ANTIBIOTICS AND ANTIBIOTIC RESISTANCE

Ola Sköld

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To the memory of J. B. Neilands, Professor of Biochemistry,
University of California, Berkeley
“The finest Gentleman of Science I ever met”
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By controlling bacterial infections, antibiotics have given us a health standard that we have become used to and which would be very difficult to lose. Antibiotics are unique among medicines in that they act selectively on bacteria, among them the pathogens, while leaving human cells and tissues unaffected. A description of how antibiotics work and of the mechanisms by which bacteria resist them often falls between the medical discipline of infectious diseases and the field of microbiology. With this book I mean to bridge this gap. It should serve as a brief handbook for physicians, veterinarians, and pharmacists and as a textbook for students in these areas of study. I also describe the rapid and very worrying development of antibiotic resistance among pathogenic bacteria, including the molecular mechanisms of this resistance and newly observed genetic principles for the spread of resistance among species.

Our ubiquitous use of antibiotics for medical purposes and for growth promotion in farm animals has been a toxic shock to the microbial world, which has responded by developing resistance. It can be looked upon as a piece of Darwinian evolution taking place right in front of us. Genes mediating resistance, mostly
of unknown origin, have been shown to be spread by means of earlier unknown and very efficient genetic mechanisms. No microbiologist can escape being astonished and impressed by the ingenuity of evolution in finding and combining molecular mechanisms to protect the bacterial world from the dramatic environmental change that our use of antibiotics has effected.

Finally, I describe the future possibilities that, under the threat of resistance evolution, can be envisioned to help maintain the health standard that antibiotics have helped us reach in controlling bacterial infections, which we have come to take for granted.

Ola Sköld, M.D., Ph.D.
CHAPTER 1

ANTIBIOTICS: THE GREATEST TRIUMPH OF SCIENTIFIC MEDICINE

The use of antibiotics has given us medical control of bacterial infections. This is a health standard that we have become accustomed to and have come to regard as self-evident. Today, it is impossible to imagine health care that is not able to cope efficiently with bacterial infections. Medical disciplines such as oncology and organ transplantation surgery would simply collapse without access to modern antibiotics.

The tremendous success of antibiotics in the field of infectious diseases for seven decades or so has led to very wide distribution and consumption of these agents. Besides their medical use for human beings and animals, antibiotics have been used in very large quantities as growth stimulants in husbandry and as prophylactic protection against plant pathogens. All this has led to the spread of millions of tons of antibiotics in the biosphere during the antibiotics epoch. This has induced a drastic environmental change, a toxic shock to the bacterial world. It has been said that “the world is immersed in a dilute solution of antibiotics.”
Bacteria have adjusted to the changed environment in the usual method used by living organisms: by evolution. The bacterial world, including human pathogens, has developed and mobilized molecular defense mechanisms for protection against the human-produced poisons that antibiotics are. This has led to increased antibiotics resistance among human pathogens, which are becoming more difficult to treat. This poses a serious threat to our health standard in that the ability of medicine to cope with bacterial infections has slowly been eroded. Medical journals and daily newspapers report on cases of infectious disease that were untreatable because of antibiotics resistance. One recent report described a young woman dying of tuberculosis despite intensive treatment. The tuberculosis bacteria causing the disease were multiply resistant and thus resisted treatment with all available antituberculosis drugs. What is happening, and what is going to happen?

Harmful cells comprise the two greatest threats to our health. In the first case, our own cells lose their growth regulation by genetic changes, thereby causing cancer. In the second, foreign organisms infect and establish themselves in the tissues of the human body, inhibiting their functions and destroying them by the action of toxins. Bacteria form the dominant part of the latter group: tuberculosis, syphilis, cholera, typhus, typhoid fever, and bubonic plague, for example. The medical treatment of cancer and that of bacterial infections are related in that both include the use of cell growth–inhibiting or cell-killing agents. Cancer cells are treated with cytostatics, which are difficult to use and must be handled by oncology specialists. This is because cancer cells originate from normal cells and are metabolically very similar to normal cells, letting cytostatics also interfere with healthy cells, such as those of the bone marrow, where the continuous growth of cells is necessary for the support of life.
SELECTIVITY

Bacteria belong to another biological world and are structurally and metabolically very different from our cells. They can be inhibited in growth and also killed by agents that do not interfere with our cells. That is, antibacterial agents, antibiotics, used for clinical purposes in medicine must act selectively on bacteria. Their handling can therefore be focused on the characteristics of the infecting bacterium.

Penicillin was discovered more than 80 years ago. Penicillin and its many followers, all with a selectively inhibiting effect on bacteria, had a tremendous impact on the treatment of infectious diseases and on their panorama of occurrence in the first decades of their ubiquitous clinical use (1950–1980). The great clinical success of antibiotics changed the attitude of the medical profession toward bacterial infections. This is reflected in a statement from 1969 by the Surgeon General, William H. Stewart, to the U.S. Congress: “It is time to close the book on infectious diseases.”

The surgeon general is the highest medical officer in the U.S. Department of Health and Human Services.

Antibiotics are unique among pharmaceutical remedies in that they do not direct their action toward our own cells but selectively toward foreign cells, bacteria coming from the outside and infecting our tissues. Their selective action means that they must target physiological and biochemical differences between our cells and bacterial cells in order to effect bacteriostatic or bacteriocidal activity. The key property of clinically useful antibacterial agents, then, is selectivity. It can be noted that in the search for new antibiotics in molds and other microorganisms, with Penicillium as an example, many selective and useful antibiotics were found (e.g., streptomycin and rifampicin), but also others with a good antibacterial effect but without selectivity, making them unusable for the clinical treatment of bacterial infections. The
latter antibiotics show inhibiting or killing activity toward both bacteria and our cells, and have in some cases (e.g., adriamycin, bleomycin, and mitomycin) found use as cytostatic agents in the treatment of cancer, and then under the usual strict oncologist control of, among other things, bone marrow function.

DEVELOPMENT OF RESISTANCE

Since antibiotics are only active against foreign cells, bacteria, and should have no effect on our cells and tissues, they are not pharmacologically active, except for side effects that occur with several of them when given in large doses. This means that they can be prescribed less strictly than other pharmaceuticals. In many patients showing signs of infection they are given simply for safety, without a strict bacterial diagnosis. This has contributed heavily to the very large consumption of antibiotics that can be estimated from sales figures, which can be used as good proxies for actual consumption (Chapter 2).

Resistance to antibiotics among pathogenic bacteria has developed within a short time and in many ways faster than could have been expected. This can be explained partially by the short generation time of bacteria, allowing them to undergo a Darwinian evolution in a much shorter time than has been possible for animals and other organisms. Furthermore, bacteria have the ability to manipulate their own genetic makeup, leading to a faster adaptation to the toxic effects of antibiotics: that is, the development of resistance. It can be looked upon as the natural genetic engineering of bacteria, including the uptake and incorporation of resistance-mediating genes from related organisms by adaptation of evolutionary old genetic mechanisms to the new environmental situation of the large presence of antibiotics. No microbiologist can escape feeling surprise and wonder as these phenomena continuously unfold.
Resistance is the dark and daunting side of the antibiotics triumph, and we are forced to realize that the health standard that antibiotics have given us is not stable. The great asset that antibiotics represent is devalued by the evolution of resistance. In a longer perspective this development is quite threatening. Many medical specialities are dependent on efficient antibiotics. Will we be able to maintain control of bacterial infections, or will our descendants look back nostalgically and talk about the time that we had both oil and antibiotics?

**SULFONAMIDE: THE FIRST ANTIBACTERIAL AGENT ACTING SELECTIVELY**

Louis Pasteur, a great French microbiologist of the nineteenth century, formulated and proposed what was called the *germ theory of disease*, the concept that infectious disease was caused by microorganisms. Later, Robert Koch at the Imperial Health Office in Berlin provided proof, with *Bacillus antracis* as an example, that there is a definite causal relation of a particular microorganism to a particular disease. From these ideas Koch formulated his postulates for characterizing a pathogenic microbe:

1. The organism is found regularly in the lesions of the disease.
2. It can be isolated in pure culture on artificial media.
3. Inoculation of this culture produces the disease in experimental animals.
4. The organism can be recovered from lesions in these animals.

Based on these basic ideas, Paul Ehrlich at the Royal Institute for Experimental Therapy in Frankfurt am Main advanced the idea of direct selective action of a drug on infecting microbes. His
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expression for this was the "magic bullet," which would exhibit a greater affinity for pathogenic bacteria than for host cells. For this selective action he coined the word chemotherapy. Ehrlich further observed that dyes stained different cell components selectively and proposed the idea that organic stains taken up, particularly by living cells, could have a therapeutic effect by interfering with bacterial infections.

In the 1930s, these ideas led Gerhard Domagk, who was working at the Institute of Experimental Pathology at the I.G. Farbenindustrie in Elberfeld, Germany, to the discovery of Prontosil rubrum (4-sulfonamide-2',4'-diaminoazobenzol, Domagk 1935) (Fig. 1.1); a chemically synthesized dye of red color, which showed an effect against bacterial infections in animals. It was, however, inactive in vitro. Jaques and Therese Tréfoüel of the Pasteur Institute in France could show that patients treated with Prontosil excreted a simpler product, sulfanilamide, which was active in vivo as well as in vitro against the growth of bacteria. This was a dramatic development since it finally established Ehrlich's principle of chemotherapeutic action. Sulfanilamide is a colorless substance and not a dye, partly contradicting the theory leading to its discovery.

Sulfanilamide was set free from the dye by hydrolysis in vivo in animal experiments. Sulfanilamide was thus the first antibacterial agent to act selectively. The first trials of Prontosil rubrum on animals were performed by Domagk in 1932. He could show that mice infected experimentally with Streptococcus pyogenes by injection into the peritoneum were protected from peritonitis with this agent. The results were published in Deutsche medizinische Wochenschrift in 1935, and sulfonamides were soon used widely for the clinical treatment of infections with streptococci, staphylococci, meningococci, and other severely pathogenic bacterial agents. Domagk's work is unjustly forgotten today but was much appreciated by his contemporaries, and at the end of
the 1930s he was nominated for the Nobel Prize in Physiology or Medicine. The Nazi regime of that time in Germany had, however, declared that it did not want to see any German as a Nobel laureate, probably because of the Nobel committee’s choice of earlier Nobel Peace Prize laureates. The German government of that time tried through its embassy in Stockholm, and also directly through the foreign office in Berlin, to interfere with the work of the Nobel committee at the Karolinska Institute in