Thirty Years of Penicillin Therapy

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Three decades have passed since the publication of the paper by our group at Oxford in which it was, for the first time, reported that the mould metabolite penicillin exhibited remarkable chemotherapeutic effects in clinical bacterial infections, including those caused by *Staphylococcus aureus*, against which no member of the only then known group of antibacterials possessing *in vivo* chemotherapeutic activity, the sulphonamides, was fully effective (Abraham *et al.*, 1941). A year earlier we had published our first paper on the chemotherapeutic power of penicillin in experimental bacterial infections in mice, which was dramatic and of unprecedented magnitude (Chain *et al.*, 1940).

The introduction into clinical medicine of penicillin and of the antibiotics stemming from it has completely revolutionised the treatment of bacterial infections in both man and animals, and rendered the large majority of them, including the most severe ones, amenable to successful therapeutic control.

The thirty years—conventionally accepted as the life span of a generation—that have elapsed since the introduction of penicillin into clinical medicine would seem an appropriate occasion to mark this event—and it is, I believe, an event well worth marking. This is the purpose of this symposium, gratifyingly organised under the joint auspices of the two leading professional scientific and medical organisations of this country, the Royal Society and the Royal College of Physicians, and, in itself, representing an historic event; for, as far as I am aware, no joint function of this kind has ever been held in the long history of these distinguished bodies.

The task of presenting the historical survey has fallen to me since I, jointly with the late Lord Florey, was responsible for the planning and initiation of the research that led to the discovery of the curative properties of penicillin and, until the present day, have been closely associated with the scientific developments in the penicillin field. It is a matter of general regret and sorrow that Lord Florey passed away suddenly and unexpectedly and is not with us for this meeting.

The development of penicillin and antibiotic therapy occurred in four well-defined phases. The first, which led to the discovery of the chemotherapeutic

power of penicillin and thus laid the foundation for the subsequent development of the antibiotic therapy, was based on a biochemical approach. The second, which started independently about the same time as the first and led to the discovery of streptomycin, came from soil microbiological studies. The third phase, strongly stimulated by the first two, was concerned with the systematic screening of micro-organisms for antibiotics; it led to the discovery of most of the clinically used antibiotics. The fourth phase, which led to the discovery of the semi-synthetic penicillins, is characterised by a combination of the biochemical and organic-chemical approach. We now find ourselves actively engaged in what might be called the fifth and ultimate phase of this development which centres on the study of antiviral substances of microbial origin. This is, again, based mainly on the biochemical approach.

Both university and industrial laboratories have made important contributions to the development of the antibiotics field. In fact, without the participation of both kinds of laboratories with their mutually complementary methods and, in some cases, without their close collaboration, the whole development could not have taken place so rapidly and so successfully as it did.

The history of the first phases of the discovery of penicillin and its curative power is surrounded by the mist of more or less romantic legends that make good material for the daily press and, I regret to say, have also appeared quite frequently in the medical press, but are as far removed as can be from the reality. Yet the reality is by no means less interesting than the legends. It is a sequence of simple, sober and logical events that are readily understandable, familiar and obvious to all those used to the way of thinking and the laboratory methods of the chemist and biochemist. As in all scientific discoveries, that of penicillin and its curative properties is based on building stones laid by others, and, as in all scientific discoveries, luck has played a very important role.

Since the beginning of my scientific career, I have been interested in the study of biological phenomena that could be explained in terms of the action of well-defined chemical substances, and in the study of the structure and mode of action of these substances. The action of toxins, the phenomenon of bacterial lysis, growth promotion and inhibition, virulence, regulation of metabolic reactions by hormones come into this category. After more than forty years of professional activity this is essentially still my personal approach to biochemical research.

As long as the activity of these substances manifested itself in a clear-cut physiological or pathological effect, amenable to quantitative measurements, the substances attracted my interest, and difficulties in their isolation, whether due to chemical instability, or their presence in the biological starting material in minute amounts only, did not deter me, nor did it matter to me whether

the active substance crystallised readily, or was of low or high molecular weight.

In 1935, a few months after his appointment to the Chair of Pathology at Oxford, I was invited by Professor H. W. Florey to join his staff at the Sir William Dunn School of Pathology. Though Florey had no specific biochemical training, he was very conscious of the importance of biochemistry to progress in all the biological sciences, and particularly his own subject, experimental pathology, and he felt that a department such as the one he intended to build up could not be fully successful without biochemical support. For this reason he had approached Sir Frederick Gowland Hopkins, the head of the Sir William Dunn School of Biochemistry at Cambridge, then one of the leading world centres of biochemical research, to advise him on the appointment of a suitable biochemist who would be able to build up a biochemical section in his new department at Oxford. Hoppy, as he was affectionately called by the members of his staff—and he was one of the most considerate and kindest human beings I ever had the good fortune to meet—very kindly suggested my name as a possible candidate. I was then a refugee from Hitler's Germany. I had left my native town, Berlin, on that fateful day, 30th January 1933, when Hitler acceded to power and Europe was temporarily plunged into a darkness in comparison to which the darkest Middle Ages now appear as a blaze of light. After a short interlude in London at University College, I had the great good fortune to be accepted by Hopkins in his department as a research worker, largely through the good offices of J. B. S. Haldane, and spent two very happy years there. One of my main scientific interests at that time was the study of the biochemical mode of action of neurotoxic snake venoms. I had found at Cambridge that some of the most potent neurotoxic snake venoms had the property of inhibiting glycolysis and alcoholic fermentation when added in very small amounts to cell-free extracts (Chain, 1937). Continuation of this work at Oxford showed that the active principle in these snake venoms was an enzyme of nucleotidase nature, which exerted its inhibitory action on glycolysis and alcoholic fermentation by destroying the essential co-enzyme of these processes, nicotinamide adenine dinucleotide, through hydrolysing of the dinucleotide into two mononucleotides (Chain, 1939). Thus, for the first time, the mode of action of a natural toxin of protein nature could be explained in biochemical terms as that of an enzyme acting on a component of vital importance in the respiratory chain.

When Hopkins asked me whether I would like to go to Oxford to join Florey's staff, I was both extremely surprised and delighted, for I never expected such exceptionally good fortune to come my way in my unsettled

condition, with a very uncertain future in front of me. He introduced me to Florey in his office immediately after our talk and I naturally accepted the offer without any hesitation. Thus, I migrated from one Sir William Dunn School to another.

When Florey and I first discussed the future possible research programme of the biochemical section in his department which I was to organise, he drew my attention to a very striking lytic phenomenon in which he himself had been interested for some years (Goldsworth and Florey, 1930). In 1924, Alexander Fleming, working at St Mary's Hospital, London, had made the observation that tears, nasal secretion and egg-white contained a substance capable of dissolving thick suspensions of a saprophytic bacterium which he had isolated from the air. The bacterium was termed by Fleming *Micrococcus lysodeicticus*. The active lytic substance had obvious enzymic properties, but the substrate on which it acted in the bacterial cell was not known, and Florey suggested to me that it would be interesting to attempt to isolate and characterise this substrate, if indeed lysozyme was an enzyme. The reason why Florey was interested in lysozyme was not so much its antibacterial power, which was of a very limited range, but the fact that, in addition to the sources I have just mentioned, it also occurred in duodenal secretions, and Florey thought at that time that it might play a role in the mechanisms of natural immunity and, in particular, could be involved in the pathogenesis of duodenal ulcers.

The study of the biochemical mode of action of a powerful bacteriolytic agent, as lysozyme obviously was, was a problem exactly representative of the kind that has always attracted my particular interest. I therefore took up Florey's suggestion with enthusiasm and started the work in 1936 with a PhD student, L. A. Epstein, an American Rhodes scholar. We were able to show that lysozyme was an enzyme of polysaccharidase nature acting on a polysaccharide which we could isolate from dried bacterial cells of *M. lysodeicticus*. We prepared the latter in batches of several hundred grams by growing the bacteria on agar surface in large Winchester bottles; for the purpose of distributing the agar evenly in these bottles we constructed a Heath-Robinson contraption for rolling them. For the first time I was confronted with the problem of producing micro-organisms in large amounts; this problem has remained with me ever since and still confronts me today. We were able to characterise the polysaccharide by showing that N-acetyl glucosamine and other sugars were its main components (Epstein and Chain, 1940). This work marked the beginning of, and laid the foundation for, a chapter of biochemical research that in recent years has become of central interest: the chemical nature of the bacterial cell wall and its enzymic hydrolysis and synthesis.

In the years 1937–8, when this work was about to be written up for publication, I surveyed the literature of bacteriolytic agents and found that several cases of lysis of one bacterial species by another had been described, apart, of course, from the bacteriophage, a powerful bacteriolytic agent on which a large literature existed. I also came across a large number of papers in which growth inhibition of one bacterial species by another, without the occurrence of actual lysis, was described. Thus, I stumbled, more or less accidentally, across the well-known phenomenon of microbial antagonism, first described very lucidly by Pasteur and Joubert in 1877. That Pasteur, who was the first to grow bacteria in culture, should also have been the first to discover bacterial antagonism is not at all surprising, for it is practically impossible for anyone growing bacteria not to come across chance contaminants with antagonistic properties. In the case of Pasteur and Joubert the contaminating organism was a strain of *Escherichia coli* from the air, and the contaminated culture was the anthrax bacillus. Pasteur and Joubert not only noticed the phenomenon of growth inhibition of the latter by the former, but immediately tried to reproduce it in *in vivo* experiments in rabbits and claimed to have attained success: the rabbits, though infected with anthrax, did not contract the disease when simultaneously they were injected with the antagonistic *E. coli* strain. On the basis of this observation, they predicted therapeutic possibilities in man that, however, were not realised.

I collected about 200 references on growth inhibitions caused by the action of bacteria, streptomycetes, fungi and yeast on one another. It was evident that in many cases the growth inhibition was caused by specific metabolites produced by the various micro-organisms. However, next to nothing was known about the chemical or biological nature of the inhibitory substances, and it seemed an interesting and rewarding field of exploration. I discussed this matter with Florey and it emerged from our talk that he knew of the existence of the field of microbial antagonism (Goldsworth and Florey, 1930) and was interested in its study. We eventually decided to undertake jointly a systematic investigation of these antibacterial substances produced by micro-organisms from the chemical and biochemical as well as the biological point of view. These substances are now known as antibiotics, a name coined in 1945 by S. A. Waksman, one of the pioneers in this field. My part of this project was the isolation and study of the chemical and biochemical properties of these substances; Florey's the study of their biological properties. There was, in addition to the scientific interest, still another potent stimulus for undertaking this work. We were then, as many laboratories are now, very short of cash—in this respect the passage of thirty years has not brought significant changes—so much so that one day in the autumn of 1938, I

received a message from Florey that, as the department had an overdraft of £500, I was to cease ordering equipment of any kind, even if it was only a piece of glass rod. I remember this message very well, and after an unsuccessful approach to the MRC for a very modest support of my research project, decided that it was essential for us, if we were to make any progress at all, to become as independent as possible of both university and government financial support and to look for private funds. I thought that a long-term project that would keep us going for a number of years would suit us best so that we would not have to go through the agonising experience of fund raising every year, and I started to think intensely about possible subjects for such a project. The systematic study of antibacterial substances produced by micro-organisms seemed ideal for the purpose.

I suggested to Florey to try our luck with the Rockefeller Foundation as this organisation had already given us some small support for equipment, and he agreed to explore the possibilities. On the first informal approach he was told that if we could submit a long-term research programme of bio-chemical, but not medical nature, it would receive favourable consideration. Florey asked me to formulate such a programme, which I did, and I included in this programme the project on antibacterial substances produced by micro-organisms, which could plausibly be expected to occupy us for quite a number of years. A few months later, we received a favourable reply: we were granted an annual sum of 5,000 dollars for five years, which at that time seemed royal generosity to us—we are considerably less modest these days.

One of the most impressive and best described phenomena of bacterial antagonism that I found in the literature was described in 1928 by the same bacteriologist who had discovered lysozyme some seven years earlier, Alexander Fleming. He had shown that a mould, a penicillium species which had settled on one of his Petri dishes, later identified as *Penicillium notatum*, had growth-inhibiting properties against a number of pathogenic bacteria. I had come across this paper early in 1938 and on reading it I immediately became interested. The reason was that, according to Fleming's description, the mould had strong bacteriolytic properties against the staphylococcus. You are all familiar with the famous Petri dish showing the antagonistic lytic action of this mould, whose photograph Fleming published in 1929, and most of you are familiar with the story, so often recounted, of the stroke of good luck that Fleming experienced and exploited, which consisted of the fact that a spore of a rather rare mould, *P. notatum*, had accidentally floated through his laboratory window and settled on his Petri dish to produce the phenomenon that led to the discovery of penicillin and its antibacterial properties. Fleming indeed had a stroke of good luck, but not in the sense of

this popular story. The phenomenon Fleming observed seems simple and straightforward enough but in actual fact it is not, and few people are aware of and understand its complexity and the fact that it needed the coincidence of several most unusual circumstances to make its observation possible. R. Hare (1970), in his book *The Birth of Penicillin*, recently analysed these circumstances. Briefly, they were as follows. Fleming had used a conventional agar plate for the study of staphylococci. These had been spread in the usual way over the agar surface of a Petri dish and grown in separate colonies over the plate. Normally, bacteriologists send away contaminated Petri dishes for sterilisation and washing-up as soon as possible after use; they are not kept in the laboratory for more than a few days. Fleming, however, for some unknown reasons must have left his Petri dish for at least, as we know now, a month or six weeks on his laboratory bench. During this time, a mould contaminant had, not surprisingly, developed on one side of the agar in the Petri dish. When Fleming, before finally disposing of the dish, looked at it once more he noticed that in the neighbourhood of the mould colony the bacterial colonies that he had seen before had disappeared; they had undergone lysis. Fleming, through his discovery of lysozyme, was prepared for lytic phenomena, and the far-reaching lysis of the staphylococcal colonies that had occurred in this Petri dish was so striking that it could not possibly have escaped his attention. He, therefore, decided to sub-culture the mould on the surface of a common culture medium, and to test the culture fluid after the growth of the mould for antibacterial action. He noted that the growth of the staphylococcus and many other Gram-positive pathogens was inhibited by this culture fluid without lysis. He gave the name 'penicillin' to the antibacterial culture fluid, as he could not know whether the antibacterial action was due to one or several antibacterial principles. Growth inhibition occurred even after diluting the culture fluid as much as 800 times. He even went further and injected as much as 20 ml of the culture fluid into rabbits and 0·5 ml into mice without noticing any toxic effect. He noticed, however, that the active principle or principles were very unstable and were lost in all attempts at purification, even during simple concentration.

Those who wish to reproduce the lytic phenomenon that Fleming observed will meet with difficulties. As we know now, penicillin has no direct lytic effect on the staphylococcus or any other sensitive bacterium. The lysis of staphylococcal colonies that Fleming noticed and that led him to the discovery of penicillin, was an autolysis that occurs only under very special conditions, depending on the physiological state of the micro-organism. The requirements essential for lysis of staphylococcal suspensions after contact with penicillin were studied in some detail by my late colleague E. S. Duthie

and myself (Chain and Duthie, 1945) because we were puzzled by Fleming's description of the lytic effect of penicillin on his agar plate, which we have never been able to demonstrate in liquid culture media. It turned out that staphylococcal cultures do indeed undergo lysis under the influence of penicillin, but only when they are suspended in a medium in which they are potentially capable of undergoing at least one division. We were able to show by turbidity measurements and haemocytometer counts that in the presence of penicillin a staphylococcal suspension of 200 million organisms in nutrient broth first swelled, without increasing in numbers, and then underwent complete lysis, while penicillin was without any effect on staphylococcal suspensions in buffer solutions, even if the suspensions were much less dense. The same requirements for lysis, i.e. the capability of the organisms to undergo at least one division, obviously also obtain for colonies growing on agar. The element of good luck that favoured Fleming was not that a strange mould settled by chance on his Petri dish. The penicillin producing *P. notatum* which he had found on his plate is ubiquitous and quite common; it can be isolated from most of the back and front gardens of London houses; it must have landed on innumerable Petri dishes of bacteriologists in this country. The unusual element in Fleming's case was that he left his Petri dish with the staphylococcal colonies on its agar layer for such a long time on the bench that the contaminating mould had time to develop and the antibacterial agent it produced reached the colonies just at the right age and physiological state when they were still capable of dividing and, therefore, were susceptible to lysis under the influence of penicillin. Fleming did not discover the growth-inhibiting effect of penicillin on bacteria directly by observing a phenomenon of inhibition of bacterial growth (which penicillin commonly exerts on many bacterial species under many growth conditions and which is, of course, the basis of its chemotherapeutic action), but through a bacteriolytic effect of penicillin which it exerts only under the very special conditions outlined above, not normally encountered in the bacteriological laboratory, and only on very few bacterial species. Fortunately, the staphylococcus on which Fleming had worked was among these. The reason that the antibacterial action of penicillin on bacteria was not discovered long before Fleming, despite its ubiquitous occurrence, was that, normally, if the bacteriologist sees that one of the plates he wishes to use for a diagnostic test is contaminated with a mould, he does not use it, and thus has no chance of observing an inhibition of bacterial growth by the contaminant; on the other hand, once his bacteriological observation is completed, he throws away the Petri dishes as soon as possible without waiting for contaminants to appear.

When I saw Fleming's paper for the first time I thought that Fleming had

discovered a sort of mould lysozyme, which, in contrast to egg white lysozyme, acted on a wide range of Gram-positive pathogenic bacteria. I further thought that in all probability the cell wall of all these pathogenic bacteria whose growth was inhibited by penicillin contained a common substrate on which the supposed enzyme acted, and that it would be worth trying to isolate and characterise the hypothetical common substrate. For this purpose it would, of course, be necessary to purify the supposed enzyme, but I did not foresee any undue difficulties with this task for which I was well prepared from my previous research experience. At that time I was actually more interested in studying the substrate of the supposed enzyme than the enzyme itself. My belief that we were dealing with an enzyme was strengthened by a paper from Raistrick's laboratory (Clutterbuck *et al.*, 1932) in which Fleming's observations on the instability of penicillin were confirmed and extended. These authors found that on extracting the acidified penicillin-containing culture fluid the active principle disappeared from the aqueous phase, but could not be recovered after evaporation of the ether. I interpreted these results as indicating a surface denaturation of the supposed active protein by ether, such as occurs readily with lysozyme. My working hypothesis was proved by my first experiments to be completely erroneous, but it was not quite as far off the mark as it may seem. As it turned out during the course of modern studies on the structure of bacterial cell walls, penicillin-sensitive bacteria do contain a common substrate of polysaccharide-peptide nature, and penicillin acts not as a hydrolytic enzyme like lysozyme but as an inhibitor of an enzyme of transpeptidase nature which brings about the last step of the synthesis of the cell wall of the mucopeptide, the cross linking of one peptide chain to the next through elimination of alanine. The study of the mode of action of penicillin and the cell wall constituents whose synthesis it inhibits has become a very active field of research.

That penicillin could have practical use in clinical medicine did not enter our minds when we started our work on it. A substance of the degree of instability that penicillin seemed to possess according to the published facts does not hold out much promise for clinical application. If my working hypothesis had been correct and penicillin had been a protein, its practical use as a chemotherapeutic agent would have been out of the question because of anaphylactic phenomena that inevitably would have followed its repeated use. From the scientific point of view, however, the problem of purifying penicillin and isolating the substrate on which I thought it acted was of interest and, hence, well worth pursuing.

I started to work on penicillin in 1938, long before the outbreak of the war. The frequently repeated statement that the work was started as a contribution

to the war effort, to find a chemotherapeutic agent suitable for the treatment of infected war wounds, has no basis. The only reason that motivated me was scientific interest. I very much doubt whether I would have been allowed to study this problem at that time in one of the so-called 'mission oriented' practically minded industrial laboratories. The research on penicillin, which was started as a problem of purely scientific interest but had consequences of very great practical importance, is a good example of how difficult it is to demarcate sharp limits between pure and applied research.

Fleming's mould, by a curious coincidence, was present in the culture collection of the Sir William Dunn School of Pathology. A sub-culture was obtained from Fleming by Florey's predecessor, the Danish bacteriologist, Dreyer, who was interested in bacteriophage and thought that Fleming had discovered a new kind of bacteriophage. I obtained a sub-culture from a former collaborator of Dreyer, Miss Campbell-Renton, who continued to work in the department for some years after Dreyer's death in 1935. I started to culture the mould in surface culture on a simple Czapek-Dox synthetic medium, such as Raistrick and his colleagues had used in their investigations, and it took me some time to get reproducible antibacterial activity in the culture fluids, which was partly due to my inexperience, partly to the great variability of Fleming's strain.

When Florey and I decided to undertake a systematic investigation of antibacterial substances produced by micro-organisms we gave penicillin, on which I had already started to work, first priority because of its scientific interest, and we added two other substances, pyocyanase, a lytic principle produced by *Pseudomonas pyocyanea*, which had already had limited clinical use in Germany in a very crude form, and actinomycetin, a lytic principle from *Actinomyces albus*. It was already evident from the literature that the actinomycetes were a rich source of antibiotics, and it was our intention to concentrate on this class of micro-organism, from which the majority of clinically useful antibiotics are derived. This was also clearly stated in our first application to the Rockefeller Foundation.

It is of interest to speculate on the course of events that would have taken place if Fleming had not had the good fortune to encounter the unusual experimental circumstances which made it possible for him to observe the bacteriolytic effect of penicillin on the staphylococcus. I believe that the field of microbial antagonism had become ripe for study when we started our own investigations in 1938. The existence of antibacterial substances produced by micro-organisms had been well documented and it was of obvious interest to study their biological and biochemical properties. We would have started our research programme on these substances even if Fleming's paper had

not been published, and if we had not done so, someone else in some other laboratory would have taken the initiative. As a result, some interesting antibacterial substances would have been discovered, as they later were, and, following this, a general screening programme for organisms capable of producing antibiotics would have started. This would undoubtedly have revealed the existence of the penicillin-producing penicillia. The development of the antibiotics field might have been delayed by a few years, but it would, inevitably, have taken place with the same final results.

As things turned out, penicillin became available during the catastrophe of the Second World War when it was most needed, and naturally the needs of the war greatly accelerated the pace of development of the antibiotics field.

In 1940, we invited one of my former collaborators, Dr N. G. Heatley, whom I had met in my Cambridge days at the School of Biochemistry, to join us. A pupil of J. Needham, he was a gifted experimentalist, with a particular flare in the devising and use of micro-methods, and I had asked Florey in 1937 to engage him as a member of my biochemical team for metabolic studies requiring the use of micro-methods. We asked him to take over the growing of the mould and the testing. He worked out a very neat and simple assay method for penicillin, which became known as the cylinder-plate test and is still widely used in modified form and has greatly facilitated our investigations.

I concentrated on the isolation and characterisation of the active antibacterial principle. My first experiments showed that penicillin was not a protein, but a low molecular substance that diffused readily through cellophane membranes. I was, at first, disappointed with the finding, for my beautiful working hypothesis dissolved into thin air, yet the fact of the instability of penicillin remained and became even more puzzling, as it could not be explained on the basis of being a protein. There was, at that time, no other known antibacterial with that degree of instability, and it became very interesting to find out which structural features were responsible for the instability. It was clear that we were dealing with a chemically very unusual substance. Only the nature of our problem had changed: instead of studying the isolation and mode of action of an enzyme with strong antibacterial properties, our task was now the elucidation of the structure of a low molecular substance that combined high antibacterial power with great chemical instability.

The first experiments, designed to test the stability of penicillin in aqueous solution at various pH, showed that it was stable only between pH 5 and 8, but was rapidly inactivated under more acid and alkaline conditions. This, of course, explained Raistrick's findings. It was, however, possible to slow

down the rate of inactivation in the acid pH range by cooling to 0°C, and on this basis we developed a method of extracting penicillin from the aqueous acidified cooled solution into an organic solvent in the form of the free acid and back into water as salt, adjusting the pH to 7 by addition of alkali. In this way a considerable concentration and purification of penicillin could be achieved, but it still was not possible to concentrate the aqueous solution to dryness without loss of activity. I then tried the method of freeze-drying which had just been introduced for the drying of blood serum by R. T. N. Greaves in Cambridge, and this proved successful.

We thus obtained a brown powder which displayed considerable antibacterial activity, in dilutions of 1 in 10^6, i.e. it was about 20 times more active than the most active sulphonamides.

A colleague of ours, Dr J. Barnes, had, some weeks earlier, carried out a mouse toxicity test with a preparation obtained by methanol extraction of the freeze-dried culture filtrate, which was less pure than the ether-extracted material, but still exhibited a high antibacterial activity. To everyone's surprise, he found this preparation entirely non-toxic in a dose of 10 mg.

As a matter of routine, and without any over-optimistic expectations, we tested the toxicity of the purer, ether-extracted material on mice and noted that it, too, despite its higher antibacterial activity, had none in a dose of 10 mg i.v. The finding of the apparent non-toxicity of an evidently highly active antibacterial substance was so novel, and the implications so far-reaching that we repeated the experiment, though at that time it was quite an enterprise to collect 10 mg with our primitive methods of production, and we obtained the same result. We also noticed that the urine of the mice had the same dark brown colour as did our penicillin preparations, and on testing the urine for antibacterial activity, we found that it was highly active. From this we concluded that as penicillin passed through the body of the mouse without loss of activity, it was probable that it would display its antibacterial activity in body fluids. Therefore, we carried out, with the help of our colleague, Dr A. D. Gardner, then Reader in Bacteriology, and his collaborator, the late Miss Orr-Ewing, who were more experienced in the handling of pathogenic organisms than any of us, a small-scale therapeutic experiment with eight mice infected with a virulent strain of *Streptococcus haemolyticus*. Four of these were treated by repeated injections of penicillin; four served as controls. After 24 hours the four controls were dead, the four treated survived, a clear-cut result.

This experiment was, in essence, the demonstration of the chemotherapeutic effect of penicillin. Everything that followed was, more or less, in the nature of a routine operation. It was evidently necessary to extend the chemo-

therapeutic experiments to other pathogens sensitive to penicillin; this needed more material. It was necessary to study the pharmacological properties of penicillin; this needed considerably more material. Dr Gardner's and Dr Orr-Ewing's help on the bacteriological aspects of the work had already been enlisted for the first chemotherapeutic experiments. In order to make progress as speedily as possible, we asked several other colleagues in the department to collaborate on the pharmacological, bacteriological, and chemical aspects. This group of people was later frequently referred to in the popular press as the Oxford team, not an appropriate term and one that renders less than justice to its nature. The group was assembled after, not before, our first chemotherapeutic experiments on our mice, for the purpose of speeding up the work, and it was not so much an organised team as a group of colleagues with different backgrounds of expertise collaborating with each other to achieve obvious objectives in the minimum of time.

With great difficulties, we succeeded in getting sufficient material for repeating our chemotherapeutic tests and confirmed our initial results. We also obtained equally successful results with a number of other Gram-positive pathogens reported by Fleming to be penicillin-sensitive; the staphylococcus and the anaerobic *Clostridium septicum* (Chain *et al.*, 1940). These experiments and the absence of any toxic effect of our preparations encouraged us to try injections into man, but unfortunately they proved pyrogenic. My colleague, E. P. Abraham, and myself, therefore set out to purify the preparation further and achieved a tenfold purification. The material proved non-pyrogenic in man, and with it our first clinical experiments were performed by Dr C. M. Fletcher and showed that severe human clinical infection responded to penicillin just as well as the experimental infections in animals (Abraham *et al.*, 1941). We thought at the time that this preparation, which showed an activity of 1 in 10^6, was near purity. Little did we suspect that our preparation contained just about 1 per cent of penicillin and 99 per cent impurities. We now know that our first preparations contained about 30 different substances, and it borders on the miraculous that none of the impurities was toxic enough to mask the non-toxicity of the active antibacterial principle, penicillin.

At this point I should like to comment on Fleming's paper. I have mentioned that Fleming had injected 0·5 ml of the penicillin-containing culture fluid into mice without any toxic effects. His culture fluid must have contained at least 1 to 2 units of penicillin per ml. It has always amazed me that Fleming did not carry out exactly the same experiment on mice infected with streptococci. No chemical knowledge was required for such experiment. Had he done this, he would certainly have got some chemotherapeutic effect that

would have been impressive enough to stimulate chemists to get the active principle in a purified form, which was not a very difficult operation. In this they would undoubtedly have succeeded, with the result that humanity would have had penicillin at its disposal ten years earlier than it did. The reason why Fleming did not even attempt to carry out this simple experiment is, in my opinion, that the whole atmosphere in the Institute where he worked, the Inoculation Department of St Mary's Hospital, was not conducive to this approach; it was, in fact, positively unsympathetic to experiments of this kind. The Director and Head of the Department was Sir Almroth Wright, who believed that antibacterial therapy could only be based on immunological techniques, and any attempt to use chemicals was doomed to failure, as all the available experience had shown that they would be more harmful to the host than to the invading bacteria. 'Stimulate the phagocytes' was his motto and dogma, (he was the model for the doctor in Bernard Shaw's play *The Doctor's Dilemma*) and he would not readily admit any other view. Fleming was his pupil and junior collaborator, and became himself a strong advocate of immunotherapy and was openly sceptical about the potentialities of chemotherapy. Most of the leading bacteriologists at that time shared this view; it was, after all, six years before the discovery of prontosil and the sulphonamides.

Despite the generally unsympathetic attitude to the possibilities of antibacterial chemotherapy that prevailed in the twenties at the Inoculation Department of St Mary's Hospital, no one in that Department, and least of all Almroth Wright, would have prevented Fleming from carrying out the simple experiment of injecting the crude penicillin-containing culture medium into a mouse infected with a penicillin-sensitive bacterium if he had wanted to. He did not perform it because he did not think that it was worth trying. I mention this aspect of the history of the discovery of penicillin as a good example of how preconceived ideas in science can stifle imagination and impede progress. It is always dangerous when any generally accepted theories or any kind of central dogmas are taken too seriously. Nonetheless, there can be no doubt that Fleming made a contribution of great importance, as measured by any standard one would like to apply, to microbiology by following up the lytic phenomenon that he observed, admittedly as the result of a most improbable combination of circumstances, and in this way discovering the growth-inhibiting properties of penicillin against many important pathogens.

The results of our chemotherapeutic experiments with penicillin on animals and man demonstrated clearly its extraordinary efficacy as a chemotherapeutic systemic agent against bacterial infections. However, the substance as

we produced it in the laboratory was no drug, but a laboratory curiosity; it was just not accessible in reasonable amounts with our methods of production. To convert this laboratory curiosity into a useful cheap drug necessitated the work of many hundreds of scientists and technologists over a period of many years, work mostly carried out in industrial research laboratories and a few government research stations, mainly in the United States. Instead of growing the mould on the surface of culture fluids in numerous small vessels, the method of submerged culture was developed, allowing the growth of the mould in stirred stainless steel fermenters in volumes that have reached over 50,000 gallons. A whole new chemical technology was developed that today is one of the main pillars of the pharmaceutical industry. Through strain improvement by genetical mutation techniques, improvements of the culture medium and improved aeration, the yields of penicillin have risen from the 1 to 2 units/ml which we obtained, to 25,000 units/ml, i.e. about 15 mg/ml; penicillin has become one of the cheapest drugs in clinical use. Why this development was almost entirely an American one is an interesting question, the answer to which would go beyond the scope of this article. The same applies to the question of why the methods of extraction and purification of penicillin were not patented in this country, and why, in consequence, this country had to pay royalties for a period of fifteen years for American know-how, instead of receiving them.

In the meantime, my colleague Abraham and myself, in collaboration with Sir Robert Robinson and Professor Wilson Baker, continued our investigations on the structure of penicillin, and the same aim was pursued in many American research laboratories. As the structural features of the penicillin molecule gradually came to light, the opinion held by the overwhelming majority of the organic chemists working on this problem was that it would be only a matter of months before the penicillin molecule would be synthesised. I never shared this optimistic view and we had some heated arguments on this question on various committees. The problem was not just one of theoretical interest, but it had very considerable practical significance, for it involved the question as to whether the pharmaceutical industry was justified in investing large sums of money in fermentation plants if the synthesis of the substance was only a matter of time. There was the danger that before the costly fermentation plants were properly started they would be made obsolete by a cheap method of synthesis. I did not believe that synthesis of the penicillin molecule was around the corner, because I could not visualise how the final cyclisation step of the open chain penicilloic acid molecule, leading to the formation of the β-lactam ring, could be achieved by any of the then known ring-closing agents in the very limited conditions under which the biological

activity of the penicillin molecule is not destroyed. My doubts were fully vindicated by events. It took over ten years to develop an entirely novel kind of ring-closing agent which made the synthesis of the penicillin molecule possible by ring closure of the corresponding penicilloic acid.

In 1943, we proposed the thiazolidine-β-lactam structure of penicillin that is now universally accepted. It was one of the last structural investigations that was carried out mainly with chemical methods, though we received considerable help from X-ray crystallographic analysis carried out by Dorothy Hodgkin. In 1945 she obtained the complete structure of the penicillin molecule by X-ray analysis and proved unequivocally the presence of the four-membered β-lactam ring which was doubted by many organic chemists. It took Dorothy Hodgkin about two years to calculate the structure of the penicillin molecule using the mechanical calculators at her disposal; today, with modern computers, the job would be completed in two to four weeks.

The thiazolidine β-lactam structure explained the instability of the penicillin molecule. It turned out, as could be expected, to possess a unique structure. In alkaline solution and under the action of penicillinase the β-lactam ring is opened. In acid solution a rearrangement to the imidazoline derivative penillic acid takes place. The penicillin molecule is capable of several other rearrangements, and, as has been shown in recent years, of polymerisation. The open chain derivative, penicilloic acid, was obtained quite early by synthesis, but all attempts at ring closure with the available ring-closing agent gave, as expected, the new stable 5-membered azlactone penicillenic acid, without biological activity. It was not until 1957 that Sheehan and Henery-Logan, using a new class of condensing agents, the substituted carbo-diimides, which act at neutral pH in aqueous solution and bring about the formation of peptide bonds from amino acids, succeeded in achieving ring closure of phenoxypenicilloic acid to phenoxypenicillin and accomplished the first total synthesis of a penicillin. The yields were minimal and even today have not been improved to the extent that penicillin synthesis can compete with the biological production method, nor is this likely to happen in the foreseeable future.

The publication of our results on the chemotherapeutic power of penicillin naturally stimulated an intensive search, by both industrial and academic research scientists, for other antibiotics. As I have pointed out, there was plenty of evidence for the existence of such substances, particularly among the group of streptomycetes. This search received an additional impulse by the discovery by Waksman and his collaborators in 1944 of the antibiotic streptomycin. Waksman was a soil microbiologist particularly interested in humus production, which is caused by the group of streptomycetes. He became an

expert in this group of micro-organisms and over the years had assembled one of the largest collections of streptomycetes in the world in his laboratory at Rutgers University, New Brunswick, N.J. Naturally, he was familiar with the antagonistic properties of many representatives of the group of streptomycetes against many bacteria. He began to study them about the same time as we started our work on penicillin and in collaboration with Woodruff isolated several strongly antibacterial substances with interesting chemical and biological properties, among them actinomycin (Waksman and Woodruff, 1940) which in later years has played an important role in the study of protein synthesis as a specific inhibitor of the transcription of DNA to messenger RNA, and streptothricin (Waksman and Woodruff, 1942). In 1944, Schatz, Bugie and Waksman described the isolation of a very much less toxic antibiotic, streptomycin, with a broader spectrum than penicillin, being active against several Gram-negative, in addition to the Gram-positive, bacteria. One of the most important properties of streptomycin was discovered by Feldman and Hinshaw (1944) of the Mayo Clinic, who found that streptomycin was active against the tubercle bacillus not only *in vitro*, but also *in vivo* in experimental tuberculosis in guinea-pigs, which it could cure. Streptomycin was subsequently shown to be effective also in various forms of tuberculosis in man, and is today still an indispensable and essential component, together with other antitubercular substances such as para-aminosalicylic acid and isoniazid, of tuberculosis chemotherapy. This therapy is still generally successful if properly applied.

The systematic screening for new antibacterial substances brought to light virtually hundreds of new antibacterial substances, many of great biochemical interest, but only very few of clinical importance. These are: chloramphenicol, the tetracyclines, the macrolides, kanamycin, lincomycin, the polymixins and the antifungal antibiotic griseofulvin. All these antibiotics were discovered in industrial research laboratories. In contrast, one of the last antibiotics of clinical importance that arose from the screening effort was discovered in a non-industrial laboratory. It was cephalosporin C produced by a cephalosporium. The organism was isolated in a small public health laboratory in Sardinia by an Italian public health officer, G. Brotzu, whose attention was drawn to it by its strong antagonistic action against *Salmonella* strains (Brotzu, 1948). Cephalosporin C was isolated and its structure determined by my former colleague Abraham and his collaborator, the late Dr Newton, at Oxford (Newton and Abraham, 1956; Abraham and Newton, 1961) and with X-ray crystallographic studies by Dorothy Hodgkin and Maslen (1961). Its structure proved similar to that of penicillin; it contains the four-membered β-lactam ring, but this is fused with the six-membered thiazin ring instead of with the

five-membered thiazolidine ring. Penicillins and cephalosporins can be interconverted by chemical methods.

If one considers the immense effort that was expended in the search for new antibiotics of clinical usefulness, the result must be considered as meagre. Many hundreds of people participated in the effort, and hundreds of millions of dollars were spent on screening micro-organisms from the air, the earth and water, with the result of a dozen or so antibiotics of clinical importance. The effort was possible only because it was distributed over a large number of independent groups that shared the risk. No centrally organised state organisation would have been able to sponsor such effort. One could not imagine the civil servant who would have had the courage to spend such very large sums of money on a project that could not be guaranteed to be successful. These simple facts should not be forgotten in discussions on the desirability of nationalising the pharmaceutical industries.

My own part in the screening effort was very limited. I did isolate a few new antibiotics, but I held the opinion that this kind of research was essentially a matter for industrial laboratories that have the organisational and financial means to this end with regard to manpower and equipment which is just not available in academic laboratories. Furthermore, it was clear to me that it was impossible to work in this field satisfactorily without fermentation pilot plant equipment of semi-industrial dimensions. I had felt, intensely, right from the beginning and throughout the course of our work on penicillin, the need of larger quantities of material for all aspects of our work; lack of material had been the most important single obstacle to progress from which we suffered all the time. With the conventional laboratory equipment at our disposal, it was just not possible to obtain the quantities required. This caused a sense of acute continuous frustration that is one of my most vivid memories of the time. An intensive struggle for material became a permanent and prominent feature of my daily activities. It was most galling to have to rely on the goodwill of others for adequate supplies of penicillin preparations so as to ensure uninterrupted progress in our own discovery and to avoid our group being squeezed out completely from a field we had opened up and which obviously had great perspectives.

It was clear to me from the earliest stages of our investigations that the lack of material from which we suffered and which placed us at a great disadvantage *vis-à-vis* other groups, particularly in the USA, working under more favourable conditions, was entirely due to our primitive and amateurish methods of penicillin production. I was convinced that the whole supply situation could be transformed rapidly and with the greatest ease by adopting in our laboratory more professional techniques and I expressed this view

clearly, frequently and persistently, but without success. The more I became involved in problems in the field of chemical microbiology, the more I became convinced of the absolute necessity—in any case, for the study of problems in which I was interested—of the availability of proper fermentation pilot plant facilities to enable the investigator to produce sufficient quantities of the substances of microbiological origin in which he was interested. However, despite a number of energetic attempts, I did not convince the authorities in this country of the correctness of my views. I was told that fermentation pilot plant facilities, as I envisaged them, did not belong in university research laboratories, but were a matter for industry. I had no success with my arguments that the size of the reaction vessels did not determine whether the character of a particular biochemical research problem to be investigated was pure or applied, that many of the biochemically most interesting substances were of catalytic nature and, therefore, by definition present only in small amounts, that many such substances of low as well as high molecular weight were still to be discovered and that micro-organisms were a particularly good source for many of them. The proposal to content myself with one single fermenter of moderate dimensions which was repeatedly made to me, was unacceptable; it seemed to me equivalent to asking the organic chemist to limit himself to the use of one Erlenmeyer flask only in his experiments.

My inability to obtain the equipment I considered essential for the successful continuation of the studies in the field of chemical microbiology in which I was interested was the reason why, towards the end of 1948, I accepted an invitation from the then director of the Italian Institute of Public Health, Professor Domenico Marotta (made to me while I was on a lecture tour in Italy for the British Council) to organise a biochemical department at that Institute and to build up, as part of it, a fermentation pilot plant with a sufficient number of fermenters of varying sizes, ranging from 10 to 500 litre capacity, and all the accessory equipment necessary for the extraction and purification of the desired microbial metabolites. This installation, started in 1949 and finished in 1951, marks the beginning of the fourth phase of antibiotic research to which I have referred. I was particularly interested in the possibility of modifying the penicillin molecule. The penicillin molecule consists of a nucleus, termed 6-amino penicillanic acid, following the nomenclature introduced by Sheehan, and a side chain. In the clinically most widely used natural penicillin, benzylpenicillin, the side chain is phenylacetic acid, but penicillins with other side chains occur in nature. Behrens and his colleagues, of the research laboratories of Eli Lilly at Indianapolis, had shown in the middle forties that the mould *Penicillium chrysogenum* possesses the property of incorporating not only phenylacetic acid but other organic acids into the

side chain if they are added to the culture medium as precursor, provided they are derivatives of acetic acid. Thus, phenoxyacetic acid is readily incorporated to give phenoxymethylpenicillin. This turned out to be acid stable and has, therefore, acquired considerable clinical importance as an oral penicillin preparation.

In the late forties and early fifties great difficulties were experienced in some hospital wards in the treatment of staphylococcal infections with penicillin. While at the beginning of the penicillin therapy the staphylococcus was one of the most sensitive organisms to penicillin, there began to appear epidemics of staphylococcal infections, often fatal for the patients affected, which were resistant to the action of penicillin. Penicillin-resistant strains of staphylococci had already been described by Fleming in 1943, and shortly afterwards the American bacteriologist, Kirby, showed that all the penicillin-resistant strains he had collected produced the enzyme penicillinase which opens the β-lactam ring of the penicillin molecule; this enzyme had been discovered in 1940 by Abraham and myself (Abraham and Chain, 1940). Kirby's findings were confirmed by many bacteriologists. In this country the late Mary Barber worked extensively in this field and demonstrated beyond doubt that the penicillin-resistance of staphylococci was due exclusively to the capability of these strains to produce penicillinase (Barber, 1947). This view was not generally accepted; the opinion was held that penicillin could render penicillin-sensitive strains resistant through induction of penicillinase. This view was not compatible with the findings of my late colleague, E. S. Duthie, and myself, according to which it was easy enough to render penicillin-sensitive staphylococcal strains penicillin-resistant by the usual technique of sub-culturing in the presence of sub-inhibitory concentrations of penicillin, but it was impossible to make these strains produce penicillinase. It was, therefore, clear to me that the capability to produce penicillinase was a genetically conditioned property, and strains either possessed it or did not. In strains genetically equipped to produce penicillinase the production of this enzyme is stimulated by a factor of up to several hundred times by the presence of penicillin in the culture medium; penicillinase is a readily inducible enzyme. For this reason it has been subjected to extensive molecular biological studies. Clinically, the ready inducibility of penicillinase aggravated the situation, for many staphylococcal strains which, when grown in the absence of penicillin, produce only small amounts of penicillinase and, therefore, respond to therapeutically attainable concentrations of penicillin, produce, when grown in the presence of sub-inhibitory concentrations of penicillin, such large amounts of penicillinase as to render them completely insusceptible to penicillin therapy. However, it was also clear to me that if the penicillin resistance of

staphylococcal strains encountered in hospitals was due entirely to penicillinase, it should be possible to change the affinity of the penicillin molecule to this enzyme if it could be chemically modified so that penicillins with either very high or very low affinity could be produced; in either case they would not be subject to the hydrolytic action of the enzyme. I had this possibility in mind for many years, and it was for this reason, among others, that I wanted the fermentation pilot plant in this country. I gave this project first priority in Rome. The penicillin in which I was particularly interested was *p*-aminobenzylpenicillin, which was known to be formed biosynthetically by addition of *p*-aminophenylacetic acid to the culture medium, but had not been obtained pure and in a reasonable yield and had not been properly studied. *p*-aminobenzylpenicillin lends itself to modifications of the side chain through the free amino group of the latter. In 1954 the directors of a British pharmaceutical firm, at the initiative of their dynamic chairman, Mr H. G. Lazell, decided to explore the possibilities of entering the antibiotics field and had approached me for advice through their chief consultant, Sir Charles Dodds. I expressed the view that the conventional screening of micro-organisms for new antibiotics, already extensively exploited, had little chance of success at this late stage, but suggested that modification of the penicillin molecule offered interesting possibilities. I told them also that work of this kind necessitated the availability of a fermentation pilot plant which involved a capital outlay of at least £50,000. They accepted my advice and agreed to have a fermentation pilot plant, modelled on my own, constructed at the earliest opportunity. To save time, it was decided to send a team of four scientists to my department in Rome to participate in our research programme on modifying *p*-aminobenzylpenicillin. They started work in 1955. As new biosynthetic penicillins cannot be expected to have the same biological activity as has benzylpenicillin, we used two assay methods: a biological and a chemical one. The ratio of chemical to biological assay in benzylpenicillin is about one. We noted that when *p*-aminophenylacetic acid was added as a side chain precursor instead of phenylacetic acid, the value of this ratio rose to 1·4. When no precursor acid was added to the culture fluid, the ratio rose still further to 3·9. The chemical method for determining penicillin is based on the behaviour of the thiazolidine-β-lactam ring, which is characteristic for the penicillin molecule. It was thus clear that there was present in the culture fluid a substance that behaved chemically like penicillin, but had no biological activity. At the time we concentrated our efforts on working out a method for the preparation of reasonable amounts of *p*-aminobenzylpenicillin, and actually succeeded in this aim so that we could start our programme on the chemical modification of this substance.

In 1956 the Beecham research workers returned to their own laboratory where the construction of their fermentation pilot plant had meanwhile been completed. A few months later four members of the Beecham team used a simple chromatographic experiment to clarify the structure of the substance formed in the culture fluid in the absence of a precursor acid which behaved chemically like penicillin but had no or little biological activity. They showed that after phenacetylation of the culture fluid benzylpenicillin was formed. Thus, it was probable that the substance was, in fact, the nucleus of the penicillin molecule, 6-aminopenicillanic acid (Batchelor *et al.*, 1959; Ballio *et al.*, 1959).

This isolation in pure state and the unequivocal proof of the structure of 6-aminopenicillanic acid was obviously the next step. This was done (Batchelor *et al.*, 1961a), using methods similar to those we had developed for the isolation and purification of 6-aminobenzylpenicillin, which had similar chemical properties. The discovery of 6-aminopenicillanic acid (6-APA) meant that our original programme of modifying the penicillin molecule could be extended beyond all expectations. We found ourselves in a situation very similar to that of Trefouëls, Bovet and Nitti in 1935 when they recognised that the active principle of prontosil was sulphanilamide. As in this case, the molecule of 6-APA made possible the synthesis of a very large number of acyl derivatives: the limit was the power of imagination of the organic chemist to invent new organic acids that could be used as side chain precursors. Finally, the organic chemist had come into his own in the penicillin field and the antibiotics field in general. Of all the antibiotics of clinical interest only chloramphenicol and many analogues of chloramphenicol had been synthesised; none of these was, however, in any way superior to the original chloramphenicol.

6-aminopenicillanic acid is produced by direct fermentation only in small yields, a fraction of the yields of benzylpenicillin (Batchelor *et al.*, 1961b) and its purification is a difficult process. It was, therefore, a great step forward when an enzyme of amidase nature was found in strains of streptomycetes which was capable of removing the side chain from phenoxymethylpenicillin with the liberation of 6-APA (Batchelor *et al.*, 1961c). Later, workers from the Bayer research laboratories as well as microbiologists from our own group found bacterial enzymes that liberated 6-APA from benzylpenicillin. At present, the yield of crystalline 6-APA from benzylpenicillin is over 90 per cent and 6-APA has thus become as readily available as benzylpenicillin.

The discovery of 6-APA and of its ready accessibility started an intensive effort to synthesise semi-synthetic penicillins by acylation of the free amino group of 6-APA, and several thousands of such penicillins were made in many

industrial research laboratories. Suddenly, after the lapse of many years, the centre of attention of the pharmaceutical industry engaged in antibiotics production was, once again, focused on penicillin, which had been given up by all of them as a research topic of no commercial interest. In fact, the prices fetched by the ordinary penicillins had reached such low levels that all but very few penicillin production plants had to close down; only the largest and most efficient survived. At the present time, there is a world shortage of penicillin, and as a result of the large demand for 6-APA, the prices are rapidly rising.

Three groups of semi-synthetic penicillins resulted from the work of the Beecham Research Laboratories and each of these has given substances with wide clinical applications. Intensive research in many laboratories of pharmaceutical companies has added many new members to these groups, but no new groups of substances with fundamentally different properties have emerged. These three groups were—

1. The acid stable penicillins, a large number of which was obtained, with a spectrum of activity not very different from benzylpenicillin, but with improved properties with regard to their binding capacity to serum proteins and their rate of excretion.

2. Penicillins resistant to penicillinase and, therefore, active against the benzylpenicillin-resistant staphylococcus. This was the primary objective of the whole exercise. It was attained very soon after the discovery of 6-APA. The first of such penicillins was methicillin, resistant to staphylococcal penicillinase because it possesses a very low affinity for the enzyme. It behaves as if the penicillinase of the staphylococcus was not present. It is unstable to acids and, therefore, is active only on injection. Although its activity is about 50 times less than that of benzylpenicillin it is clinically very effective. It was followed by the iso-oxazolylpenicillins, of which oxacillin, cloxacillin and diecloxacillin are representative members. These are acid stable and possess in the test-tube about one sixth of the activity of benzylpenicillin. Their penicillinase stability is based on the fact that their affinity to the enzyme is very high so that the action of the enzyme is inhibited.

3. Penicillins with a broader spectrum of activity than benzylpenicillin. Not many such compounds have become available. One of these is ampicillin in which the side chain is an amino acid, phenylglycin. Ampicillin has a spectrum of activity very similar to that of chloramphenicol and the tetracyclines. It has about the same activity against strains of *E. coli* and *Salmonella typhimurium*, but is much less toxic and does not cause the disturbances of the intestinal flora that result in an overgrowth of *Candida*, which very frequently

occurs after the administration of the tetracyclines and causes very unpleasant symptoms. Against many strains of *Proteus* ampicillin is much more effective than the tetracyclines and chloramphenicol. Ampicillin today is one of the most widely used antibiotics.

Another example of the third group of semi-synthetic penicillins with a broader spectrum of activity than benzylpenicillin is carbenicillin. This substance, containing a phenylmalonic acid side chain, is active against *Pseudomonas pyocyanea* against which no other antibiotic is active except the polymixines which are toxic, and, in particular, show nephrotoxicity.

The most remarkable property of the penicillins is their almost complete lack of toxicity. Despite this fact, there existed a very real danger in penicillin therapy right from the beginning. About five per cent of the patients treated reacted with strong allergic symptoms, urticaria and even anaphylactic shock. Penicillin was found to be one of the most powerful allergens, the allergenic nature being based on its property to react with proteins. This was first thought to be due to the property of the penicillin molecule to undergo rearrangement to penicillenic acid which, as an azlactone, could readily react with free amino groups. However, it was shown that the thiazolidine-β-lactam ring system can also react directly with proteins, as 6-APA, which is not able to undergo the penicillenic acid rearrangement, can react with proteins forming peptide links. With the availability of the new semi-synthetic penicillins the hope arose that it might be possible to obtain penicillins with reduced capability to react with proteins. This hope was regrettably not realised. However, intensive research has led to a much better understanding of the mechanism of penicillin allergy and to progress in the practical prevention of this condition.

Batchelor and his colleagues (1967) of the Beecham laboratories found that the normally used benzylpenicillin contained small amounts of a penicilloylated protein, deriving from the fermentation process that was carried through all the steps of purification to the final product. This protein was found to be highly allergenic, only fractions of a microgram being required to kill sensitised guinea-pigs with anaphylactic shock.

It is easy to sensitise rabbits with commercial benzylpenicillin, in contrast to guinea-pigs for which, for obscure reasons, benzylpenicillin is a very toxic substance. Rabbits sensitised to commercial benzylpenicillin preparations react to very small amounts of the penicilloylated protein contaminant with typical allergic increase of capillary permeability and, in slightly larger amounts, but still only fractions of a microgram, with anaphylactic shock.

It is possible to remove the protein impurity from the commercial

benzylpenicillin by various methods, for instance filtration through sephadex columns. Such purified penicillins do not produce allergic reactions in rabbits sensitised to either commercial benzylpenicillin or the penicilloylated protein impurity, not even in large doses. Nor do they sensitise rabbits, which shows that the anaphylactic reactions are not due to homologous penicilloylated proteins if such are formed. In man, benzylpenicillin freed from the protein impurity does not react in the skin test in 90 per cent of penicillin-sensitive patients. Thus, it seemed that the problems of penicillin hypersensitivity in man were largely solved by removing the protein impurity from commercial preparations. However, penicillin chemistry never ceases to produce surprises. It appeared that aqueous solutions of purified benzylpenicillin on standing started to react again in penicillin-sensitised rabbits, and this was found to be due to the formation of polymers with molecular weights up to about 3,000 (Grant *et al.*, 1962; Smith and Marshall, 1971). These polymers were shown not only to react as haptens, i.e. to produce an immune response in rabbits sensitised against penicilloylated proteins, but to be able to combine with proteins co-valently through a reaction mechanism not as yet fully understood and to elicit immune response, even of the anaphylactic type (Munro *et al.*, 1971). The penicillin polymers can be removed by gel filtration. As they appear only on standing in aqueous solution, it is important to use penicillin solutions as soon as possible after preparing them (Dewdney *et al.*, 1971). Anaphylactic shock is observed only in very rare cases after oral administration of the penicillin. The allergic symptoms after oral use of ampicillin manifest themselves in the form of a rash which can, on occasion, be very severe. Recent clinical trials have shown that when ampicillin freed from polymers is used, the incidence of rashes is reduced to that of the control level.

The last problem that I wish to mention briefly is the present state of the sensitivity of pathogenic bacteria to the penicillins. Is it true, as one frequently reads in the daily press, but sometimes also in the medical press, that penicillin therapy is less effective today than it was thirty years ago when it was introduced into medicine? Let us first consider the Gram-positive and Gram-negative cocci. Neither the pneumococcus nor the haemolytic streptococcus have lost their sensitivity to penicillin to any degree. Pneumonia caused by the pneumococcus is entirely a disease of the past, so that the large majority of young physicians have never seen a typical case. The causative agent of endocarditis lenta, a disease fatal without exception before the introduction of the penicillins, is as sensitive to penicillin as it was three decades ago and endocarditis lenta responds as well to penicillin therapy today as it did then. The same is true for the meningococcus. In most countries the vast majority

of gonococcal strains are still highly penicillin sensitive. However, in recent years a few gonococcal strains resistant to penicillin concentrations (0·5 to 5 μg) readily achievable under clinical conditions have been isolated in certain countries. Whether these have increased in numbers as the result of indiscriminate use of penicillin through gradual elimination of the penicillin sensitive strains, or whether they have always been present in the gonococcal population at the same small percentage but have escaped attention because of inadequate bacteriological examinations, is impossible to establish with certainty. Fortunately, they are sensitive to some of the synthetic antibacterials and therefore have not presented a clinical problem. Moreover, so far there is no evidence that they have spread to any other countries.

The problem of the penicillinase producing benzylpenicillin-resistant staphylococcus has already been discussed. It has been solved for all practical purposes by the introduction of the penicillinase-resistant semi-synthetic penicillins. It is true that staphylococcal strains have been isolated which are resistant to these penicillins but usually these strains are a mixture of mainly sensitive and a few resistant organisms, and so far do not represent a serious clinical problem, except in cases where the natural immunity of the host organism is so weakened that it cannot cope even with mild infections. There have been reports that in cases of advanced cancer, Hodgkin's disease, advanced lupus erythematosis and cardiac insufficiency, staphylococcal strains resistant to the action of the penicillinase-resistant penicillins have been a contributory factor to death, but this is to be expected as in such cases many factors that have no effect on the normal host organism can cause a deterioration in the condition of a very sick host organism. However, what is important in this respect is that epidemics, such as have occurred in hospital wards with the benzylpenicillin-resistant penicillinase producing staphylococcus, have never been observed with staphylococcal strains that are resistant to the semi-synthetic penicillinase-resistant penicillins.

But, there is no room for complacency and the situation has to be carefully watched. There have been reports from hospitals in some countries, for instance Denmark, of difficulties that were experienced in the treatment of some infections with staphylococcal strains resistant to the penicillinase-resistant penicillins. Fortunately, these infections usually respond to treatment with other antibiotics such as kanamycin, or to synthetics such as trimethoprim.

The situation is different and less favourable in the case of the Gram-negative rods. Ampicillin is so far one of the few semi-synthetic penicillins that is active against this group of pathogens. As yet no strain of *S. typhimurium* has been isolated that is not sensitive to ampicillin, and no clinical cases

of typhoid fever have been reliably reported that have not responded to ampicillin therapy. In contrast to *S. typhi*, many ampicillin-resistant strains of *E. coli* have been isolated. Their resistance is not due to penicillinase production, but to another, hitherto not understood, mechanism. The majority of *E. coli* infections of the urinary tract respond to ampicillin, but there are cases that do not respond. It is possible that in the closed space of a hospital ward such ampicillin-resistant *E. coli* strains can spread by cross infection from patient to patient, and thus cause epidemics that cannot be treated with ampicillin. Under these circumstances other antibiotics have to be tried, but frequently *E. coli* strains resistant to ampicillin are as resistant to the tetracyclines and chloramphenicol. In such cases, which fortunately are rare, the chemotherapeutic treatment encounters difficulties. Sometimes antibiotics of the second line of defence such as kanamycin, the polymixins or synthetic preparations such as trimethoprim, have been used successfully, but there is a great need for new kinds of antibacterial chemotherapeutic agents against *E. coli*.

In connection with the problem of the resistance of Gram-negative rods, such as *E. coli*, the phenomenon of the so-called infective resistance has to be discussed. Some years ago Japanese workers found that Gram-negative bacteria resistant to antibiotics have the property of transferring their resistance to sensitive strains. This transfer can be effected through phage action, or by a para-sexual recombination. In both cases a part of the nucleic acid system forming part of the genetic apparatus of the micro-organism, and responsible for its resistance to antibiotics, is transferred to and incorporated into the genetic apparatus of the sensitive micro-organism. The process is termed transduction in the case of the phage or conjugation in the case of the para-sexual recombination. The nucleic acid system transferred is known as episome. Transduction and conjugation are thus new methods of obtaining new strains of bacteria, in this case antibiotic resistant mutations; the classical methods used were radiation with ultra-violet or X-rays or treatment with chemical mutagens. The phenomenon of infective resistance is of great theoretical interest. The practical significance for the treatment of bacterial infections is at the moment not clear or may not exist. It has been postulated that through the veterinary use of antibiotics resistant strains of *S. typhimurium* could be obtained which are the causative agents of food poisoning. These would then not respond to chemotherapy. This fact in itself would not matter very much, as attacks of food poisoning by *S. typhimurium* are usually acute and short, and are not normally treated by antibiotics. It has, however, been postulated that these resistant strains of *S. typhimurium* could transfer their resistance to sensitive strains of *S. typhimurium*, and would thus render this

infection resistant to treatment with antibiotics. On the basis of these postulates it has been suggested that all antibiotics used in human medicine for veterinary use should be banned, and such suggestions have frequently been strongly supported by the daily press following the report of any failure of the chemotherapeutic treatment of an infection with a Gram-negative rod. Theoretically, everything is possible, but there is no evidence that the sequence of events of resistance transfer, as postulated, does in fact occur or, if it does occur, has practical significance.

Tetracycline and chloramphenicol therapy of Gram-negative infections has existed since 1947, and the veterinary use of the tetracyclines for the last twenty years. Despite this, the resistance of *S. typhimurium* has not increased and the clinical treatment of typhoid fever responds as well to chloramphenicol as it ever did. As the resistance transferred by transduction or conjugation is of multiple character, it would have been expected, if infective resistance did play a significant role, that the resistance to ampicillin of *S. typhimurium* would have increased. As was pointed out above, there are no well-documented cases of typhoid fever which did not respond to ampicillin therapy. On the other hand, ampicillin-resistant strains of *S. typhimurium* were isolated long before the introduction of this antibiotic into veterinary practice.

In any case, infective resistance is a process that occurs independently of the use of antibiotics though it can be facilitated by antibiotics that create an increased pressure of populations of naturally resistant bacteria through the process of eliminating the sensitive ones by Darwinian selection. In summary, the problem of infective resistance is of great scientific interest and must be watched from the practical point of view, but there is not the slightest evidence to justify panic measures, such as the complete ban of clinically used antibiotics in veterinary practice. Recent legislation in this country has, in fact, not banned this use, but has restricted it by making a prescription from a qualified veterinary surgeon compulsory.

Finally, it can be stated that the effectiveness of penicillin therapy has not diminished significantly over a period of thirty years. On the contrary, through the introduction of the semi-synthetic penicillins its possibilities have been greatly extended. The conquest of the benzylpenicillin-resistant staphylococcus is a significant advance in bacterial chemotherapy, and so is the successful treatment of infections caused by Gram-negative rods, such as *E. coli*, salmonella and proteus by ampicillin and *Pseudomonas pyocyanea* by carbenicillin.

It would seem safe to assume that in the foreseeable future the penicillins will continue to play an important and irreplaceable role in the chemotherapy of bacterial infections.

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