

ERNST BORIS CHAIN (1906-1979) AND PENICILLIN

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“In the whole history of medicine few achievements can match that of Ernst B. Chain. His success in preparing the first efficient antibiotic, penicillin, produced a real revolution in medicine. The use of penicillin, some of its chemical modifications, and the many other types of antibiotics developed as a result of Chain’s success has virtually wiped out the most dreaded bacterial epidemics, such as plague, cholera, typhoid, bacterial pneumonia, and many frequently fatal streptococcal infections, such as scarlet fever. History is full of reports of how whole countries, or even parts of a continent, were devastated by epidemics. Chain’s contribution initiated a new era in medicine. The number of people whose lives were saved by antibiotics in the past few decades is probably several times as large as that of the people killed during World War II (55 million). The genius of Paul Ehrlich, the father of chemotherapy, searching for compounds capable of destroying pathogenic bacteria without hurting the host organism, could not have foreseen the almost incredible efficiency and the wide scope of antibiotics in the fight against bacterial infections. In Ehrlich’s time, biochemistry was, of course, in its infancy. Chain is not only an excellent and competent biochemist; in addition he has a rare combination of abilities; not only does he explore the chemical properties of biologically active and important compounds, but he makes every effort to use his discoveries for the benefit of medicine. Thus the success with penicillin was not incidental. It was the outcome of his fundamental approach to his research.” (D. Nachmansohn, *German-Jewish Pioneers in Science*, 1979).

For his part in the development of penicillin, Boris Ernst Chain was honored in 1945 by the award of the Nobel Prize in Physiology and Medicine, shared together with Alexander Fleming and Howard W. Florey.

Chain:

Ernst Chain was born in Berlin in 1906. In 1930 he earned his Ph.D. from the Pathological Institute of the Charité Hospital in Berlin for his research on the optical specificity of esterases. Because he was a Jew, when Hitler came to power in 1933 Chain left

Germany for England.

Chain was recommended to Prof. Gowland Hopkins by Prof. J.B.S. Haldane, both of whom had seen copies of Chain’s Ph.D. thesis. Haldane was later to write that this was “what posterity may regard as the best and most important action of my life”. Chain was forever grateful to these two great men for having extended to him kindness when he was a refugee without means in a strange country. The adjustment to England was particularly painful as Chain had left behind in Germany his mother and sister. They were last seen alive at Theresienstadt. Chain earned a second Ph.D. in 1935 under Prof. Hopkins at Cambridge for his work on the acid-base properties of the phospholipids lecithin and cephalin. Chain received a grant from the London Jewish Refugees Committee and the Liberal Jewish Synagogue. This was arranged by Dr. Redcliffe N. Salaman, a trustee of Jews’ College, the plant virologist and the great expert on the potato. Years later Chain was to say, “I owe to this country - in particular the Anglo-Jewish Community, a great deal - in fact my existence.”

Hopkins recommended Chain to Prof. Florey for a position at Oxford, saying in part, “I feel that if his race and foreign origin will not be unwelcome in your department, you will import an acceptable and very capable colleague in taking him”. In 1935 Ernst Chain joined the staff of Prof. Florey at the Sir William Dunn School of Pathology at Oxford University, with the task organizing a biochemical section in his department.

Chain: “We discussed the plans for the future organization of the Department of Biochemistry, which Florey wanted me to organize in his institute. He had been convinced for some years that biochemistry was of very great importance for the development of pathology and that in fact all pathological changes had at their basis biochemical phenomena, and he told me that I would have a completely free hand in developing that section. The only problem that he himself suggested was that I should become interested in elucidating the mode of action of the bacteriolytic substance lysozyme. He himself had been interested in this substance for some years”. Alexander Fleming had discovered lysozyme in 1922.

In 1938, in the final stages of a project on lysozyme, Chain in the course of a literature search on antibacterial substances, came across Fleming's 1929 paper about penicillin.

Prof. Alexander Fleming, a bacteriologist at St. Mary's Hospital, London, observed in 1928 that around a spot of contaminating mould on one of his petri dishes a large circumference of the colonies of staphylococci had been killed and dissolved away (lysed). Fleming coined the term penicillin as a short way of referring to the antibacterial culture fluid, the word penicillin subsequently taking on the meaning of the active molecules that were present in the medium. The mould was later identified as *Penicillium notatum*. After the work by Fleming and also by others, nobody believed that penicillin was of any use in therapeutic medicine.

Chain: "I told Florey about my finding in the literature of penicillin. Though he never mentioned the word penicillin to me during our frequent conversations, he appeared to be familiar with the substance and asked me whether I was aware that in 1933 a group of well-known and successful natural product chemists, Raistrick and two of his colleagues, had worked on it, but could not make any progress because of its instability. I had not heard of this paper, but read it immediately after my talk with Florey. The paper showed that the active substance disappeared from the culture fluid after extraction with ether at acid pH, but could not be recovered after evaporation with ether. I thought at the time that in those experiments penicillin was very similar in character to lysozyme. Lysozyme disappeared from the culture after shaking with ether, but this was due to surface denaturation to which lysozyme was particularly sensitive. The reading of the paper by Raistrick and his colleagues only increased my interest in Fleming's penicillin. I told Florey that we would certainly find a method for at least partially purifying penicillin despite lability. He agreed. So we started our work on the isolation and purification, not in the hope of finding some new antibacterial chemotherapeutic drug, but to isolate an enzyme which we hoped would hydrolyse a substrate common on the surface of many pathogenic bacteria. The motive for this work was therefore a general biological one. There was nothing in Fleming's

paper which justified the hope that his penicillin was a substance or mixture of substances of extraordinarily high therapeutic power, which for some reason, was neglected by everyone for many years."

Isaac Berenblum (who in 1950 joined the Weizmann Institute of Science and set up the department of experimental biology and was Chairman of the Israel society for the fight against cancer) recalls a tea-time meeting at that time at which Florey and Chain were present:

"The talk, he says, turned to antiseptics, and then to those that could not be used internally. At this point Florey first recalled Fleming's discovery of penicillin and then mentioned Raistrick's experiments in which penicillin had appeared to be unusually unstable. Chain, says Berenblum, overheard the conversation and, in character, chipped in with the opinion that in that case Raistrick could not be such a very good chemist. It must, he is remembered as adding, be possible to produce it in a stable form. This assertion, it can be claimed with considerable plausibility, played its part, if not indeed the vital part, in leading to the work from which penicillin finally emerged as the life-saver it was."

Chain's part of this project was the chemical and biochemical properties of the substances. Florey's part was the study of their biological properties.

Chain: I concentrated on the isolation and characterization of the active antibacterial principle. The first experiments which I carried out showed that penicillin was not a protein, but a low molecular substance which diffused readily through cellophane membranes. I was, at first, disappointed with the finding, for my beautiful working hypotheses 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 antibacterial with that degree of instability known, 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, and thus it was of obvious interest to continue the work. 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 which 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 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 10⁶, i.e. it was about 20 times more active than the most active sulphonamides".

Chain decided to ask his friend Barnes to do the first preliminary crucial experiments showing that penicillin was not toxic to mice. As to the above experiment, Chain was later to say: "The barriers were removed to our hopes and our dreams, and the fears that our purified extract would be harmful were all banished. "...[It was] the crucial day in the whole development of penicillin and the day on which everything became possible to us."

On May 25 1940, Florey carried out the experiment of injecting eight Swiss albino mice with a virulent strain of streptococcus haemolyticus (the bacteria that was causing the death of two mothers in every thousand childbirths from puerperal fever). After 16 ½ hours, the four control mice were dead and the four mice that were treated with penicillin were alive. Penicillin was now a known life-saver.

From May 26 through June 4 took place the evacuation from Dunkirk of three hundred and forty-five thousand mostly British and other allied troops.

On June 14, 1940 the Germans occupied Paris. In 1940 the British feared an invasion of England. Secretly, Chain, and other members of the group smeared the linings of their clothes with the mould. If the Germans were successful, it was hoped that one of the group would manage to escape and carry on the work.


On August 24, 1940, the now classic paper by Chain, Florey, Gardner, Heatley, Jennings, Orr-Ewing, and Sanders appeared in *Lancet* reporting on the effectiveness of penicillin in vivo against pathogenic organisms.

The failure of some cultures of *Penicillium notatum* to produce penicillin led Chain to look for an enzyme produced by contaminating bacteria that inactivated the antibiotic. In 1940 Abraham and Chain reported such an enzyme which they called penicillinase that blocked the activity of penicillin. Penicillinase was one of the causes of bacterial resistance.

Due to the difficulties of carrying out development in wartime England, major parts of the project were transferred to the Americans. Clinical trials of penicillin were carried out. By 1944 methods of producing the mould in quantity were developed. The first massive use of penicillin was made on June 6, 1944, D-day, where it saved thousands of lives.

The correct chemical structure of penicillin was proposed independently at Oxford (Abraham, Chain, Robinson, Baker) and by the Merck laboratories. It was verified by x-ray techniques by the group led by Dorothy Hodgkin at Oxford in 1945. Due to availability of computers very soon after, Chain was later to comment that the work on the determination of the penicillin molecule might very well have been the last case whereby classical methods of organic chemistry were employed to determine the structure of a natural product.

Due to the military importance of obtaining a synthesis of penicillin, there was a ban both in Britain and the United States on publication of the chemical work from 1943 until after the war. During 1944 and 1945, more than a thousand chemists at 39 laboratories in both England and the United States worked on a synthesis, but failed to achieve their goal. A synthesis was to be achieved in 1957 at MIT after ten years work by John C. Sheehan and his colleagues.



Chain considered a large fermentation plant essential for continued work on antibiotics, the funds for which he was not able to obtain in Britain. He accepted a position in Rome where he remained from 1948 until 1964. At that time he accepted the Chair of Biochemistry at Imperial College, from which he retired in 1973.

The generally held medical opinion up until the discovery of the therapeutic use of penicillin was that any substance that was lethal to bacterial cells also had to be to some extent toxic to that of the host. We now understand that penicillin inhibits the final assembly of the peptidoglycan molecule that is essential for the stability of the bacterial cell wall, so that the cell wall disintegrates. Animal cells lack cell walls and so are not affected. Antibiotics are now known that work by other mechanisms.

Chain (1971): “To convert this laboratory curiosity into a practical useful cheap drug, necessitated the work of many hundreds of scientists and technologists over a period of many years, and this work was mainly carried out in industrial research laboratories, 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 which allowed the growth of the mould in stirred stainless steel fermenters in volumes which have reached over 227,000 l. A whole new chemical

technology was developed which today is one of the main pillars of the pharmaceutical industry... Through strain improvement by genetic mutation techniques, improvements of the culture medium and improved aeration, the yields of penicillin have risen from 1-2 units/ml which we obtained, to 25,000 units/ml; penicillin has become one of the cheapest drugs in clinical use.” Penicillin is today still manufactured mainly by fermentation methods”.

Chain was associated with many Jewish and Israeli organizations. He was particularly active on behalf of the Weizmann Institute of Science, where he was a member of the Board of Governors and of the executive council. He was a member of the world executive of the World Jewish Congress.

According to Selman Waksman, who was awarded the 1952 Nobel Prize for his discovery of streptomycin, the first antibiotic effective against tuberculosis, “Man in his historical progress has utilized various animals and plants inhabiting the earth in order to increase his food supply, to improve his health, and to provide shelter for himself. He domesticated these animals and plants and developed them for his needs. More recently, man developed special varieties of microbes which yield life-saving drugs. Thus, man has brought about a series of domestication processes, including now also the antibiotic-producing microorganisms.”