
Str. faecalis	often > 40
C. diphtheriae	0·004 — 1·5
Diphtheroid bacilli	0·005 — > 0·08
Staph. aureus and *albus*	0·005 upwards often 0·02—0·06, highest reported 1,000
Actinomyces bovis	0·005 — > 1·5
N. meningitidis	0·016 — 1·25
Clostridia	0·016 — 0·6
Erysipelothrix rhusiopathiae	0·02 — 0·16
Streptobacillus moniliformis	0·001 — 0·1
Trep. pallidum, *Trep. pertenue*, *Borrelia vincenti*, *F. fusiformis*, *Spirillum minus*	highly sensitive *in vivo*

[1] E. P. ABRAHAM, E. CHAIN, C. M. FLETCHER, H. W. FLOREY, A. D. GARDNER, N. G. HEATLEY and M. A. JENNINGS, Lancet, *2*, 177 (1941).

[2] M. E. FLOREY and H. W. FLOREY, Lancet *1*, 387 (1943).

[3] H. W. FLOREY and H. CAIRNS, Investigation of War Wounds. Penicillin. Preliminary report to the War Office and Medical Research Council. War Office (A.M.D. 7), 1943.

2. *Moderately sensitive*

	Minimum and maximum inhibition reported: units per cc.
Haem. ducreyi	0·13 — 0·25
Haem. influenzae	0·5 — > 5
Haem. pertussis	1·0
Br. abortus	0·125 — 16
Br. melitensis, *Br. suis*	0·25 — >16
Leptospira icterohaemorrhagiae	0·11
Listerella	0·7
B. anthracis	2·5
Trep. recurrentis, Viruses of ornithosis	Some effect *in vivo* from large doses.

3. *Resistant*

	Minimum and maximum inhibition reported: units per cc.
Salm. typhi	1 — 50
Salm. enteritidis Gärtner	3 — 20
Salm. paratyphi	3 — 400
Proteus	3 — 500
Shigella shigae	9 — 30
Shigella flexneri	9 — >100
Salm. schottmülleri	10 — 15
Bact. coli	15 — 300
Shigella sonnei	27 — >100
Salm. typhi-murium Aertrycke	30
Past. septica	30
Bact. aerogenes	30 — >100
Bact. friedländeri	95
Ps. pyocyanea	>60
Mycobact. tuberculosis	1,000 without effect
Past. pestis	without effect
Yeasts	,, ,,
Fungi	,, ,,
Protozoa	,, ,,
Viruses — majority	,, ,,

I would like to call your attention to the fact that there is considerable strain variation in sensitivity, for instance amongst the staphylococci some 5—10 per cent at least have been found in large series to be relatively insensitive. For this reason it is necessary in cases which do not respond to treatment as expected to investigate the sensitivity of the organism.

You will note among the insensitive organisms the bacterium of tuberculosis and also many of the Gram-negative organisms which produce pus. I need not spend time on emphasizing that diseases caused by these organisms do not respond to penicillin.

Mode of Action. It is necessary to consider here what is known of the mode of action of penicillin. FLEMING'S original description of the action of *Penicillium notatum* on staphylococci was that lysis occurred, but he later spoke of an action which was mainly bacteriostatic. At Oxford we thought that it was a bacteriostatic substance, since experiments done in the Warburg apparatus with 24 hour broth cultures of staphylococci showed that even strong concentrations of penicillin did not interfere with the respiration of the bacteria. Upon this evidence the conclusion was drawn that penicillin was a bacteriostatic and not a bactericidal agent. These experiments however, only revealed part of the truth. It was HOBBY, MEYER and CHAFFEE[1] (1942) working in America, who first discovered the condition under which penicillin is bactericidal. This condition is that the organisms should be multiplying actively. There is now much work both from America and England which fully substantiates the view that penicillin is bactericidal to dividing or "feeding" organisms but has no effect on organisms in the resting state. For some organisms penicillin is also bacteriolytic—the staphylococcus is the most notable example. Unfortunately penicillin does not appear able to effect complete sterilization of broth cultures, some living organisms are always left, a fact first pointed out by HOBBY, MEYER and CHAFFEE[1] and later brought into prominence by BIGGER[2] (1944) who gave the name "persisters" to these organisms. If the same thing happens in the body it may perhaps explain why relapse may occur unless treatment is continued for some time after the active infection has apparently been eliminated.

General Administration. You will remember that from the early animal experiments it was found that if penicillin was injected into a vein or intramuscularly or subcutaneously it would be absorbed into the bloodstream. It was rapidly excreted by the kidneys. It was rapidly destroyed in the stomach and rectum, though if these could be passed without loss, for instance by a duodenal tube, it was absorbed from the intestine. For such a rare drug as we were handling in the beginning injection methods were the obvious choice, at first intravenous either intermittent or continuous, replaced later by intramuscular injections at 3 or 4-hourly intervals. More recently continuous infusion into a muscle has come into use and has been found to be one of the most satisfactory methods.

The ordinary blood transfusion apparatus can be used to infuse 500 to 1,000 cc. in 24 hours into a suitable muscle such as the *rectus femoris*. In order to reduce the bulk of the fluid certain types of apparatus have been designed to give a much slower steady continuous flow, but the ideal has yet to be reached.

In all methods involving the slow administration of penicillin the solutions pass through rubber tubes.

[1] G. L. HOBBY, K. MEYER and E. CHAFFEE, Proc. Soc. exp. Biol. N. Y. *50*, 281 (1942).

[2] J. W. BIGGER, Lancet *2*, 497 (1944).

It has been found that some rubber tubing particularly if it is made of synthetic rubber, causes fairly rapid destruction of penicillin, though other samples do not do this. It is therefore necessary to select a suitable type of rubber tubing by careful tests.

Attempts in a different direction aim at lengthening the time for which a single injection is effective. The most successful so far appears to be that devised by ROMANSKY, MURPHY and RITTMAN[1] (1945), who emulsify the required dose of penicillin up to say 300,000 units of the calcium salt, with pea-nut oil containing up to 6 per cent of beeswax.

Another possibility which is being explored is that of keeping the penicillin in the body by blocking its exit from the kidneys. There is evidence that penicillin is excreted by the tubules of the kidney and it occurred to RAMMELKAMP and BRADLEY[2] (1943) to try to interfere with this excretion by giving a substance also excreted by the tubules which would compete with the penicillin for passage through the tubule cells. They used diodrast and obtained evidence that more penicillin was retained in the body when diodrast was given. BEYER[3] and his colleagues (1945) were able to produce a similar effect with para-amino-hippuric acid. These latter results have been to a large extent obtained on animals and the method while interesting, does not seem likely to be used in the clinic.

Now that penicillin is more plentiful there is a revival of interest in giving it by mouth. Up to the present no entirely satisfactory method has been described. Attempts are being made to pass penicillin through the stomach in various vehicles such as inorganic buffers, egg, oils, pea-nut oil, beeswax mixture and in capsules of various kinds. Some workers consider water to be as good a vehicle as oil or gelatine capsules. Aluminium hydroxide which adsorbs penicillin and slowly liberates it has been said to possess advantages (WELCH[4] *et al.*, 1945). Nearly all observers agree that considerably more penicillin—about 4 times as much—is needed for oral administration in comparison with injection methods, and again most observers record relatively low blood-levels even after large doses.

Dose. I think penicillin is unique in being the only drug which can be used without fear of toxic symptoms being produced, so that one is not looking for the maximum amount of the drug which can be tolerated before toxic symptoms set in, but at least while material is scarce, for the minimum effective dose. From the outset of the clinical work it was considered necessary to give injections sufficiently frequent so that an amount of penicillin was always present in the blood-stream capable of completely inhibiting a sensitive staphylococcus, and this principle still holds good. Our earliest curves showed that in most people after the intravenous or intramuscular injection of 15,000 units penicillin could be detected in the blood for from 2½ to 3 hours. We therefore arrived at a standard basic dose for an adult of 15,000 units every 3 hours. With that dosage we felt that for no substantial time during the treatment would the penicillin level in the blood-stream fall below that necessary for complete inhibition of the growth of the organism. For a considerable portion of the time it would be well above that level.

Independent work in America brought them also to a similar conclusion, that the standard basic dose was between 15 and 20,000 units every 3 to 4 hours. Figures 10, 11 and 12 show some of the penicillin levels obtained after various doses and administration in various ways.

It is certain that by giving much larger doses than those that have been usual hitherto, higher levels can be reached in the serum. This may bring into therapeutic range diseases caused by some relatively in-

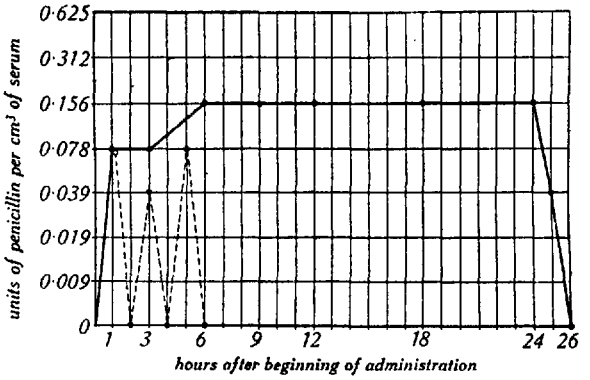

Fig. 10 (HIRSCH and DOWLING[1], 1945) compares in a semi-diagrammatic way the median blood-levels from a similar total dose given i. m. intermittently and continuously into the muscles.

Continuous intramuscular drip using 200,000 units penicillin in 24 hours.

o Intermittent intramuscular injection using 20,000 units penicillin every two hours.

sensitive strains of bacteria, but such very large doses will not be practicable for general use till penicillin is very much more plentiful.

Though, as I have said, the treatment of most medical patients is fairly straightforward, such is not the case with surgical patients. Here a great deal depends on the skill of the surgeon and the intelligence with which he uses a drug capable of controlling serious

[1] M. J. ROMANSKY, R. J. MURPHY and G. E. RITTMAN, J. Amer. med. Ass. *128*, 404 (1945).

[2] C. H. RAMMELKAMP and S. E. BRADLEY, Proc. Soc. exp. Biol. N. Y. *53*, 30 (1943).

[3] K. H. BEYER, W. F. VERWEY, R. WOODWARD, L. PETERS and P. A. MATTIS, Amer. J. med. Sci. *209*, 608 (1945).

[4] H. WELCH, C. W. PRICE, V. L. CHANDLER, J. Amer. med. Ass. *128*, 845 (1945).

[1] H. L. HIRSCH and H. F. DOWLING, Amer. J. med. Sci. *210*, 435 (1945).

sepsis. All one can say in general terms is that with the aid of penicillin the surgeon can safely undertake the manipulation, excision or removal of tissues infected with pyogenic cocci without fear of subsequent local spread or general invasion of the blood-stream.

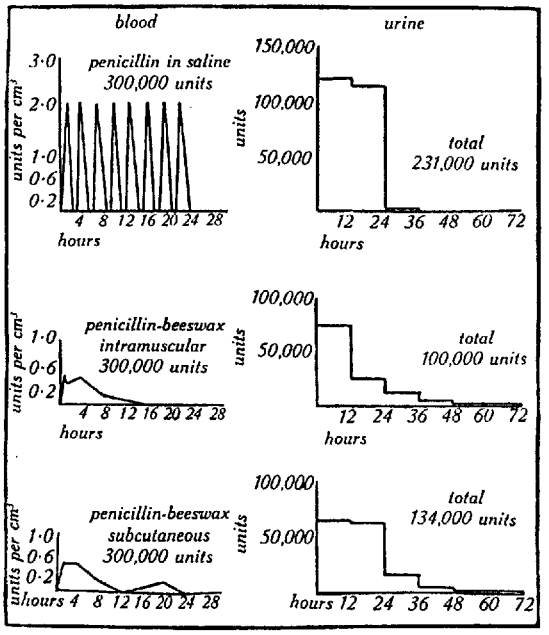

Fig. 11 (KIRBY[1] *et al.*, 1945) compares the blood-levels and urinary excretion of the same total amount (300,000 units) given in eight divided doses in saline or in a single dose in a beeswax-peanut oil base, intramuscularly or subcutaneously.

Local treatment. Though for many purposes penicillin can only be used by parenteral administration for the obvious reason that it is impossible to reach the diseased parts except by way of the blood-stream, there is a big field for treatment by local application. The local application of penicillin calls for more ingenuity and skill than generalized use, but besides its advantage in economy of material it often repays the trouble by the intimacy with which high concentrations can be brought into contact with infected parts. In certain positions it is to be preferred, for instance penicillin does not pass freely through the serous membranes so that infections in the pleural or cerebrospinal space or other serous cavities are by preference treated by local injection, supplemented by systemic treatment if there is a spread outside the space. Similarly, penicillin does not penetrate freely into the eyeball.

Again, in skin diseases local application has a very wide field, and recently good results have been reported from the use of oral pastilles in mouth and throat infections by VINCENT's organisms or the streptococcus.

Results. Though the saving of life by penicillin is very dramatic, it concerns relatively few cases. Among the chief benefits penicillin confers is the reduction in the duration of many non-fatal illnesses and the improvement of function consequent on the early elimination of sepsis, which so often causes the formation of excessive amounts of scar-tissue. One may perhaps illustrate this simply by a comparative series of infected hands treated locally only by M. E. FLOREY and WILLIAMS[1] (1944). They showed in 35 cases treated by penicillin the saving of 1,000 working days in comparison with 35 similar cases treated by orthodox methods. The effect can probably be attributed to two factors, firstly the supression of infection reduces fibrosis and tissue reactions to a minimum and secondly in the absence of infection healing is more rapid. In the same way, on a greater scale, the elimination of sepsis from war wounds saved many men from serious and lasting disabilities.

It must be stressed that the elimination of infecting bacteria by penicillin confers no immunity on the patient. If he has an open wound or burn it can easily be reinfected when penicillin treatment is stopped, or infected with Gram-negative organisms even during penicillin application, so that the strictest asepsis is required in dressing wounds. One of the main advantages of the early suture made possible by penicillin is that it diminishes the chance of secondary infection.

Causes of Failure. Some cases fail to respond and these failures can usually be attributed to lack of attention to one or more of the following points: —

1. Dead tissue such as slough or sequestrum is present and is forming a focus of infection. Such tissue

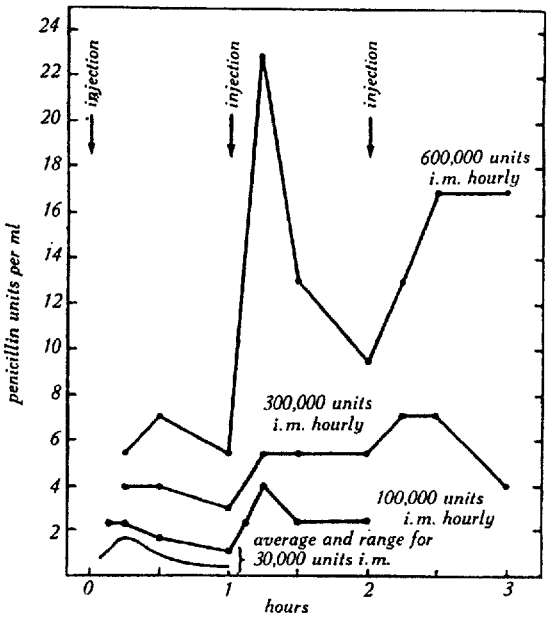

Fig. 12 (LOURIE[2] *et al.*, 1945) shows the high blood-levels which can be reached if very large doses, up to 600,000 units, are given hourly.

[1] W. M. M. KIRBY, W. LEIFER, S. P. MARTIN, C. H. RAMMELKAMP and J. M. KINSMAN, J. Amer. med. Ass. *129*, 940 (1945).

[1] M. E. FLOREY and R. E. O. WILLIAMS, Lancet *1*, 73 (1944).

[2] E. M. LOURIE, H. O. J. COLLIER, A. O. F. ROSS, D. T. ROBINSON and R. B. NELSON, Lancet *2*, 696 (1945).

should have been removed at the beginning of treatment or in any case as early as possible.

2. An infected area is not being reached by the drug. In systemic treatment a large or thick-walled abscess, or an infected serous cavity will form such an area; good surgical access and drainage followed by local injection of the drug will be called for. In a local infection there may be unsuspected sinuses or other extensions of infected tissue, and these are an indication for further surgical intervention to enable the drug to reach every infected piece of tissue.

3. Treatment is not carried on long enough.

4. The dose is too small or application too infrequent.

5. The principal bacteria causing the infection are not sensitive to penicillin.

6. The preparation of penicillin has lost potency and no longer contains the stated number of units. Whether this is so can only be ascertained by a fresh assay carried out by someone experienced in assay work.

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Prof. FLOREY, der gemeinsam mit Dr. E. CHAIN und Prof. A. FLEMING für seine Arbeiten über Penicillin mit dem Nobelpreis für Medizin 1945 ausgezeichnet wurde, hielt Ende Februar dieses Jahres in verschiedenen Schweizer Städten den hier veröffentlichten Vortrag, welchen er uns freundlicherweise zur Publikation zur Verfügung gestellt hat. Er schildert darin besonders die Arbeiten der Forschergruppe, die unter seiner Leitung stand, und zeigt in überaus anschaulicher Weise den weiten Weg, der zurückgelegt werden mußte, um von der einfachen Feststellung der antibakteriellen Wirkung von *Penicillium notatum* durch FLEMING zum heutigen Chemotherapeutikum von überragender Bedeutung, dem Penicillin zu gelangen. *Die Redaktion*

Der chemische Aufbau des Holzes

Von A. v. WACEK, Wien[1]

Das Holz wurde ursprünglich für ein einheitliches chemisches Individuum gehalten. Mit der Entwicklung der organisch-chemischen experimentellen Technik gelang es aber schon vor etwas über hundert Jahren, einzelne Konstituenten zu isolieren, und seit der Mitte des vorigen Jahrhunderts hat sich die heute noch übliche Anschauung allgemein eingeführt, wonach das Holz aus drei Hauptbestandteilen, der Zellulose, den Hemizellulosen und dem Lignin besteht.

Mit dem Fortschreiten der Methodik und der konstitutionschemischen Aufklärung erkannte man bald, daß die isolierten Bestandteile in qualitativer wie in quantitativer Hinsicht je nach dem Gewinnungsverfahren verschieden ausfielen. Man hat sehr viel Mühe darauf verwendet, die *begrifflich* definierten Konstituenten auch in Substanz in ideal reinem Zustand zu isolieren und quantitativ zu bestimmen, wobei man diese Forderung im Sinne der klassischen organischen Chemie erfüllt sehen wollte. Dieses Ziel ist nicht erreicht worden und ist wohl auch unerreichbar, wenn man ihm auch, wenigstens für die Zellulose, durch Verfeinerung der Arbeitsweisen in den letzten Jahren recht nahegekommen ist. Die einzelnen Holzbestandteile sind makromolekulare Gebilde mit beschränkten Löslichkeitseigenschaften; eine Trennung in der Art, daß man die einzelnen Anteile unverändert und quantitativ wiederbekommt, ist undurchführbar. Da sie auch gegenüber chemischen Umsetzungen, ganz besonders solange der Holzverband noch intakt ist, großenteils recht resistent sind, muß man immer einen Teil der Holzsubstanz zerstören, um andere Anteile freilegen und isolieren zu können. Dabei ist ein gewisser Angriff auf alle Anteile nicht zu vermeiden, und es ist klar, daß sich die isolierten Komponenten von den nativen und auch untereinander mehr oder weniger unterscheiden werden. Das soll allerdings nicht dazu führen, daß jede Variante als eigenes Holzbauelement betrachtet wird — denn dann müßte man unzählige Zellulosen und Lignine annehmen —, oder im Extremfall, wie es in den letzten Jahren mehrfach geschah[1], dazu, die Existenz eines dem isolierten Lignin mindestens sehr ähnlichen Körpers im Holz überhaupt anzuzweifeln und dieses *nur* als Kunstprodukt chemischer Isoliermethoden zu betrachten. Gerade an diesem Widerstreit der Meinungen ist zu erkennen, daß eine klare, eindeutige und den heutigen Ergebnissen angepaßte Definition dessen, was beim Holz als Zellulose, als Hemizellulose und als Lignin zu bezeichnen ist, unumgänglich notwendig wäre, denn viele Divergenzen beruhen darauf, daß aneinander vorbeigeredet wurde.

Kann nun auf Grundlage der experimentell sichergestellten Tatsachen eine solche Definition, die voraussichtlich auch einer künftigen Entwicklung standhalten wird, gegeben werden, oder entspricht die übliche Unterscheidung der drei Hauptbestandteile nicht mehr und sollte sie durch eine andere ersetzt werden? Dazu ist zu sagen, daß sich die bisherige begriffliche Unterteilung als durchaus berechtigt erwiesen, sich besonders bei unzähligen technisch-analytischen Methoden bestens bewährt hat und auch so eingebürgert ist, daß ein Abweichen davon unpraktisch wäre. Allerdings muß man sich dabei klar sein, daß als Kriterium für die Reinheit eines Holzanteils — bzw.

[1] I. Chem. Univ.-Laboratorium der Universität Wien, Organische Abteilung und Abteilung für Chemie des Holzes.

[1] Arbeiten von R. S. HILPERT und Mitarbeitern, bes. Berichte *68*, 16, 380 (1935). — F. SCHÜTZ und P. SARTEN, Cellulosechemie *21*, 35—48 (1943); *22*, 1, 114 (1944).