BACTERIOLOGY

Phage Factor

Long ignored by mainstream researchers, the viruses that infect bacteria have a role to play in modern medicine, Vincent Fischetti says

Interview by Brendan Borrell

INSIDE A THIRD-FLOOR OFFICE A FEW BLOCKS FROM THE HUDSON RIVER IN YONKERS, N.Y., a small biotechnology company called ContraFect prepares to test a remarkable new way to kill bacteria in humans. Antibiotics, after many years of use and overuse, have lost their edge against rapidly evolving bacteria, with everything from staph infections to tuberculosis becoming more devastating, deadly and difficult to treat. Whereas traditional antibiotics have mostly been derived from chemicals produced by soil bacteria and fungi, ContraFect has found an alternative in bacteriophages: viruses that infect bacteria and hijack their internal machinery. In nature, phages produce enzymes called lysins, causing the bacteria fall to pieces and new phages to tumble out by the hundreds. ContraFect believes it can harness these lysins to treat bacterial infections in humans.

IN BRIEF

WHO
VINCENT FISCHETTI

VOCATION | AVOCATION
Microbiologist

WHERE
Rockefeller University

RESEARCH FOCUS
Finding an alternative to overused antibiotics.

BIG PICTURE
Could viruses that attack bacteria be used to treat and prevent infections?

The first trials for patient safety are expected to start this year. It is a moment that Vincent Fischetti, a 71-year-old microbiologist at the Rockefeller University, has been approaching for decades. A child of working-class parents on Long Island, he once thought he would be a dentist before getting hooked on microbiology as an undergraduate. Studying for his master's degree by night and paying his bills as a technician on a scarlet fever project by day, he became fascinated by phages. After years of work, he demonstrated, in 2001, that lysins could help mice fight strep throat infection. The military also sees potential in lysins, which could be administered before surgery to prevent infection or spread over surfaces to clean an area contaminated by an anthrax attack.

More broadly, researchers are showing renewed interest in delivering cocktails of phages to treat stubborn infections. That strategy was nurtured in the former Soviet Union and all but ignored stateside. Some technical and practical challenges stand in the way of their widespread adoption in human therapeutics,

Photograph by Spencer Heyfron

although several U.S. companies have Food and Drug Administration approval to include *Salmonella*- and *Escherichia coli*–killing phages in packaged meats and other food products.

Scientific American spoke with Fischetti to learn more about the promise and peril of phages in human health. Excerpts follow.

Scientific American: How did you first become interested in science?

FISCHETTI: I grew up on Long Island, and my family had a landscaping business right next to a pond. When I was around 12, my parents bought me a microscope. There was no Internet or anything to distract me, so I would take water samples from the pond and spend evenings looking at the microbes swimming around in the water samples: *Euglena, Paramecium* and all kinds of things. I spent hours just fooling around with that. When I took my first microbiology course at Wagner College on Staten Island, I realized this is really what I love to do, and I stayed with it.

When did you first learn about phages?

In my first job, I was a lab technician at Rockefeller working with John Zabriskie, a physician-scientist. At that time, scientists at New York University had recently discovered that pertussis toxin—the toxin that causes whooping cough—was produced by a bacteriophage carried by a bacterium. We wondered whether the toxin that caused scarlet fever was also controlled by a bacteriophage. We found that it was. In this case, the *Streptococcus* bacterium carries a bacteriophage that has the gene for the scarlet fever toxin. When the phage replicates inside a streptococcal organism that has infected a person, it produces the toxin, which causes the reddening of the skin and high temperature associated with scarlet fever. We now know phages are responsible for most of the toxin-associated diseases.

How important are phages in the environment?

Every gram of soil, every cubic centimeter of water, has at least 10 million to 100 million phages. Phages are the most numerous biological entities on earth. They are in everything we touch, we eat, we drink. We ingest phages all the time. They are found in our gut, on our mucous membranes, everywhere in our body. Bacteriophages continuously infect and kill bacteria. Then resistant bacteria grow out again, and the process continues. Every two days half the bacteria on earth are killed by bacteriophages.

It's a hugely dynamic process, where both bacteria and bacteriophages need each other to survive. And it's my view—and I don't know if anyone actually believes this view—that because there are 10 times more bacteriophages than there are bacteria, what's really in control of the planet are the bacteriophages. They control everything.

When did scientists realize that phages could be used in medicine?

About 100 years ago, when bacteriophages were first identified, antibiotics did not exist, and it was felt that here was the substance that kills bacteria—we could now harness this to kill bacteria causing infection. In the U.S., Pfizer was one of the first companies to start developing phages as a therapeutic, and it had a facility in Brooklyn to grow bacteriophages for controlling infection. But right around the same time, antibiotics were discovered, and we dropped bacteriophages as a means for controlling infection here in the U.S. We went with the antibiotic approach.

And the Soviet researchers went the other route?

That's right. A couple of institutes, including one in Tbilisi, Georgia, still have an active bacteriophage program. People who have infections, mostly diabetic foot ulcers, not cured by ordinary antibiotics can go there and be treated with a cocktail of bacteriophages. It works, but it's really a boutique-type treatment. Unlike antibiotics, which can kill lots of different organisms, bacteriophages are unique in that they kill only specific bacteria. Basically, when you go to Tbilisi, they'll culture the bacteria in your foot, they'll develop a cocktail of phages that will target those bacteria, and you will be treated there for several weeks. In the U.S., Randall Wolcott of the Southwest Regional Wound Care Center in Lubbock, Tex., has also been using bacteriophages to treat resistant bacteria in wound infections.

Is the rise of antibiotic resistance contributing to renewed interest in phage-related therapies?

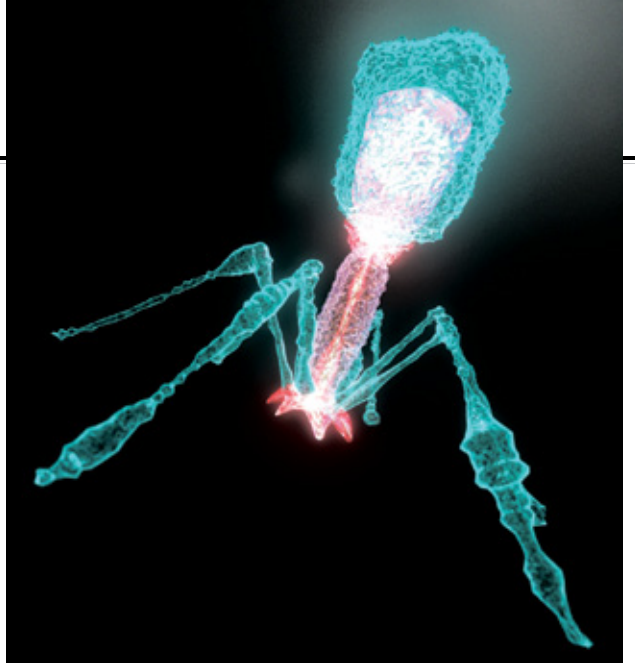
Yes. Antibiotic resistance is a very serious problem that presents two issues. First, bacteria are now becoming resistant to multiple current new-generation antibiotics. The biggest problem right now is methicillin-resistant *Staphylococcus aureus,* or MRSA, and vancomycin-resistant staph bacteria are emerging. It's already a problem for patients undergoing surgery who have compromised immune systems. But it won't be long before you and I could go into the hospital for a minor procedure, get infected by one of these organisms and become seriously ill. There's not much that can be used to treat you, and this type of infection is becoming more prevalent not only in the hospitals but also in the community.

The second issue is large drug companies are no longer in the antibiotics business. It's too expensive for them to develop an antibiotic for which the organism will become resistant very rapidly. This is disturbing because they are the best equipped to develop antibiotics, and I think it's their duty to continue.

What are the obstacles to phage therapy?

First off, the successes in Russia [and the former Soviet Union] have not been well documented. Where two individuals have a similar type of wound, the wounds are not necessarily treated with the same exact phage. So it's difficult to document a success in a true, scientific way.

Another problem is you need to use a cocktail of phages to kill a single organism. Complex mixtures may have trouble receiving FDA approval. Bacteriophages also pick up DNA from bacteria, so the FDA will want to know what DNA they are picking up. Phage therapy companies are trying, and that's not to say it'll never



PHAGE is a virus that infects bacteria. It has a capsid, or head (*top*), tail (*pink*) and tail fibers (*bottom blue appendages*).

PHOTO RESEARCHERS, INC.

be achieved, but they really have an uphill battle to try to get phage therapy approved for human use.

Are there any other ways to take advantage of phages in medicine?

We've developed one way, which is to use phage lytic enzymes. When phages enter a bacterium, they take over the cell to produce new virus particles. At the end of the cycle, the bacteriophages have to get out of the bacterium. They do this by producing a lytic enzyme that degrades the bacterial cell wall, causing the bacteria to explode. We've purified that enzyme, and we add it back to our bacterial cells. It will drill a hole in the cell wall, causing the bacteria to die virtually instantly. In humans, lysins can be applied directly on the skin or mucous membranes or injected into the blood. Because they are quickly cleared from the body and cannot break down human tissue, we anticipate that they will be safe.

How did you realize that these enzymes could be used therapeutically?

I purified one of these lysins for my Ph.D. thesis about 40 years ago. At that time, I used this enzyme to degrade the cell walls of *Streptococcus* bacteria to study surface proteins, but my real medical breakthrough came around 10 years ago. I had mice with group A strep throat. When I delivered the lysin in the throats of these mice, I found it killed the strep quickly. Then I realized that these enzymes could be used in a therapeutic way. It was an aha! moment. This was the first time anyone had ever used a lysin in an animal model and showed a therapeutic effect.

Since then, we have used lysins in lab animals to treat endocarditis, an infection of the heart valves, and we have used them to study meningitis, an infection of the brain. We also have used lysins to treat pneumonia, group B streptococcal infection and bacteremia, a blood infection. These enzymes are very stable and can be frozen or dried for many years and still retain their activity.

That's impressive. Did other scientists see that same therapeutic potential?

It was tough. People said, "That's interesting but—" The pharmaceutical industry was worried our immune systems would make antibodies to these lysins and neutralize them. Also, it was concerned we had enzymes that were very specific: the strep enzyme killed only strep, the pneumococcal enzyme killed only *Pneumococcus* and the anthrax enzyme killed only anthrax. People said, "You know, these are too targeted. We need broadly active enzymes."

We now have enzymes that have fairly broad activity, but broad activity is not the way to go, because you kill too many good bacteria. When you kill good organisms, you run into other problems. You're better off only killing the organisms that you want to kill without collateral damage and killing the organisms that are necessary for health and well-being. I think that everything is starting to turn in that direction: to try to kill only what you want to kill without destroying everything that you have in your body.

And these lysins can be used in other ways to protect human health?

That's right. We developed an enzyme that kills anthrax. It took 10 years for the government to realize that if there's an anthrax terrorist event, where anthrax spores are spilled in a city, it will take decades to safely remove all those spores from that environment. And to do so, you need to use corrosive materials. What we've been able to do in the lab is take the anthrax lysin and combine it with a natural chemical that tricks anthrax to germinate. Within 20 minutes, you can kill 99.99 percent of the spores. It's all-aqueous, it's all very safe, and so it could be used to decontaminate wide areas of contaminated surfaces of spores.

You can imagine this could be used for killing bacteria in agriculture or for controlling MRSA in hospitals by swabbing patients before and after surgery. Bacteriophages are also being used to kill bacteria on packaged meats.

Couldn't bacteria develop resistance to lysins?

So far we haven't found any resistant bacteria to these enzymes. I think it's really based on the way these enzymes have evolved over billions of years to stick to parts of the bacteria that the bacteria can't change. Never say never, but it would be a very rare event for resistance to develop.

The first ContraFect clinical trial against MRSA begins this year using a lysin you discovered, CF-301. Is that the first human trial with lysins?

Exactly. That will be the first time lysins will be used in humans.

I guess you're pretty excited about that?

Very excited. It took 10 years of hard work to get to this point. SA

Brendan Borrell, *based in New York City, writes frequently for* Scientific American *and* Nature.

MORE TO EXPLORE

View animations depicting how bacteriophages work: http://tinyurl.com/btxzr2f

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Read more about Fischetti's research at ScientificAmerican.com/aug2012/phage