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Penicillin

Nobel Lecture, December 11, 1945

I am going to tell you about the early days of penicillin, for this is the part of the penicillin story which earned me a Nobel Award. I have been frequently asked why I invented the name "Penicillin". I simply followed perfectly orthodox lines and coined a word which explained that the substance penicillin was derived from a plant of the genus *Penicillium* just as many years ago the word "Digitalin" was invented for a substance derived from the plant *Digitalis*. To my generation of bacteriologists the inhibition of one microbe by another was commonplace. We were all taught about these inhibitions and indeed it is seldom that an observant clinical bacteriologist can pass a week without seeing in the course of his ordinary work very definite instances of bacterial antagonism.

It seems likely that this fact that bacterial antagonisms were so common and well-known hindered rather than helped the initiation of the study of antibiotics as we know it today.

Certainly the older work on antagonism had no influence on the beginning of penicillin. It arose simply from a fortunate occurrence which happened when I was working on a purely academic bacteriological problem which had nothing to do with antagonism, or moulds, or antiseptics, or antibiotics.

In my first publication I might have claimed that I had come to the conclusion, as a result of serious study of the literature and deep thought, that valuable antibacterial substances were made by moulds and that I set out to investigate the problem. That would have been untrue and I preferred to tell the truth that penicillin started as a chance observation. My only merit is that I did not neglect the observation and that I pursued the subject as a bacteriologist. My publication in 1929 was the starting-point of the work of others who developed penicillin especially in the chemical field.

Penicillin was not the first antibiotic I happened to discover. In 1922, I described lysozyme - a powerful antibacterial ferment which had a most extraordinary lytic effect on some bacteria. A thick milky suspension of bacteria could be completely cleared in a few seconds by a fraction of a drop of

human tears or egg white. Or if lysozyme-containing material was incorporated in agar filling a ditch cut in an agar plate, and then different microbes were streaked across the plate up to the ditch, it was seen that the growth of some of them would cease at a considerable distance from the gutter.

But unfortunately the microbes which were most strongly acted on by lysozyme were those which do not infect man. My work on lysozyme was continued and later the chemical nature and mode of action was worked out

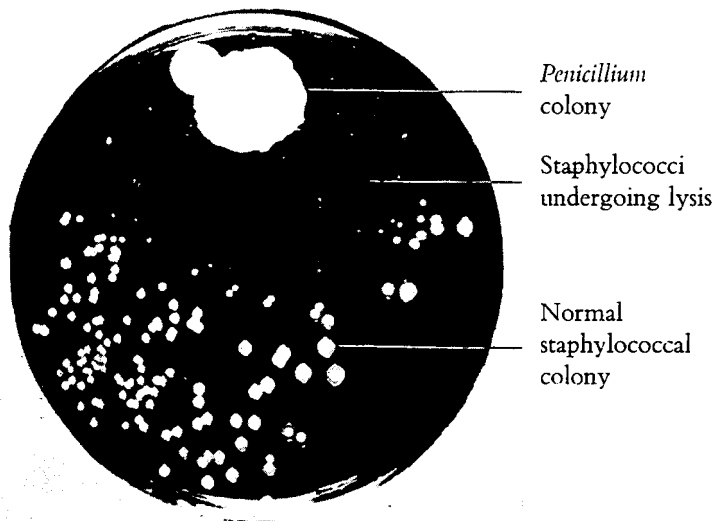


Fig. 1. Photograph of a culture-plate showing the dissolution of staphylococcal colonies in the neighbourhood of a *Penicillium* colony.

by my collaborators in this Nobel Award - Sir Howard Florey and Dr. Chain. Although lysozyme has not appeared prominently in practical therapeutics it was of great use to me as much the same technique which I had developed for lysozyme was applicable when penicillin appeared in 1928.

The origin of penicillin was the contamination of a culture plate of staphylococci by a mould. It was noticed that for some distance around the mould colony the staphylococcal colonies had become translucent and evidently lysis was going on. This was an extraordinary appearance (Fig. 1) and seemed to demand investigation, so the mould was isolated in pure culture and some of its properties were determined.

The mould was found to belong to the genus *Penicillium* and it was even-

tually identified as *Penicillium notatum*, a member of the *P. chrysogenum* group, which had originally been isolated by Westling from decaying hyssop.

Having got the mould in pure culture I planted it on another culture plate and after it had grown at room temperature for 4 or 5 days I streaked different microbes radially across the plate. Some of them grew right up to the

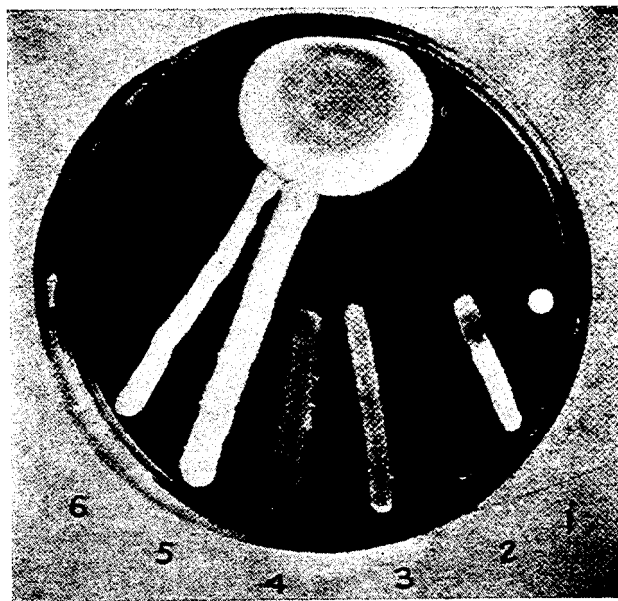


Fig. 2. Different bacteria streaked radially to a four-day-old colony of *Penicillium notatum* on agar.

The bacteria are: (1) *Staphylococcus*; (2) *Streptococcus* (haemolytic); (3) *B. diphtheriae*; (4) *B. anthracis*; (5) *B. typhosus*; (6) *B. coli*.

mould - others were inhibited for a distance of several centimetres. This showed that the mould produced an antibacterial substance which affected some microbes and not others (Fig. 2).

In the same way I tested certain other types of mould but they did not produce this antibacterial substance, which showed that the mould I had isolated was a very exceptional one.

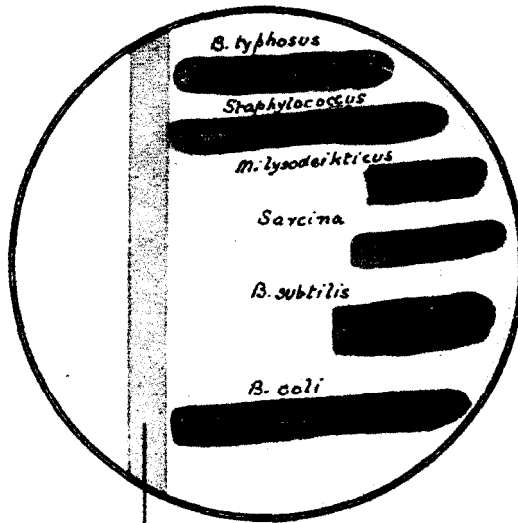
Then the mould was grown on fluid medium to see whether the antiseptic substance occurred in the fluid. After some days the fluid on which the mould had grown was tested in the same way that I have already figured for

Penicillin



Penicillin embedded in agar:

Lysozyme



Lysozyme embedded in agar.

Fig. 3. Differential inhibition of bacteria by penicillin and lysozyme embedded in a gutter in an agar plate.

Streak of mixture of *Staphylococcus*
and *B. violaceus*.

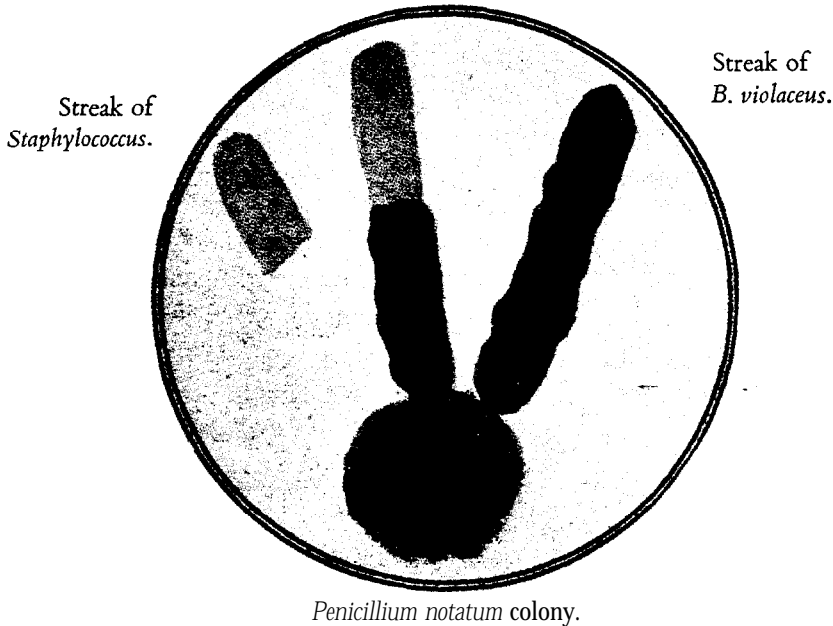


Fig. 4. Effect of penicillin on the mixture of *Staphylococcus* and *B. violaceus*.

lysozyme - by placing it in a gutter in a culture plate and then streaking different microbes across the plate. The result shown in Fig. 3 is very similar to that observed with lysozyme with one very important difference, namely that the microbes which were most powerfully inhibited were some of those responsible for our most common infections.

This was a most important difference.

By this method and by the method of serial dilution I tested the sensitivity of many of the common microbes which infect us and found exactly what is illustrated in Fig. 2 - that many of the common human pathogens were strongly inhibited while many others were unaffected.

This led us to our first practical use of penicillin, namely in the preparation of differential culture medium. There was such a sharp distinction between the sensitive and insensitive microbes that by adding penicillin to the culture medium all the sensitive microbes were inhibited while all the insensitive microbes grew out without hindrance. This made it very easy to isolate microbes like the whooping-cough bacillus and Pfeiffer's influenza bacillus

which are normally found in the respiratory tract in association with large numbers of cocci which are sensitive to penicillin.

In those early days also I used penicillin to show up bacterial antagonisms in a dramatic manner and I combined this with the use of a method which I had developed for growing chromogenic bacteria. If a disc of paper is laid on agar in a culture plate the nutrient material diffuses into the paper and supports the growth of bacteria planted on the surface. If these bacteria are chromogenic such as *Staphylococcus aureus*, *B. prodigiosus* or *B. violaceus* they develop their colours beautifully on the white paper.

Fig. 4 shows the result obtained when mixtures of *Staphylococcus aureus* and *B. violaceus* are planted on such a paper disc on which *Penicillium notatum* has been grown for four days. The mould has made penicillin which has diffused out for a considerable distance and inhibited the staphylococcus. The staphylococcus beyond the reach of the penicillin has completely inhibited the *B. violaceus* which being insensitive to penicillin grows out luxuriantly as soon as the staphylococcus is inhibited by the penicillin.

Table 1. Sensitivity of bacteria to penicillin.

<i>Sensitive</i>	<i>Insensitive</i>
<i>Staphylococcus aureus</i>	<i>Enterococcus</i>
<i>Staphylococcus epidermis</i>	Non-pathogenic gram-negative cocci found in the mouth
<i>Streptococcus</i> (haemolytic)	
<i>Streptococcus</i> (viridans)	<i>B. pyocyaneus</i>
<i>Pneumococcus</i>	<i>B. proteus</i>
<i>Gonococcus</i>	<i>B. friedländeri</i>
<i>Meningococcus</i>	<i>B. coli</i>
<i>M. catarrhalis</i>	<i>B. typhosus</i>
Diphtheria group	<i>B. paratyphosus</i>
<i>B. anthracis</i>	<i>B. dysenteriae</i>
Air-borne micrococci	<i>Vibrio cholerae</i>
<i>Sarcina</i>	<i>Pasteurella</i>
<i>Actinomyces</i>	<i>Brucella abortus</i> and <i>melitensis</i>
<i>B. welchii</i>	<i>B. tuberculosis</i>
<i>Vibrion septique</i>	
<i>B. oedamatiens</i>	
<i>B. tetani</i>	
<i>Spirochaetes</i>	

N.B. Those below the line have been added since my original paper in 1929.



Fig. 5. Comparison of diffusibility of penicillin and some other antiseptics. Discs of blotting paper soaked in antiseptic imbedded in agar plate inoculated with *Staphylococcus*.

The same paper culture method has enabled me to prepare excellent permanent specimens of *Penicillium notatum* and other mould cultures. The mould is grown on the paper disc on the surface of a suitable culture medium. When the colony has developed the paper disc is removed, sterilized in formalin vapour and then mounted. I would like, Mr. Rector, to present you with such a culture.

But to return to the properties of penicillin. We had established its specificity. We found that it was of such strength that the culture fluid could be diluted 1,000 times and it would still inhibit the growth of staphylococci. In this connection it is well to remember that phenol loses its inhibitory power when it is diluted more than 300 times. So that in this respect the crude culture fluid on which the mould had grown was three times as potent as phenol.

Then as to its action on the microbe. All the experiments I have cited showed that it was bacteriostatic, that is, it inhibits the growth of microbes. But I showed also that it was bactericidal - that it actually killed them. Then the very first observation of penicillin showed that it induced lytic changes in the bacteria. Thus it was bacteriostatic, bactericidal, and bacteriolytic - properties which have since been shown to be possessed by the purified penicillin.

The first observations on penicillin to which I have alluded showed that penicillin is freely diffusible in agar. In this it differs from the older antiseptics. This is brought out in a striking manner in the following experiment.

With a cork borer, discs are cut out of an agar culture plate. Discs of filter paper soaked in antiseptics are placed at the bottom of the holes thus formed and the holes are then filled with melted agar. The surface is then planted with staphylococci. On incubation the staphylococcus grows over all the older antiseptics but is inhibited through a considerable distance by the penicillin, thus showing that penicillin is the only one of these substances which is freely diffusible (Fig. 5). I consider this diffusibility an important property in any substance for use as an antibacterial agent inside the body.

I had since the war of 1914-1918 been interested in antiseptics and in 1924 I described what I think is probably the best experiment I ever did. This showed up in a dramatic fashion the relative activity of a chemical on bacteria and on human leucocytes.

Normal human blood has a strong bactericidal power on the ordinary cocci, e.g. staphylococci and streptococci, but this power is completely lost if the leucocytes are removed from the blood. If defibrinated blood is infected with a small number of staphylococci (say 4,000 per cc.) and incubated in a capillary space - a slide cell or a capillary tube - the cocci which survive grow out into colonies which can easily be enumerated. But only about 5 per cent grow out. If however, phenol is added to B concentration of 1 in 600 all the cocci grow out freely. Here the phenol in a concentration which does not interfere with bacterial growth has destroyed the leucocytes which constitute one of our most powerful defences against infection (see Fig. 6).

I had tested all the chemicals which were used as antibacterial agents and they all behaved in the same way - in some concentration they destroyed leucocytes and allowed bacteria to grow. When I tested penicillin in the same way on staphylococcus it was quite a different story. The crude penicillin would completely inhibit the growth of staphylococci in a dilution of up to

1 in 1,000 when tested in human blood but it had no more toxic effect on the leucocytes than the original culture medium in which the mould had been grown. I also injected it into animals and it had apparently no toxicity. It was the first substance I had ever tested which was more antibacterial than it was antileucocytic and it was this especially which convinced me that some day when it could be concentrated and rendered more stable it would be used for the treatment of infections.

Had I been an active clinician I would doubtless have used it more extensively than I did therapeutically. As it was, when I had some active penicillin I had great difficulty in finding a suitable patient for its trial, and owing to its instability there was generally no supply of penicillin if a suitable case turned up. A few tentative trials gave favourable results but nothing miraculous and I was convinced that before it could be used extensively it would have to be concentrated and some of the crude culture fluid removed.

We tried to concentrate penicillin but we discovered as others have done since that penicillin is easily destroyed, and to all intents and purposes we failed. We were bacteriologists - not chemists - and our relatively simple

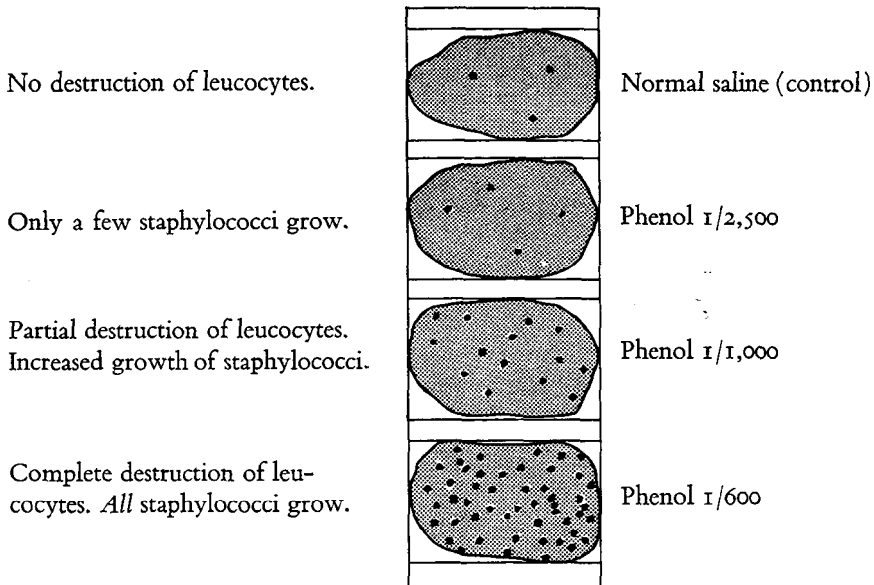


Fig. 6. Experiment illustrating the greater toxicity of phenol to leucocytes than to bacteria. (Each cell contains human blood + 50 staphylococci.)

procedures were unavailing, which is not surprising in view of the trouble which the chemists have had with penicillin in recent years.

However, I preserved the culture of the mould and used penicillin habitually for differential culture.

In 1929, I published the results which I have briefly given to you and suggested that it would be useful for the treatment of infections with sensitive microbes. I referred again to penicillin in one or two publications up to 1936 but few people paid any attention. It was only when some 10 years later after the introduction of *sulphonamide* had completely changed the medical mind in regard to chemotherapy of bacterial infections, and after Dubos had shown that a powerful antibacterial agent, *gramicidin*, was produced by certain bacteria that my co-participators in this Nobel Award, Dr. Chain and Sir Howard Florey, took up the investigation. They obtained my strain of *Penicillium notatum* and succeeded in concentrating penicillin with the result that now we have concentrated penicillin which is active beyond the wildest dreams I could possibly have had in those early days.

Their results were first published in 1940 in the midst of a great war when ordinary economics are in abeyance and when production can go on regardless of cost. I had the opportunity this summer of seeing in America some of the large penicillin factories which have been erected at enormous cost and in which the mould was growing in large tanks aerated and violently agitated. To me it was of especial interest to see how a simple observation made in a hospital bacteriological laboratory in London had eventually developed into a large industry and how what everyone at one time thought was merely one of my toys had by purification become the nearest approach to the ideal substance for curing many of our common infections.

And we are not at the end of the penicillin story. Perhaps we are only just at the beginning. We are in a chemical age and penicillin may be changed by the chemists so that all its disadvantages may be removed and a newer and a better derivative may be produced.

Then the phenomenal success of penicillin has led to an intensive research into antibacterial products produced by moulds and other lowly members of the vegetable kingdom. Many substances have been found but unfortunately most of them have been toxic. There is one, however, *streptomycin*, which was found by Waksman in America which will certainly appear in practical therapeutics and there are many others yet to be investigated.

But I would like to sound one note of warning. Penicillin is to all intents and purposes non-poisonous so there is no need to worry about giving an

overdose and poisoning the patient. There may be a danger, though, in underdosage. It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body.

The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant. Here is a hypothetical illustration. Mr. X. has a sore throat. He buys some penicillin and gives himself, not enough to kill the streptococci but enough to educate them to resist penicillin. He then infects his wife. Mrs. X gets pneumonia and is treated with penicillin. As the streptococci are now resistant to penicillin the treatment fails. Mrs. X dies. Who is primarily responsible for Mrs. X's death? Why Mr. X whose negligent use of penicillin changed the nature of the microbe. *Moral:* If you use penicillin, use enough.

I have told you of the beginnings of penicillin. How a mould which was not wanted, contaminated one of my culture plates. How it produced an effect which demanded investigation. How I investigated its properties and found that while it had a powerful effect on many of the common microbes which infect us it was apparently quite non-poisonous to animals or to human blood cells. How it was an unstable substance and how we failed to concentrate and stabilize it.

I will now leave Sir Howard Florey to continue the story of penicillin.