Experiment 5: Koch's postulates and experimental evidence: A lesson in correlation vs. causation

All people are scientists. We all make observations, look for correlations, develop hypotheses, and then test them. From the beginning of human existence people have been teasing apart the world and advancing the knowledge of how things work by searching for relationships between events. This is such a common part of our everyday lives that many of us do not realize we are doing it. We notice, for example, that when the soil looks dry, our tomato plants wilt- a simple observation. So we develop a hypothesis, such as lack of water causes plants to wilt. We might do an experiment to see if watering the tomato plants prevents wilting. An essential step is observing a simple correlation, but the experiment establishes that the lack of water is causal to wilting. If the water does not reverse the symptoms, we look elsewhere for cause of the wilting.

In the example in the previous paragraph, the answer was pretty clear because we all know that plants need water, but the obvious answer is not always the right answer. It is all too easy to be misled by assuming that a relationship is causal based on a simple correlation. One of the most difficult challenges in biology is to determine whether events that appear to be related are causally associated. Just because two things happen in the same place or at the same time does not mean that one causes the other. For example, there may be an association or correlation between the number of telephone poles in a geographical region and the frequency of cancer in that region, but that does not mean that telephone poles cause cancer. Careful experimentation is needed to separate causation from correlation. An instructive example of such experimentation is the story about the discovery of the role of microbes in causing disease.

To understand the story, we must examine the context in which the experiments were done. It is not always easy to assimilate new discoveries into our body of knowledge and beliefs. Conversely, it can also be difficult for us to try to imagine what it must have been like in the past when people did not have some of the knowledge that we now take for granted, such as the concept that germs cause disease. It was only through clear evidence from thoughtfully designed experiments that we came to adopt what is now referred to as the germ theory of disease.

By the mid l9th century, the French scientist Louis Pasteur had conducted extensive studies of the role of bacteria in fermentation, and he had shown conclusively that while germs could travel through the air, they were not capable of spontaneous generation. There was also a prevailing assumption at the time that microbes were in some way connected with disease, but whether their presence was a requirement for disease or a result of disease was not clear. Furthermore, many infected tissues contained more than one type of microorganism. This made it difficult to define with certainty the role played in disease by any particular type of bacterium.

The work of Pasteur and others, improved techniques in microscopy, and, perhaps most important, the discovery of semi-solid culture media all paved the way for a German physician, Robert Koch, to demonstrate for the first time in 1875 that a specific type of bacterium was responsible for a specific disease.

Koch had been studying anthrax disease in sheep, and he noticed that certain rod-shaped bacteria and their spores were characteristically found in the tissues of the sick livestock. He meticulously isolated these bacteria, which he named *Bacillus anthracis*, and grew pure cultures of them in a culture medium consisting of the aqueous humor of cattle or rabbit eyeballs. Next, he introduced the bacteria from the cultures into healthy rabbits. When the rabbits subsequently developed symptoms of anthrax, Koch again isolated the bacteria from the tissues of the rabbits and observed them under the microscope to confirm that they were indeed the same ones he had seen in his original culture.

The steps he used are now known as Koch's postulates. Meeting the criteria laid down by Koch is referred to as "satisfying Koch's postulates" and is considered the standard evidence required to show that a microorganism plays a causal role in a particular disease.

Koch's postulates

1. Observe a consistent association between the disease condition and the presence of a specific microbe.
2. Isolate the microbe and grow it in pure culture outside of the original host.
3. Inoculate a healthy, susceptible host with the pure culture and observe disease symptoms that are the same as those in the original host.
4. Isolate the microbe from the inoculated host and demonstrate that it is the same as the microbe from the original diseased organism.

Note: These criteria must be met to firmly establish that a microbe causes disease.

Resources

Koch’s postulates are described in most microbiology textbooks. Often they can be found in an introductory chapter discussing the history of microbiology.
An overview of the germ theory of disease and Koch’s contributions to microbiology can be found at the following websites.
Germ Theory: http://ocp.hul.harvard.edu/contagion/germtheory.html
Koch: http://ocp.hul.harvard.edu/contagion/koch.html

Key concepts

Specific microbes cause specific diseases in plants.
Satisfying Koch's postulates provides rigorous evidence that a specific microbe is responsible for a particular disease.

Challenge

You will be provided with a diseased fruit or vegetable and a healthy fruit or vegetable, a microscope and slides, and petri plates with media on which to grow cultures of microbes from the fruits and vegetables. The growth media include potato dextrose agar (PDA), which favors the growth of fungi, and LB or T-soy agar, which favor the growth of bacteria. Design an experiment based on Koch's postulates to identify the microbe that is responsible for the disease.

Key questions

- What is disease? How can it be distinguished from other conditions?
- What was the cause of the symptoms in your unhealthy plant? What evidence do you have?
- Why couldn't you simply grind up some of your initial plant tissue, spread it on a second (healthy) host and see if the disease appeared? Assuming it did appear, what would that show and what would that fail to show?
- Why is it important to grow a pure culture and then inoculate with bacteria from a single colony?
- What if the disease-causing pathogen does not grow outside of the host? How might this have changed Koch's understanding of infectious agents? Can you think of any examples?

This lab is adapted from Handelsman J., Houser B., Kreiger H. *1997*. Biology Brought to Life. Times Mirror Higher Education Group, Dubuque, Iowa.