A Brief Look at the History of Antibiotics

Streptomycin, sulfa drugs, and penicillin were the first antibiotics to move from the laboratory into general clinical use. Subsequently, scientists have developed many new forms of these antibiotics and are currently looking for new types of antibiotics with targets different from those of the older antibiotics, but as will become evident, this quest is not as easy as it was in the early years of antibiotic discovery and development.

Purifying Soil and Beautiful Earwax

Antimicrobials have not always been as user-friendly as they are now. Today’s antibiotics have undergone extensive testing and approval procedures. As mentioned in the first chapter, the earliest antibacterial compounds, such as mercury and the derivatives of arsenic, were almost as toxic for us as for the bacteria. This early approach to therapy was based on what has been called the poison principle. That is, known poisons were administered in limited doses in the hope that the bacteria causing the infection would be killed before the person being treated.

Rene Dubos, a research scientist at Rockefeller University, was the first to take a very different view of how antibacterial compounds should work. His view emerged naturally from his lifelong study of soil microbes. Dubos had an almost religious reverence for the purifying properties of soil. In an experiment that was destined to make history, he targeted the bacterium *Streptococcus pneumoniae*. *S. pneumoniae* is the most common cause of bacterial pneumonia and has been a major killer for centuries. Dubos’ strategy was to mix a laboratory culture of *S. pneumoniae* with an aqueous extract from soil. His theory was that there must be bacteria in soil that could kill or inhibit the growth of *S. pneumoniae* because the ecological balance would be maintained only if such microorganisms existed. His idea made sense because *S. pneumoniae*, despite its ability to
colonize the human body and cause serious disease, has not taken over the world and is very uncommon in soil.

Dubos isolated a soil bacterium, *Bacillus brevis*, which produced a substance that was antagonistic to the growth of *S. pneumoniae*. Dubos may not have realized it at the time, but he was on the verge of a new paradigm for fighting bacterial infections and a new era of medicine.

Unfortunately, in the beginning of his quest, all was not sweetness and light. The initial form of his antibacterial substance had some rather unappealing characteristics. As Rollin Hotchkiss, a colleague of Dubos, later described it in the book *Launching the Antibiotic Era* (Rockefeller University Press, 1990) the antibacterial compound was a “crude brownish material [that] . . . congealed into a sticky mass as unpleasant as so much uncouth earwax. But it was a powerful wax all right.” An extract from this “uncouth earwax” was able to inhibit the growth of such bacteria as *S. pneumoniae*. Ultimately, Hotchkiss and others isolated the active component of the uncouth earwax, a compound we now know as gramicidin.

Gramicidin is a peptide that forms channels in bacterial membranes. Because the cytoplasmic membranes of bacteria and humans are very similar in composition, gramicidin proved to be too toxic for internal use in humans, although not so toxic as mercury and arsenic. It is still used as an ingredient in topical antibacterial preparations. The importance of the discovery of gramicidin was that it directed attention to soil microbes as a possible source of antibacterial compounds. Subsequently, soil bacteria and fungi proved to be rich sources of antibiotics, such as penicillin and tetracycline, that were much more human friendly than gramicidin.

**The Sulfa Drugs**

Parallel to the quest by Dubos and his colleagues for natural products of soil microbes, another line of research was emerging. This approach was to modify compounds that kill bacteria to make these compounds less toxic for humans. This approach had been tried with arsenic, but the derivatives of arsenic were still too toxic and were not very effective against bacteria. As chemists became more sophisticated, however, they experienced their first success: synthetic compounds called sulfonamides.

The discovery of sulfonamides arose from the observation that a red dye, Prontosil, could cure some cases of pneumonia. In the early 1930s, scientists discovered that the active component in Prontosil was a compound that was converted by human cells into an antibacterial compound called sulfanilamide. Sulfanilamide was not nearly as toxic to humans as
mercury and arsenic. Thus were born the drugs that came to be known as “sulfa drugs.”

We now know that sulfanilamide and other sulfa drugs mimic para-aminobenzoic acid, a precursor of the vitamin folic acid. Bacteria make their own folic acid, whereas humans obtain preformed folic acid from their diet and thus do not have to synthesize it themselves. Because of this difference in metabolism, chemical mimics of para-aminobenzoic acid affected bacteria adversely by inactivating an enzyme the bacteria needed to make folic acid, but they had no effect on human cells, which do not have to produce folic acid from such precursors. From these two parallel lines of research emerged a new principle: the principle of selective toxicity, toxicity against bacteria but not against humans.

Penicillin Is Discovered (Almost by Accident)

There has been a longstanding debate about who actually discovered penicillin, the antibiotic that, despite the early successes of the sulfa drugs, unquestionably gave the antibiotic revolution its huge momentum. Some historians credit Alexander Fleming, at Oxford University in the United Kingdom, as the discoverer of penicillin. His contribution arose from a series of experiments with a bacterium that was a notorious cause of life-threatening wound infections, *Staphylococcus aureus* (Fig. 2.1). Fleming

![Figure 2.1](image_url)  
*Figure 2.1  Staphylococcus aureus*, a cause of serious wound infections, as seen with an electron microscope. (Courtesy of Janice Carr, CDC, Atlanta, Ga.)
noticed that on some agar plates that had been inoculated with *S. aureus*, which normally forms colonies over most of the surface of the plate, there was an inhibitory zone in which no bacteria grew. This zone had developed around a colony of what turned out to be a fungus, *Penicillium notatum*, which was later identified as the producer of penicillin. However, this discovery was not as intentional as textbooks tend to imply.

Alexander Fleming was a microbiologist-physician who was interested in a more effective treatment for wound infections. Fleming had focused on *S. aureus* because of its role in war wound infections. He had some interest in new compounds that might be used to control bacterial infections, but his primary focus had been on known antibacterial compounds such as arsphenamine, a derivative of arsenic that had attracted much attention because of its success in curing some cases of syphilis. However, arsphenamine was a rather toxic compound.

Fleming knew that some of the bacteria with which he was working could be dangerous. Accordingly, he discarded used agar plates containing colonies of *S. aureus* into trays filled with a disinfectant that was supposed to kill the bacteria. Like some microbiologists of the time, however, he could be careless with his discarded specimens.

Read an account of what actually happened, written by Norman Heatley, one of the early workers in the area of antibiotic research (*Launching the Antibiotic Era*).

In the summer of 1928, Fleming goes on holiday, unaware that he has been chosen by the Fates to take the first steps in introducing the antibiotics to mankind. Having made a wise choice of their agent, the Fates also arranged that one of his plates, inoculated with staphylococci but not incubated [at 37°C], should be contaminated with a spore of [the fungus] *Penicillium notatum*, and that the weather conditions during the subsequent weeks should provide the sequence of rather narrow temperature ranges required to produce the penicillin effect [killing of surrounding bacteria]. Fleming returns from his holiday and goes through the pile of used plates on his bench, looking at them and discarding them into the tray of disinfectant. The plates are numerous and soon they pile up, above the disinfectant. But what is this? Gracious heavens, he has discarded the plate! All is not lost, for the Fates have a messenger on hand in the form of Fleming’s colleague, D. M. Pryce. Pryce makes his entrance, they chat about staphylococci and to make a point, Fleming picks up some of his discarded plates. The Fates hold their breath. Yes! He picks up the plate, looks at it, and says “That’s funny . . .” [See what Fleming saw in Fig. 2.2]. How fortunate that trays rather than buckets were used for discarded cultures and that D. M. Pryce was on hand at the critical moment.
Although Heatley and Fleming clearly viewed Fleming’s careless laboratory practices as a gift from the gods, microbiologists today would see that carelessness differently. The events described in Heatley’s account, particularly the stacks of plates rising out of the disinfectant, would have gotten Fleming in a lot of trouble if he were practicing microbiology today, possibly leading safety officers to shut down his laboratory until he cleaned up his act. Laboratory safety is taken a lot more seriously today than it was in Fleming’s time. In fact, a book on legally mandated safety procedures for microbiological laboratories, which is published by the Centers for Disease Control and Prevention, now exceeds 200 pages.

Does this mean that bureaucratic interference is currently stifling great scientific discoveries? Obviously not, since the pace of progress in microbiology has increased a lot since Fleming’s day. It just means that scientists now have a better appreciation for the safety of fellow laboratory workers and the public than they did in the early days of microbiology. Also, good laboratory procedures make for more reliable, more believable scientific results. The experience of microbiologists in the ensuing decades has convinced us that the kind of sloppiness exhibited by Fleming is far more likely to lead to bad science than to great discoveries.

Although Fleming is often credited with the discovery of penicillin,
many would argue that the scientists who deserve credit for the real successes of penicillin were an Australian scientist, Howard Florey, and his assistant, Ernst Chain. Florey and Chain were scientists who did not see penicillin as merely another microbiological curiosity, as Fleming did initially, but instead dedicated themselves to discovering how to produce enough penicillin to make the drug widely available. Prior to their discoveries, penicillin was available only in very limited quantities. Without their intervention, penicillin would have had only modest impact. Only when it was produced in large scale did penicillin begin to lead to mass cures of diseases from wound infections to syphilis to bacterial pneumonia.

It is comforting to see that in some ways virtue is rewarded. Even if Florey has not gotten the credit some people think he deserves, his fellow Australians have recognized his contribution in a very material way. His picture appears on the Australian 50-pound note. Even the Queen of England merits only the 5-pound note.

In the race to produce enough penicillin to meet military and civilian demand, scientists resorted to a variety of growth vessels, ranging from glass bottles that had originally contained popular drinks to bedpans. The goal was to grow large amounts of the fungus *P. notatum* in order to harvest the culture liquid that contained the antibiotic secreted by the fungus. The main problem was not just volume, however, but that culture supernatants from *Penicillium* had very low activity.

Later, Chain realized that the low potency of the culture fluid was due to the fact that the initial cultures of *Penicillium* were contaminated with a strain of the common bacterium, *Escherichia coli*. This particular *E. coli* strain produced an enzyme that degraded penicillin. Thus, even at the earliest steps in the discovery of what would ultimately become one of the most important antibiotic classes ever discovered, the penicillin family of antibiotics, scientists also saw the first evidence that bacteria could become resistant to penicillin. Uh oh.

**Contaminating the Miracle: The Tuskegee Study**

Discovering antibiotics and producing them in quantities sufficient to be useful to the population at large were only a part of the process. Making sure that these products were equitably distributed was equally critical. Anyone who has been present at graduation ceremonies for medical students about to enter their profession can hardly fail to be moved by the Hippocratic oath, an important part of which is “above all, do no harm.”
What happens when physicians forget this oath? Unfortunately, we have an answer to this question in the form of the so-called Tuskegee study.

Syphilis was widespread in the southern United States in the 1940s, as it was in many other parts of the world. The discovery that arsenicals could successfully treat some cases of syphilis pointed to a breakthrough in the treatment of this horrible disease. The success of the arsenicals was spotty, however, so it is perhaps understandable that although those rich enough to afford this new treatment were able to get it, there was not a massive public health assault on the disease in communities where people were too poor even to afford visits to doctors. Then penicillin came along, offering a gentle cure that was highly effective, and it was soon cheap enough to allow a massive campaign to eradicate syphilis, even among impoverished populations.

To make a long sordid story short, physicians associated with the U.S. Public Health Service, the precursor of the current U.S. National Institutes of Health, decided that there was not enough information on the pathology of syphilis to complete the textbook description of the disease. Their solution to this problem was to create a study in which a group of syphilitic African American sharecroppers in Alabama would not be given the new antibiotic treatment. Instead, they would be offered free “health care” that consisted mostly of collecting blood samples and cataloging the deterioration of their health. Since these men had never had any health care at all, they were easily convinced that they were lucky to be included in a study where at least they saw a nurse on a regular basis. It was only during the 1970s that this shameful study was exposed and the few men in the study who were still alive were finally treated and compensated for their suffering. Many classic political cartoons appeared when this outrage was revealed. A number of them are included in the book Bad Blood by J. Jones (Simon and Schuster, 1993).

To withhold effective antimicrobial therapy from sick people would be unthinkable today, right? Well, perhaps not. Most of the people suffering from tuberculosis in the world today will not receive the (very cheap) antibiotics that could cure them because they cannot afford those antibiotics and no one with money cares enough to provide them.

Perhaps the highest profile recent controversy in this area, however, has been the one surrounding azidothymidine (AZT). AZT is an anti-human immunodeficiency virus (HIV) compound that does not cure HIV infection but at least slows the progression of the infection to AIDS and has prolonged the survival of many AIDS patients. In particular, short-
term AZT administration to pregnant women about to give birth is now known to prevent the transmission of HIV from the infected mother to the infant. We know this because of the results of a very controversial set of clinical trials.

The short-term AZT therapy was tested in Africa and Thailand, but any ethics panel in a developed country would not have approved the design of this clinical trial. Two populations were compared. One group of women was given the short-term course of AZT, and a second group, the control group, was given a placebo. In the United States and Europe, the comparison would have been between women given the new short-term AZT regimen and women given the much longer-term regimen now used in developed countries to prevent mother-to-infant transmission.

The AZT trials in Africa and Thailand caused a storm of international protest and raised the inevitable suggestion of parallels to the Tuskegee study. Unquestionably, the two studies differ vastly from each other. The AZT trials were designed and carried out by physicians and scientists who were dedicated to helping prevent HIV transmission and who had the health of the subjects in the study very much in mind. However, the AZT trial controversy shows that the ethics of drug testing can still be a contentious issue, especially when trials are conducted in poor countries.

It is now very expensive to test new antibiotics for safety and efficacy in countries like the United States. It is hard to imagine that any reputable pharmaceutical company would try to cut costs in the way they were curtailed in the case of the short-term AZT trials, but only constant vigilance by the scientific community will ensure that the testing and distribution of precious, life-saving antimicrobial drugs will be equitable and ethical.

Issues to Ponder

1. Who really does deserve the credit for the discovery of penicillin: Fleming or Florey and Chain? This is actually a fairly deep question about assigning credit for a scientific discovery. The credit for such a discovery usually goes to the person or persons who first noticed a phenomenon and performed the first experiments to test the hypothesis the phenomenon suggested. However, effective delivery of such insights to the public is also an issue for all of us. In the case of a work of art, the curators and exhibitors do not get the same degree of credit as the artist who created the work, but in the case of a medical treatment like penicillin, how useful
is a drug that remains a curiosity and never makes it to the people who need it?

2. As will be described in chapter 9, the declining profitability of antibiotics has made them less and less desirable to pharmaceutical companies. Also, antibiotics are old news to the scientific community. Accordingly, research on such promising but so far nonproductive therapies as gene therapy and stem cell research have taken the publicity foreground in recent years. No one disputes the importance of giving high billing to promising areas of research that need all the encouragement they can get. However, the relative lack of interest in antibiotic discovery on the part of scientists and the public may not be such a good idea if the goal is to encourage development of new antibiotics to meet the challenge of increasingly resistant bacteria. What should be the relative interest in and coverage of exciting new, but still unproven, therapies compared to older successful but no longer as exciting therapies? This is not a simple question, because it asks about the balance between encouraging innovation and supporting the continuation of past successes.

3. Today, there is no question that the Tuskegee study was a moral abomination. Nonetheless, it is still troubling that it happened at all, and, even more disturbing, that the controversy raised by Tuskegee was echoed, however faintly, in the recent AZT trials. In medical school programs, lectures on ethics are now de rigueur, but is the Tuskegee case part of that training? In most medical schools, the answer has been “no,” and it is uncommon to find medical students today who even know this story. There are two schools of thought on the issue of whether to include this historical account in a microbiological curriculum. One, which we advocate and thus will present first, is that the Tuskegee experience is such a stark example of how highly trained scientists can go astray that it grabs the attention of students, making them, one hopes, more sensitive to ethics discussions that have more subtle shadings. The countervailing view is that the Tuskegee experience was so starkly an example of criminal behavior that it will never be repeated and thus does not merit discussion today. What do you think? Remember, medical students are being bombarded with an expanding mass of new medical information, which makes time available for coverage of any subject more and more precious.