



chest wall decompression phase, resulting in decreased coronary perfusion pressures and lower survival rates.

The American Heart Association recommends 12 to 15 breaths per minute in patients with secured airways during the performance of CPR by professional rescuers. Aufderheide, in an analysis of 13 consecutive adults receiving CPR in Milwaukee, found the average ventilation rate was about 30 breaths per minute, with positive pressure in the lungs found 47% of the time.

“Our work says further study is warranted to determine how widespread this phenomenon is,” Aufderheide said. “The study demonstrates there is a way health care providers perform CPR in a classroom and another way at a scene

of cardiac arrest. Ventilation rate is not the only issue—duration and allowing the chest wall to completely recoil is also important.”

Paul Pepe, MD, professor and chair of the University of Texas Southwestern Medical Center in Dallas, said slowing the frequency of ventilation given by rescuers goes against human nature. But overzealous efforts to provide patients with oxygen are often harmful, and, in some cases, probably fatal.

In an animal-model study of 8 pigs moderately hemorrhaged to systolic blood pressure of less than 65 mmHg, Pepe and colleagues found that with respiratory rates lowered from 12 to 6 breaths per minute, the average systolic blood pressure rose to 84 mm Hg

while the animals maintained adequate oxygenation and acid-base status. In contrast, with respiratory rates elevated to 20 and 30 breaths per minute, blood pressure, oxygenation, and acid-base status all decreased.

Based on these findings, the researchers argue that current resuscitative protocols involving assisted positive pressure ventilation should be reexamined as a potential exacerbating factor in compromised hemodynamics and as an underrecognized confounding factor thwarting resuscitation efforts.

“Educators were well intentioned to tell paramedics to put more oxygen in patients—it’s a reasonable conjecture,” Pepe said. “But it’s not clear that was based on scientific grounds.” □

Set a Microbe to Kill a Microbe Drug Resistance Renews Interest in Phage Therapy

Paul D. Thacker

AS GARY SCHOOLNIK, MD, WAS growing up during the late 1940s in Seattle, he had his first memorable encounter with a virus.

“My mother had typhoid fever and was sick in the hospital for about a month,” said Schoolnik, of Stanford University Medical School in Stanford, Calif. Antibiotics such as chloramphenicol were not available at the time, but his surgeon father recalled reading an interesting study in the *Journal of Bacteriology*. A group of physicians in the Los Angeles area were using bacteriophage (abbreviated as phage), a virus that infects bacteria, to treat typhoid.

“My father requested the phage and administered it to my mother,” said Schoolnik. “She became afebrile in about 48 hours and was cured.”

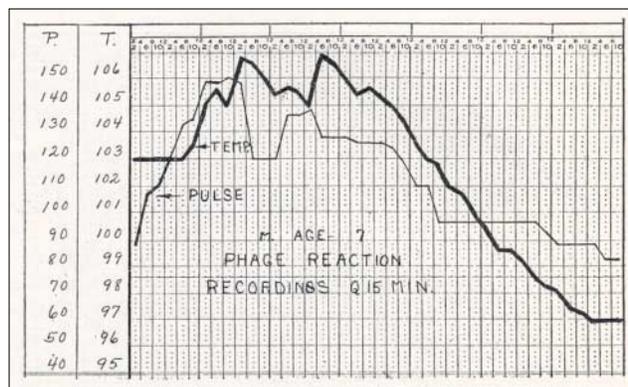
The concept is fairly simple: a bacterial virus targets specific bacteria, usually a specific bacterial strain, ignoring other bacteria, as well as nonbacterial cells. Some are “lytic” phages that in-

fect a bacterium and immediately begin replicating, lysing the cell a short time later. Others are “temperate” phages that often integrate their genetic material into that of the host bacterium and are copied (along with the bacterial genes) when the bacterium divides; later, the viruses reemerge, replicating and, ultimately, lysing the host cell.

In some cases, when a temperate phage extricates itself from the bacterial DNA, it carries with it host genes; when it reinfects another bacterium, it may insert those acquired genes into the

genetic material of its new host. In some cases the purloined genes encode a virulence factor (such as a toxin) that can transform a benign bacterium into a pathogenic one. For example, phage ferry genetic instructions into cholera bacilli that encode the toxin that causes the disease.

Phage therapy predated antibiotics by decades, but was largely supplanted when these drugs became available. Now, however, the emerging threat posed by antibiotic-resistant pathogens is spurring a resurgence of inter-



In the 1940s, physicians at Los Angeles County General Hospital used bacteriophage therapy for patients with cholera. This chart shows pulse and temperature readings from a 7-year-old boy treated with phage. (*JAMA*. 1946;132:134-138)



A. Sulakvelidze, PhD



Pioneers in bacteriophage therapy, Félix d'Herelle (seated) and Giorgi Eliava (right), at a bacteriophage research center founded by the latter in 1923 in Tbilisi, Georgia (then a part of the Soviet Union). The Eliava Institute remains in operation today.

est in phage, as a potential therapy to cure or prevent infections and as a tool to kill food-borne pathogens.

HISTORY OF A BACTERIA EATER

Perhaps the earliest reference to bacteriophage dates back to 1896, when British chemist E. H. Hankin noticed that water from the Ganges and Jumna rivers could kill cholera bacteria (*Ann Inst Pasteur*. 1896;10:511). But it was not until two decades later that two scientists working independently, Frederick W. Twort, a British bacteriologist, and Félix d'Herelle, a self-taught biologist working at the Pasteur Institute in Paris, concluded that some mysterious entity was capable of killing bacteria.

d'Herelle went on to become the first champion of phage, traveling around the world to isolate phage from the feces of individuals recovering from illnesses. Although he sensed phage's promise, his understanding of phage biology was limited.

"You have to understand the time period that we're talking about," said Ry Young, PhD, a phage researcher at Texas A&M University, in College Station. "He thought the body was producing these things and that it was a major immune response."

d'Herelle later helped establish the Eliava Institute in Tbilisi, Georgia (then part of the Soviet Union), which retains a flourishing practice in phage therapy. Scientists in other parts of the world also experimented with phage therapy. In India, for example, a Brit-

ish medical officer used phage during the 1930s as prophylaxis against cholera epidemics. But certain villages refused to cooperate with British colonials, becoming unwitting controls in what Young calls the first large-scale phage "trial." Few died in villages that accepted phage, but areas that refused treatment recorded hundreds of deaths due to cholera.

Australian physicians studied phage therapy, as did their counterparts in the United States. The likely source of the phage that saved Schoolnik's mother was Los Angeles County General Hospital, which successfully treated typhoid with phage for well over a decade (*JAMA*. 1946;132:134-138). Pharmaceutical companies also embraced phage therapy. Eli Lilly & Co sold "Staphylo Jel;" E.R. Squibb & Sons, Abbott Laboratories, and others marketed other phage products.

But most studies of phage therapy were anecdotal or qualitative. In 1934, the *Journal of the American Medical Association* published a report stating that phage therapy was unreliable (*JAMA*. 1934;132:1769-1939). Enthusiasm waned, and with a few exceptions, phage therapy was largely abandoned with the introduction of antibiotics after World War II.

NOT READY FOR PRIME TIME

"The problem with phage [therapy] is that it was a premature technology," said Young, who is also executive director of GangaGen, a fledgling biotechnology

company based in San Francisco and Bangalore, India. "It wasn't ready for prime time back in the '20s, and this led to a lot of negative history that has nothing to do with phage itself."

Early practitioners' lack of knowledge caused many mishaps, said Carl R. Merrill, MD, chief of the biochemical genetics section at the National Institute of Mental Health in Bethesda, Md.

"They often didn't check to make sure the phage attacked the specific pathogen associated with the infection that they were trying to treat," he said. Inadequate purification techniques failed to eliminate toxins or cellular debris from the phage cocktail. Some researchers even failed to realize that if the material was not refrigerated, phage activity would be lost.

Now that scientists have a much more sophisticated understanding of microbial genetics, culture techniques, and the like, some believe it may be time to take another look at phage therapy's potential.

"Given the increasing antibiotic resistance, I think we have to explore other options," said biologist Bruce Levin, PhD, of Emory University, in Atlanta, who has found that phage can eradicate bacterial infection in mice. "But I wouldn't bet on phage anytime soon," cautioned Levin, who studies the pharmacokinetic properties of phage.

One vocal proponent of phage in medicine is Elizabeth Kutter, PhD, professor of biophysics at Evergreen State College, Olympia, Wash. While studying phage genomics in the early '90s, she spent a sabbatical at the Eliava Institute.

"At first I was extremely skeptical [about phage therapy], until I saw phage being used and treated as nothing special." Kutter said that phage therapy was once used widely by the Soviet Army, and that it remains a highly useful treatment for certain wounds, such as diabetic ulcers, in which poor circulation hinders the use antibiotics.

Researchers hoping to move phage studies in the clinical realm face the increased regulatory scrutiny by the Food and Drug Administration (FDA) that



accompanies any testing of a biological therapy in humans, notes Sankar Adhya, PhD, chief of the developmental genetics section of the National Cancer Institute's Laboratory of Molecular Cell Biology in Bethesda. Because of this, some companies studying phage therapy are opting to focus on other applications.

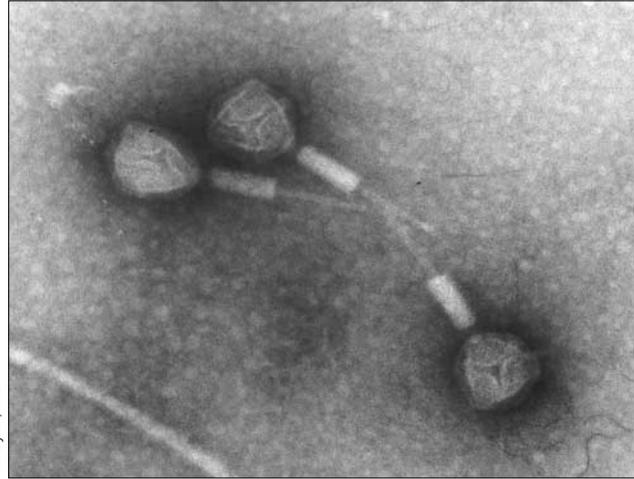
The FDA regulations could kill any company that tries to bring phage to market, said Alexander Sulakvelidze, PhD, a researcher at the University of Maryland School of Medicine and a founder of Intralytix, Inc, a biotechnology company, both in Baltimore. "We realized that it would be hard to survive as a company and get through the FDA," said Sulakvelidze. "So we . . . focused on agribusiness."

Working with US Department of Agriculture researcher Britta Leverentz, PhD, Intralytix scientists demonstrated that phage can successfully clean fresh-cut fruits and vegetables of *Listeria* and *Salmonella* (*J Food Prot.* 2001; 64:1116-1121 and *Appl Environ Microbiol.* 2003;69:4519-4526).

"Once you remove the peel, you make a lot more nutrients available to pathogens," said Leverentz. Unlike chemical treatment or heating, phage treatment does not harm the fruit; it also targets specific pathogens rather than wiping out the whole microflora and giving any subsequently introduced bacteria an open field on which to grow.

Last summer, the Environmental Protection Agency granted Intralytix permission to test phage on nonfood surfaces. Other laboratories are exploring the use of phage in food processing to kill *Salmonella* and *Campylobacter* on chicken and *Escherichia coli* O157:H7 in beef. And some researchers are studying the use of phage to improve food safety by ridding food animals such as oysters and beef cattle of bacteria that are pathogenic to humans.

Researchers at Rockefeller University, New York, led by Vincent Fischetti, PhD, are exploring a different approach to phage therapy: treating bacterial infections not with live phage but with the phage enzymes that lyse bacteria. The enzymes, like the phage



Bacteriophage, viruses that target specific bacteria, are attracting renewed interest as a potential therapy against antibiotic-resistant bacteria. This electron micrograph shows bacteriophage, isolated from Baltimore's Inner Harbor, that kill *Salmonella* bacteria.

from which they are derived, target specific bacteria. After isolating the phage enzymes, Fischetti and colleagues sequence the genes that encode these proteins; recombinant technology is then used to make large quantities of the enzymes, which can be administered via a nasal spray.

Certain pathogens such as *Staphylococcus aureus* colonize human skin and membranes, and can be a particular problem among hospitalized patients with catheters or open wounds that facilitate entry of bacteria into the bloodstream. Fischetti suggests such pathogens could be removed from patients using phage as prophylactic "smart bombs" designed for pathogens that pose this kind of threat.

"We usually wait for an infection and then treat it," Fischetti said. "But now we can remove the exact organism you want before it becomes a problem."

Merril is also developing a prototype diagnostic test of the sort that would be needed if phage therapy reaches the clinic. Because a given phage only works against certain bacteria, a physician would need to be able quickly determine which phage to prescribe. A patient specimen containing the pathogenic bacteria would be incubated in a multiwell plate, each well seeded with different types of phage; if the patient is infected with a bacterium that is the host for one of the phages in the test, a "reporter gene" in the phage produces a color

change as the phage infect the bacteria and reproduce.

REGULATORY HURDLES

Many phage researchers say that bureaucratic and regulatory hurdles present a considerable challenge to moving phage-related advances into the clinic. It is not even clear, for example, which section of the FDA will supervise phage-related products. Different offices of the FDA might regulate different aspects of phage, depending on whether it is used as a topical agent, given intravenously, or sprayed on vegetables. For the moment, the FDA doesn't appear to have a clear plan for evaluating new phage-based products.

But Merrill says if the need arises for the agency to form a group to specifically oversee this area, they might call on scientists in the academic community who've had years of experience using phage in basic molecular and microbiological research for guidance.

"People have to start paying attention because there's so much antibiotic resistance," Merrill said. Many phage researchers have founded or joined startup biotechnology companies, hoping to capitalize on the research when the regulatory tangle is unsnarled.

But for Schoolnik, proof of phage therapy's value came 60 years ago when his mother was saved by phage. "I think it's important to understand that this can work," he said. "There are proofs of principle that cannot be ignored." □