

Volume 1


Article 1

10-5-2011

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Recommended Citation

Dworkin, Martin (2011) "Robert Koch: From Obscurity to Glory to Fiasco," *Journal of Opinions, Ideas, & Essays*: Vol. 1, Article 1.
Available at: <http://pubs.lib.umn.edu/joie/vol1/iss1/1>

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Abstract

Robert Koch went from an obscure country physician to be the discoverer of the etiology of anthrax, the inventor of the technique of pure culture bacteriology and with that to the isolation of the tubercle bacillus and its identification as the etiological agent of tuberculosis. These successes propelled him to world-wide glory. In his search for a cure for tuberculosis, he proposed that tuberculin was such a cure. Unfortunately this turned out to be false, and his continued advocacy was a fiasco. Nevertheless his formulation of the germ theory of disease transformed medicine and led to a remarkable series of successes that clarified the etiology of a large number of infectious diseases

Keywords

microbiology, tuberculosis, germ theory of disease, anthrax, tuberculin, Pasteur

ROBERT KOCH: FROM OBSCURITY TO GLORY TO FIASCO

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The Early Years

Nunquam otiosus (21)

Robert Koch was born in 1843 in Clausthal, Germany, a small mining city in Lower Saxony. His father was a mining engineer and Robert was one of eleven surviving children. During his early education he decided to become a teacher but always had an inclination toward natural science. Shortly after entering the University of Gottingen, one of Germany's leading universities, his interactions with such great scientists as Henle, Wohler, and Meissner convinced him that natural science was to be his destiny. However, practical considerations led him to study medicine. While it became quickly clear that he had a talent for experimentation, Koch was a practical man and his impending marriage to Emmy Fraatz made it clear that a steady source of income was a reality.

After completing his medical studies in 1866, Koch took and passed the state medical examination and became licensed to practice medicine. During the next six years he had five different positions as a practicing physician. But none of them provided him and his new wife Emmy and their young daughter Gertrud either the financial security that Koch as a young father sought, nor the intellectual stimulation that this incipient young scientist craved. Finally, he applied for and was appointed *Kreisphysikus* or District Health Officer in Wollstein near the Polish-Russian border. Thus began the decade that led to Koch's worldwide fame. Koch's official duties as District Medical Officer required him to be responsible for the District's public health matters such as smallpox vaccinations and overseeing the local hospital. But in addition, to bolster his modest income he established a small medical practice. He was apparently an excellent general medical practitioner and was admired and respected by his patients. Accordingly, his practice grew. Nevertheless, he was still able to find time for his favorite hobby,

archaeology. He participated in local digs, displayed the artifacts he found at the Berlin Museum and was eventually made a member of the Berlin Gesellschaft (5). By now, his beloved daughter Gertrud was seven years old and Koch's life was full. Nevertheless, the urge for experimentation was strong.

Koch was a naturalist from the time of his childhood, and it was this inclination in the context of his medical and public health career that eventually evolved into the ecology of infectious disease. He was never to be interested in the mechanisms of disease, only in clarifying its causes and thus eventually its cures. So, for example his interest in the natural history of anthrax and *Bacillus anthracis* was the root from which his understanding of the etiology of the disease sprang. And from this emerged the new science of bacteriology.

Three months to Anthrax

Koch was a genius. But a very particular kind of genius. He had the ability to gather up the threads that others had spun and to weave them into a beautiful fabric. He was never responsible for formulating a new conceptual paradigm in the way that Pasteur was. He was an extraordinarily talented experimentalist, with a logical, linear and aggressive approach to solving practical problems. It is particularly ironic that in his lifetime, while his work did lead to placing disease and microbes firmly into the cause and effect relationship conceptually formulated by his archrival Pasteur, it did not result in a single cure.

Koch's urge for experimentation began to crystallize when in 1875 he took a grand trip to attend a number of scientific and medical meetings, and to visit a number of the major German and Austrian medical research laboratories. His interests had gradually been focusing on infectious disease, and this trip catalyzed his determination to begin his active scientific efforts in this area. And from this moment on, in a period of six years, from 1876 to 1882, Robert Koch went from an obscure country physician to an internationally reputed biomedical scientist.

Ideas about infectious disease were at this time in a state of flux. Pasteur had proven that microbes could be the cause, rather than the result of chemical change and it was not a great conceptual leap to conclude that the process of infectious disease was analogous to chemical change. Indeed, there were numerous provocative experimental clues that bacteria were associated with certain infectious diseases. But the convincing causal relationship between the two resisted conclusive proof.

The vehicle for Koch's efforts was the infectious disease anthrax (*Milzbrandkrankheit*). This is a disease that affects cattle and sheep with high mortality and could also infect humans, sometimes fatally. It had been shown by Pollander in 1855 (42) that blood from infected animals contained numerous rod-shaped foreign objects, and Davaine in 1863 had been able to infect healthy cows with blood from an infected animal, which contained what he referred to as "bacteridia" (11). Koch confirmed Davaine's findings and found, in addition, that the bacteria contained endospores. Ferdinand Cohn, who was to become Koch's mentor and enthusiastic supporter had earlier discovered and described, for the first time, endospores formed by the bacterium he named *Bacillus subtilis*, which is the non-pathogenic counterpart of the organism that was to become *Bacillus anthracis* (10).

Koch must have realized that this effort was going to be more than just a dabbling avocation, and he purchased a better microscope (figure 1) (at the expense of a carriage that he sorely need for his increasing medical practice). At this point his efforts shifted into high gear. The timing of what happened during the next few months is nothing short of astonishing. Koch's life-changing trip to scientific meetings and research laboratories took place in the fall of 1875. A few days before Christmas he began his anthrax experiments. Working through Christmas eve and Christmas day until January 1, he accomplished the following: 1) worked out the life cycle of sporulation, germination and outgrowth of *B. anthracis* (named by Cohn); 2) established a new paradigm in infectious disease, namely that human infectious disease could occur not only by transmission from person to person, but also via an inanimate substance such as soil, and 3) proved that *B. anthracis* was unequivocally the etiological agent of anthrax.

While serving as District Health officer Koch did the simple, crude experiment of showing that material scraped from the hide of a sheep that had died of anthrax, and containing bacteria, was infectious for an experimental healthy rabbit. An autopsy showed that the lymph gland of the rabbit was teeming with bacteria. Koch then made the remarkable and fortuitous discovery that the bacteria would grow in the corneal fluid expressed from the rabbit's eye. This allowed him to set up slide cultures inoculated with the bacteria, which he then carefully observed microscopically. They soon formed intracellular, refractile spheres, which he immediately recognized as the "Dauersporen" or resting spores that had earlier been described by Cohn (10). In addition, Koch had a culture of Cohn's *B. subtilis* and did the important control of showing that it did not cause anthrax when inoculated into a healthy rabbit.

He was able to observe in the slide culture not only the vegetative growth of the bacteria, but also the intracellular appearance of the spores, as well as their germination to vegetative cells. All these were beautifully illustrated in a composite figure (22) used both by Cohn and Koch but appearing in Cohn's paper, preceding Koch's in the same issue (figure 2). The transmission of infectious disease was, until now, believed to take place only by passage from one individual to another. The insights drawn from this part of the study allowed Koch to understand how a bacterium can lay fallow in the soil for extended periods of time and then be able suddenly to infect a sensitive host. At this point, Koch the physician took over from Koch the naturalist. He proceeded to carry out a series of successive inoculations of mice (20 in all). Bacteria from each infected animal served as the inoculum for the next. In every case the results were the same, death of the animal, with a swollen spleen containing large numbers of vegetative rods of *B. anthracis*. Koch concluded, "We thus see that anthrax tissues, regardless of whether they are relatively fresh, putrefying, dried or many years old can produce anthrax when these substances contain bacilli capable of developing spores of *Bacillus anthracis*." (22) Q.E.D.!

It is interesting that the work was done before Koch had developed his plating technique for colony growth and was thus done without having isolated pure cultures. But fortunately, the blood and internal organs of the infected animals contained what must have been a pure culture of the anthrax bacillus, thus allowing Koch to draw his conclusions. However, before publishing his work, as a newcomer to professional experimental science, Koch needed assurance that his work was likely to be received positively. Accordingly he sought a meeting with the acknowledged leader in this new

field, Ferdinand Cohn, who just a few years previously has published the first scientific book on bacteria (9). Koch in his old age was to become an arrogant and authoritarian *geheimrat*; however, the youthful Koch wrote a touchingly humble letter to Professor Cohn:

“Honored Professor!

I have found your work on bacteria, published in the *Beiträge zur Biologie der Pflanzen*, very exciting. I have been working for some time on the contagion of anthrax. After many futile attempts, I have finally succeeded in discovering the complete life cycle of *Bacillus anthracis*. I am certain now, as a result of a large number of experiments, that my conclusions are correct. However, before I publish my work, I would like to request, honored Professor, that you, as the best expert on bacteria, examine my results and give me your judgment on their validity....

Therefore, I earnestly request that you permit me to visit you in your Institute of Plant Pathology for several days, so that I may show you the essential experiments. If this request is agreeable to you, perhaps you might inform me of a suitable time that I might come to Breslau.

Very sincerely yours,

Dr. Koch, *Kreisphysicus*” (17).

A small digression is appropriate at this point. It is important to emphasize the role played in Koch’s career by the eminent botanist Ferdinand Cohn, who viewed this young, rising star not as a competitor but as a colleague, and served as his mentor and patron until Koch’s career solidified with his appointment in Berlin. Cohn was a Jew, and as such, had found difficulties in academic Germany in the early part of his career. He was not permitted to study for a doctoral degree at the University in Breslau, but the more liberal University of Berlin allowed him to do so. He then returned to Breslau to begin his work in plant pathology, and after 20 years, the University grudgingly appointed him Director of a new Institute of Plant Biology. He emphasized the study of living plants and initially focused his efforts on studying algae. In the 1860s he turned his efforts toward the bacteria, and the science of bacteriology began, ironically to be brought to an exponential stage of development by Robert Koch, then Cohn’s acolyte.

Imagine the exalted Herr Professor, Director of the Institute receiving such a request from an unknown country physician. Yet Cohn immediately agreed to see Koch, and invited him to Breslau. Koch arrived with all of his data and demonstrations. Cohn was immediately excited and enthusiastic about the work and invited his assistants and colleagues to view the work. Included among these was Julius Cohenheim, Director of the Institute of Pathology at the University. Cohenheim’s own prescient words to one of his colleagues describe his response to Koch’s results.

“Now leave everything as it is, and go to Koch. This man has made a magnificent discovery, which for simplicity and the precision of the methods employed, is all the more deserving of admiration, as Koch has been shut off completely from all scientific associations. He has done everything himself and with absolute completeness. There is nothing more to be done. I regard this as the greatest discovery in the field of pathology, and believe that Koch will again surprise us and put us all to shame by further discoveries” (47).

Koch's work was published in Cohn's new journal, *Beiträge zur Biologie der Pflanzen* (22) and one of his figures showing germination of spores of *B. anthracis* was actually prepared by Cohn. Koch acknowledged Cohn in a footnote:

"I...lay great importance on the fact that, at my request, Professor F. Cohn, to whom I owe special thanks for his trouble, tested, and in every respect affirmed my statements regarding the life cycle of *B. anthracis*" (22).

Despite the immediate and enthusiastic acclaim for the work, there continued to be objections and criticisms both of the work and of the very idea that bacteria were the causes of disease. The matter was to be settled categorically and conclusively by Koch's canonical work on the etiology of tuberculosis, but before that was to happen, it was necessary for Koch to invent pure culture bacteriology and along with that, many of the routine tools of laboratory bacteriology.

The modus operandi of bacteriology

"The pure culture is the foundation of all research on infectious disease" (24)

Back in Wollstein, resonating to the success of his work on anthrax and its enthusiastic reception, Koch sought new worlds to conquer. The problem he set for himself was to work out the etiology of wound infections. It was quite clear, based on the work of the English scientist Joseph Lister and others that sepsis of wound infections was almost certainly a bacterial process. But once again, the specific etiologies were unclear. Nor was it even clear that there might have been more than one etiological agent responsible for the various septic processes. Unfortunately, what had worked so effectively with anthrax was ineffective with septic wound infections. The diseased tissue was usually inhabited by mixed cultures, and successive passaging of this material in experimental animals simply resulted in increasingly selective enrichment cultures, so that what emerged after numerous passages bore little resemblance to the initial etiological agent. But during this work Koch perfected his microscopic techniques and pioneered and refined the use of aniline dyes, use of the Abbe condenser to achieve increased resolving power and the use of the immersion lens to effectively increase magnification.

Nevertheless, Koch was increasingly dissatisfied with his isolation in Wollstein from the scientific community, which in the late nineteenth century was in the midst of new discoveries, new paradigms and most of all for Koch, new opportunities. He made frequent visits to Breslau to confer with Cohn and his colleagues, and Cohn in turn sought for an opportunity to bring Koch to Breslau. He managed to obtain for Koch an appointment at the University as a sort of adjunct professor with few perquisites and even less salary. To bolster this he also arranged for Koch to be appointed to a position of *Gerichtliche Stadtphysikus*, a kind of public health director for the city, and Koch and the family moved to Breslau. Alas, it quickly proved unsatisfactory. He had, of course, given up his private practice in Wollstein and its important supplementary income; his family was unhappy in Breslau and back they went to Wollstein. But shortly thereafter, opportunity emerged in Berlin, which was to become Koch's venue for the rest of his life and career.

When the various German states were unified into the German Empire in 1871, one of its early administrative acts was to establish the Kaiserliche Gesamtheitsamt or Imperial Health Office. A new bacteriological research institute was established and Koch was the obvious candidate to head it. He was then 37 years old (Fig.3). Koch's two assistants were Friedrich Löffler who subsequently was to isolate and identify the etiological agent of diphtheria and Georg Gaffky who was to do the same for typhoid. The year following Koch's appointment, the Institute began publication of a new Journal, *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, and the first paper in the first volume was the paper (24) that Brock has described as the one publication most significant for the rise of microbiology (5). This paper takes off from the point where Koch's work on anthrax ended and describes the technology for obtaining pure cultures and for more effectively using microscopy to study bacteria.

Three years earlier, Joseph Lister had published a beautiful paper on an organism that carried out a lactic acid fermentation, probably a *Lactobacillus* (34). One might wonder why a surgeon interested in wound and surgical sepsis would be interested in studying the lactic acid fermentation. He chose it as a model system, amenable to experimentation and as a vehicle for working out techniques of bacteriology. He described a method for obtaining the organism in pure culture by means of dilution. It was a simple, elegant albeit cumbersome technique, but it worked. He described the various mundane, necessary precautions that are now routine for bacteriological procedure, such as flaming vessel apertures, the use of sterile media and glassware, sterile cotton pugs, inoculating needles etc. It is not clear whether or not Koch was aware of this paper. If he was, he did not cite it in his paper.

The prelude to Koch's technique of obtaining a pure culture was a paper published in 1872 by Joseph Schroeter, a student of Ferdinand Cohn (43). In it he described the appearance of colonies of bacteria on the surface of slices of boiled potato that had simply been exposed to laboratory air. He used this technique to obtain some pure cultures but did not pursue the matter. Koch was obviously aware of Schroeter's work, but again, did not cite it. He recognized that each colony was the accumulated progeny of a single cell and thus a pure culture. He realized the potential of the technique and proceeded to exploit it successfully. What an incredible moment! It was what Thomas Kuhn has referred to as a new paradigm (33), and it transformed bacteriology into a science. It made it possible, in a critical fashion, to attribute a specific pathological, physiological, ecological or chemical change to a specific, single microorganism. It made it possible to count viable bacteria accurately, and of course, later on, made it possible to do bacterial genetics. But it was soon obvious that a slice of boiled potato was far from an appropriate or convenient bacterial growth medium. Koch then formulated the idea of solidifying a nutrient medium by the addition of 2.5-3% gelatin. While this was a huge improvement over potato slices, i.e., the medium was transparent and could contain any necessary nutrients, it had its own drawbacks. It liquefied at temperatures necessary to grow most pathogens, i.e., 37°C, and was degraded and thus liquefied by many bacteria.

Walther Hesse, who had briefly worked in Koch's laboratory, was carrying out experiments attempting to quantitate the bacteria in air, and his work was hampered by the limitations of gelatin as a solidifying agent. His wife Fannie, who assisted him by preparing his bacteriological media, was familiar with the use of agar-agar from Japanese seaweed (*Gelidium corneum*) which she used as a gelling agent in her cooking. She

suggested it to her husband, and it was as if it had been specifically designed for bacteriological use. He communicated this discovery to Koch who recognized its utility and promptly made it his own. No publication describing its use or properties was ever made but the history of its discovery has been described (19).

The other bacteriological stand-by introduced to common use by Koch is the Petri dish, devised by R.J. Petri in 1887 (40). Petri was a minor Prussian functionary, and it is ironic that his name is routinely uttered by biologists overwhelmingly more frequently than that of Koch.

It is interesting that if Koch were to walk into a bacteriological laboratory today he would see essentially the same inoculating loops, Petri dishes filled with agar media, stains, slides, cover slips and flasks stoppered with cotton plugs (albeit most of it now disposable) that he used or devised 250 years earlier. But he'd be thrilled and astonished by the sophisticated microscopic and photomicroscopic equipment he'd see.

Koch used a Siebert microscope, an excellent albeit simple instrument (figure 1). It was monocular, with coarse and fine focusing knobs, a simple condenser and a swiveling mirror. Its magnification was as high as 700 \times , enough to view the large cells of *B. anthracis* easily, and barely enough for most other bacteria. Koch dried and heated the bacteria on cover glasses and stained them with aniline dyes such as methyl violet and methylene blue.

In his constant pursuit of more effective images of his bacteria, Koch also incorporated the Abbe condenser, which allowed a more focused entrance of light into the objective lens and thus a higher resolving power, and oil immersion lenses which allowed magnifications of up to 1000X. But he was frustrated by the need to laboriously draw by hand any examples of the bacteria he was studying. Photomicroscopy was the obvious solution, but not one that was easily and conveniently available in Koch's time. Photomicroscopy today is a relatively simple process. Set a slide on the microscope, set the focus and push a button. In the 19th century it was a far more arduous and involved procedure. The lighting of Koch's preparations was by sunlight – household electric power was not yet available. His wife Emmy would be stationed outside the laboratory window to inform him of the appearance of clouds that might affect the exposure; he referred to her as his “*wolkenschieber*” or cloud chaser. Sunlight through the window was directed by a heliostat onto the microscope mirror, and the shutter was on the window rather than on the photocamera, so as to prevent any undue change in the focus when the shutter was activated (figure 4). When the slide was in place and properly focused, the photographic plate had to be quickly prepared. A glass plate was coated with a thin film of iodized collodion, which was then immersed in a bath of silver. The wet collodion plate, impregnated with silver iodide was rushed to the microscope and, if the sun had cooperated, the photo was taken. The results of Koch's efforts were the first photomicrographs of bacteria ever taken and were published in Koch's second paper (23) (figure 5).

It is not an overstatement that these relatively simple technical innovations represented the beginning of bacteriology as a quantitative, analytical science.

The great white plague

“All these facts taken together lead to the conclusion that the bacilli that are present in the tubercular material not only accompany the tuberculosis process but are the cause of it.”
(17)

In the early nineteenth century, tuberculosis was one of the major causes of death in western Europe and the United States. About one person in four died of it and almost all Europeans were infected with it (48). In his country practice, Koch must have seen it many times, and as Head of the Institute for Bacteriological Research in the Imperial Health Office he was certainly preoccupied with the disease as a major public health problem. While it had become increasingly clear that the disease was an infectious process – Villemin had already shown that the disease could be transmitted to healthy animals by the injection of the sputum of a diseased animal (45) – critical proof was absent. In fact, Rudolf Virchow, one of the world’s leading pathologists believed that the disease was not infectious but was caused by a tumor. It was obvious to Koch that it was necessary not only to demonstrate unequivocally that the disease was infectious, but also to isolate the infectious agent itself.

The first step in the process was to demonstrate that the putative infectious organism was present in all cases of the disease. Before Koch had developed the technique for isolating pure cultures, this could only be done microscopically. However, not only is the tubercle bacillus extremely small, but Koch’s first attempts to stain the bacillus were also unsuccessful. Due to its waxy outer coat, the tubercle bacillus could not be stained by the ordinary aniline dyes Koch used at the time. An investigator less confident than Koch might have given up at this point and concluded that no bacteria were involved in the process. But Koch persisted. The suspected tissue was immersed in an alcoholic solution of methylene blue, treated with potassium hydroxide, heated, and the surrounding tissue counterstained. The tubercle bacillus stained a beautiful blue against a background of brown stained tissue. Koch saw the tubercle bacillus in all samples of infected tissue that he examined. What had begun as a setback emerged as an advantage, as the tubercle bacillus, which we now know as an acid-fast bacterium, could now be detected with a differential stain that allowed it to be distinguished from other bacteria. “On the basis of my extensive observations, I consider it as proved that in all tuberculous conditions of man and animals there exists a characteristic bacterium which I have designated as the tubercle bacillus, which has specific properties which allow it to be distinguished from all other microorganisms.” (26).

Thus, the first criterion of what were to become known as “Koch’s postulates” had been satisfied. Next it was necessary satisfy the second postulate, to isolate the putative etiological agent and cultivate it in pure culture so as to eliminate any adhering diseased material that could be thought of as playing a role in the disease. And here Koch made use of his newly devised technique for the cultivation of bacteria on a solid surface. As indicated earlier, gelatin as a solidifying agent was unsatisfactory, and while he was now aware of the use of agar as a solidifying agent, he chose to use instead coagulated cow or sheep blood serum. He used it in the form of what we now know as slants in a test tube rather than Petri dishes, and spread a bit of a single tubercle over its surface. When

isolated colonies appeared they were subcultured; thus Koch was able to forestall the objection that disease resulting from the injection of the infectious material was due to some adhering non-microbial material.

Here, as happened frequently in Koch's publications, his willingness to cite the work of others whose observations or innovations were precursors to his own work was somewhat lax. He made no mention of Schroeter's work on the appearance of colonies on potato slices, no mention of Lister's dilution method for obtaining pure cultures, no mention of Petri's dishes, no mention of Hesse's suggestion of the use of agar, nor any mention of Joseph Tyndall's use of coagulated serum as a growth medium.

Koch then used the pure cultures to inoculate healthy guinea pigs, and was able to demonstrate that these experimentally infected animals developed the characteristic tubercular nodules and died within four to six weeks. Thus, the third of the postulates was satisfied.

Koch explicitly stated his postulates (albeit in somewhat incomplete form) in his first tuberculosis paper (26) as follows:

“To prove that tuberculosis is brought about by the tubercle bacillus, and is a parasitic disease that is caused by the growth and reproduction of the bacilli, it was first necessary to isolate the bacteria from the body; to grow them in pure culture until they are free of any diseased tissue from the infected animal that might have adhered to them; and then by introducing the pure culture of the bacilli to animals, produce the same pathological condition which can be obtained by infecting with spontaneously generated diseased material.”

The postulates as thus stated, do not include the requirement that the responsible organism be present in all cases of the disease. The complete set of postulates was formally stated in a subsequent paper by Löffler, Koch's assistant (35).

It is commonly acknowledged that the conceptual basis for the postulates was formally stated in 1840 by Jacob Henle, one of Koch's teachers. Henle stated that the etiological agent of infectious disease is likely a living organism, a “*contagium animatum*”, and laid out three criteria, essentially identical to “Koch's postulates” that must be satisfied to define the etiology of the disease (18). Although Koch acknowledged his general debt to Henle as one of his teachers, he never cited Henle's formulation of the postulates.

Before Koch published his work, it was presented orally at the Physiological Society at Humboldt University in Berlin, and this meeting has become an important historical event. It was an occasion preceded by controversy, and for Koch, filled with anxiety. There was, at the time, no society for bacteriology, and the Berliner Pathologische Gesellschaft, the dominant organization of pathology would have been the logical place for Koch's presentation. It was, however, dominated by Rudolf Virchow, the Lion of Pathology, who had for the past thirty years imposed his dogma of the non-infectious nature of tuberculosis, and which had been firmly implanted as gospel. He viewed the establishment of The Imperial Health Office as a personal affront, had for years criticized Villemin's work as deluded and misleading, and was in no mood to welcome this upstart doctor with his new-fangled ideas. Accordingly, it was arranged for Koch's work to be presented to the Physiological Society, an equally prestigious organization, albeit somewhat removed from the newly emerged field of bacteriology.

The presentation took place in a small meeting room crowded with the cognoscenti of the German biomedical profession, as the word had gotten out that this was to be an important and contentious event. Koch's nemesis Virchow, the protagonist of dualistic theories of infectious disease was there, and there must have been great expectation that Koch's presentation would be followed by a devastating refutation of the work.

Koch had prepared well. He brought an extensive array of demonstrations with him; over 200 slides, a microscope, test tube slants, plate cultures on Petri dishes and liquid cultures in Erlenmeyer flasks. He proceeded slowly and methodically. The inexorable logic and clarity of his experiments led in a crescendo to the final clincher – fulfillment of the third of the postulates – the induction of disease in healthy animals infected with the pure culture that had undergone multiple transfers. Löffler, who had helped organize and was present at the meeting describes that when Koch finished there was a stunned silence. There was no applause, no questions, and no discussion. Virchow was silent. Members of the audience rose to shake Koch's hand, and to examine his preparations. Löffler later commented, "It was quite an evening." (36). Paul Ehrlich, who was present, said, "Everybody who attended the lecture was deeply affected, and I have to say that the evening has remained in my memory as my greatest scientific occasion" (12).

His work was published two weeks later in the *Berliner Klinische Wochenschrift* (26) and followed two years later by a more comprehensive study (28). It is interesting that the same issue contained papers by two of Koch's assistants, Löffler, on the etiology of diphtheria (34), and Gaffky, on the etiology of typhoid (13).

What were the consequences of Koch's discovery of the tubercle bacillus? It is interesting that the discovery that *Mycobacterium tuberculosis* was definitively the etiological agent of tuberculosis had no substantial effect on the rate of decrease in the death rate from tuberculosis, that had been steadily decreasing over the past forty years (figure 6-figure 22.2 Brock). However, as the vehicle for the germ theory of disease, it did lead to therapies and preventive measures for a host of other infectious diseases, e.g., diphtheria, tetanus, cholera, typhoid and wound infections, that were more amenable to treatment and prevention than the complex disease tuberculosis. It is also interesting that to this day, we still don't understand the mechanism of pathogenesis of the tubercle bacillus.

Koch's discovery did, however, free us from the mysterious and magical miasmatic theories of disease. In Koch's own resounding words: "If we ask further what significance belongs to the results gained in this study of tuberculosis it must be considered a gain for science that it has been possible for the first time to establish the complete proof of the parasitic nature of a human infectious disease, and this of the most important one... It may be expected that the elucidation of the etiology of tuberculosis will provide new viewpoints for the study of other infectious diseases... Tuberculosis has so far has been habitually considered to be a manifestation of social misery, and it has been hoped that an improvement in the latter would reduce the disease. Measures specifically directed against tuberculosis are not known to preventive medicine. But in the future the fight against this terrible plague of mankind will deal no longer with an undetermined something but with a tangible parasite whose living conditions are for the most part known and can be investigated further." (41)

Cholera

The success of his work on the etiology of tuberculosis brought Koch authority and prestige. Confidently, along with a team of assistants, he was sent to Egypt in 1883 to confront a plague of cholera and to conquer yet another demon of infectious disease. Koch and his team arrived in August and immediately set to work trying to isolate the cholera bacillus. They were sure that the disease was an infectious one and that the etiological agent would be a bacterium. But their initial efforts were unsuccessful. Failure followed failure.

There were essentially three obstacles initially in the hunt for the cholera bacillus. Unlike the anthrax bacillus which existed in the blood stream of the host in an essentially pure culture, and the tubercle bacillus, enclosed alone in the tubercular nodule, the cholera bacillus existed side by side with thousands of other bacteria in the infected intestinal tract. Thus, microscopic examinations were useless.

There was no selective medium for the cholera bacillus. As a result, its presence amidst a zoo of other intestinal bacteria resulted in its being overgrown after relatively brief periods of incubation in rich media.

There was no experimental animal that could be infected with the cholera bacillus. Inoculation of mice with intestinal contents of stricken patients had no pathological effect on the animals. Thus, the critical third postulate could not be satisfied.

By September, the epidemic in Egypt had ended. But once again, Koch's genius for determination, adaptability and organization came to his aid. His team transferred their efforts to India, where cholera was endemic. Within days of their arrival they were able to isolate a pure culture of a comma-shaped bacillus, morphologically identical with the organism seen in all cases of the disease. The apparent key to their sudden success was that the organism was isolated from a corpse only a few hours after death and presumably soon enough so that the cholera bacillus was still a dominant organism in the intestine. Despite the inability to fulfill the third postulate, and despite the skepticism of many, including Pasteur, Koch was confident that he had captured his prey.

However, in the absence of such definitive proof, Koch turned his attention to a more epidemiological and public health approach. He was able to show convincingly that contaminated water was the source of the infections and thus confirmed, although once again did not cite John Snow's earlier demonstration that the public water pump on Broad Street was the source of endemic cholera in London (8). Koch and his team returned to Germany in triumph. The details of this remarkable expedition are described in a volume edited by Koch's assistant Gaffky with the collaboration of Koch himself (14).

In 1892, a cholera epidemic arose in Hamburg, and again Koch and his team were called to deal with it. This time, armed with the results of the earlier expedition to Egypt and India, they were able relatively easily to isolate the cholera vibrio and to demonstrate convincingly that the means of transmission was from river water contaminated by the release of urban sewage. In a real sense this marked the beginning of scientific, microbiological epidemiology.

The Koch-Pasteur controversy

“Of these conclusions of Pasteur on the etiology of anthrax there is little that is new, and that which is new is erroneous. ...Up to now, Pasteur’s work on anthrax has led to nothing.” (25)

“Koch acted ridiculous and made a fool of himself.” (39)

The interaction between Pasteur and Koch was infamous. One commentator went so far as to refer to their relationship as “hateful” (37). There seem to have been four underlying bases for this tension. First, there existed a mutual interethnic contempt between the French and Teutonic cultures, reflecting a Dionysian sensual France versus a rational, self-disciplined Apollonian Germany. By the Germans, a reluctant envy of French cuisine on the one hand, coupled with a view of French government in general and French science in particular as bumbling and disorganized; the converse view by France of German science, personified by Koch, as arrogant, rigid and authoritarian. Pasteur never forgot the contemptuous rejection by Leibig, Berzelius and Wohler of his germ theory of fermentation, ridiculing the very idea that a yeast cell could actually transform sugar into ethanol. Second was the humiliating defeat of France by Prussia in the Franco-Prussian war of 1870-1871, when the French armies were defeated by Prussia in the period of a few weeks. This resulted in the acquisition by Prussia of the provinces of Alsace and Lorraine, and was followed shortly thereafter by the unification of the German states into the single German Empire. This was personified for Pasteur by the appointment of Koch as Director of the newly formed Kaiserlichen Gesundheitsamte, a single, powerful national Institute of Hygiene and Disease.

Pasteur was passionate in his hatred of the Prussians. He vowed that “Each of my efforts until my last day will carry an epitaph “Hatred of Prussia. Vengeance, vengeance!”(44). Pasteur declined the award of the Prussian order Pour le Mérite (the prestigious “Blue Max”), and in 1871 he returned in protest an honorary MD degree awarded to him in 1868 by the University of Bonn. His accompanying letter read: “...I obey a call of conscience in requesting you to erase my name from the archives of your faculty and to take back this diploma as a sign of indignation which the barbarism and hypocrisy instills in a French scientist from those who, to satisfy a criminal need, insist on the massacre of two great nations.”

The Dean of the Medical School of the University of Bonn responded: “Sir, The undersigned, the present Dean of the Faculty of Medicine of the University of Bonn, is charged to answer the insult which you have dared to make to the German nation in the sacred person of its noble emperor, the King William of Prussia, in sending you the expression of all his contempt! Signed Dr. Maurice Naumann.” (44).

The third basis was a conflict between two egotistical geniuses, giants of microbiology, jockeying for priority and recognition as the first to have solved the problem of the etiology of anthrax. Both were eager to be recognized as the true power behind the immense new paradigm of the germ theory of disease. Koch stated his position clearly:

“Although I have no interest in priority disputes, these matters are so obvious that I cannot ignore them. I can only answer Pasteur’s claim by referring to my publication of 1876 that describes the generation of anthrax spores and their relation to the etiology of anthrax. Pasteur’s first word on anthrax was published one year later in 1877. This requires no further comment.” (27). Koch’s position is accurate.

Fourth was a series of criticisms leveled by Koch against Pasteur’s experimental strategies and techniques. The conflict broke into the open at a meeting of the IVth International Congress for Hygiene and Demography in Geneva in September 1882. Pasteur, stung by Koch’s criticisms, spoke, challenging Koch to debate the matter there and then publically. Koch rose afterwards and declined Pasteur’s invitation, indicating that he would respond later, but in print. He did so in a brochure published in 1882 (27) in which he leveled a series of devastating criticisms of Pasteur’s work in particular, as well as of Pasteur’s general scientific deficiencies. “So the methods followed by Pasteur must be called full of mistakes and cannot lead to successful results because they lack microscopic examinations, involve the use of impure substances and use unsuitable experimental animals.” (27). In addition to the criticisms mentioned above he assailed Pasteur for failing to observe the logical proofs we now know as Koch’s postulates. These focused mainly on Pasteur’s failure to use microscopy to demonstrate the presence of the putative etiological agent in all cases of the disease, and his failure to use pure cultures to fulfill the third of the postulates. He also castigated Pasteur for his tendency to withhold data that contradicted his biases as well as those that would compromise the commercialization of his work.

In the following year, Pasteur responded to Koch’s violent attack on his work (39). To assure that the reader knew what he was responding to, Pasteur’s reply was preceded by a French translation of Koch’s brochure. Pasteur spent much of the essay emphasizing the role of his work on the germ theory of fermentation as a conceptual precursor of the germ theory of disease. He quotes the prescient comment of Robert Boyle the eminent 17th century chemist:

“...He that thoroughly understands the nature of ferments and fermentations shall probably be much better able than he that ignores them, to give a fair account of divers phenomena of several diseases (as well as other fevers) which will perhaps never be understood without an insight into the doctrine of fermentation.” (39).

Pasteur referred to his work on fermentation where he was indeed able to use a pure culture of yeast, despite the fact that pure cultures of bacteria were essentially unobtainable until Lister’s and Koch’s contributions. He justified his work on attenuation and immunization. He refers to Koch’s comments with such terms as “ludicrous”, “flippant”, “hasty”, “thoughtless”, “noisy”, “disdainful”, “fantastic”, and “childish”, emphasizing, in passing, his work in 1856, when Koch was 13 years old.

If anything, the juxtaposition of these two brochures illustrates the unbridgeable gap between these two giants- each arguing past the other, neither convinced by the arguments of his opponent, waiting for posterity to judge their respective roles.

If there was a conceptual leap that sprang essentially unaided by preceding work, it must be ceded to Pasteur’s concept of the germ theory of fermentation. It made everything that followed attributing to microbes the cause of chemical and pathological change possible. And Koch acknowledged that. But it remained for Koch, reflecting his

inimitable experimental genius, to demonstrate the cause and effect relationship convincingly.

The tuberculin fiasco

Koch's brilliant successes in elucidating the etiologies of anthrax and tuberculosis were followed quickly by his work on cholera, which opened the entire area of the epidemiology of infectious diseases. In 1884, his assistants Löffler isolated the diphtheria bacillus, and Gaffky the typhoid bacillus. Koch's work generated a euphoric optimism that the ravages of infectious disease would soon be at an end. German science and Germany's new nationalism were flourishing. And in 1885 Koch received the prestigious appointment as Professor of Hygiene in the Medical Faculty at the Friedrich-Wilhelm University of Berlin. But the newly created Institute of Hygiene with Koch as its Professor was not the blessing it seemed to be. The Institute had been formed by the government against the wishes of the University and was opposed by the Faculty of Medicine. Koch was beset by administrative responsibilities and teaching obligations in addition to his personal marital problems. The years between his triumph over cholera in 1884 and 1890 saw no significant publications, and in fact Koch's productive research had ceased. The spectacular successes of Pasteur's anthrax and rabies vaccines and of the Pasteur Institute stood in striking contrast to the fact that while Koch's towering achievements on the etiology of infectious diseases had led to some non-specific measures of preventive hygiene, they had resulted in no specific therapies- not a single cure. Tuberculosis still ravaged the world.

During this period, Koch's research had focused sharply on finding, if not a cure for tuberculosis, at least an effective therapy. But a number of experimental strategies had failed. Following Pasteur's successes, he was unable to accomplish an attenuation of the virulence of the tubercle bacillus by continuous laboratory cultivation (29). A series of experiments testing various substances for their ability to affect the course of the disease in experimental animals were unsuccessful. Koch never published these results (38). In 1886 Koch refocused his efforts on an alternative strategy for finding a cure. This work culminated with Koch's spectacular announcement at the 10th International Medical Congress in Berlin that "...I ultimately found substances that halted the growth of tuberculous bacteria not only in test tubes but also in animal bodies." (30). This announcement of "substances" that were effective not only in vitro but also in vivo electrified the audience and quickly the entire world.

No details as to the nature of the "substance" were released and in a subsequent paper Koch stated: "As regards the origin and preparation of the remedy, I am unable to make any statement as my research is not yet concluded; I reserve this for a future communication. The remedy is a brownish, transparent liquid."(31).

Thousands, including tubercular patients, physicians, reporters and opportunists descended upon Berlin, creating a remarkably serious public health threat in the city. Tributes poured in. Even Louis Pasteur congratulated Koch (5). Requests for tuberculin far exceeded the limited supply, and requests that Koch release details for its production were met with Koch's refusal to do so. He argued that the difficulty in preparing the

material could lead to ineffective or dangerous substitutes (5). Finally, under considerable pressure Koch released details for the production of the “substance” which he now referred to as “tuberculin”. It consisted of a crudely purified solution of antigens of the tubercle bacillus present in the culture supernatant of a heat-killed culture of the tubercle bacillus (32). By early 1891 it had become clear that tuberculin had no merit as an effective therapy for tuberculosis (1,2,3,15). Successes were minimal and in the absence of careful double blind studies may even have been non-existent. Side effects were often severe. Nevertheless, Koch was to persist in his experiments with tuberculin for the rest of his life.

Although it took over fifty years – tuberculin continued to be used therapeutically - careful, systematic laboratory analysis eventually replaced the almost hysterical response by an eager world for a cure. Tuberculin is now widely used to reveal a present or previous infection by *Mycobacterium tuberculosis*, manifested as a delayed-type hypersensitivity (T-cell mediated) to the tubercular antigens, i.e., tuberculin.

What possibly could have induced a careful, cautious investigator such as Koch to commit such a scientific blunder? Burke (6) has suggested a variety of pressures, both external and internal that led Koch into this fiasco. During the late 1880s, Koch made the mistake of sharing with some government officials news of his tentative successes. An awareness of what Louis Pasteur’s work had done for the prestige of France and French science could not have escaped the officials. It has been reported that days before the 1890 10th International Medical Congress in Berlin, Koch was being directly pressured by officials in the government to announce his discovery (20). Koch was in the midst of negotiations with the government to agree to found a new Institute of Infectious disease with himself as Director. It is not unreasonable to conclude that he succumbed to their pressure.

To further cloud his judgment, Koch’s ongoing rivalry with his French colleague for international recognition must have been exacerbated by Pasteur’s successes with the anthrax and rabies vaccines. And internally, within Koch’s own Institute for Hygiene, his colleagues were doing exciting work. Von Behring’s work on diphtheria toxins, for which he was to receive the first Nobel Prize in Physiology and Medicine, Paul Ehrlich, Kitasato, Wasserman, Proskauer, were all on the way to becoming well known figures in the microbiology of infectious diseases. Koch was unaccustomed to playing second, third or fourth fiddle.

Some authors have suggested that Koch was motivated by pecuniary interests, and in fact profited from tuberculin. This matter is a contentious one and both sides of the question have been argued in the literature, e.g.:

“With the development of his supposed cure against tuberculosis, tuberculin, Koch attempted to give his research a new direction, earn a fortune with the profits and become more independent of Prussian government officials who, up to that point had had a major influence on his career.” (16). On the other hand:

“There is absolutely no evidence that Koch received personal gain, either from tuberculin or from any of his other discoveries.” (5).

Postlude and summing up

Bacteriology could not have developed as a quantitative science until Robert Koch was able to grow bacteria as pure clones on the surface of a solid medium. Koch must have realized that he had made a new science possible. Nevertheless, he died in 1910 frustrated that his remarkable invention of pure culture bacteriology had still not led to a cure for tuberculosis.

“What has been achieved by all the arduous labour that has been invested in the study of bacteria?” (30). It was implied by Koch, and hoped by the world when he presented his convincing description of the etiology of tuberculosis, that this presaged the end of the terrible white plague. But it was not to be.

It is ironic that had Koch not chosen tuberculosis as his battlefield – a disease manifesting multiple forms, caused by an extremely slow growing microbe, surrounded not only by its own tough wall but walled off by the body’s own defense – but instead had chosen the typhoid, cholera or diphtheria bacilli, his discovery could have been followed by therapeutic or preventive measures. Nevertheless, his work did lead to a new conceptual paradigm, that infectious diseases were not caused by miasmas, evil spirits or cellular malfunctions (46), but rather by tangible, definable microbes, and could be countered by science and rational measures.

But perhaps even more importantly, pure culture bacteriology led to an attitude and a canon about how one solved problems in microbiology. The ability to deal with a single pure culture made possible an intensely reductionistic approach to clarifying the cause and effect relationship between bacteria and their chemical and pathological effects. And for over a hundred years that approach was effective and powerful (Fig. 7; Table 1, Brock , page 290).

Koch’s invention of the use of solid media for the cultivation of bacteria, not only allowed the emergence of pure culture microbiology, but along with the canonization of Koch’s postulates reified an attitude about the microbial causation of disease. Like the later biochemical dogma of “one gene, one enzyme”, the idea of one bacterium, one disease, while effective for a hundred years, froze microbiology into a restricted posture when it came time to deal with complex microbial flora, multiple interactions within the flora, and complex host or environmental responses. At one time, working with mixed cultures would have been grounds for bacteriological excommunication. Microbiology is only now struggling to figure out how to deal with the complexity of those diseases or ecological processes that involve multi-membered microbial populations.

Koch’s development of photomicroscopy freed experimentalists from the necessity of demonstrations and witnesses to validate the accuracy of subjective drawings. His development of effective staining techniques, incubation chambers, and the use of experimental animals were part of his invention of the mundane but requisite tools of bacteriology. And Koch’s work on the etiology of cholera led to the emergence of the field of microbial epidemiology and to the public health measures that in most countries have resulted in the eradication of the disease.

Gradmann (16) has pointed out that one of the results of Koch’s work, even of his unsuccessful and misleading work on tuberculin, was the entrance of government into the realm of big science, and the increasing role of government in the establishment of prestigious research institutions. It is ironic that at that time, it reflected a gross exaggeration of the impact of the new bacteriological science on the solution of the

problems of infectious diseases. Far more important were the derivative fields of immunology and public health, reflecting the work of von Behring on diphtheria toxin and anti-toxin and the successful public health measures against cholera. For Koch, tuberculin made it possible for him to leave the directorship of the Institute of Hygiene, a position that had brought him no personal or professional satisfaction, and to become director of a truly scientific institute created specifically for him. The tale of how that happened is worth telling and bears on the question of his pecuniary gain from tuberculin.

In the euphoric aftermath of Koch's momentous announcement at the 10th Berlin Congress, that he had found a cure for tuberculosis, Koch proposed to Friedrich Althoff, an official at the Prussian Ministry of Culture, that an institute for continuing work on tuberculin be established. He also proposed that the proceeds from the sale of tuberculin be assigned to him and his collaborators. The government was to bear the risk for the venture, and profits would be turned over to them after six years. Koch estimated that the profits would amount to approximately 4.5 million marks per year (5). The Prussian government viewed these demands as inappropriate and proposed instead a direct, single payment to Koch, a proposal he rejected. The Prussian Chancellor put an end to the negotiations fearing the political fallout if it were to be revealed that an individual was profiting from this potentially humanitarian discovery. His ostensible justification for the veto was that other such institutes in Prussia prohibited personal gain by members of the institute. Koch's demands collapsed when it began to be clear that tuberculin, as a cure was ineffective, and construction on the new institute was stopped. The government, nevertheless, was eager for the new institute to be formed. In an address to the National Representative Assembly, Althoff said:

“There is a patriotic side to the affair as well. We have here a case of honor for German science. German research – and I have already mentioned the men- can take particular credit for detecting the cause of infectious diseases. Other states had preceded us with similar or related institutes.... We do not want to fall too far behind, we want to keep pace with them, we want you to enable German science to conclude what it has started, to harvest what it has planted.”(16).

The government agreed to establish Koch's salary at the enormous level of 20,000 marks per year; Koch accepted the proposal and the Institute for Infectious Diseases was completed.

The last 20 years of Koch's life, while free of the exciting and tempestuous highs and lows of the first 40 years, saw the success of his Institute of Infectious Diseases, the training and encouragement of a generation of important bacteriologists and immunologists such as Löffler, von Behring, Ehrlich, Pfeiffer, Kitasato, Gaffky, Wasserman and many others. Koch's personal life was enriched by his loving relationship with his second wife, Hedwig Freiberg, and he died peacefully at home in 1910.

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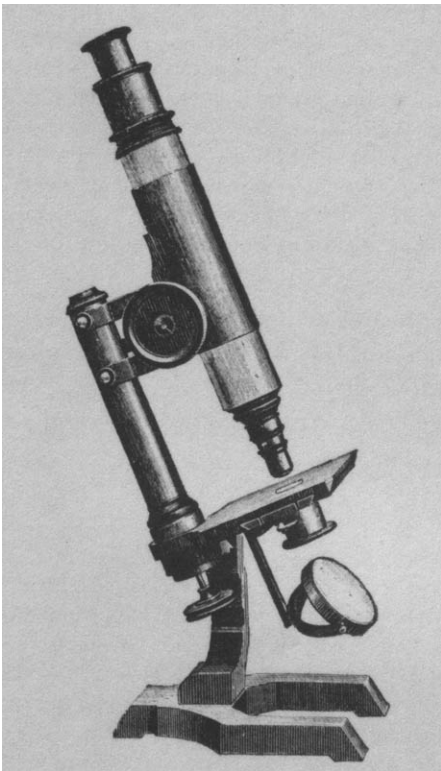


Figure 1 Koch's Siebert microscope



Figure 2 Stages in the life cycle of Bacillus anthracis. (22).



Figure 3. Robert Koch



Figure 4. Koch's photomicroscopy setup

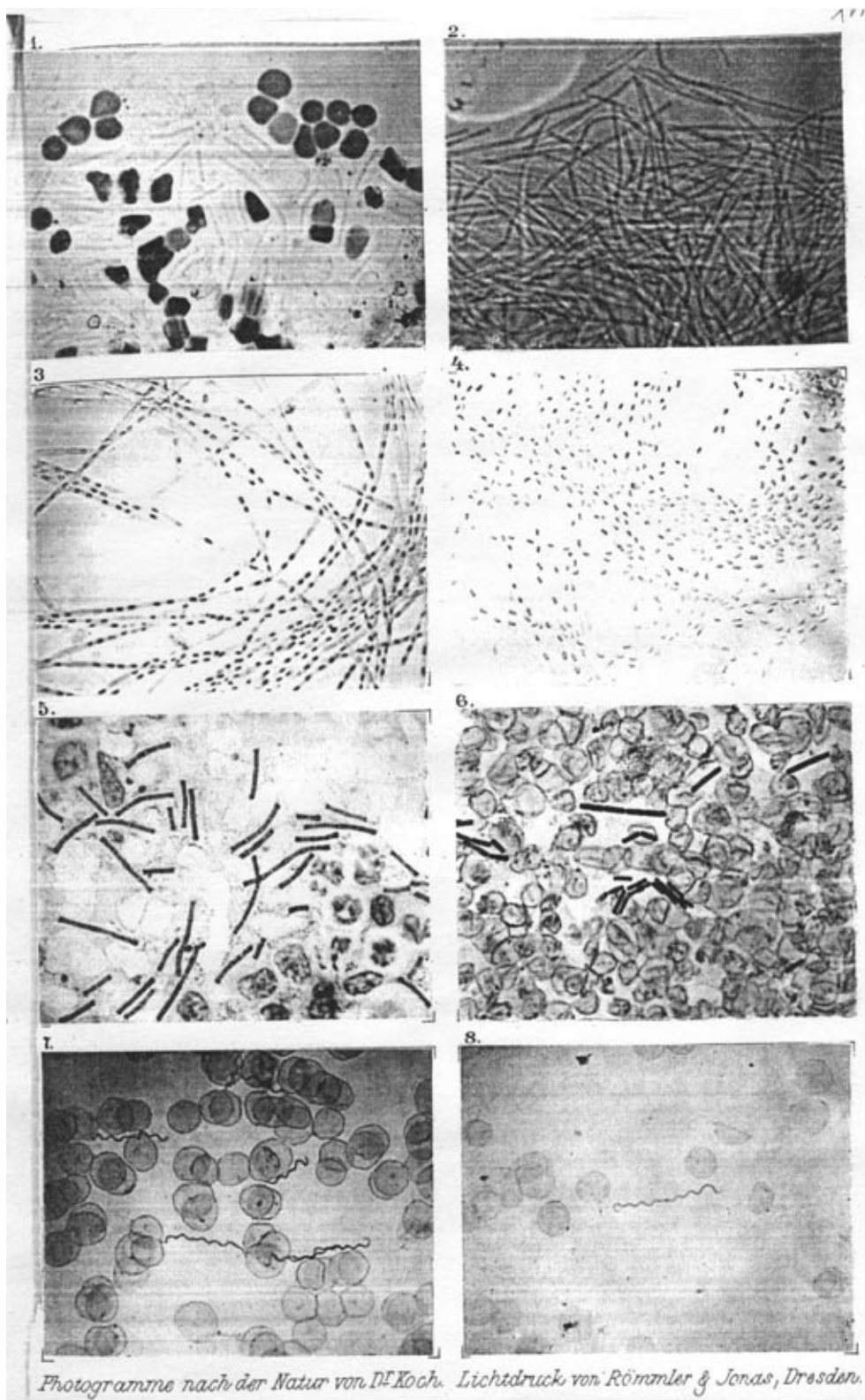


Figure 5. Koch's photomicrographs of *Bacillus anthracis* (23).

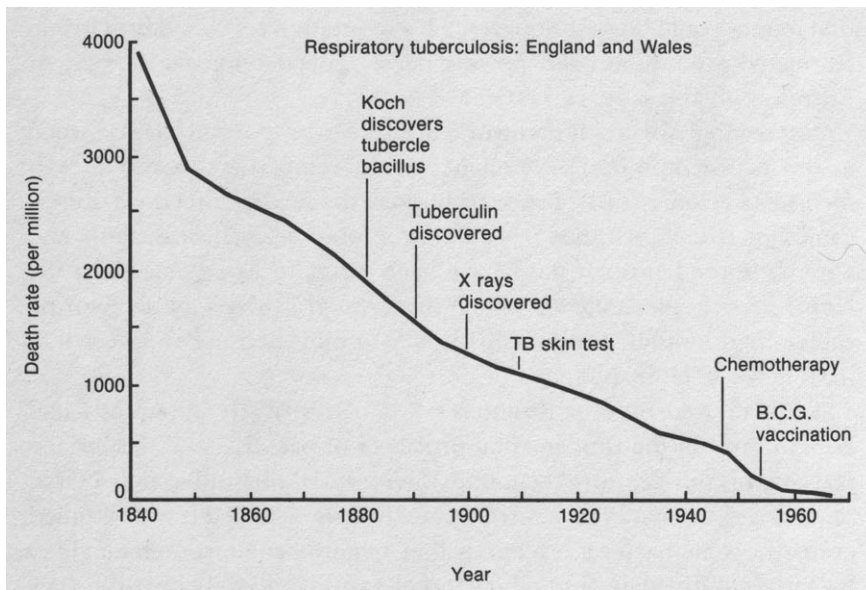


Figure 6. Death rate from tuberculosis in England and Wales as a function of time (5).

Koch's Legacy: The Discoverers of the Main Bacterial Pathogens

Year	Disease	Organism	Discoverer
1877	Anthrax	<i>Bacillus anthracis</i>	Koch, R.
1878	Suppuration	<i>Staphylococcus</i>	Koch, R.
1879	Gonorrhea	<i>Neisseria gonorrhoeae</i>	Neisser, A.L.S.
1880	Typhoid fever	<i>Salmonella typhi</i>	Eberth, C.J.
1881	Suppuration	<i>Streptococcus</i>	Ogston, A.
1882	Tuberculosis	<i>Mycobacterium tuberculosis</i>	Koch, R.
1883	Cholera	<i>Vibrio cholerae</i>	Koch, R.
1883	Diphtheria	<i>Corynebacterium diphtheriae</i>	Klebs, T.A.E., Loeffler, F.
1884	Tetanus	<i>Clostridium tetani</i>	Nicholaier, A.
1885	Diarrhea	<i>Escherichia coli</i>	Escherich, T.
1886	Pneumonia	<i>Streptococcus pneumoniae</i>	Fraenkel, A.
1887	Meningitis	<i>Neisseria meningitidis</i>	Weischselbaum, A.
1888	Food poisoning	<i>Salmonella enteritidis</i>	Gaertner, A.A.H.
1892	Gas gangrene	<i>Clostridium perfringens</i>	Welch, W.H.
1894	Plague	<i>Yersinia pestis</i>	Kitasato, S., Yersin, A.J.E. (independently)
1896	Botulism	<i>Clostridium botulinum</i>	van Ermengem, E.M.P.
1898	Dysentery	<i>Shigella dysenteriae</i>	Shiga, K.
1900	Paratyphoid	<i>Salmonella paratyphi</i>	Schottmüller, H.
1903	Syphilis	<i>Treponema pallidum</i>	Schaudinn, F.R., and Hoffmann, E.
1906	Whooping cough	<i>Bordetella pertussis</i>	Bordet, J., and Gengou, O.

Figure 7. Successes emerged from Koch's work.