Chapter 1: Introduction to Antibiotics and Antibiotic Resistance

In the everchanging landscape of medical treatments of bacterial infections, a rising issue and complication within potential avenues of curing ailments, is the once unstoppable antibiotics, now not functioning as well against pathogens that they used to wipe the floor with. But why is that? Over the course of this textbook that's the question we will be setting out to explain and cover, the structure of bacteria, differences between gram negative and gram positive, and much more. This information is the basis for how we treat infections with **antibiotics**, which are being defined in this textbook as any class of organic molecule that inhibits or kills microbes by specific interactions with bacterial targets, without any consideration of the source of the particular compound or class.[1] After there is a firm understanding of bacterial function, we will discuss antibiotics in specific, their structures and what systems in a bacteria they target to disable and cause the death of the pathogen. This information and more will be discussed within the upcoming chapters, however for the time being, to better understand the relevance of antibiotics in the modern period we will be discussing some of the history of medical treatments before we were aware of the nature of what causes infections and how they can be cured. Afterwards, some of the events through history that led to the initial discovery of penicillin and the further refinement into a usable antibiotic.

The Balancing of Humors, And Other Outdated Treatments

Practitioners of medicine before the 19th and 20th century quite literally had no inclination of knowing about tiny microorganisms that cause infections and disease in a host's body. Because of this, most treatments of diseases and infections were by today's standards barbaric and widely ineffective. Some of the earliest treatments were based more around ideology of life, since health is a representation of balance, disease must be an imbalance in a person and that it could be remedied by trying to fix this imbalance. A second century Greek physician Galen of Pergamon identified four humors: Blood, phlegm, yellow bile, and black bile. (Humor coming from the Greek word for juice, chymos). Excessive bile was linked to fevers, and phlegm was linked to epilepsy. The way these four humors were balanced was by bleeding the patient, inducing vomiting, and causing large blisters to form.[2 (page 7)] This practice was used throughout history and was even recommended by Sir William Osler, one of the four founding members of the John Hopkins School of Medicine in a 1923 publication *The Principles and Practices of Medicine*[2 (page 8)]. This treatment method was thought to be the best way to cure a diseased patient and was used on notable figures such as George Washington and Napoleon Bonaparte, neither of which survived their respective bouts of "treatment". Part of the reason for this prevalence and staying power of this theory was lack of a better one, as men of science only had the tools and the notions of their times, there weren't thoughts about unseen organisms that cause disease within a person. These doctors would have to make assumptions based on what they could actually see, practitioners were definitely skilled at what they knew how to do, which was to bleed and induce vomiting in patients, but they lacked the understanding of what causes disease. One of the major keys to understanding how to properly treat something is to know what needs to be treated.

Other treatments emerging in the sixteenth century that were meant to be a replacement for what some physicians saw as an ineffective method of treatment for patients thought of a

much more effective and brilliant treatment method, mixtures of salt, sulfur, and most importantly mercury. This treatment pioneered by Philippus Aureolus Theophrastus Bombastus von Hohenheim, or Paracelsus prescribed mercury to patients as a method of treatment which caught on with other doctors most likely because while mercury is toxic to living things, it does kill some disease causing pathogens[2 (page 9)].

While specific antibiotics and functions weren't known about, there were writings recorded documenting the usefulness of moldy breads in application to sites of infection and noting a marked improvement in the patient, these accounts were found in multiple parts of the world such as Egypt, China, Serbia, Rome, and Greece. Also of note tetracycline, a more modern antibiotic was found in trace amounts in the bones of Nubian people dating back to 350-550 AD. This was found by fluorescent tagging of the bones.[3] Although common people and even physicians didn't know the why or how these things cured certain ailments, there have been other effective treatments aside from the ineffective four humors and giving mercury to patients.

The Inklings of Bacterial Discovery

In the later half of the 19th century, a scientist by the name of Robert Koch was studying a deadly disease that was heavily affecting grazing animals (Anthrax), he was trying to answer multiple questions: What caused it, how it was transmitted from sick to healthy animals, and if it could be prevented or cured. His methodology wasn't very sophisticated in the sense of equipment, he was working in a village rather than a laboratory. He used fluids from the spleens of dead cows and sheep placing it on slivers of wood and inoculating mice with it. He figured that since the wooden slivers were still infecting the mice, and that the hosts were dead, it couldn't be the blood and that it had to be something within the blood[2 (page 23)]. Using this information and home-made equipment he successfully purified the pathogen from the blood and he was able to cause it to proliferate in the watery fluid of the eye of an ox, then using this fluid injected it into healthy animals and infecting them[2 (page 23)]. For the first time in history a direct link between an object smaller than the eye can see unaided and causing an infection was forged. Other scientists who doubted his work recreated his experiments and found the same results, but adding to the findings that anthrax, which was thought to be killed by exposure to oxygen didn't end up dying, but going into a dormant mode since it had no food source to consume and proliferate off of[2 (page 26)].

This discovery was Koch's first claim to fame, afterwards he was able to move to a more advanced lab and continue his work, where he discovered better growth mediums for bacteria, eventually creating the agar growth medium used in labs worldwide today. His assistant Julius Richard Petri designed the petri dish within this lab as well [2 (page28)]. Over the next few years Koch went on to discover the bacterium that caused tuberculosis in 1882 and the bacterium responsible for cholera in 1885. Lastly Koch created four postulates in relation to pathology and linking a single pathogen to a single disease, they are as follows:

1. A pathogen must be found in all organisms that are a disease's victims, but not in any healthy organisms

2. The microorganism must be isolated from a diseased organism and multiply in a culture like agar
3. The cultural microbe must continue to cause disease in a healthy host
4. The reisolated microbe would be the same as the original one[2 (page 28-29)]

These four statements maybe sound confusing at first, but essentially the first postulate states the bacteria, for example cholera, needs to be found in those who are sick with cholera symptoms, and it shouldn't be found in someone not presenting with cholera symptoms. While this doesn't exactly work when thinking of carriers of diseases, this wasn't something that was common knowledge at the time. The second is saying that for a pathogen to be identified as one, it needs to be able to survive and reproduce itself outside of its current host. Thirdly, the disease in question needs to cause a continual disease within the host, continual more meaning for an extended period of time, and finally that the microbe of cholera doesn't change into something that isn't cholera upon further isolation. While the four postulates don't currently cover completely how we diagnose and treat pathogens it was revolutionary in it's time.

The Breakthrough of Alexander Fleming

Alexander Fleming is a man of many accomplishments within the scientific community, and a major player in the start of the antibiotic revolution. He was an accomplished doctor in his own right and during World War 1, he went to a British military hospital and showed that covering battle wounds increased the rates of infections from bacteria like gangrene, tetanus, and peritonitis[2 (Page 87)]. Mostly by chance after the war, in 1922 Fleming also discovered a very common protein involved in the immune response of an animal, a lysozyme, while plating some bacteria cultures he had accidentally dripped mucus onto the agar plating and happened to drip the mucus into a bacterial culture that had lysozyme sensitivity, most bacteria and a lot of dangerous pathogens aren't affected by the enzyme on its own[2 (Page 87)]. While these discoveries aren't of importance to antibiotics it is some of his additions to scientific knowledge about pathology and immunology, which commonly goes hand in hand with antibiotic resistance. The breakthrough in question as mentioned in the title of the section, is the discovery of penicillin. Dr. Fleming seems to have a track record of chance discoveries as when he left a window in his lab open before departing for a trip and some unsecured plated cultures, when he came back weeks later, he found that the bacteria he cultured *staphylococci* had a curious fungal growth within the center, and a ring of no bacteria around the outside of it. This showed Fleming that something about the fungus killed that bacterium. Upon further research Fleming found the fungus named *Penicillium notatum*, which as stated is a fungus, but more commonly it's a mold, a mold that produces a substance that was able to kill the staph bacterium, which he named penicillin. Sadly though, after the initial publishing of his discovery the grounds on which penicillin were useful just weren't there, it was hard to create a pure version of it without other compounds present, its shelf life was sometimes days, and sometimes a few hours. Due to the struggle of accomplishing any worthwhile production of the antibiotic, Fleming would pursue more research into the lysozyme discovery he made years prior. While Fleming was unable to successfully create a better production method for the antibiotic this initial discovery was a cornerstone for future scientific efforts.

The Dunn School Efforts Towards Penicillin

Located within Oxford, the Dunn School focused on scientific research efforts, but for the purpose of this book, the focus will be on Howard Florey, a PhD leader of research at the school. When he took over the head position in 1935, he took particular interest in the unknown mechanisms of lysozyme within the gastrointestinal tract[2 (Page 103)]. The more important part of this are those who Florey recruited for this endeavor, Ernst Chain, and Norman Heatly, Chain a distinguished chemist, and Heatly an outstanding engineer, a MacGyver of making homemade laboratory equipment. This trio figured out the specific substrate of lysozyme was a polysaccharide which showed that the lysozyme was a polysaccharidase, or it broke down the polysaccharide molecules, of which certain bacteria like E. coli have coating their outer walls[2 (Page 110)].

Around this time Florey and his team were planning to catalogue all the antibacterial productions produced by microorganisms, an ambitious goal for the time to be sure, the study planned to include dozens of different bacteria strains, but also fungi; Florey had obtained the papers written by Fleming roughly ten years prior, prompting his interest in the commonly thought dead end discovery, and so the *Penicillium* mold was included in this survey. The surveys focus was still rooted in working on the understanding of lysozymes, and in the aspect of *Penicillium* they thought it was a mold lysozyme of sorts acting on bacterial cell walls. Though they still faced the issues that Fleming did, creating consistent amounts of the compound. Chain and Heatly worked together on the issue to create a way to reliably obtain penicillin but for cheap, Heatly is a MacGyver with the odds and ends around the lab and school due to the lack of funding that they had, Florey was their spokesman for the project, trying to squeeze out necessary funding for their team from various support foundations such as the Medical Research Council, and the Rockefeller Foundation[2 (Page 114)]. Eventually after many trials and tribulations the team was able to produce stable amounts of penicillin that was stable and able to last for close to two weeks at room temperature, still the mixture was still essentially a broth that wasn't a pure compound. Following this, the team began to experiment with lab animals, which quickly dismantled their still held belief that penicillin was a lysogenic enzyme, they found that when injected into the abdomens of mice it was shown to breakdown unlike how a protein would, and the they showed no noticeable immune response which would be an expected response to the injection of such a protein[2 (Page 118)]. However, the team did find solace in the mice having no adverse reaction which did point to the substance being potentially safe for use and they found that the substance would be excreted in urine and would retain its potency against bacteria during this process. On May 25th, 1940, Florey gave eight mice, *Streptococcus pyogenes* a pathogen causing strep throat at the least and at the worst, necrotizing faciitis or death of the bodies soft tissue. He also injected four of the mice with penicillin at varying dosage amounts and follow up doses, giving two mice 10 milligrams of penicillin and two 5 milligrams of penicillin. Within twenty-four hours the control group of mice were dead, and the four mice given the treatment of penicillin were still alive and well[2 (Page 118)]. Once again, at this point and time the solution of penicillin being used was not even remotely pure, showing the raw strength of this antibiotic even amongst many other impurities.

Turning Towards Human Treatment And Beyond

Following the success of this experiment they went on to test this on multiple other pathogens finding that the penicillin was still effective, as they were doing these tests Chain worked with other chemists to continue refining the production to remove impurities. After their findings were published it garnered attention from multiple countries like Switzerland and Germany. However, due to the timing of the world, it was in the middle of World War 2 that these discoveries were being found and learned of, giving the team pause to outsource production and give information on the drug that could help the enemy, leading them to continue their research efforts without outside aid[2 (Page 128)]. With the ingenuity of Heatly he was able to produce a contraption that would create larger amounts of penicillin than they were previously using, so much so that it allowed Florey and his team to move to potential treatment of humans. After some issues with impurities in the penicillin causing adverse immunological reactions in humans, mostly due to pyrogens, or inflammatory response promoters, they further refined it to remove those impurities, and they finally went from an eighty percent purity to a one-hundred percent purity, completely benign penicillin[2 (Page 129)]. Although they were able to produce enough penicillin to treat a few people and they did so with positive responses, they ran into supply issues due to the rate of production compared to the amount needed for continued human treatment, it was estimated that Florey's team would need one kilogram of pure penicillin and at the time they weren't producing enough for that; during their first round of tests they were reusing excreted urine from the patients to recover penicillin for further treatment. Due to this lack of production quantity, Florey turned to the United States due to its production value of agriculture, which would be the perfect place for mold farms to grow more and more penicillin.

Florey was able to get a trip to the US during the wartime and brought Heatly with him, and successfully obtained a partnership with the US to work on making larger quantities and stronger batches of penicillin. The duo made their way to a lab in Peoria, Illinois where years before the both of them even came to the US, scientists at the lab were conducting their own research with the *Penicillium* mold, and found three key discoveries, firstly they discovered and identified the best producers of penicillin from the molds, secondly a method to increase production rate, and thirdly a better method of fermentation for quicker production[2 Page 135]. During these critical time periods the manufacturing methods of penicillin became more and more refined and even Florey noted that it was beginning to shift to America[2 (Page 141)]. During the 1940s the midst of World War 2 is when penicillin saw much success resulting in Florey being able to go to many countries teaching about the administration of the antibiotic and how to properly close wounds and through tubing disperse the antibiotic's solution to help with killing the bacterial pathogens.

Summary

Throughout history there have been treatment methods steeped in speculation and some in actual useful practice, the methods of balancing the four humors and using mercury were obviously not effective treatment methods, but due to the knowledge of the time it was the best information they had to go on. Following some chance discoveries in the 20th century Alexander Fleming planted the seeds of the discovery and mass production of powerful antibiotics that are widely used today. This leads into further discovery as time progresses.

References

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