

Proof in Medicine: The Four Postulates of Dr. Robert Koch

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*“Does disease follow bacteria, or do bacteria follow disease? We still do not know.”
Dr. Karl Thiersch, 19th century*

The cause of disease has long been a significant question for philosophers, religious leaders, scientists, and physicians. During biblical times, Jewish communities considered leprosy to be a punishment from God for sins committed by an individual. Traditional Chinese Medicine has maintained for centuries that disease results from an imbalance of the flow of the body's life energy. Greek scholars of antiquity, including Aristotle and Galen, similarly believed diseases resulted from an imbalance of the four humours: phlegm, blood, yellow bile and black bile (Sutter). Although modern society accepts without question that many illnesses result from the side effects of an infection, it was not until the 19th century and the work of Dr. Robert Koch that the germ theory of disease became widely accepted among scientists. In addition to unequivocally confirming the germ theory of disease, Koch further provided researchers with a series of four requirements that could be used to link individual bacterial species with certain diseases. This checklist of requirements is known as Koch's Postulates, and continues today to be the most rigorous and effective formulation of proof in medicine (Herman).

Dr. Koch developed this system for identifying causal agents of disease while studying the bacterium *Bacillus anthracis*, presently well known as the cause of anthrax, and the effects of this pathogen in cattle. In many respects, Koch's Postulates hold similarities to the fundamental characteristics of the axiomatic method used in the study of geometry. Before any part of this

comparison is addressed, however, a presentation of the postulates is in order. Koch's Postulates, taken in their modern form from *Brock Biology of Microorganisms* (Madigan, Martiko and Parker), can be summarized by the following four conditions:

1. To establish a bacterium as the cause of a particular disease, a microorganism must be shown to be present in animals suffering from the disease, and this organism should not be present in healthy individuals.
2. This bacterium must then be isolated and grown in a pure culture away from the animal's body.
3. When cells from this culture are inoculated into healthy animals, the characteristic disease symptoms must appear in the newly infected individuals.
4. The bacterium must then be reisolated from this second set of animals and cultured again, and the new organism must be identical to the first.

Similar to the axiomatic method presented in Greenberg's text, these postulates (axioms) are as elementary as possible and must be accepted as true for the methodology to be effective. If someone disagrees with the first postulate, and instead argues that microorganisms need not be present in a diseased host, then the method of proof loses all validity for that individual. Koch's Postulates also make use of undefined terms, specifically the concepts microorganism and disease. Webster's Dictionary contains entries for these nouns, just as it carries definitions for point, line, incidence, etc., but each term has yet to be explained in its entirety. Examples of each can be provided, just as a teacher can draw a representation of a line on the blackboard for his or her students, but until every microorganism and disease have been characterized, the actual definition of each term remains beyond our grasp.

Koch's Postulates are not entirely akin to the axiomatic method, however. Unlike geometric proofs, where a single axiom may be used to justify a logical step, Koch's Postulates are only an effective means of proof when utilized as a unit. Simply demonstrating that a bacterium is found in a diseased individual (Postulate 1) is not adequate proof that this bacterium is responsible for the disease, as the organism could instead be a result of the disease. Likewise, observing disease symptoms in an animal injected with an unknown bacterium is not adequate evidence the disease resulted from the bacterium, since the symptoms could be the effect of a different opportunistic infection. Finally, if the culture of the bacterium is not pure, which means there is evidence of only one species' presence in the culture, then it is impossible to prove that the disease resulted from the suspected bacterium and not one of the contaminant species.

If the methodology of Koch's Postulates is carefully scrutinized, one will quickly note that the collected evidence is somewhat circumstantial, since the effects of pathogenic bacteria on living cells are not directly observed. Because bacteria can only be clearly visualized with electron microscopy, which requires the cells be chemically fixed (and therefore dead) before viewing, the step-by-step interactions between the bacterium and host cannot be observed, and 'circumstantial' evidence is all that is available to scientists. Proof is instead inferred by the strong correlation between the bacteria's presence and the subsequent disease symptoms: Postulate 2 ensures that only the bacterium under investigation is used for inoculation, Postulate 3 requires appearance of characteristic disease symptoms, and Postulate 4 ensures that the bacterium was present in the subject during the onset of the symptoms.

A second difference between the geometric axiomatic method and Koch's Postulates (as well as scientific investigations as a whole) is that mathematical proofs require 100% confidence. There is no room for a margin of error in geometric proofs: if one discrepancy or contradiction is

noted, the entire proof is considered invalid (Greenberg). Biological studies do not require such precision, however. Joseph Herman states in his article on proof in medicine that “absolute proof of anything...is probably not attainable outside of the confines of Euclidean geometry, where men have made all the rules.” Because identical results are rarely observed in any biological study, for reasons that will be discussed shortly, empirical findings are instead analyzed statistically. By convention, findings with a 95% significance or greater are generally accepted as valid proof in scientific studies. In studies following Koch’s Postulates, this means there is at most a 5% chance that the observed disease occurred by chance, and researchers can be 95% confident that the disease resulted from infection by the microorganism. It is therefore expected that not every infected subject will respond identically, but if such incongruities are minor and the sample size is large, the overall results will still maintain validity. As mentioned previously, this differs from mathematics, where the confidence interval is required to be 100%.

Geometric proofs often begin with the statement “Take an arbitrary line” (or triangle, model, etc.), and the desired outcome being deduced from this initial arbitrary representation. Because the object was non-specific, conclusions made during the proof are taken as true for all comparable objects, and repetition is unnecessary--it would yield an identical theoretical proof. Investigations in medicine and the sciences, however, adhere to the scientific method, and therefore require repetition of any finding before proof can be established, not only with more than one subject, but often also in more than one laboratory under the supervision of more than one researcher. To attempt to draw a parallel to mathematics, scientists cannot “take an arbitrary mouse and expose its respiratory tract to the bacterium *Streptococcus pneumoniae*” to ascertain whether the mouse develops pneumonia, using this one individual to support or reject the hypothesis pneumonia is caused by an *S. pneumoniae* infection. Instead of proving that *S.*

pneumoniae infection always leads to pneumonia, researchers aim to demonstrate that *S. pneumoniae* causes pneumonia a significant amount of the time (95% confidence interval), and can therefore be accepted as a cause of pneumonia.

There are myriad reasons why 100% confidence cannot be expected in the biological investigations. First, the variation that exists in biological organisms and even individual cells is unfathomable, and it would be imprudent to automatically assume two animals should respond identically to the same pathogen. Think, for example, about the common cold. As this infection passes through group of friends, coworkers or family members, each individual may exhibit different symptoms. One person may have an intensely sore throat, while another primarily has a runny nose, a third develops a combination of the two, and the fourth appears entirely healthy. Scientists have identified numerous reasons that account for these observed differences. Most importantly, the mammalian immune system has the capacity to destroy pathogenic cells, and it often prevents infections from occurring even though the pathogen has the capacity to cause an infection. Individuals also do not express the same susceptibility to infectious agents due to a variety of factors, including age, state of the immune system at the time of exposure, other illnesses, genetics, etc. For example, although HIV is extremely contagious under certain elements of exposure, like shared intravenous needle use, yet certain individuals do not become infected with HIV despite repeated exposure to the virus. Scientists have hypothesized that this resiliency is due to the expression of certain genes that are not found in the general population, but which prevent infection by HIV when present in an individual's DNA. Clearly there are many variables present in biological investigations beyond the control of the researcher, and findings--including those that link a microorganism with a particular illness--cannot be based on investigations conducted in only one individual.

Koch's Postulates have proven to be such an unprecedented and powerful methodology for proof in medicine that they have been adapted for purposes beyond the study of bacteria, both in medical and biological investigations. Koch's postulates can be used as a mechanism for proof in virtually any bacterial study, and are frequently used by plant pathologists to study disease in plants. Originally, the postulates were modified for use in identifying protozoa (which are also single-celled organisms but with many distinctly different cellular characteristics from bacteria) and viruses (which are not living cells at all and require the presence of a host in order to multiply) as disease-causing agents. Protozoa and viruses both require conditions different from bacteria in order to be cultured, and viruses cannot be maintained in a truly pure culture because they require the presence of cells in order to replicate. Koch's Postulates also form the basis criteria for linking cause-and-effect in many biological situations that do not involve disease at all. For example, modified forms of the postulates are used to prove cellular proteins perform particular functions in the cell, like communicating a signal between nerve cells or participating in a communication cascade from the cell's exterior to interior (Sutter). In these cases, the postulates are modified into the following three questions (Sutter):

- 1) Is the protein present in the cell or tissue under investigation?
- 2) Is it released or its concentration increased upon appropriate stimulation of the cell?
- 3) Does artificial release of the protein evoke the anticipate effect?

A modified form of Koch's Postulates is also used to indicate potential effectiveness of replacement therapy in individuals suffering from a dysfunctional organ or gland. Instead of requiring the positive identity of an infections agent in the diseased individual, the first postulate instead requires that the organ in question appear diseased and lacking in some particular protein.

Then, rather than requiring a culture of the microorganism and injection of the infectious agent in a healthy individual, replacement therapy expects that the protein in question be isolated from healthy cells, and administration of this protein into another diseased individual should relieve symptoms of the disease. A well-known example of replacement therapy is the provision of the compound dopamine to Parkinson's patients, who have been shown to lack proper amounts of dopamine in their brain. This artificial administration of dopamine temporarily relieves many of the symptoms associated with Parkinson's disease.

Robert Koch was certainly not the first scientist in his century to suggest a link between disease and the presence of microorganisms, but he was the first to outline so thorough a means of collecting circumstantial information that it could be accepted as direct proof. Following the careful and repeated fulfillment of Koch's Postulates, the scientific and medical community can logically accept a microorganism as the causal agent of a particular disease. Modified forms of Koch's Postulates are also essential to biological studies because the method of proof is so complete and comprehensive. Much of the authority associated with Koch's Postulates, and the reason they have persisted in medicine despite modern technology, is because the postulates are structured similarly to the highly esteemed axiomatic method used in the study of mathematics.

Works Cited

Marvin J. Greenberg, *Euclidean and Non-Euclidean Geometries*, 3rd ed., W.H. Freeman & Co., New York, NR, 1993.

Joseph Herman, Proof, *Perspectives in Biology and Medicine*, 40 (1997), 592-596.

Michael T. Madigan, John M. Martinko, and Jack Parker, *Brock Biology of Microorganisms*, Prentice Hall, Upper Saddle River, NJ, 2000.

Morley C. Sutter, Assigning Causation in Disease: Beyond Koch's Postulates, *Perspectives in Biology and Medicine*, 39 (1996), 581-592.