International Journal of Infectious Diseases 16 (2012) e470–e473

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

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

Perspective

The discovery of viruses: advancing science and medicine by challenging dogma

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ARTICLE INFO

Article history:
Received 7 March 2012
Accepted 7 March 2012

Corresponding Editor: William Cameron, Ottawa, Canada

Keywords:
Viruses
Koch's postulates
Paradigm shift

SUMMARY

The discovery of viruses in the final years of the nineteenth century represented the culmination of two decades of work on tobacco mosaic disease by three botanical scientists. Eventually their discovery led to a paradigm shift in scientific thought, but it took more than 20 years to appreciate its implications because it was inconsistent with the prevailing dogma of the time—Koch's postulates. Although these 'rules' were actually conceived of as guidelines upon which to establish microbial causality and their implementation resulted in many new discoveries, they also had the unintended effect of limiting the interpretation of novel findings. However, by challenging existing dogma through rigorous scientific observation and sheer persistence, the investigators advanced medicine and heralded new areas of discovery.

1. Introduction

The latter part of the nineteenth century witnessed major developments in medicine with the inception of microbiology as a distinct science. Although microbes had been identified in standing rain water, sea water, and human teeth scrapings by the Dutch textile merchant and amateur scientist Antonie van Leeuwenhoek using his handcrafted lenses nearly 200 years earlier, the significance of these "little animalcules" to human health and disease was unappreciated at the time.[1] Over the ensuing two centuries, through the observations and empiric efforts of individuals such as Ignaz Semmelweis, working to prevent puerperal sepsis in Austria; John Snow, mapping the epidemic curve of cholera in South London and in so doing, becoming the first 'shoe-leather' epidemiologist; and Miles Berkeley, solving the fungal etiology of the socioeconomically devastating Irish potato blight, the concept that microbial organisms caused human diseases—the "germ theory of disease"—gained adherents.[2]

2. The inception of dogma

In 1857 Louis Pasteur, an industrial chemist by training, demonstrated that microorganisms were responsible for the fermentation of fluids.[3] A few years later, he debunked the extant theory of spontaneous generation, proving instead that microbial agents were the cause of putrefaction. These events essentially established microbiology as a distinct science. Shortly thereafter, Robert Koch, a Prussian physician and scientific contemporary of Pasteur, revolutionized this new discipline with his rigorous studies that established the bacterial causes of diseases such as anthrax, cholera, and tuberculosis.

It was in the context of his isolation of the causative agent of tuberculosis in 1882 that Koch proposed a set of criteria for assigning a microbial etiology to a specific disease. These 'rules' would rapidly disseminate and become dogma throughout the scientific world. "Koch's postulates", although attributed to the great microbiologist and probably derived to some degree from earlier work by the famous German anatomist Jakob Henle, were actually first articulated in published form by his assistant Friedrich Loeffler, who would become a renowned bacteriologist in his own right. In Loeffler's 1883 paper on the bacterial etiology of diphtheria,[4] he described the criteria for establishing microbial causality, stated and refined by Koch himself in the context of his tuberculosis work: the pathogen must be found in every case of the disease and must account for its clinical and pathological features; the pathogen cannot be found in other disease states as a non-pathogen; and after isolation from diseased tissues and repeated passage in pure culture, the pathogen can induce the same disease in animal models.[5] Some contemporary reviewers added the additional caveat that the same pathogen must be re-isolated from the experimental host.

Although Koch himself appears to have realized the limitations of portions of his postulates as applied to specific organisms, the scientific community rapidly embraced them.[4] They provided a framework for scientists to experimentally ascribe medical importance to microbes, spurring a period of rapid discovery of the bacterial etiologies of many of the most important diseases of the era and leading to the general belief that an identifiable microorganism could be assigned to each infectious disease. However, the strict devotion to these nascent principles also had

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1201-9712/$36.00 – see front matter
http://dx.doi.org/10.1016/j.ijid.2012.03.005

the unintended effect of hampering the recognition, interpretation, and understanding of novel scientific findings.

3. The botanical scientists and filterable agents

Five years before Koch began his landmark work on tuberculosis in Berlin, Adolf Mayer (Figure 1), a German agricultural chemist, was appointed as the inaugural Director of the Agricultural Experiment Station and Professor of Botany at the new Agricultural School in Wageningen, a small town in the west-central Netherlands near the German border.[6] Beginning in 1879 and for the next 10 years thereafter, Mayer's laboratory research was devoted to a disease of tobacco plants that had plagued local farmers in the region. In recognition of the heterogeneously pigmented spots on the diseased leaves, he had named the affliction ''tobacco mosaic disease'' (Figure 2).

In an attempt to satisfy Koch's postulates, which had already become accepted as necessary requirements to prove a microbial etiology of a particular disease by the time the Director had become seriously engaged in mosaic disease research, Mayer performed the usual microbiologic experiments. Although unable to identify microbes in diseased plants, he proved that the disease was transmissible by replicating the disorder upon inoculation of healthy plants with sap expressed from the leaves of diseased plants.[7] However, unlike bacteria, which were too large to pass through laboratory filters, the agent of tobacco disease remained infectious after filtration. Mayer recognized the novelty of his observations, but unable to reconcile them with the prevailing dogma required to prove causality, he hypothesized the etiologic agent to be a ''soluble, possibly enzyme-like contagium''.

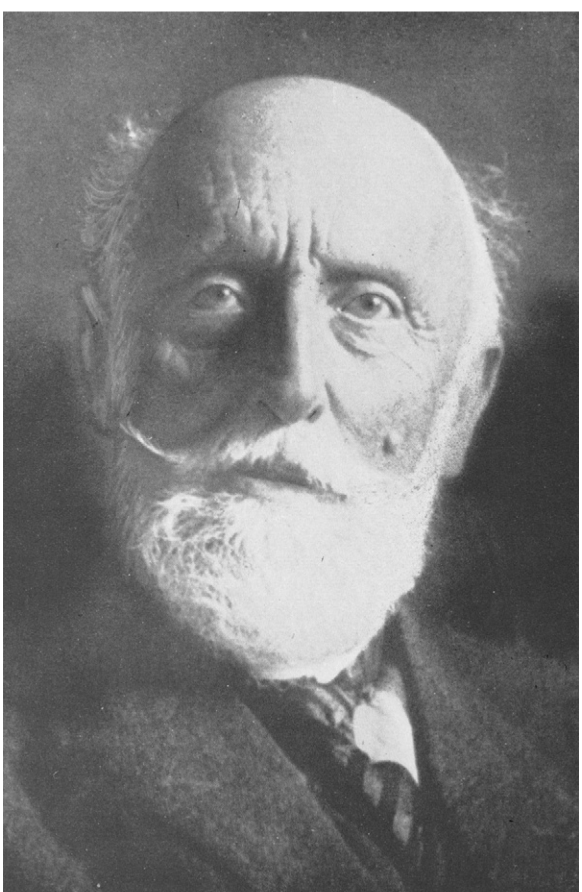

Figure 1. Adolf Mayer (1843–1942). Reprinted from Phytopathological Classics, No. 7, 1942 (reprinted 1968), American Phytopathological Society, St. Paul, MN.

He shared his findings with a younger colleague at the Agricultural School, Martinus Beijerinck (Figure 3), a chemist with an interest in botany who, with Mayer, had founded the local Natural Science Society.[6] After reviewing Mayer's experimental data and performing additional experiments, Beijerinck was also unable to demonstrate the presence of microbes, attributing this to his own lack of bacteriologic expertise.[8] Although Mayer would subsequently rethink his ''soluble contagium'' position in favor of a bacterial etiology, he remained aware that his findings failed to satisfy Koch's postulates regarding causality.

In 1892, more than 1300 miles away from Wageningen, a young botanical sciences student, Dmitri Ivanowsky (Figure 4), presented his research results on epidemic tobacco mosaic disease to the Academy of Sciences of St. Petersburg, Russia. Like Mayer, he too demonstrated the 'filterable' nature of the transmissible disease and appropriately concluded that the filtrate was infectious.[9] But as with Mayer, he too interpreted his findings in the context of the scientifically accepted supremacy of Koch's postulates, proposing that his findings represented either laboratory error due to defective filters or were due to the presence of other filterable materials such as bacterial toxins in the diseased sap.

By 1895 Beijerinck had returned to academia after leaving the Agricultural School for a 10-year stint in industrial microbiology in Delft, the South Holland birthplace of van Leeuwenhoek, one of the founding fathers of microbiology.[6] During his first years at the Technical University of Delft, Beijerinck resumed the research on tobacco mosaic disease that he had started while working with Mayer. Even then, he had appreciated that the affliction was microbial in nature, although he felt that the actual agents had yet to be discovered.[8] Beijerinck's investigations at Delft proved fruitful; he not only confirmed the infectivity of the *contagium vivum fluidum*—soluble living germ—despite filtration, but he importantly demonstrated that unlike bacteria, the culprit of tobacco disease

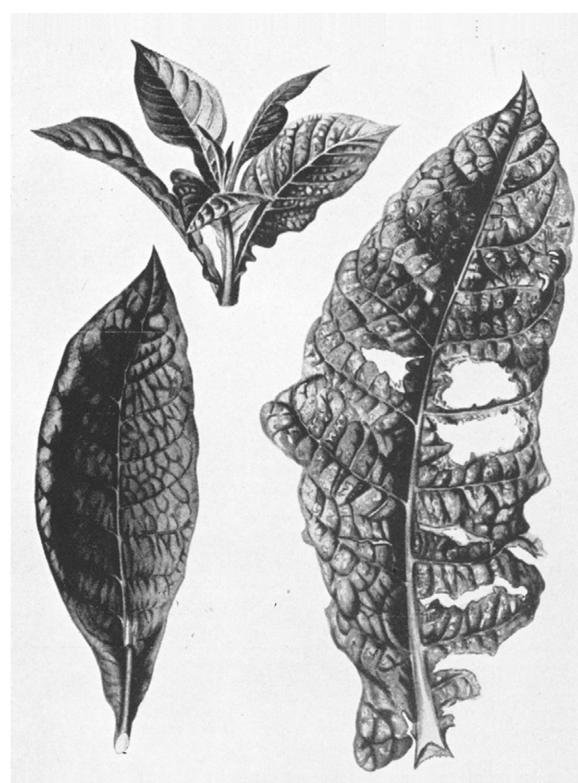

Figure 2. Tobacco leaves infected with tobacco mosaic virus. Reprinted from Phytopathological Classics, No. 7, 1942 (reprinted 1968), American Phytopathological Society, St. Paul, MN.

Figure 3. Martinus Beijerinck (1851–1931). Reprinted from Phytopathological Classics, No. 7, 1942 (reprinted 1968), American Phytopathological Society, St. Paul, MN.

Figure 4. Dmitri Ivanowsky (1864–1924). Reprinted from Phytopathological Classics, No. 7, 1942 (reprinted 1968), American Phytopathological Society, St. Paul, MN.

of plants was incapable of independent growth, requiring the presence of living, dividing host cells in order to replicate.[8]

Through his investigations and building upon the works of Mayer and Ivanowsky, Beijerinck definitively established that a member of a new class of infective agents, one that would come to be known as 'viruses', caused tobacco mosaic disease. But largely because of extant dogma—Koch's postulates—it took nearly two decades, beginning with Mayer's work, for the scientific world—and the investigators themselves—to accept this novel truth. Once established, their discovery unleashed a torrent of scientific investigation that, as with Pasteur and Koch two decades earlier, again revolutionized medicine.

By the end of the first quarter of the twentieth century, essentially the same 20-year timeframe that it took to establish the existence of viruses and their involvement in disease, more than 65 diseases of animals and humans had been attributed to these filterable agents.[10] Furthermore, the fledgling field of virology, launched by the work of the three botanical scientists, laid the foundations that galvanized the modern molecular era and also ushered in the 'golden age of vaccinology', leading directly to the prevention of polio, measles, and other scourges of nature.[11]

4. Challenging dogma as a route to advance science and medicine

Mayer, Ivanowsky, and Beijerinck were neither the first nor will they be the last in any field to challenge existing dogma. Examples of this abound in the histories of science and medicine. The notion that germs causing lethal puerperal ('childbed') fever were transmitted to hospitalized, parturient women through the contaminated hands of obstetricians was considered heresy by the medical establishment when proposed by Semmelweis in 1847 Vienna.[2] His thesis failed to gain acceptance despite the demonstration that hand washing by physicians and medical students between patient contacts significantly reduced the mortality from the disease. Two decades later Lister introduced similar concepts of disinfection, sterility, and antisepsis into surgical practice in Glasgow.[12] The medical community rapidly embraced these practices, in large part because the intellectual environment had changed during the 20-year interval between the work of Semmelweis and Lister. Pasteur had proven the germ theory of disease, thus moving the field forward in a radical way and establishing a new paradigm—a framework upon which to interpret such novel discoveries.

As scientific tools and technology evolved and knowledge expanded throughout the twentieth century, Koch's postulates were revisited and revised. Their first major reassessment was necessitated by the discovery of viruses and its scientific aftermath:

> "At the time when they were formulated Koch's postulates were essential for the progress of knowledge in infectious diseases; but progress having left behind old rules requires new ones which some day without doubt will also be declared obsolete. Thus, in regard to certain diseases, particularly those caused by viruses, the blind adherence to Koch's postulates may act as hindrance instead of an aid."[13]

Koch's postulates—originally established as guidelines for establishing microbial causality—have since undergone reevaluations multiple times in response to novel paradigms that were precipitated by new technologies and new knowledge. Most recently, this has been occasioned by the development of genotype-based microbial discovery.[14] Common to all such 'episodes' of reevaluation is a shift in scientific thinking that moves the field forward.

Kuhn, in his landmark treatise on the nature of scientific progress and discovery, characterized this process as comprising intervals of "normal science"—incremental advances based on past achievements—punctuated by periods of revolutionary changes in scientific thought.[15] These 'game-changing' episodes of discovery represent revolutions that challenge dogma and inevitably cause upheavals within the scientific community. But these episodes eventually lead to new paradigms that change thinking and galvanize further, novel discoveries and in doing so, advance the field. Although Kuhn's thesis was based on examples from the physical sciences, it clearly resonates in the biological arenas as well.

The story of the discovery of viruses vividly illustrates the kinetics of paradigm shifts in scientific thinking, and how these changes lead to new discoveries and potentially downstream shifts that may amplify the discovery process further. Novel or unexpected findings, whether originating from the clinic or the laboratory bench, should rightly force physicians and scientists to question their experimental and intellectual processes and to reevaluate their approach. However, careful, reproducible, clinical or experimental observations that cannot be explained by existing paradigms or that are not consistent with existing dogma should not be disregarded. Instead, as with the experimental findings of Mayer, Ivanowsky, and Beijerinck, such discoveries may only be reconciled through the development of novel paradigms.

Conflict of interest: No conflicts of interest to disclose.

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