Koch's Postulates—Then and Now

Amid challenges, his principles remain useful for confirming microbial roles in diseases and other processes

D. Jay Grimes

This story begins in the early 1880s. The germ theory of disease was fairly well established, although some experts continued to challenge it. For example, Theodor Billroth (1829–1894) of Vienna, often called the founding father of abdominal surgery, did not immediately accept the germ theory, but later embraced the concept and became very successful in many pioneering surgeries. Another critic was Max von Pettenkofer (1818–1901) of Bavaria, a noted experimental hygienist. In 1892, Pettenkofer openly challenged Robert Koch (1843–1910) on his theories about *Vibrio cholerae* by drinking the contents of a flask containing the cholera bacillus. The flask had been given to Pettenkofer by Koch himself, but Pettenkofer did not contract cholera! Perhaps the biggest critic was the famous physician Rudolph Virchow (1821–1902) of Berlin. Virchow believed that diseased cells arose only from other diseased cells, and strongly opposed the germ theory of disease. Other skeptics could be cited, but the important point is that Koch was working in a controversial area of medicine and experimental research in the late 1800s.

Summary

- When Robert Koch was framing his postulates about infectious disease, several contemporaries published similar ideas, while prominent skeptics argued strongly against the germ theory of disease.
- Koch, who studied cholera, and others, from his era and our own, recognized exceptions to his postulates, particularly in dealing with species-specific pathogens for which suitable animal models are difficult, and sometimes impossible, to identify.
- The principles underlying Koch's postulates sometimes are being applied, for example, in environmental settings to address questions of microbial causality that are far afield from infectious diseases.

Koch's Early Focus on Tuberculosis Led Him To Frame Postulates

In 1880, Robert Koch at age 37 moved his family to Berlin, where he joined the staff of the Imperial Health Office. He had conducted his anthrax research about a decade earlier and in Berlin turned his attentions to the dreaded disease tuberculosis. He developed many techniques in his own laboratory, including how to isolate pure cultures along with many applications of microscopy, photomicroscopy, and staining. He also fitted his microscope with the oil-immersion lens that Ernst Abbe (1840–1905) at the Carl Zeiss Company had recently developed.

At about this time, Edwin Klebs (1834–1913), a student of germ theory antagonist Virchow, published a paper that delineated what would soon be established as Koch's postulates (see box, p. 224). Klebs's three rules are strikingly similar to those of Koch and his students, but Klebs could not verify his procedures mainly because he was unable to isolate pure cultures, a central requirement for both his procedure and Koch's postulates.

Koch published his discovery of the tubercle bacillus in 1882, describing an exacting approach that laid the framework for his postulates. He observed and isolated the microbe in pure culture from numerous patients, then introduced the microbe into

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Procedure developed by Edwin Klebs to demonstrate causal relationship between "germs" and disease

1. Careful microscopic study of the diseased organ
2. Isolation and culture of the germ associated with the disease
3. Production of the same disease by inoculation of this cultured germ into healthy animals

guinea pigs, successfully induced disease in them, and then reisolated the microbe from those animals. This work made Koch an instant hero and his name a household word.

In 1883, Friedrich Loeffler (1852–1915), one of Koch's assistants, published a paper on diphtheria (Fig. 1). Loeffler stated that three postulates had to be fulfilled to prove that diphtheria is a disease caused by a specific microorganism (see box, below). He argued that these steps were necessary "to demonstrate strictly the parasitic nature of a disease." His three postulates and the procedures described by Koch a year earlier are nearly identical. Based on the work of Klebs and Loeffler, one can question whether Koch's postulates were his!

Koch's Formalized His Postulates between 1884 and 1890

In 1884, Koch published a definitive paper on tuberculosis and more fully outlined his methodology. In the first pages, he described how he discovered the anthrax bacillus and defined its role in causing anthrax, explaining that this approach laid the framework for his research on tuberculosis. "[W]hether the organisms have constant characteristics. . ." is the logic underlying his first postulate, and he insisted that this question should be asked during each investigation of an infectious agent.

Further in the same text, Koch discussed the basis for his second postulate—asking whether "the parasite" is the cause of the illness or a symptom. The answer to this question, he added, can be established only under circumstances "in which the parasite is completely separated from the diseased organism and from all symptoms of the disease." He then developed the basis for his third postulate by stating that "by introducing the isolated parasite into the healthy organism the disease can be produced again with all its usual characteristics." Over the next 80 pages, Koch further used his anthrax discovery to explain the etiology of tuberculosis.

Thus, Koch explained his postulates. However, he did not publish them in the form that we recognize today until 1890 (see. box, p. 225). Since then, the postulates have been used extensively to elucidate many diseases and are presented in all beginning textbooks of microbiology. Whether Koch merely compiled or genuinely conceived them, he now is credited for articulating these simple but powerful tenets of infectious disease etiology.

Early and More Recent Exceptions to Koch's Postulates

During the next few decades, others uncovered many exceptions to Koch's postulates. Indeed,

FIGURE 1

Friedrich Loeffler and Robert Koch.

Loeffler's Postulates (see p. 424 in Loeffler, 1883)

1. The organisms must be shown to be constantly present in characteristic form and arrangement in the diseased tissue.
2. The organisms which, from their behavior appear to be responsible for the disease, must be isolated and cultivated in purity.
3. The pure cultures must be shown to induce disease experimentally.

Koch's Postulates as We Know Them Today

1. The same organism must be present in every case of the disease.
2. The organism must be isolated from the diseased host and grown in pure culture.
3. The isolate must cause the disease, when inoculated into a healthy, susceptible animal.
4. The organism must be reisolated from the inoculated, diseased animal.

he described one of those first exceptions himself! In 1884, when Koch published his findings on cholera, he noted that, with no animal model for this disease, he could not satisfy postulate three. Today, cholera models do exist, including the rabbit ligated ileal loop assay. Moreover, modern molecular biological techniques and epidemiology often help to overcome seeming obstacles to Koch's postulates for those analyzing the etiology of infectious diseases.

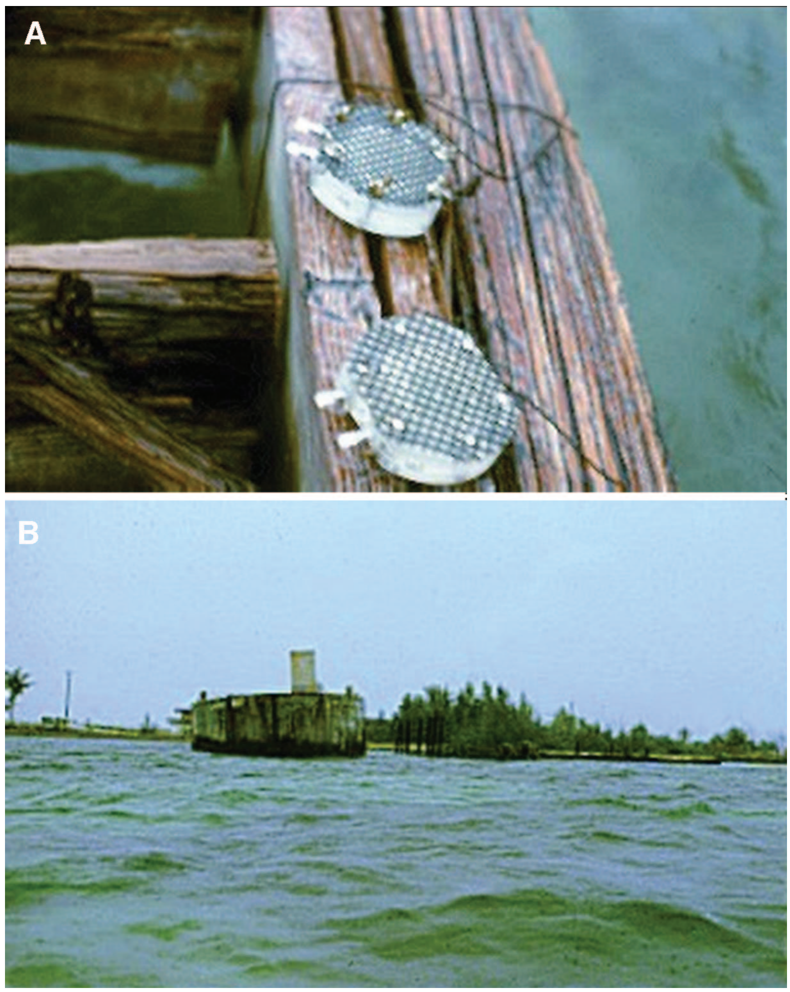
Other seeming exceptions to Koch's postulates include many of the diseases attributable to viruses, animal parasites, rickettsia, and chlamydia. In most of these cases, host specificity appears confined to humans, making it difficult if not impossible to verify the third postulate in an animal model. Although cell cultures now are available for many viruses, the disease itself cannot be recreated in cells, even if cytopathogenic effects can be observed. As for animal parasites, intermediate hosts are often available, but human disease symptoms are not always manifest in these animals. Like viruses, rickettsia and chlamydia are obligate intracellular parasites, and lack animal models.

Polymicrobial causes of diseases have long been suspected, and today this is an emerging field of study. These disease processes, such as soft tissue abscesses and periodontal disease caused by mixed infections with anaerobes, cannot be recreated in other species. However, as microbiologists become more successful in producing functional mixed cultures, for whatever purpose, it will no doubt become possible to satisfy Koch's postulates for polymicrobial diseases.

Lysogenic bacteria provide another example of exceptions to the postulates. For instance, Loeffler's diphtheria findings might have looked very different had the *Corynebacterium diphtheriae* strain that he discovered been cured of their prophages before they were reintroduced into susceptible hosts. Although we now realize that lysogenic bacteria cause many diseases (or intoxications), their etiologies are not always immediately apparent.

Another exception worth noting is contaminated cultures, and hog cholera provides a good example. Originally thought to be caused by the

FIGURE 2



Membrane chambers (A) containing *Escherichia coli* H10407 were attached to a sunken barge (B) in Nixon's Harbor, South Bimini.

bacterium *Salmonella choleraesuis*, hog cholera is now known to be caused by a virus—the hog cholera virus (HCV). HCV belongs to the family *Flaviviridae*, genus *Pestivirus*, and infections with this virus can give rise to swine fever. In the late 1890s, hog cholera was thought to be caused by the *S. choleraesuis*, because the HCV is filterable and was therefore carried along with bacterial "pure cultures" in swine-challenge experiments. Marion Dorset (1872–1935) of the U.S. Department of Agriculture uncovered the viral cause of this disease in 1903.

Fulfilling Koch's Postulates Can Still Prove Challenging

Countless numbers and types of bacteria cannot be cultured with currently available techniques and media. Some of these nonculturable bacteria are considered dormant, and sometimes are called "somnicells." Somnicells (sleeping cells) include those bacteria that, when stressed, become dormant and cannot be cultured on available media.

Some bacteria are more prone than others to enter into this "growth stage" or survival strategy, including human pathogens such as *Legionella pneumophila, Vibrio cholerae,* and *Helicobacter pylori*. Sometimes pathogens are isolated from an infected tissue only to be "lost" during successive culture attempts. This phenomenon is responsible for many of the difficulties in culturing these and other etiological agents.

Not-yet-cultured microbes likely constitute 99% of the microbes thought to exist in the biosphere. *L. pneumophila* was in this category before the 1980s. Other yet-to-be-cultured, disease-causing microbes are *Treponema pallidum*, which causes syphilis, and *Mycobacterium leprae*, which is responsible for leprosy, also known as Hansen's disease.

Koch's Postulates—Simplified

1. Universal presence of the microbe(s)
2. Isolation of the microbe(s) in pure (mixed) culture
3. Use the isolate(s) to recreate the process
4. Observe and re-isolate the microbe(s)

Two decades ago, Rita Colwell and I relied on a variation of Koch's postulates to show that *Escherichia coli* can form somnicells. We suspended viable, culturable cells of *E. coli* H10407 (an enterotoxigenic strain) within membrane chambers in Nixon Harbor, South Bimini, Bahamas (Fig. 2). Over a 13-hour period, cells in the membrane chamber became nonculturable. Several days later, the somnicells were placed into rabbit ileal loops for 36 hr. These loops, which developed a toxic response, were removed from the rabbits. The loop contents contained culturable *E. coli* H10407 that, on the basis of marker plasmids, derived from recovered cultures.

Without doubt, these steps vary from—but also comply with—Koch's postulates. Thus, *E. coli* H10407 is well known for causing gastroenteritis. We placed a pure culture into a natural aquatic environment, we recovered cells, and we produced the disease in an animal model; and, finally, we recovered the culture from those diseased animals. These experiments also provided evidence that an enteric pathogen can survive in seawater to cause disease in a suitable host.

Applying Koch's Principles To Address Broader Microbiological Issues

Gary Sayler of the University of Tennessee, Knoxville, and his collaborators relied on a variant of Koch's postulates to demonstrate in situ bioremediation of naphthalene-, anthracene-, and phenanthrene-enriched soil. They introduced a *lux* gene cassette into a biodegradative plasmid of *Pseudomonas fluorescens*, enabling this strain to emit light after it was added to polyaromatic hydrocarbon-enriched soil (Fig. 3). Although not a subject involving infectious diseases, this field trial relied partly on Koch's postulates, to show that a genetically engineered microbe could degrade polyaromatic hydrocarbons in situ.

Because many microbes are yet to be discovered, we cannot fully understand biogeochemistry. Surely, many of those undiscovered bacteria, archaea, and viruses play important roles in the carbon cycle, the nitrogen cycle, and other important elemental cycles. "Simplified" versions of Koch's postulates (see box, p. 226) that embody his principles may help us to address such challenges. For instance, some biogeochemists claim that microbes interact with each of the chemical elements. Those principles can help to guide experimental approaches for testing which elements are subject to microbially mediated oxidations and reductions.

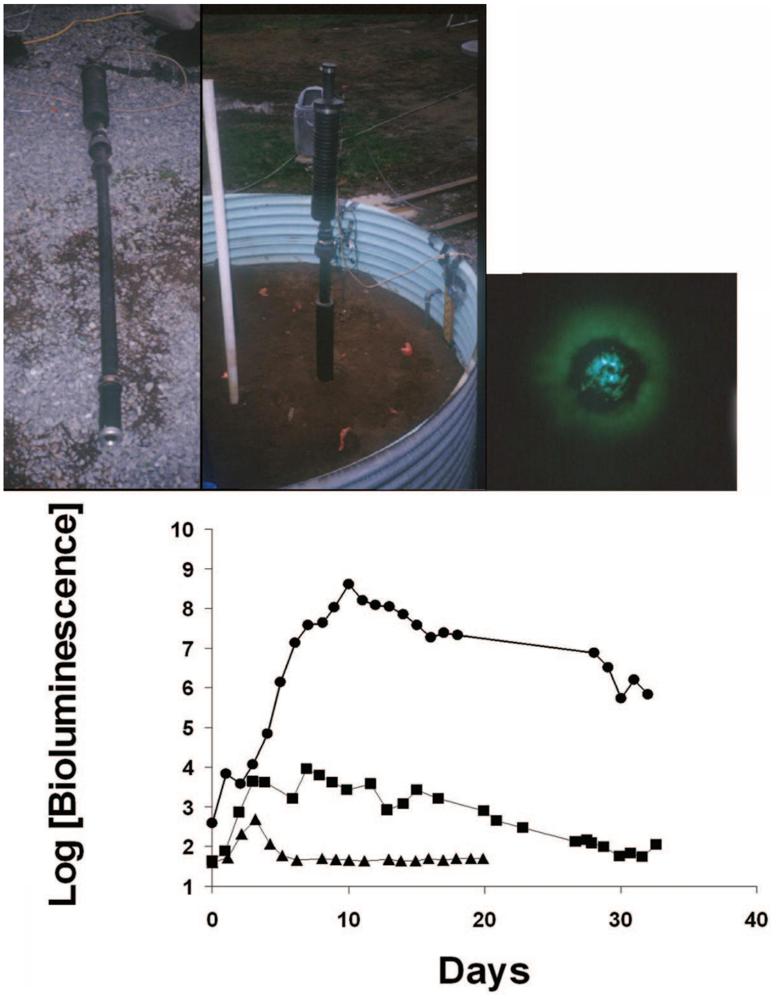
Microbes are used industrially, including to produce many beverages and foods, and these processes often are carefully controlled and monitored. For example, cheeses are often made with special starter cultures. Here again, this reliance on particular microbes reflects Koch's principles at work. Thus, appropriate microbes were isolated from cheeses, placed into culture, and reintroduced into milk to make cheese reliably like the prototype batches.

Perhaps the ultimate misuse of Koch's postulates is to subvert them to serve bioterrorism and biowarfare purposes. Many of the disease agents that raise such concerns are available in cultures, are candidates for genetic manipulation, and can readily be introduced into suitable hosts—ranging from humans to crops and livestock. One of them, *Bacillus anthracis*, which Koch described in the 19th century, was the agent used for bioterrorism late in 2001 (Fig. 4). This deadly unsolved crime cost the U.S. government more than $1 billion to mitigate.

Koch received the Nobel Prize in Medicine in 1905 for his research on tuberculosis. That work led him to frame a simple but powerful set of tenets that continue to help us better understand and mitigate infectious diseases. While those postulates have not proved readily applicable to every human disease, often our scruples about using human subjects are the main—and entirely appropriate—obstacle. It simply is not acceptable to test dangerous infectious disease agents in humans, and thus we find other means for determining which agents cause some diseases. Moreover, in other cases when Koch's postulates did not seem to apply, investigators subsequently learned by other means why the postulates did not seem to fit, and then typically reached the desired outcome following other routes.

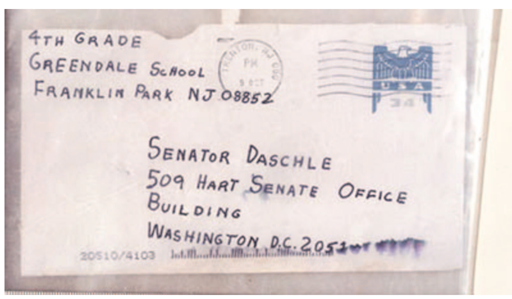
When Koch framed his postulates, they were bold and visionary. Now, these same principles are being applied to many other types of problems as microbiologists continue to use them, either knowingly or not, to address problems far outside the realm in which Koch once worked.

FIGURE 3



Monitoring bioluminescene of *Pseudomonas fluorescens* HK44 in naphthalene contaminated soil.

FIGURE 4



Photograph of an envelope containing anthrax spores that was sent to Senator Tom Daschle in 2001.

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This article expands on a talk given during the symposium "From Postulates to Posterity: Why Robert Koch Is Still Revered and Reviled," that honored the 100th anniversary of Robert Koch's Nobel Prize in Medicine. I am grateful to Joan Bennett for asking me to participate in that symposium during the 105th ASM General Meeting, held in 2005 in Atlanta, Ga.; to Tom Brock, who always writes good books; and to Rodney Rogers for first introducing me to Koch's postulates in 1966. Special thanks are extended to Phyllis Jestice for translating relevant passages from Koch (1884) and Loeffler (1883).

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