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## *1 The Lonely Search*

I have often been asked why I undertook to synthesize penicillin after the most extensive and intensive effort in the history of organic chemistry, indeed of all medical science, had failed. Only the Manhattan Project leading to the development of the atomic bomb equaled the efforts of the Office of Scientific Research and Development during World War II to produce a synthetic penicillin. When the penicillin project failed to accomplish this goal, most research directors in the pharmaceutical industry and academic chemists in the universities were led to the conclusion that the synthesis of penicillin was an impossible problem.

For several years a synthetic penicillin had seemed just within reach. In the early days of the research program, chemists optimistically thought that because penicillin was a small molecule, synthesizing the compound would pose no special problems. However, although chemists working in the United States and Great Britain did in time describe the characteristic chemistry of penicillin and, after much disagreement and confusion, even worked out the structure of the penicillin molecule, they failed in their primary goal: to design a rational process for synthesizing penicillin. The penicillin molecule proved to be trickier than that generation of chemists had suspected. After many years of intensive research, most investigators involved in the study of penicillin felt that even if a successful synthesis were finally designed, that feat would probably never amount to more than a clever scientific stunt. In any event, according to most authorities on penicillin in the late 1940s and early 1950s,

the naturally fermented penicillins would continue to dominate the field. They were plentiful and, as far as anyone knew at the time, they probably could not be surpassed.

Mine was a lonely search for the right combination of materials and methods by which to make a penicillin in the laboratory. After their own tentative efforts to continue penicillin research were frustrated, most other synthetic chemists in the field abandoned penicillin research to me. How I laid the groundwork for the synthesis of penicillin, how I recast the problem, the new tools I devised for the job are the subjects of my book.

While this is the story of penicillin, it is also a personal story of frustration and discovery. Accounts of scientific discoveries often oversimplify the bewildering complexities of research. In movie versions of science, for instance, one sees the moment at which the scientist finds the long-sought microbe under the microscope or finally discovers the hoped-for product in the test tube. In all discovery there is a moment of what psychologists call the Aha! experience. That triumph occurs in the privacy of the creative mind. Artists and scientists share that particular joy. However, there are the long periods during which the work must go on before that moment of high enthusiasm. And after the discovery, other complexities intrude themselves. If the discovery is an important one, the public soon becomes aware of it. The private triumph becomes a personal and social responsibility at that point. In my case, the struggles after I had found synthetic penicillin in my test tube were at least as arduous as the labors that preceded that exciting moment. The scientific triumph led to the development of a whole family of life-saving antibiotics. Just as important for the scientist, the successful synthesis of penicillin reaffirmed the triumph of reason in a world of disorder.

For thirty years after the discovery of a natural penicillin by Sir Alexander Fleming, the source and the nature of the penicillins changed only slightly. From the time Fleming

published his discovery in 1929 through the 1930s very little new information was developed about penicillin. Today we are familiar with the wonders of antibiotics, but fifty years ago the unprecedented benefits of enlisting the penicillin molecule in the battle to destroy disease-producing organisms were only dimly perceived. Even medical experts, who might have known better, considered penicillin a minor laboratory curiosity. Despite early efforts, ten years after Fleming's discovery of penicillin in 1928, the chemical structure of penicillin had yet to be established; the substance was not available in appreciable amounts for medical therapy or for scientific research; and few people thought that antibiotics had much of a future.

World War II changed all that. The military emergency raised the medical problems of treating battlefield injuries and disease from the level of academic research to national crisis. The sulfa drugs, themselves only recently developed, were one important means of treating disease chemically; but they were limited. The sulfa drugs had a very narrow spectrum of activity; many diseases could not be treated successfully with them. Some bacteria were capable of developing resistance to sulfa drugs with alarming rapidity. Finally, the sulfa drugs could interfere with the body's own natural defense against infection. As one response to the pressing needs, the United States government undertook a large-scale effort to produce penicillin in therapeutically useful quantities. Exploratory research conducted in Great Britain by Sir Howard Florey, Dr. Ernst B. Chain, Dr. N. G. Heatley, and Dr. E. P. Abraham, all working at Oxford University, had revealed the exciting potential of penicillin. Although the Oxford team had produced only small amounts of concentrated but still impure penicillin in their laboratory, they had successfully demonstrated the value of penicillin in treating a variety of otherwise intractable diseases and had thereby extended the pioneering work done by Fleming.

As part of the wartime drive instigated by Florey and others toward improved biological production of naturally fermented penicillin, the U.S. government initiated a massive effort organized by the Office of Scientific Research and Development (OSRD), Committee on Medical Research (CMR) to determine the chemical structure of the penicillin molecule and to try to synthesize the drug by purely chemical means. The government-organized effort to produce penicillin went in two directions: one toward the maximum production of naturally fermented penicillin; the other toward the chemical synthesis of penicillins. The two projects were closely related, of course, for the raw materials of the chemical work were made possible only by the successes of the biological work.

At the height of the effort during World War II, more than thirty-nine major laboratories were involved in the efforts to synthesize penicillin. At least one thousand chemists were involved in the project. Failure was mounted upon failure, despite this massive investment by the U.S. government, private commercial interests, and academic institutions. The total chemical synthesis of penicillin came to be known—and with some justification—as the impossible problem.

The general impression current among people familiar with the history of penicillin is that British scientists deserve the full measure of credit for initial insights into the virtues of the *Penicillium* mold as well as most of the credit for describing the fundamental chemical properties of the substance penicillin. According to this popular, albeit mistaken, view of the history of penicillin, Americans were parvenues who came into penicillin research only after the German bombing of Britain made British research in penicillin difficult and industrial production practically impossible.

This view of history assigns American scientists a relatively minor role in the development of penicillin. The belief was current as early as the beginnings of the penicillin devel-

opment (*British Medical Journal*, August 5, 1942, p. 186; Levaditi, 1945). The British were given credit for the scientific insight; the Americans were thanked for their industrial ingenuity.

One of the American pioneers in penicillin research tried to correct this misconception at the source. He agreed that the work of the British researchers should be recognized, but, he wrote, "I would not be satisfied with my attempt to review the historical development of penicillin if I failed to correct the impression, which prevails to some degree, that scientists in this country were not intensively interested in penicillin before our government agencies, stimulated by Florey's visit [June 1941], became actively engaged in sponsoring its development" (Herrell, p. 8).

At least three major scientific projects involving penicillin were already in progress before representatives of the Oxford group enlisted the aid of the Americans in the summer of 1941. In 1930, Roger D. Reid, working at the Pennsylvania State College, compared cultures of twenty-three molds with subcultures of Fleming's original *Penicillium notatum* "to find others than the one isolated by Fleming which would produce a similar inhibitory substance" ("Some properties of a bacterial-inhibitory substance produced by a mold," *Journal of Bacteriology* 29(1935):215-221). None but Fleming's mold did. In 1940 and 1941, workers at Beth Israel Hospital and Columbia Medical College in New York and the Mayo Clinic in Rochester, Minnesota, had begun serious studies of the chemistry of penicillin and its chemotherapeutic action.

The general impression is mistaken in another respect as well. One would be led to believe that a cadre of American technicians, mobilized by the United States government during the hectic preparations for war and motivated by the industrial instinct for profit, had nothing more sophisticated to do than scale up the small laboratory procedures developed by the British to full-blown industrial production.

This impression is far from what actually happened in those difficult years of the early 1940s.

Even when the time came for the industrial production of penicillin, countless questions remained unanswered. What was the structure of the penicillin molecule? What were the most effective ways of isolating penicillin from the fermentation broth in which it was produced? What were the most appropriate methods for growing the mold? And, ultimately, could the drug be synthesized?

Of course commercial interests influenced the development of penicillin. Those same commercial interests, however, required the most sophisticated fundamental research into the microbiology of the *Penicillium* mold and the chemistry of its most important product.

It was known that the penicillins were relatively small molecules, with molecular weights of about 350. That was encouraging, for the relatively low molecular weight put penicillin well within the range of molecules that had already been synthesized by industrial processes. Unfortunately, we soon realized that the size of the molecule was the least of our problems in working with penicillin. From the point of view of the organic chemist, penicillin was a molecule that was far ahead of its time. The fact that a thousand of the best chemists in the United States and Great Britain could not come up with a definitive synthesis of penicillin did not reflect upon their unquestioned abilities. Rather it indicated to me that the appropriate techniques and reactions for putting together the penicillin molecule simply had not yet been discovered.

The essential portion of the penicillin molecule, in the words of R. B. Woodward, one of the outstanding organic chemists of our time, was "a diabolical concatenation of reactive groups" that defied all the chemists' most subtle approaches or brutally direct frontal attacks. As we were to discover, a seemingly enchanted ring of chemically active centers, one of them a beta-lactam ring, put the synthesis

of penicillin beyond the reach of the most advanced methods available to chemists in the 1940s.

The history of chemical research on penicillin is, by and large, a history of controversy concerning the beta-lactam. The beta-lactam structure was unknown in natural products at the time. A few beta-lactams had been made in the laboratory, but these were well shielded by large groups on the molecule and were not nearly as reactive as the beta-lactam found in penicillins. Therefore, few chemists at the time thought that a beta-lactam could be the heart of the penicillin molecule. Conventional wisdom forbade the presence of the structure and even more forcibly prohibited the presence of a beta-lactam along with other known portions of the penicillin molecule.

The beta-lactam ring is the critical part of the molecule. With that ring structure intact, the molecule possesses antibiotic properties. When the ring is disturbed—and many conditions can disturb it—the desired antibiotic properties disappear. Chemists were faced, therefore, with the difficult problem of working with delicate and reactive groups on the penicillin molecule, groups with which they had had little prior experience, and with chemical tools that were simply too crude for the job. All efforts failed to close the beta-lactam ring or to protect it while performing other chemical operations. At the time of my successful synthesis of penicillin in 1957, I compared the problem of trying to synthesize penicillin by classical methods to that of attempting to repair the mainspring of a fine watch with a blacksmith's anvil, hammer, and tongs.

In contrast to the synthesis program, efforts to master the natural fermentation of penicillin were completely successful. The delicate and chary *Penicillium* mold was coaxed by science and industry into producing more and more precious penicillin. Scientists learned increasingly efficient ways of isolating, concentrating, and purifying the product. The project to elucidate the chemistry of penicillin, however,

could boast of far fewer successes. At the end of the wartime penicillin program, penicillin had not been synthesized, and confusion reigned among chemists even about the molecular structure of the compound. After years of study, chemists had accumulated a great deal of information about the chemistry of penicillin; but the more we learned, the more complicated the penicillin problem became.

I was particularly intrigued by the challenge of penicillin synthesis. I had worked with Dr. Max Tishler at Merck & Company in Rahway, New Jersey, on producing streptomycin, and earlier I had studied organic synthesis with Werner Bachmann at the University of Michigan. Perhaps my most important work with Professor Bachmann, while I was a post-doctoral fellow at the University of Michigan, was to develop a commercially feasible synthesis of RDX, the explosive known as cyclonite. Our work, which made large quantities of this high explosive available to the Allies, revolutionized submarine warfare and gave the Allies the advantage at sea. RDX was the explosive that made possible the development of the bazooka and the blockbuster. *Plastique*, as it was known by the French Resistance, changed guerrilla warfare. And so when I met Max Tishler I was already experienced in applying the arcane knowledge of organic syntheses to the solution of real-life problems. Having participated in this work, I was eager to join in the efforts to develop the antibiotics.

Perhaps I was motivated by my vivid recollection of the year I had spent struggling against pneumonia and mastoiditis. That struggle nearly cost me my life. If my doctors had had a course of treatment as effective as that made possible by penicillin, I would probably not have lost that year. As a problem in organic chemistry, moreover, the synthesis of penicillin was a significant challenge. Many had attempted the synthesis. Some had come close; none had succeeded.

Why was the penicillin molecule so difficult to synthesize? Some investigators had believed that the difficulty was due

to the source. The lay public and even some professionals believed that because penicillin came originally from a natural source, only living organisms could produce it. We may have difficulty giving this argument much credit today, but such vitalist notions were still current only a few decades ago. And these scientific prejudices occasionally interfered with the rational study of penicillin.

A second difficulty was that old chemical notions interfered with the discovery of new solutions demanded by the penicillin problem. Many prominent chemists doubted that the penicillin molecule could ever be assembled in the laboratory. At one point as many as ninety different structures were proposed for the small penicillin molecule. Eventually, however, the field was narrowed to two: the beta-lactam and the oxazolone-thiazolidine. Evidence mounted in support of a structure for penicillin containing the beta-lactam ring, but no naturally occurring substance was known to contain this structure. The alternative oxazolone-thiazolidine formula was more familiar to chemists. One of the many ironies of the penicillin story is that at least two different reactions to synthesize penicillin that were directed by what turned out to be the wrong formula did actually produce minute quantities of penicillin. On the contrary all efforts to synthesize penicillin by what turned out to be the correct formula proved too difficult and failed.

After studying the results of those early efforts, I reached two conclusions that shaped the course of my own career in chemistry and incidentally changed the course of penicillin research. One was that penicillin was a difficult molecule but, like any other natural substance, could be synthesized by a rational chemical process. The second conclusion was that penicillin could not be synthesized by any combination of techniques known to chemistry at that time. New methods were needed.

My moment of decision was 1948. When I moved from Merck to MIT, I set about devising new methods that would

be useful not only in the limited matter of synthesizing penicillin and related compounds but also in solutions to broader synthetic problems encountered in modern organic chemistry and biochemistry.

Many of my friends openly questioned the wisdom of getting involved with penicillin again. The synthesis of that compound was widely considered not only a difficult problem but an elusive one. From the earliest days of the penicillin research, major scientific and technological breakthroughs were continually believed to be just around the corner. But isolation, purification, production, and the chemical identification of penicillin all proved to be inordinately difficult.

Scientific work is ultimately objective; on the way to those ultimate goals, however, scientific work is an art. Intuition, personalities, and luck all play important roles. The laboratory is orderly, the glassware is clean, and the notebooks are pristine and to the point. But the orderly laboratory is a privileged and magical place surrounded by a jungle of disorder. One sympathetic colleague at MIT turned out to be secretly working for a patent adversary. Old friends became acrimonious scientific rivals. Old rivals proved to be surprisingly supportive. In the complicated story of penicillin they all played major roles.

The story of Fleming's stroke of genius is well known. Alexander Fleming had been searching since World War I for antibacterial agents that would kill bacteria selectively without damaging the tissues of the host. In 1928, Fleming found what he was looking for. He noticed that a contaminant in one of his culture dishes was killing the once-thriving colony of staphylococcus bacteria. Fleming accepted this fateful invitation and took up the study of the world's first safe systemic antibiotic. He identified the contaminant as a variety of the mold *Penicillium* and named the antibiotic substance penicillin.

I became involved in penicillin research in a similarly fortuitous manner. After I had been at Merck for about a year, I had some good results to show for my work on calcium pantothenate, streptomycin, vitamin B<sub>6</sub>, and a few other projects that had turned out rather well. One afternoon Dr. Randolph Major's secretary called me to set up a meeting with the boss. Dr. Major was at that time director of research for the entire group at Merck, not only the chemical division but the microbiological division and others as well. In an administrative sense he was at least two notches higher than I was. I was a group leader. My immediate superior was Dr. Max Tishler, above him was Dr. Joseph Stevens, and then came Dr. Major. So he was considered to be at the top of the scientific pyramid at Merck.

Although I had had some social contact with Dr. Major while at Merck, we had not had much professional contact. I was rather puzzled about why he would want to talk with me.

When I entered his office, Dr. Major said in his characteristically diffident way that he had been noticing from our research reports that I was making rather good progress. He expressed his pleasure.

"You have worked with Professor Bachmann," Dr. Major said to me.

"Yes," I said.

"He is well known for his steroid work," said Dr. Major. Again I said yes, but that I had not done any work with him on steroids. We had had frequent group seminars and so I was familiar with the literature and current work being done on the steroids. But I could not pretend to be an authority.

"Well," said Dr. Major, "we have two problems coming up that we think are going to be important. One has to do with the steroid cortisone. If you would like to work on that problem, I will assign it to you."

When I told Dr. Major that I would feel comfortable working on that problem, he interrupted me to say, "We do, however, have someone coming from Princeton, from

Everett Wallis's laboratory, who has done some work on steroids. His name is Lewis Sarrett."

Dr. Major then went on to say that Merck had decided to work on another interesting problem, namely penicillin. I had heard a little bit about penicillin. I knew that it was supposed to be a remarkable drug but very difficult to work with chemically.

"Yes, that's right," said Dr. Major. "But we think that it is so important we should start on it as soon as we can." He paused for a moment. "So, I will give you your choice. Which one would you like to work on, cortisone or penicillin?"

After a moment I said, "If it is all right with you, Dr. Major, I'll take the penicillin."

Lewis Sarrett eventually synthesized cortisone and later became president of Merck Sharp & Dohme Research Laboratories. I have sometimes joked with him about what would have happened if we had reversed roles. My own guess is that we both would have failed.

Merck had begun developing methods for the production of another antibiotic, streptomycin. Karl Folkers was deeply involved in the research to determine the structure of streptomycin, but he kept running into the problem of purifying the compound for his own research and for general distribution. The drug was produced by fermentation. It was purified by adsorption on charcoal and then elution to remove the material from the carbon. In spite of Folkers's best efforts, most of the streptomycin produced in this way contained impurities that resembled histamine and produced in patients histaminelike allergic reactions—elevated blood pressure, pain, and allergic rashes. Consequently, Merck felt that the streptomycin was still too impure and dangerous for general release.

Max Tishler's group had been working for about six months on the purification of streptomycin. About the same

time, my mother had contracted a difficult urinary tract infection. I realized that she was not responding to any of the treatments then attempted and felt that streptomycin might be just the drug she needed. Even though the drug was still in its developmental stages, I thought it was worth the chance. I went to Max Tishler.

Max heard my plea with his usual sympathy and compassion. "Yes, John," he said, "we can make enough streptomycin available to you through our medical department. We can get it to your mother's doctors and see how it works." Then, almost as an afterthought Max said, "You know, John, we've been having a lot of trouble lately in producing pure streptomycin."

"Yes," I said. "I had heard something about the problem."

"Well," Max said, "you know something about this histamine problem. I would appreciate it if you and your group would work on it. The problem is getting very serious."

I asked Max for all the written reports on the histamine problem and for about 100 grams of the impure streptomycin. The reports were impressive. Tishler's and Folkers's groups had tried just about every conventional adsorbent and nearly all the solvents one would normally think of. They even tried chromatography methods. All to no avail. The impurities could not be separated from the streptomycin.

I realized that streptomycin is an amino sugar, very much like cane sugar (sucrose). It is extremely soluble in water, so that I could easily make up a 25 percent solution of streptomycin, just as one could make a sugar solution. The streptomycin solution resembled honey in consistency. I also realized that although amino sugars are soluble in water, they are virtually insoluble in certain organic solvents that are immiscible with water. This extreme solubility in water and insolubility in solvents that do not mix with water struck me as properties worth exploiting in the purification of streptomycin.

Then I remembered an old German process for purifying cane or beet sugar. The major portion of the sugar could be crystallized directly from a concentration of the juice. After removing as much sugar as possible by crystallization, a substantial portion of the impurities remaining in the molasses could be separated from the residual sugar by extraction with liquid phenol. The two problems were similar.

When I mixed phenol and water with the dark brown mixture of streptomycin and its impurities in a separatory funnel, the brown color went almost immediately into the phenol layer. The water layer was clear. I separated the two layers, washed the water layer with ether to remove traces of the phenol, degassed to remove traces of the ether, and then freeze-dried (lyophilized) the remaining product.

Freeze-drying is a slow process. I let it go overnight. In the morning I found the most beautiful colorless solid product at the bottom of the flask. "Now have your people run a test on this," I said to Max. In a few hours, he came running back to say, "That is the best streptomycin we have ever seen."

That problem took me a day to solve. The penicillin problem took nine years.

My friends were probably right in trying to steer me away from penicillin. I had just arrived at MIT, and a young academic chemist is usually dissuaded from undertaking a problem in which his progress is likely to be painfully slow. Because he is bound to be subjected to periodic review by faculty tenure committees, a young chemist is better advised to try dazzling them with a blaze of flashy experiments and a trail of scholarly publications. I settled for a number of smaller victories. There was a whole series of papers with the running title "The Synthesis of Substituted Penicillins and Simpler Structural Analogs" that gave us a lot of running room. We started out with the simple compounds that we could make and gradually worked our way into the more difficult areas of penicillin research.

In all this work, I had one advantage over chemists work-

ing in the government-sponsored project during the war. I had, in my view at least, unlimited time. The chemists working on penicillin during the war were under the most severe time constraints. If the program was to continue, successes had to be almost immediate. I decided that I would keep trying until penicillin was synthesized even if I became professor emeritus in the attempt.

My group and I worked out a series of simpler compounds, foothills on the way to the peak. For example, by the time we began our work, the beta-lactam was known to be a key element of the penicillin molecule. By the end of the penicillin project, it was well recognized that the business end of the penicillin contained the baffling beta-lactam ring. Anything that destroyed the ring also destroyed the antibiotic properties of the compound. We made some very simple beta-lactam compounds. This in itself was not much of a trick if one stuck to the older methods. Such beta-lactam rings had been made in the laboratory before. But the older methods had not worked in the synthesis of penicillin. The importance of our early work was that we devised at least four different new methods for making beta-lactam rings under the mild conditions required for a penicillin synthesis.

Once I decided that penicillin was an important problem, and one that had a solution, I never re-evaluated my position. No matter how discouraging the laboratory work turned out to be, I simply went back in and tried more approaches. I went back to the library and read more research reports. I thought more about the problem. As long as I could avoid asking myself the defeating question "Should I really be in this?", I remained immune to the anxieties that accompany scientific research. For me it was always forward march, never halt, never retreat.

Not all scientific projects can be handled in this way. This is certainly not the case in industry, for example. There a research project is reviewed frequently and may be terminated abruptly if the managers of the laboratory feel that

progress is too slow or if they simply decide that it is no longer an interesting or profitable research problem for the company to support. At MIT, on the other hand, I was a research committee of one. I could make the decision to spend the rest of my life on the penicillin problem; it was only my career that was on the line.

I did eventually reach a point when I began to believe that sooner or later we would come up with a rational synthesis of penicillin. During the early 1950s, I was sure that we had developed adequate methods for the delicate synthesis and that the end was in sight. I could not have predicted exactly what methods or materials would be involved in the reactions, but I did begin to feel that these were relatively minor details to be cleaned up. The difficult work of conceiving the general plan of the synthesis and of discovering the singularly appropriate coupling agent for closing the beta-lactam ring had already been accomplished.

It also became apparent in handling the penicillin molecule and some of our simpler analogs that the penicillin structure was not nearly as sensitive as it had been generally believed to be. An inspection of the literature—and I must add my own contributions to that literature as well—emphasized the great sensitivity of penicillin to degradation by acid, base, heat, and other factors. I took few pains to dispel this belief. Certainly it was to my advantage that chemists believe that penicillin was a tough molecule to work with and that anyone working with it should realize that he had a tiger by the tail.

One of the remarkable features of the penicillin work is that there was no competition from the years 1948 to at least 1957. It is essentially unparalleled in modern organic chemistry to have such green pastures entirely to oneself. I had always assumed that I would have lots of competition. The penicillin molecule, after all, had a glamorous history and promised an equally glamorous future.

I once asked R. B. Woodward why I was allowed to approach the penicillin problem all by myself. He said, "John, we all knew that it was in good hands and that you would get it eventually." I believe it is more likely that other chemists were simply tired. They had worked for years on the problem and no amount of talent or money or time could bring them closer to a solution. In fact, it sometimes appeared that the more they knew about penicillin, the farther they moved from understanding it.