Following the Inner Light

Glow genes provide revealing pictures of infections

By JOHN TRAVIS

A few years ago, Christopher H. Contag attended a meeting at which investigators presented data obtained with the latest techniques for detecting viruses. As he listened to each speaker, the Stanford University virologist realized how frustrated he had become with the tools available to his profession.

Consider the way researchers usually establish the normal course of a bacterial or viral infection, remarks Contag. They infect dozens, if not hundreds, of animals, usually mice. As the infection proceeds, the investigators kill groups of the mice and, using a variety of means, painstakingly examine the animals' tissues to determine how far the microorganisms have spread.

Such experiments are costly and laborious; they also raise the ire of animal rights activists. Contag felt there had to be a better way. "If we can track the trajectory of a bullet in space, we must be able to track a pathogen in a living animal," he says.

Contag began to take a look at bioluminescence, the natural emission of light by organisms such as fireflies and certain bacteria. These creatures all owe their glow to an enzyme called luciferase.

For more than a decade, scientists have made use of luciferase or the genes that encode it. For example, with the aid of this light-producing enzyme, they've discovered and studied the genes that establish circadian rhythms (SN: 8/12/95, p. 108). Pamela Contag, also at Stanford, has popped luciferase genes into salmonella bacteria to better study how the microbes interact with animal cells.

After reading an article about detecting light that passes through tissue, Christopher Contag wondered if bioluminescent microorganisms inside living animals might be observable from the outside. Fortunately, David Benaron, a medical imaging researcher at Stanford, was nearby. The virologist strolled over to Benaron's office and outlined his proposition.

"I thought it was an unusual mixture of ideas. But I have to tell you my first reaction was, that's impossible," says Benaron.

Benaron's main concern was that the greenish yellow glow emitted by the standard luciferase-induced chemical reaction would be mostly absorbed and scattered by the animal tissues.

That reservation didn't stop him from agreeing to give the idea a try, however. Benaron and the two Contags decided to test first whether bioluminescent microbes could shine through dead tissue. "The easiest source of tissue was the grocery store, so we picked up some chicken breast, beef liver, and lamb kidney," laughs Christopher Contag.

In their initial experiment, the investigators placed a vial of Pamela Contag's glowing salmonella inside a thawed chicken breast. The bacterial light had to penetrate about half a centimeter of meat to reach a highly sensitive photon detector. "Sure enough, light came out," says Benaron.

That glimmer has since led to what bacteriologist Gordon Stewart of the University of Nottingham in England calls "some of the most important work in microbiology in a decade."

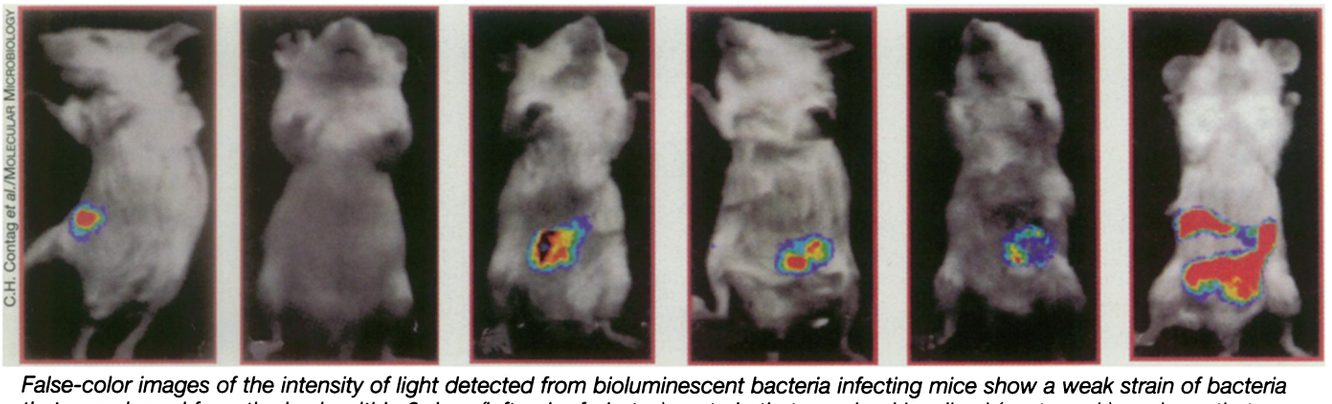
After their grocery store foray, the Stanford investigators moved on to test their idea in live animals. Success wasn't guaranteed, notes Benaron, because the optical properties of living tissues, determined in part by their warmer temperature and the presence of blood and oxygen, differ significantly from those of dead tissue.

The researchers added the genes for bioluminescence to three different strains of salmonella and inoculated mice with the glowing bacteria. As they reported in the November 1995 MOLECULAR MICROBIOLOGY, Benaron and his colleagues had little difficulty imaging the bacteria with a digital camera from a colleague's lab.

More important, they were able to follow the course of each infection and distinguish among the different salmonella strains. The bioluminescence vanished as the mice quickly eliminated the weakest strain.

A somewhat stronger strain emitted a persistent, weak glow as it established a low-level infection. The most virulent strain spread rapidly through tissues in the mouse, according to the images produced by the camera.

By illuminating the same patterns of bacterial infection that had been observed with the more laborious, traditional techniques, these initial luciferase experiments established the power and accura-



C.H. Contag *et al.*/MOLECULAR MICROBIOLOGY

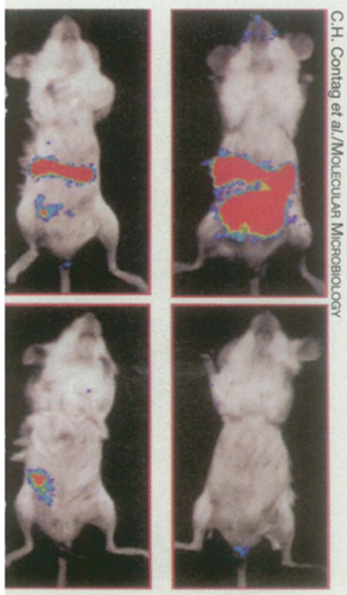
False-color images of the intensity of light detected from bioluminescent bacteria infecting mice show a weak strain of bacteria that was cleared from the body within 6 days (left pair of photos), a strain that remained localized (center pair), and one that spread through the gut (right pair).

cy of the new technique, says Christopher Contag. One surprise did emerge, however, suggesting that the technique will provide new insights into the progress of infections.

The images indicated that the bacteria establish colonies in the cecum, a large structure at the junction of the small and large intestines. If the infection is not controlled by the mouse's immune response, the bacteria appear to spread out from that reservoir.

"It appears that the cecum is a site that may be a contributing factor to the persistent infections and to the progressive infections. Previously, that hadn't been documented," notes Christopher Contag.

Additional salmonella studies demonstrated that the imaging of internal bioluminescence can enable investigators to gauge the effectiveness of drug treatments. The digital camera allowed the Stanford group to watch as antibiotics successfully rid the mice of an infection, or failed to do so. Indeed, Benaron suggests, that ability to visualize the potency of new drugs in living animals might be particularly attractive to companies developing treatments for AIDS. "What has really been missing is the ability to track the effect of new drugs or treatments on an agent in vivo," he says.



C.H. Contag *et al.*/Molecular Microbiology

Researchers plan to gauge the effectiveness of new drugs by monitoring bioluminescent bacteria inside live animals. Untreated infections spread rapidly (top photos), but antibiotics kill most bacteria (bottom).

Christopher Contag notes that researchers can probably engineer bioluminescence into most bacteria. To light up salmonella, the investigators actually added five genes. Two genes serve to make the subunits of luciferase, while the other three encode enzymes that synthesize the material that luciferase combines with oxygen to produce light.

Adding bioluminescence to viruses is more challenging. Many viruses are simply too small to hold five or so extra genes.

"You either have to engineer a larger virus that accepts foreign genes, or you have to engineer an animal to emit light when a virus is in a cell," says Christopher Contag. To explore the practicality of the latter idea, the investigators have been experimenting with genetically engineered mice created by John D. Morrey of Utah State University in Logan.

Every cell in these mice contains the luciferase genes hooked up to a genetic sequence, known as LTR, found in HIV, the AIDS virus. This particular sequence, which is involved in turning on other HIV genes, is triggered by the virus when it enters a cell.

Because HIV does not infect rodents, the Stanford researchers have used a chemical called DMSO to turn on LTR in various tissues within the mice. The simulated HIV infection, as planned, activated the luciferase genes.

"You see light coming from inside the animal. You can detect gene expression from deep tissues. The colon, for example, becomes luminescent," says Christopher Contag.

Another field where imaging luciferase activity in live animals might prove useful is gene therapy, suggest Benaron and his colleagues. Gene therapy often involves removing blood cells from a body, adding a gene to them in the laboratory, and returning the modified cells to the body. If the luciferase system were also added to those cells, researchers might be able to track where the cells migrate in the body and how long they persist.

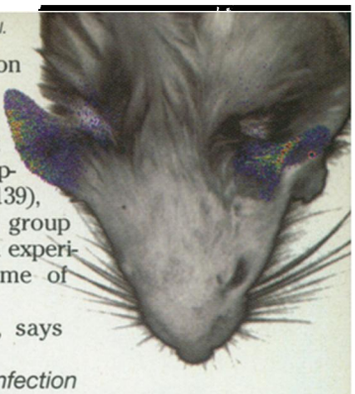
Alternatively, investigators might be able to design the procedure so that the luciferase genes produce light only when the added gene makes its protein. That would allow them to monitor whether the protein is being synthesized in the appropriate sites throughout the body, says Christopher Contag.

Cancer investigators might also find this new research technique a boon, the researchers speculate. Very often, they note, an initial tumor isn't dangerous. It's when tumor cells spread, or metastasize, to other sites, such as crucial organs, that cancer becomes most deadly. Researchers studying metastasis in mice and other animals might one day watch the spread of bioluminescent cancer cells, says Christopher Contag.

Perhaps the main advance needed to bring these grand notions to fruition is the development of luciferases that stimulate the emission of a redder light. Scattering and absorption diminish the greenish yellow light of standard luciferase by 90 percent for every centimeter of tissue it traverses. Red or infrared light, Benaron notes, travels 10 times farther before it suffers a similar reduction in brightness.

Luciferases generating redder light have been developed (SN: 8/31/96, p. 139), and the Stanford group has already begun experimenting with some of them.

With red light, says Benaron, "one could actually use large animals and see through 10 centimeters of tissue. You would open a new window into the body." For example, red-light-producing luciferase might enable investigators to follow how infections of SIV, the monkey version of HIV, progress in primates.



C.H. Contag *et al.*

A simulated HIV infection causes this mouse's ears to emit light. The colors mark different amounts of light detected.

The ability to image microorganisms, and the activity of their genes, as they spread throughout the body of a living animal is almost a dream come true, say researchers familiar with the work of the Stanford group. "I think it will be revolutionary. I would certainly like to use [the technique] in my research," observes Andrew Camilli of Tufts University School of Medicine in Boston, who studies how the *Vibrio cholera* bacterium infects the small intestine.

Stewart, who has used luciferase to study bacteria for many years, ruefully admits that he has a camera similar to that used by the Stanford group but never dared to think of imaging glowing germs in live animals. Indeed, notes Stewart, if the idea had come to him in a grant proposal, he would probably have rejected it as infeasible.

A convert now, Stewart has confirmed the Stanford group's success at imaging bioluminescent bacterial infections in mice. He plans to link the activity of the luciferase gene to that of genes in salmonella bacteria. In particular, Stewart intends to have his bacteria light up only when they turn on some of their virulence genes, which they employ only at certain times during the infection of a host (SN: 12/2/95, p. 382).

Among other duties, the proteins produced by these genes help bacteria set up colonies in host tissue and evade the host's immune response. Furthermore, it's often the activity of these virulence genes that has the greatest effect on the health of an infected animal. By linking the virulence genes to the luciferase gene, Stewart hopes to resolve difficult questions, such as exactly when various virulence genes switch on and in what environments.

"The beauty of the new approach is that you can watch what happens, almost hour by hour, in the same animal and in a noninvasive manner. Wow!" he exclaims. □