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### Robert Koch: Mastering Tuberculosis

On the evening of March 24, 1882, eyes were wide open and minds were in a great reverence as the man who would later be known as "The Father of Bacteriology" presented his discovery of *Mycobacterium tuberculosis* to the audience of scientists. This man, Robert Koch, an aspiring German physician and scientist, isolated and identified the bacteria of tuberculosis, which annihilated one-third of the middle-age population in the 19<sup>th</sup> century in Europe and the United States. In 1905, Koch was presented a Nobel Prize in Physiology or Medicine for his findings of the tuberculosis bacterium (Press Release: The 1905 Nobel Prize in Physiology or Medicine). The discovery of *Mycobacterium tuberculosis*, as well as other significant revelations in bacteriology and serology attained by Koch, laid the foundations for the discovery of other disease-causing bacteria, allowing scientists to pave the road for a healthier future by identifying the agents of several diseases.

Although bacteriology was a fairly recent science in the 19<sup>th</sup> century, several fundamental implements aided in the exploration of basic science. The compound microscope, invented in 1665, allowed the human eye to observe the intricate details of cellular organisms, among them the multitudinous bacteria. In 1877, a student of Koch devised a special glass dish called the Petri dish in which most of Koch's analysis of bacteria took place. A basic scientific principle that disease-causing bacteria invade the body of a host was first observed by an 18<sup>th</sup> century Italian anatomist and pathologist, Battista Morgagni, who discovered the relationship between disease and physiological

changes (Columbia University Press).

A bacterium, in all its simplicity, is a unicellular organism that lacks a nucleus, thereby classified as a prokaryote. Its DNA is suspended within the cell in a coil-like structure. Prokaryotes are known to reproduce by fission, through an asexual process. Unicellular organisms, like prokaryotes, have a tendency to form in dense films, known as biofilms, instead of existing as clouds of individual cells. A biofilm will form once the bacteria contacts a solid surface, in which it will contrive a gel-like polysaccharide matrix and trap other bacteria (Purves et al. 532-533). In 1839, staining bacteria with certain dyes was first initiated in microbes. Then in 1872 it was discovered that chromogenic, or color-forming, bacteria would grow on solid substrates and that these colonies were able to reproduce colonies of the same color, forming bacteria of the same type. These discoveries would be the starting point for Koch's pure-culturing techniques.

Empowered with this knowledge, Koch set to work: to find the bacteria responsible for the deaths caused by tuberculosis. The methods of identifying any bacteria were rather incomplete, since microbiology in itself was a new science, unexplored in great depths. The concept of finding the right technique for staining bacteria was a key in distinguishing specific bacteria. As Koch went along to isolate and identify bacteria, he perfected staining and culturing techniques. Koch believed that if a microbe could be isolated, then the disease could be controlled. Isolating some bacterial strains and cultivating them in a liquid culture medium, similar to broth, was achieved earlier by Louis Pasteur. However, problems arose when the bacteria in the liquid appeared distorted under microscopic view. Koch devised a medium that could be used in hot temperatures and had the ability to maintain solid. Koch identified bacteria by preparing

thin layers of bacteria on glass slides and fixing them with gentle heat. He invented the drop technique, where microorganisms could be cultured in a drop of nutrient solution on the inside of a glass slide. He would stain bacteria with an aniline dye, and record the minuscule images of bacteria on film with a photographic apparatus he built to his own likening (Porter). These experiments, developed with precision and ingenuity by Koch, demonstrated the complete life cycle of bacteria (Enersen).

Koch's next aspiration was to cultivate a set of procedures to extract and analyze the causing agent of a microbial disease. Although disease-causing bacteria were acknowledged in the 18<sup>th</sup> century, previous scientists were unaware that specific bacteria stimulate a specific disease in an organism. Koch formalized four rules which question the validity of disease-causing micro-organisms, universally known as Koch's Postulates. To indicate that a certain microorganism causes a particular disease, it must always be found in individuals with the disease and can be taken from the host and grown in pure culture. Once the microorganism is grown in a culture, a sample of the culture should produce the disease once it is injected into a new, healthy host that had not yet been invaded with the bacteria beforehand. Then, the infected host should produce a new, pure culture of microorganisms identical to those identified in the previous pure culture. Only then can a certain microorganism be labeled as disease-causing (Purves et al. 332-333).

With certain principles and techniques established to identify and isolate bacteria, Koch was ready to classify disease-causing bacteria, such as the tuberculosis bacterium. But before his final discovery of *M. tuberculosis* was achieved, Koch strenuously worked with the anthrax bacterium, *Bacillus anthracis*. Although *B. anthracis* had been discovered earlier in the century, Koch proved scientifically that *B. anthracis* causes

anthrax. He extracted the bacterium from the spleen of an animal that had died of anthrax and purified the bacterium. He then inoculated the purified bacteria into healthy livestock and saw the disease paralyze the body. When he examined the blood of the inoculated animals, he was able to re-isolate the same bacterium. He repeated this isolation, infection, and disease cycle until it was evident to him that he had discovered the anthrax agent (Coberly). In other studies related to *B. anthracis*, he observed differences in pathogenicity for different species of hosts and demonstrated that an animal's body is a great mechanism for the cultivation of bacteria. His anthrax experiments were the first genuine evidence of a relationship between a bacillus and a particular disease (Enersen). To detect organisms substantially smaller than *B. anthracis*, like *M. Tuberculosis*, Koch equipped his microscope with Ernest Abbe's new condenser and oil-immersion system, made by Carl Zeiss.

Koch devoted the next several years to isolating and identifying the infective agent of tuberculosis. He extracted the tissue of a thirty-six year-old man who was in excellent health three weeks prior to his death. Four days after his death, the man's body was covered in yellowish tubercles. Koch used the man's infected tissue and injected it into the eyes of rabbits and under the skin of guinea pigs. Consequently, he cut off a piece of the man's infected tissue and smeared it onto a glass slide, which he placed under a microscope. Several days later, Koch still could not find anything under the microscope. The bacteria responsible for the formation of tubercles is extremely tiny, approximately one-third the size of *B. anthracis*, and because it congregates in small numbers, it is even more difficult to see. This is the main reason why previous microbiologists had failed to discover its existence. But Koch came up with an ingenious idea: stain the bacteria with

several dyes. Although this technique required hours of soaking the bacteria, the tiny blue-colored rods of the bacterium were finally perceivable through Koch's microscope. With this experiment, Koch was certain he had identified the bacteria responsible for the formation of yellowish tubercles.

Koch continued to test his findings by obtaining more infected tissue from bodies of patients who had died from tuberculosis and injected it into animals of various structures. After all his animals died, Koch observed a unique pattern: only when animals developed the disease could Koch identify the tiny blue-stained rods. He concluded that the bacteria could never be found in healthy animals. Koch's main goal now was to grow the bacteria in pure cultures and inoculate it to produce infection in a new host, as per Koch's Postulates. When Koch inoculated his solid media with the infected tissue of the tubercle bacterium, the bacteria in the medium did not flourish. Koch did not surrender there. He reasoned that the growth of the bacillus was rapid and used a medium composed of blood serum by heat sterilization from animal blood (Enersen). He then used the infected tissue of a guinea pig's lung to inoculate his medium. Several days passed until he observed colonies of the bacteria settled on the medium. He then performed a series of experiments in which he grew the bacilli and infected the guinea pigs to verify his findings. To further investigate the tuberculosis bacilli, Koch injected dead bacilli onto a person who subsequently received a dosage of living ones. After he observed the reaction, he concluded that this method might be useful as a cure in the bacilli's early stages. In Koch's studies, a sterile liquid produced from cultures of the bacilli was used as an active agent. It was then, after verifying his findings on the bacterium, that Koch publicized his discovery in his famous lecture in Berlin on March

24, 1882, and added a new meaning to bacteriology in the 19<sup>th</sup> century (Press Release: The 1905 Nobel Prize in Physiology or Medicine).

After his discovery of the bacterium, Koch dreamed of suppressing tuberculosis. He came up with what he thought was a plausible cure for tuberculosis: tuberculin. Tuberculin consisted of cultures from the tubercle bacilli. It was observed that once tuberculin was injected into a person who had tuberculosis, the skin of the tuberculous patient would produce a reddish allergic reaction at the spot within the course of forty-eight hours. This reaction, known as Koch's phenomenon, allows doctors today to determine whether a patient is a victim of tuberculosis, even before symptoms of tuberculosis start to develop. Unfortunately, Koch's invention of tuberculin was not accepted by scientists, for it did not work effectively to cure tuberculosis. He spent the last years of his life focusing on a potent cure for tuberculosis. After he discovered *M.tuberculosis*, Koch continued to further analyze how tuberculosis spread. His studies revealed that tuberculosis could spread from person to person through the air and that the bacterium responsible for animal tuberculosis is not identical to the bacterium responsible for human tuberculosis.

Succeeding Koch's discovery of *M. tuberculosis*, many attempts to chemically suppress tuberculosis proceeded. In 1935 a German scientist formed an organic compound containing sulfur called Prontosil, which was used as a treatment of bacterial infections. Prontosil proved to suppress tuberculosis, but only in dosages too poisonous for human consumption. Later on in the early 20<sup>th</sup> century, a fungus was discovered that produced an antibiotic substance, streptomycin. Although streptomycin proved to work in the beginning, the tuberculosis bacilli became resistant and did not respond to the

antibiotic. Streptomycin was later combined with another chemical called aminosalicylic acid (PAS) and prevented the tuberculosis microbe from becoming resistant (Press Release: The 1905 Nobel Prize in Physiology or Medicine). However, it took a while until tuberculosis would be suppressed in the body. In 1921, the Bacille Calmette - Guerin (BCG) vaccine was first administered to a child whose mother died of tuberculosis. Although BCG is safe and cheap, it is known to prevent tuberculosis infection in brains only among children. Some of the most important drugs introduced in the 1940's and 1950's include isoniazid, rifampin, and ethambutol. Most tuberculosis drugs today are combined in order to prevent the emergence of drug resistance (Coberly). At present, tuberculosis is reappearing in HIV victims and biochemists are devising of a drug strong enough to suppress tuberculosis without the formation of drug resistance (Iseman).

Koch's astronomical accomplishments in the 19<sup>th</sup> century still continue to be felt today. From Koch's Postulates to his fundamentals of Microscopic Pathology, Koch's procedures are presently used in hospitals and in research facilities. Robert Koch promoted an era in which the scientific world excelled in its findings by implementing his bacteriological techniques and celebrated in discoveries of its own.

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