International Journal of Infectious Diseases 14 (2010) e744–e751

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Review

Robert Koch and the 'golden age' of bacteriology

Steve M. Blevins *, Michael S. Bronze

Department of Medicine, Sections of General Internal Medicine and Infectious Diseases, University of Oklahoma Health Sciences Center, 825 NE 10th Street, Suite 4300, Oklahoma City, Oklahoma 73104, USA

ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form 28 November 2009
Accepted 3 December 2009

Corresponding Editor: William Cameron, Ottawa, Canada

Keywords:
Robert Koch
Anthrax
Tuberculosis
Cholera
Bacteriology
Medical history

SUMMARY

Robert Koch's discovery of the anthrax bacillus in 1876 launched the field of medical bacteriology. A 'golden age' of scientific discovery ensued. A century after Koch's death, we remember his life and work.

1. Introduction

The year 2010, which marks the centennial of Robert Koch's death, is a propitious year to reflect on the life and work of a physician–scientist whose studies launched a new field of scientific inquiry—the field of medical bacteriology. The magnitude of Koch's achievement, well recognized by his contemporaries, appears no less extraordinary today. His discoveries ushered in a 'golden age' of scientific discovery and a new era of public health. Today, his postulates are part of the medical vernacular and his techniques of microscopy are used throughout the world. Almost every step in the development of bacteriology bears his mark, from artificial culture to disinfection and sterilization.

In 1987, Koch's most important scientific papers were published in English.[1] Thomas Brock's landmark biography was published the following year.[2] It remains the only substantial biography of Koch printed in English. These works allow us to marvel at the accomplishments of a physician whose scientific career began inauspiciously outside the academic world, and whose guiding principle, *nunquam otiosus* (never idle), led him to the summit of scientific achievement.

2. Early years

Robert Koch was born on December 11, 1843, in Clausthal, a silver-mining town in northwest Germany. The son of a mining engineer and the third of thirteen siblings, he was a precocious reader with an aptitude for science and math. During visits to the country with his uncle, he developed an interest in nature. He chose a career in medicine while studying at the University of Göttingen, but natural science was his passion, one that would dominate his professional life. In Göttingen, he learned under the tutelage of Jacob Henle, George Meissner, Friedrich Wöhler, and Wilhelm Krause.[3,4] While a student, he won a research prize for his study on neuronal innervation of the uterus. This allowed him to travel to Hanover where he encountered Germany's most renowned physician, Rudolf Virchow.

He graduated from the university *cum extrema lauda* in 1866, was married the following year, and saw the birth of his only child, a daughter, fourteen months later. His first microscope, a gift from his wife, was a source of great joy. He lived peripatetically until serving as an army doctor in the Franco-Prussian war. After the war, he moved to Wöllstein, in modern-day Poland, where he established a successful clinical practice.

In 1875, he visited many of Germany's great scientific research centers, which attuned him to the emerging world of microbial science. Louis Pasteur had discovered that bacteria cause putrefaction; Joseph Lister had developed techniques of antiseptic

* Corresponding author. Tel.: +1 405 271 3445; fax: +1 405 271 4273.
E-mail address: Steve-Blevins@ouhsc.edu (S.M. Blevins).

1201-9712/$36.00 – see front matter © 2010 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijid.2009.12.003

surgery; and Jacob Henle, Koch's anatomy teacher in Göttingen, was defending the idea of *contagium animatum*, which held that disease could be caused by living transferable entities.[5] The 'germ theory' was hotly debated, and the role of bacteria in contagious disease was uncertain.

3. Anthrax

In Wöllstein, Koch was appointed district medical officer. While maintaining his clinical practice, he began investigating a major health problem—anthrax. In four years, the disease had killed 528 people and 56 000 livestock.[6] Studies had shown that rod-shaped structures were present in the blood of afflicted animals and that the disease could be transmitted by inoculating healthy animals with blood from diseased animals.[7] Certain pastures were known to be 'dangerous' to grazing livestock and could remain so for years. Yet, little was known about the rod-shaped structures or about the nature of soil-derived disease.

Koch designed elaborate inoculation studies using mice, guinea pigs, rabbits, dogs, frogs, and birds.[8] He discovered that inoculating a mouse with blood from a sheep that had died of anthrax caused the mouse to die the following day. At autopsy, rod-shaped structures were present in the blood, lymph nodes, and spleen. Inoculation of a second mouse with splenic blood from the first mouse produced the same result. By repeating these inoculations, Koch could propagate anthrax rods over dozens of generations. The rods varied in length. Long rods were often indented, as if ready to divide. Koch hypothesized that these were living bacteria, which propagated by elongation and fission. He noted that their presence was required for disease transmission. Yet, bacteria-laden blood lost its ability to cause disease after a few days. Such blood could not explain the prolonged toxicity of certain soils.

Koch developed techniques of artificial culture that allowed him to observe changes in bacteria over time. He found that inoculating the cornea of a rabbit with bacteria-laden fluid caused changes in the aqueous humor: the translucent fluid became turbid with bacteria. The aqueous humor was, he discerned, an effective medium for 'culturing' bacteria. He developed extraordinarily refined techniques of microscopy. By placing a piece of infected splenic tissue in a drop of aqueous humor and sealing it on a concave slide, he created a 'living' environment that allowed him to observe bacterial growth over days. Using a petroleum lamp, a humid chamber, an incubator, and vegetable oil for a seal, he could control the temperature, humidity, and aeration of his specimens.

He discovered that under optimal conditions – a warm, moist, aerated environment – the bacteria would swell, elongate, and form long filaments. The filaments acquired granules, which developed into refractile spheres. The filaments then decomposed, but the spheres remained. When the fluid was dried and then reconstituted with aqueous humor, bacteria emerged from the spheres. Koch hypothesized that the spheres were spores, resilient structures that appear in harsh environments. He demonstrated that these spores, in the absence of bacteria, could cause anthrax. Their formation explained how contaminated soil could remain toxic for years. Having discovered the importance of spores in the pathogenesis of disease, Koch recommended that diseased animals be burned or buried in soil cold enough to preclude spore formation.[9]

Although the germ theory of disease long preceded him, Koch became the first to link a specific bacterium with a specific disease. In a makeshift laboratory in the backroom of his house, he had elucidated the 'life cycle' of the anthrax bacillus (Fig. 1). He presented his experiments to Ferdinand Cohn, Germany's most renowned botanist. Deeply impressed, Cohn offered to publish Koch's paper in his own botanical journal. That paper was published in 1876, when Koch was thirty-two years old. The 'golden age' of bacteriology and its impact on medicine had begun.

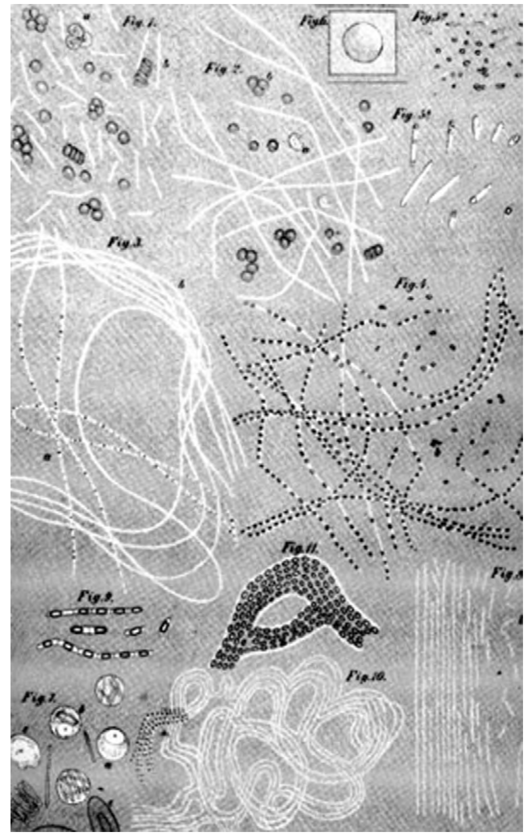

Fig. 1. Koch's drawing of the anthrax bacillus at various stages of development. (Reference: Koch R. Die Ätiologie der Milzbrandkrankheit, begründet auf die Entwicklungsgeschichte des Bacillus Anthracis. *Beiträge zur Biologie der Pflanzen* 1876;**2**:277–310.).

4. Technological progress

Microscopy was challenging because of poor illumination and because bacteria were transparent and mobile in fluid. Koch confronted each of these problems. He discovered that he could 'fix' bacteria to a slide by drying them in liquid solution. By applying aniline dyes – eosin, fuchsin, safranin, and methyl violet – he could see bacteria more easily and detect subtle morphological traits. He began working with microscope developers on methods to improve lighting and resolution (Figs. 2 and 3). He became the first physician to use an oil immersion lens, the first to use a condenser, and the first to publish photographs of bacteria. These photographs appeared in 1877 (Figs. 4 and 5).[10] Thus, the routine application of light microscopy to bacteriology is attributed largely to his efforts.

The following year, Koch began his studies of wound infections in animals.[11] With his new techniques for identifying bacteria, he distinguished various disease states – septicemia, gangrene, abscess – at the microscopic level. He advanced the theory that bacteria exist as distinct species, each producing a unique clinical syndrome, and he discredited the popular notion that bacteria with different morphologies were derived from the same species.[12] In doing so, he demonstrated the futility of generalizing about all infectious diseases. Only by studying a *specific* pathogen could a specific disease be understood.

In 1879, Koch moved to Breslau, in modern-day Poland, to become district physician. Unhappy there, he moved to Berlin the following year at the invitation of the Imperial Government. There

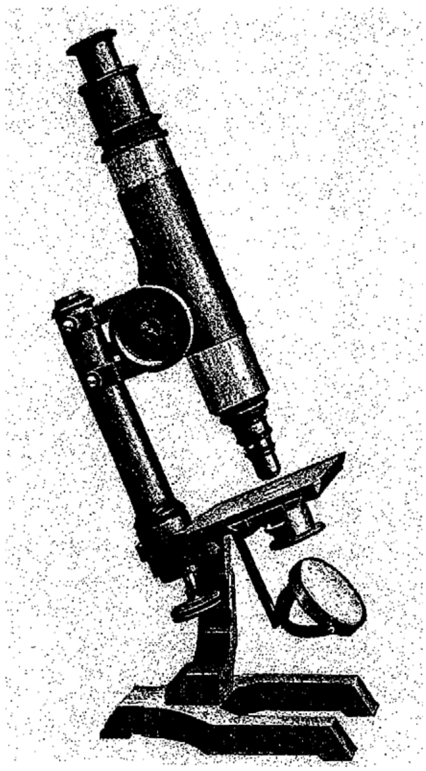

Fig. 2. A Seibert microscope of the type Koch used to study the anthrax bacillus. (Reference: Brock TD. *Robert Koch: a life in medicine and bacteriology*. Washington, DC: American Society of Microbiology Press; 1999. p. 55.).

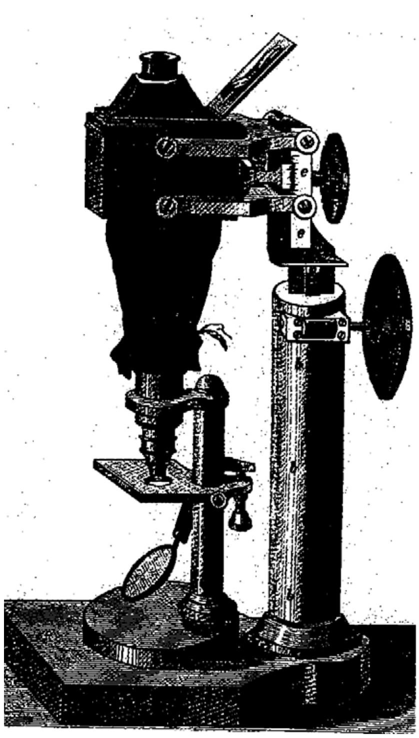

Fig. 3. Vertical photomicroscopic apparatus of the type Koch used to photograph the anthrax bacillus. (Reference: Brock TD. *Robert Koch: A life in medicine and bacteriology*. Washington, DC: American Society of Microbiology Press; 1999. p. 57.).

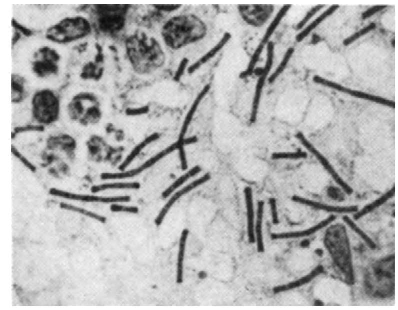

Fig. 4. First published photographs of the anthrax bacillus. (Reference: Koch R. Verfahren zur Untersuchung, zum Conservieren und Photographiren der Bakterien. *Beiträge zur Biologie der Pflanzen* 1877;**2**:399–434.).

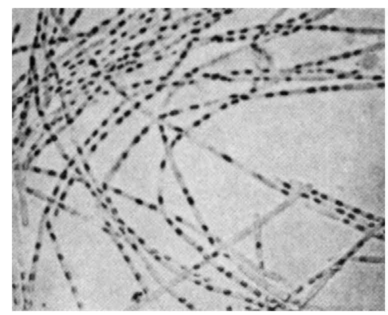

Fig. 5. First published photographs of the anthrax bacillus. (Reference: Koch R. Verfahren zur Untersuchung, zum Conservieren und Photographiren der Bakterien. *Beiträge zur Biologie der Pflanzen* 1877;**2**:399–434.).

he joined the staff of the Imperial Health Office, where he worked to develop his plate technique for generating 'pure' cultures of bacteria. This technique would be one of his greatest contributions to bacteriology. 'Pure' cultures, which were essential to the study of bacteria, were difficult to obtain by conventional means. Klebs and Lister had devised techniques using liquid media, but these were time-consuming and often unreliable.[13]

Koch's development of solid culture media was a major turning point. He began by observing fungal 'colonies' growing on a slice of potato. Each colony was 'pure', containing similar organisms. By placing liquid cultures in gelatin and then cooling the solution, he was able to produce a clear, smooth, homogeneous culture medium. In it, the bacteria would multiply to form visible colonies. One of his post-doctoral assistants, Walter Hesse, aided by his wife, Fannie Hesse, discovered that agar, a substance derived from seaweed, was an effective medium because of its higher melting point. Koch experimented with agar. Meanwhile, another of his assistants, Julius Petri, designed a shallow, covered dish into which media could be poured, cooled to solidity, and protected from contamination.

Koch now had all the tools for his 'plate technique'. He could grow bacterial colonies and subject them to steam and various chemicals, thus advancing the fields of disinfection and sterilization. He learned that while certain chemicals killed bacteria, others merely inhibited them – a distinction that would be important in the antibiotic era. In a manuscript published in 1881, he described his plate technique in exquisite detail. Others could now replicate his studies. Koch's paper became the 'Bible of Bacteriology'.

5. Tuberculosis and the application of Koch's postulates

Koch traveled to London, where he presented his plate technique to Louis Pasteur and Joseph Lister. Soon the application of his techniques for culturing bacteria became widespread. Koch

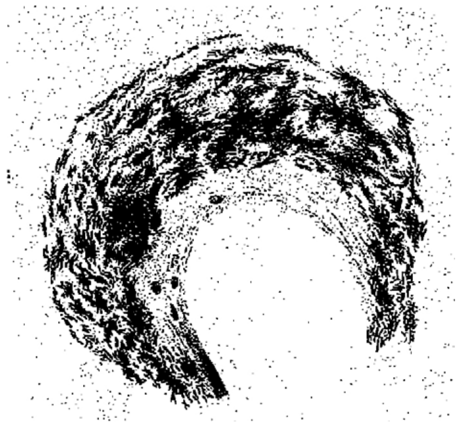

Fig. 6. Koch's drawing of tubercle bacilli in the arterial wall of a patient with miliary tuberculosis. (Reference: Koch R. Die Aetiologie der Tuberculose. *Berliner Klinische Wochenschrift* 1882;**19**:221–30.).

was promoted to Senior Medical Officer. His assistant, Friedrich Loeffler, discovered the glanders and diphtheria bacilli in 1882 and 1884, respectively. George Gaffky, also a pupil of Koch, discovered the typhoid bacillus in 1884.[14]

Yet, the most feared disease, tuberculosis, remained enigmatic. In Europe, tuberculosis was responsible for one out of seven deaths.[15] Preliminary studies had established its transmissibility, but a causative agent had not been found.[16,17]

Suspecting that tuberculosis was caused by bacteria resistant to conventional stains, Koch experimented with new stains.[15,18] Using Ehrlich's methylene blue stain, he detected a few tiny rods in tuberculous tissue. When he added a brown counterstain for photographic contrast, he 'uncovered' more bacteria. He then noticed that 'old' stains were more effective than fresh stains, and hypothesized that the old stains had absorbed a useful chemical from the air. He surmised that the chemical was ammonia, which had alkalinized the methylene-blue stain. Thus, he began adding caustic potash to his stains to achieve a similar effect.[19] Now countless bacteria were visible. Koch had discovered the tubercle bacillus (Figs. 6 and 7). The bacilli were always present in tuberculous disease, but not in normal states. Moreover, they always preceded tubercle formation, appearing before the arrival of giant cells and caseation.[20] They were numerous when the tuberculous process was incipient or progressive, and rare when it was quiescent.

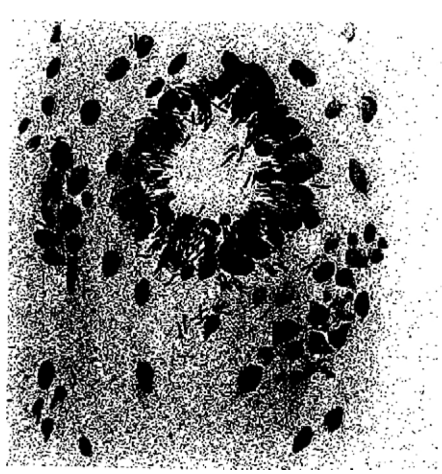

Fig. 7. Koch's drawing of tubercle bacilli surrounding a giant cell in the lung. (Reference: Koch R. Die Aetiologie der Tuberculose. *Berliner Klinische Wochenschrift* 1882;**19**:221–30.).

Koch believed these bacilli caused tuberculosis, but he needed more evidence. He formalized a set of 'postulates', derived from the work of Loeffler, Klebs, and Henle, for establishing causation in bacterial disease.[21] These postulates required coincidence of bacteria and disease, isolation of bacteria in pure culture, and induction of disease by inoculation with bacteria from pure culture. Yet, culturing the bacteria proved difficult. After experimenting with different media at different temperatures, he found that he could grow colonies on coagulated blood serum at 37–38 °C (Fig. 8). The colonies were dry and scaly, and appeared only in the second week of incubation. No other bacterial species had this appearance or grew so slowly.

To test whether these bacteria caused disease, he inoculated 217 animals with bacilli from pure cultures. In every case, tubercles appeared in numbers proportional to the size of the inoculum. Tubercles did not appear after injection of non-tuberculous tissue. Moreover, bacilli from different sources – spontaneous disease, induced disease, and artificial culture – produced tubercles of similar appearance. Koch concluded that the tubercle bacillus was the cause of disease and its defining characteristic. The bacillus – not the tubercle – was the *sine qua non* of tuberculosis. Its presence in consumption, miliary disease, caseous pneumonia, intestinal tuberculosis, and scrofula meant that these were all forms of the same disease caused by the same organism.

Koch detected the bacilli in the sputum and lung cavities of consumptives. He found that he could induce disease in healthy animals by inoculating them with infected sputum. He concluded that sputum was the principal source of transmitted disease, and that patients with laryngeal or pulmonary tuberculosis who expectorated large quantities of bacilli were particularly infectious.[22] Although the bacilli could not multiply outside a living host, in dried sputum they retained their pathogenicity for weeks. Proper disposal of infected sputum and decontamination of the environment were, therefore, essential to disease prevention. Tuberculosis was now recognized as a public health problem requiring strategies to prevent its transmission. To that end, sterilization of clothes and bed sheets and clothing was adopted, and spitting in public places was restricted.[19]

On March 24, 1882, Koch presented his findings on tuberculosis at a meeting of the Berlin Physiological Society. That demonstration, which included more than 200 microscopic preparations, is now regarded as one of the most influential presentations in medical history. Paul Ehrlich, who attended the lecture, was inspired by Koch's work. He would later refine Koch's staining techniques and influence Hans Christian Gram, as well as Franz Ziehl and Friedrich Neelsen, after whom the Gram and Ziehl–Neelsen stains are named.

Koch's paper on the etiology of tuberculosis was published the following month. As news spread worldwide, Koch became internationally famous. His discovery was celebrated in the USA, where in 1904 the National Tuberculosis Association was founded. The following year, Koch was awarded the Nobel Prize.

6. Koch–Pasteur controversy

Koch's relationship with Louis Pasteur deteriorated in the years after their first meeting.[23–25] The most famous Frenchman of his generation, Pasteur was a chemist with a broad philosophical interest in microbial science. Koch, by contrast, was a physician principally interested in microbial (especially bacterial) causes of human disease. While Pasteur worked to protect individuals through immunization, Koch worked to protect communities through better hygiene and public health. Although Koch's initial encounter with Pasteur was cordial (they met in London, where Koch presented his plate technique), he soon began attacking his

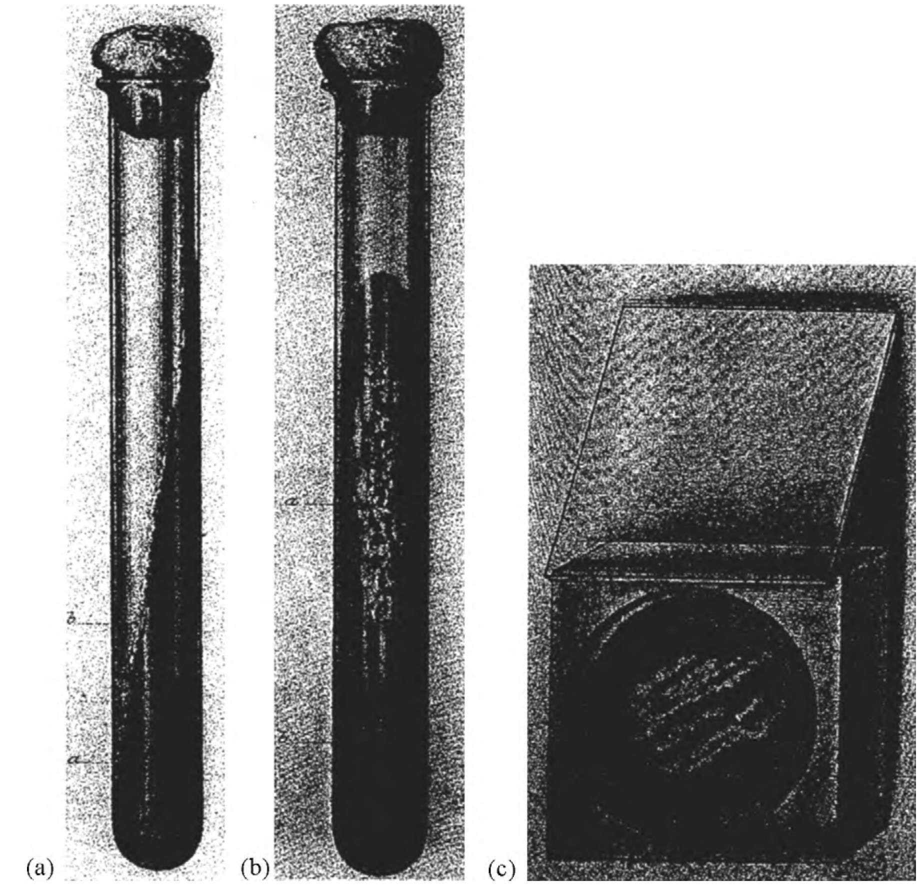

Fig. 8. Koch's drawing of tubes and plates for culturing the tubercle bacillus. (Reference: Koch R. Die Aetiologie der Tuberculose. *Berliner Klinische Wochenschrift* 1882;**19**:221–30.).

rival in writing. In particular, he criticized Pasteur's work on anthrax attenuation, and accused him of using impure cultures and of conducting faulty inoculation studies. This infuriated the French public. Pasteur responded by sending his assistant, Louis Thuillier, to Prussia to demonstrate his anthrax inoculation techniques. The experiment was successful, and Pasteur's method was widely accepted in Germany. Thuillier would write, "Koch is not liked by his colleagues... [He] is a bit of a rustic, and is ignorant of parliamentary language."[26] Koch would write, "Pasteur is not a physician, and one cannot expect him to make sound judgments about pathological processes and the symptoms of disease."[27]

The Koch–Pasteur rivalry had both harmful and beneficial effects. Although the rivalry delayed acceptance of Koch's culture techniques in France and Pasteur's rabies vaccine in Germany, French and German competition, animated by an intense desire to garner the highest accolades of scientific achievement, led to indisputable accomplishments that would outlive the rancor.

7. Cholera

A year after his famous Berlin lecture, Koch began his studies of cholera.[28] Cholera was endemic in India and had spread to Egypt, causing fear among Europeans of an imminent pandemic. The German government appointed Koch to lead a scientific expedition to Egypt. The team, comprised of four scientists, began its work in Alexandria, but later traveled to Calcutta once the Egyptian epidemic had subsided.

Koch began by examining the intestinal mucosa of the deceased.[29] In uncomplicated cases – those with little or no epithelial damage – a morphologically identical organism predominated: a comma-shaped bacillus. Using different media, Koch cultured and characterized the organism as motile, aerobic, and fast-growing. He noted that in intestinal fluid, it grew rapidly, before receding as the medium 'decayed'. It was susceptible to acids and desiccation, and produced no spores. With nourishment it could survive outside the body.[30]

He conducted almost a hundred autopsies, and found the bacilli in every case. They were especially numerous in the distal small bowel, where intestinal disease was greatest. In other diarrheal conditions, they were absent. He also noted that, when incubated with red blood cells, the bacilli caused the cells to die. He attributed this to a 'poison', which explained how bacteria could cause disease with little or no penetration of the intestinal wall.[31]

Because cholera is largely restricted to humans, Koch's animal inoculation studies failed and his postulates remained unfulfilled. (Later, during a European outbreak of cholera, he would successfully infect guinea pigs.[21]) Nevertheless, he understood the limitations of his postulates and the value of epidemiological analysis in determining disease causation. He traced seventeen cases of cholera to a nearby water tank, which had been used by local residents for drinking, washing, and waste disposal.[29] Comma-shaped bacilli were present in the tank at the height of the epidemic, but not afterwards. Examining linens belonging to the first cholera victim – linens that had been washed in the tank – he again found comma-shaped bacilli. Clearly these bacilli were the cause of disease.

Koch's epidemiological analysis was meticulous. He noted that there were no 'spontaneous' epidemics outside India. Only in the Ganges Delta was the disease predictable in its periodicity: here was the origin of recurring pandemics. River flooding produced swamps, where vegetation was abundant. Refuse from densely populated areas supplied bacteria, which grew readily in the moist, fertile environment. Intestinal exposure to contaminated water caused disease in susceptible hosts. Through excrement, the bacteria would return to the water supply. Pilgrimages and navigation spread disease throughout the country and to distant shores.

Koch's discovery meant that access to clean water was necessary to prevent the spread of cholera. To that end, filtered water lines were placed in Calcutta. Soon the incidence of disease fell. Koch's discovery was a public health triumph.

His work in Calcutta resonated in Germany eight years later. In 1892, cholera caused more than 8000 deaths in Hamburg. The adjacent town of Altona was spared. Koch noted that water filtration had been implemented in Altona, but not in Hamburg, because the water running into Altona was visibly dirty. By analyzing the water in both towns, he was able to demonstrate that water filtration had unintentionally protected Altona from disease. This affirmed the importance of water 'purification' and made water analysis a cornerstone of public health.

8. Tuberculin and the world

Upon his return from India, Koch was honored by Kaiser Wilhelm I and by Chancellor Otto von Bismarck. The following year, he was appointed Professor of Hygiene at the University of Berlin and Director of the Hygiene Institute. This would be the only academic position he would ever hold.

In 1890, he began his search for a cure for tuberculosis.[21,32,19,18] Working quietly in his laboratory with a glycerin extract of tubercle bacilli, which he named tuberculin, he found that subcutaneous inoculation of the substance in guinea pigs with tuberculosis caused a reaction not seen in healthy animals. In humans, he noticed no reaction (or, at most, a mild reaction) in healthy individuals, whereas in patients with active tuberculosis, a severe reaction occurred, characterized by fever, chills, and skin inflammation leading to necrosis. Koch believed that, in afflicted individuals, tuberculin produced a reaction that slowed or halted disease.[33] He also believed that the reaction provided diagnostic evidence of acute tuberculosis.

When he announced, unceremoniously, that tuberculin had this beneficial effect, the public response was stupendous: countless patients and doctors traveled to Berlin to obtain the remedy. Hospitals and sanatoria were overrun with consumptives. Koch resigned his professorship and began working full-time on tuberculin. He was appointed head of the new Institute of Infectious Diseases, which eventually would be renamed the Robert Koch Institute. Modeled on the Pasteur Institute in Paris, it was comprised of a hospital-affiliated department, where tuberculin was administered to patients, and a research department dedicated to the study of tuberculin. The Institute attracted luminaries like Emil von Behring, who discovered diphtheria antitoxin and who co-developed serum therapy for diphtheria and tetanus (he received the Nobel Prize in 1901); Shibasaburo Kitasato, who co-discovered the bacterium causing bubonic plague and co-developed serum therapy for tetanus; Paul Ehrlich, whose work on hemolysis, auto-immunity, and anti-bacterial chemotherapy won him the Nobel Prize in 1908; Richard Pfeiffer, who co-discovered bacteriolysis and pioneered the typhoid vaccine; and August von Wassermann, who established a complement fixation test for syphilis.

Tuberculin, however, would prove ineffective as a therapeutic agent. As enthusiasm for the putative remedy waned, Koch's reputation declined. His personal and professional life tumbled. After twenty-six years of marriage, he divorced his wife and married a twenty-year old art student. (Eventually, a modified version of tuberculin, administered intracutaneously, would become the standard for diagnosing latent tuberculosis. Koch had demonstrated, but not recognized, the phenomenon of delayed hypersensitivity and cellular immunity).

A long period of international travel ensued. In Italy, Indonesia, and New Guinea, he studied malaria, establishing guidelines for its prevention. In Trier, he studied typhoid fever, elucidating the 'carrier state'. In India, he studied plague; in East Africa, sleeping sickness. At the invitation of the British Government, he visited Rhodesia (now South Africa) to study rinderpest.[34,35] There he also studied malaria, sleeping sickness, horse-sickness, and relapsing fever. In 1908, he traveled to the USA to visit relatives and to raise money for the study of tuberculosis. A banquet was held in his honor at the Waldorf Astoria in New York. In attendance was Andrew Carnegie, who had given 500 000 marks to the Robert Koch Foundation for the Conquest of Tuberculosis. After New York, Koch traveled to the Midwest to visit two of his brothers. (One lived in St. Louis, the other in Keystone, Iowa).

He then traveled to Japan, but his visit was cut short when he accepted an invitation to attend the International Tuberculosis Congress in Washington. The Congress was convened to discuss the relation of human and bovine tuberculosis.[36–41] Koch's opinion was sought because he had previously stood against the prevailing notion that bovine tuberculosis was harmful to humans. At the meeting, Koch maintained that bovine tuberculosis did not play a major pathogenic role in human disease. Opposing him were those who wished to prevent disease transmission by eliminating human consumption of infected meat and milk. They sought mandatory inspection of cattle, pasteurization of milk, and purging of infected livestock. Still, Koch refused to advocate these public health measures. As a result, his reputation, already injured by the tuberculin debacle, took another blow. Ultimately, the US health establishment distanced itself from Koch and moved towards universal pasteurization of milk. A pasteurizing temperature was ultimately chosen sufficient to kill tubercle bacilli.

Koch's health declined soon afterward. On May 27, 1910, at age 67, he died of heart disease in Baden-Baden, Germany. His body was cremated and the remains were placed in a mausoleum in the west wing of the Koch Institute.

9. Conclusion

A panoramic view of Koch's life reveals an array of unprecedented achievements intermixed with a few notable failures. Koch's principal failures – his belief in the therapeutic potential of tuberculin, his error regarding bovine tuberculosis, and his acerbic treatment of opponents – do little to mar the legacy of a physician whose revolutionary discoveries reset the ballast of medical science.

While the breadth of Koch's achievements can be gleaned from biographical sources, his original scientific papers most effectively convey the depth of his ingenuity. Koch was meticulous with respect to scientific methodology. Today's proponents of evidence-based medicine will find in his papers a near-contemporary understanding of experimental design and analysis. Controls, bias, reproducibility, sample size – all these are central to his work.

His essays were largely polemical. He positioned himself as both prosecutor and defender of his own hypotheses. (The defense wins only after assiduous *self*-cross-examination). He confronted his opponents, exposed their errors, expounded his own theories, and buttressed them with inescapable logic.[42–44] His disdain for sloppy thinking was overt; his style, often contentious.

Koch derided faulty reasoning and imparted an uncompromising demand for careful analysis. Yet, whether eristic or descriptive, he enthralls the reader with his relentless drive and dogged pursuit of scientific truth. His experiments evince his perseverance: he propagated the anthrax bacillus for more than *fifty* generations and inoculated more than *two hundred* animals with tuberculous tissue – all done to render his conclusions irrefutable.

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

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

Fig. 9. The 'golden age' of bacteriology. (Reference: Brock TD. *Robert Koch: a life in medicine and bacteriology*. Washington, DC: American Society of Microbiology Press; 1999. p. 290.).

His postulates – a legacy of his indebtedness to Loeffler, Klebs, and Henle – have been examined and re-examined in the light of evolving microbial science. They have been modified repeatedly to accommodate organisms with special traits: those that cause subclinical, 'slow', or chronic diseases; those that are restricted to human hosts; those that do not grow in cell-free culture; those that may exist as commensals or as pathogens; those that require co-infection with other agents; and those that cause disease through immune-mediated mechanisms.[45–47] Knowledge of host and environmental effects on disease, the emergence of microbial genetics, even the discovery of infectious proteins, have invited reappraisal and revision of the postulates.[48,49] Yet, in every case, they remain the touchstone for investigating infectious disease causation.

Koch's studies inspired a generation of scientists. In the span of just 30 years – from 1876 to 1906 – the principal bacterial pathogens of human disease were isolated (Fig. 9). His discovery of the anthrax bacillus had ushered in a 'golden age' of discovery; his work on tuberculosis and cholera had awakened the world to the marvels of microbiological research; and his institute, dedicated to the study of infectious diseases, attracted scientists of extraordinary ability and achievement – scientists who, like Koch, would advance medical science immeasurably Fig. 10.

Fig. 10. Robert Koch.

Conflict of interest

No conflict of interest to declare.

References

1. Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987.
2. Brock TD. Robert Koch: a life in medicine and bacteriology. Washington, DC: American Society of Microbiology Press; 1999.
3. Schwentker FN. Robert Koch (1843–1910). *Invest Urol* 1964;**2**:300–1.
4. Lakhani SR. Early clinical pathologists: Robert Koch (1843–1910). *J Clin Pathol* 1993;**46**:596–8.
5. Jay V. The legacy of Robert Koch. *Arch Pathol Lab Med* 2001;**125**:1148–9.
6. Koch R. The etiology of anthrax, founded on the course of development of Bacillus anthracis (1876). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 14.
7. Conn HW, Conn HJ. Bacteriology, 2nd ed., Baltimore: Williams and Wilkins Company; 1924. p. 30–4.
8. Koch R. The etiology of anthrax,;1; founded on the course of development of Bacillus anthracis (1876). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 1–17.
9. Williamson F. Microbes. 4. Robert Koch: anthrax bacilli isolated. *Nurs Times* 1973;**69**:1652–3.
10. Koch R. Verfahren zur Untersuchung, zum Conservieren und Photographiren der Bakterien. *Beiträge zur Biologie der Pflanzen* 1877;**2**:399–434.
11. Koch R. Investigations of the etiology of wound infections (1878). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 19–56.
12. Penn M, Dworkin M. Robert Koch and two visions of microbiology. *Bacteriol Rev* 1976;**40**:276–83.

13. Conn HW, Conn HJ. Bacteriology, 2nd ed., Baltimore: Williams and Wilkins Company; 1924. p. 41–44.
14. Sakula A. Robert Koch (1843-1910): founder of the science of bacteriology and discoverer of the tubercle bacillus. *Br J Dis Chest* 1979;**73**:389–94.
15. Koch R. The etiology of tuberculosis (1882). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 83–96 .
16. Villemin JA. Etudes sure la tuberculose. Paris: J.B. Bailliere; 1868. p. 529–72.
17. Cohnheim JF, Salomonsen CJ. Inoculations into the anterior chamber of animals. *Jahresbericht der schlesische Gesellschaft für vaterländische Kultur* 1877;**55**:222f.
18. Gradmann C. Robert Koch and the pressures of scientific research: tuberculosis and tuberculin. *Med Hist* 2001;**45**:1–32.
19. Sakula A. Robert Koch: centenary of the discovery of the tubercle bacillus, 1882. *Thorax* 1982;**37**:246–51.
20. Koch R. The etiology of tuberculosis (1884). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 129–50.
21. Kaufmann SH, Schaible UE. 100th anniversary of Robert Koch's Nobel Prize for the discovery of the tubercle bacillus. *Trends Microbiol* 2005;**13**:469–75.
22. Daniel TM. Robert Koch and the pathogenesis of tuberculosis. *Int J Tuberc Lung Dis* 2005;**9**:1181–2.
23. Baxter AG. Louis Pasteur's beer of revenge. *Nature Rev Immunol* 2001;**1**:229–32.
24. Zetterstrom R. Robert Koch (1843-1910): investigations and discoveries in relation to tuberculosis. *Acta Paediatr* 2006;**95**:514–6.
25. Mollaret HH. Contribution a la connaissance des relations entre Koch et Pasteur. *NTM* 1983;**20**:57–65.
26. Frank RM, Wrotnowska D. Correspondence of Pasteur and Thuillier concerning anthrax and swine fever vaccinations. Tuscaloosa: University of Alabama Press; 1968. p. 121–3.
27. Koch R. On the anthrax inoculation (1882). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 100.
28. Howard-Jones N. Robert Koch and the cholera vibrio: a centenary. *Br Med J Clin Res Ed* 1984;**288**:379–81.
29. Koch R. Lecture at the first conference for discussion of the cholera question (1884). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 151–70.
30. Koch R. On cholera bacteria (1884). In: Carter KC, translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 171–7.
31. Finkelstein RA. The cholera enterotoxin—Robert Koch revisited. *Zentralbl Bakteriol Orig A* 1976;**235**:13–9.
32. Shapiro E. Robert Koch and his tuberculin fallacy. *Pharos Alpha Omega Alpha Hon Med Soc* 1983;**46**:19–22.
33. Green GM, Daniel TM, Ball Jr WC. Koch centennial supplement. 100th anniversary of the announcement of the discovery of the tubercle bacillus by Robert Koch, March, 24, 1882. *Am Rev Resp Dis* 1982;**125**(3 Pt 2):1–3.
34. James T. Professor Robert Koch in South Africa. *S Afr Med J* 1970;**44**:621–4.
35. Blumberg L. Robert Koch and the rinderpest. *S Afr Med J* 1989;**76**:438–40.
36. Grange JM. Koch's tubercle bacillus. *Zentralbl Bakteriol Orig A* 1982;**251**:297–307.
37. Packer RA. Veterinarians challenge Dr. Robert Koch regarding bovine tuberculosis and public health. *J Am Vet Med Assoc* 1990;**196**:574–5.
38. Maulitz RC. Robert Koch and American medicine. *Ann Intern Med* 1982;**97**:761–6.
39. Koch R. The relations of human and bovine tuberculosis. *JAMA* 1908;**51**:1256–8.
40. Smith AE. Report on the International Congress on Tuberculosis. *Ill Med J* 1909;**15**:303–10.
41. Smith T. Koch's views on the stability of species among bacteria. *Ann Med Hist NS* 1932;**4**:524–30.
42. Koch R. On the etiology of anthrax (1881). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 57–81.
43. Koch R. Critical discussions of publications challenging the significance of tuberculosis bacilli (1883). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 117–27.
44. Koch R. On the anthrax inoculation (1882). In: Carter KC; translator. Essays of Robert Koch. Connecticut: Greenwood Press; 1987. p. 97–115.
45. Fredricks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clin Microbiol Rev* 1996;**9**:18–33.
46. Evans AS. Causation and disease: the Henle–Koch postulates revisited. *Yale J Biol Med* 1976;**49**:175–95.
47. Madeley CR. "Is it the cause?" – Robert Koch and viruses in the 21st century. *J Clin Virol* 2008;**43**:9–12.
48. Walker L, LeVine H, Jucker M. Koch's postulates and infectious proteins. *Acta Neuropathol* 2006;**112**:1–4.
49. Falkow S. Molecular Koch's postulates applied to microbial pathogenicity. *Rev Infect Dis* 1988;**10**:S274–6.