

Biological Warfare

Scientists once again advocate pitting viruses against bacterial infections

By JOHN TRAVIS

When dysentery struck a cavalry squadron resting in Paris in 1915, Felix d'Hérelle, a young bacteriologist at the city's Pasteur Institute, noticed something remarkable: The bloody stool samples of a few of the soldiers contained microscopic agents that could destroy the dysentery bacteria.

In a 1917 report on his finding, d'Hérelle labeled the unseen killers as bacteriophages, or eaters of bacteria, and concluded that they were viruses that infect bacteria. This brash verdict, though ultimately proved correct, ignited fierce debate.

D'Hérelle wasn't the first scientist to describe bacteriophage activity publicly—English physician Frederick W. Twort published such a report in 1915. But d'Hérelle sparked great interest in bacteriophages, or phages, by confidently proclaiming that the viruses could cure dread bacterial diseases sweeping the globe. "He traveled all around the world trying to treat bubonic plague and cholera," says Carl R. Merrill of the National Institute of Mental Health Neuroscience Research Center at St. Elizabeths in Washington, D.C.

D'Hérelle even inspired the Sinclair Lewis classic *Arrowsmith*. In the 1925 novel, Martin Arrowsmith is an idealistic young physician who travels to the West Indies to treat bubonic plague with phage therapy. *Arrowsmith*, which challenged the motives of medical researchers, won a Pulitzer prize, and its author became the first person in the United States honored with the Nobel prize in literature.

Unfortunately, the novel's success was greater than that of phage therapy itself. "It was frustrating. . . . The problem was it worked some times and didn't work other times," says Merrill.

Largely because of its inconsistent nature, phage therapy never caught on in the United States. Then in the 1940s, physicians embraced penicillin and other newly discovered antibacterial drugs.

"Antibiotics took over, and phage therapy got dropped like a stone," says Richard M. Carlton of Exponential Biotherapies, a biotech firm in New York.

Phage therapy is still practiced today, but only by a few physicians in Europe and the former Soviet Union, and then usually as a last resort, notes d'Hérelle biographer William C. Summers of Yale University School of Medicine.

Some scientists now argue that d'Hérelle's dream deserves another look, especially as drug-resistant bacteria pose an ever-increasing threat (SN: 2/6/93, p. 90). A few deadly strains of bacteria today are susceptible to only one antibiotic, vancomycin, and investigators don't expect it to remain effective for very long, Merrill notes.

In Merrill's opinion, modern researchers now have the knowledge and tools to avoid the pitfalls of past phage therapy.

"This is just too good an agent to abandon. Phage is no longer the black box it was to d'Hérelle. If it doesn't work in some cases, we can now understand the molecular basis of why it fails. We should be able to engineer and manipulate phage to make it into a highly effective antibacterial agent," he says.

The destructive ability of a phage stems from the way it reproduces. After injecting its genetic material into a bacterium, the virus usually commands the internal machinery of the microbe to mass-produce copies of itself. The new phages flood the interior of the bacterium and eventually lyse, or burst, its cell wall, spreading to other bacteria and repeating the cycle.

Phage is the only antibacterial agent—actually, the only drug of any kind—that makes more of itself as it works, observes Carlton. As a result, a single dose of phage should, theoretically, be all that is required to defeat an infection. Under the right conditions, a single phage produces more than a million copies of itself in a day. (This exponential growth inspired the name of the phage therapy company Carlton now heads.)

So what prevented the success of phage therapy? Modern investigators cite a number of obstacles. D'Hérelle and other scientists of his era didn't have the technology to purify phages completely, notes Merrill. Even recent investigators may not have taken enough care to purify their phages, he contends.

Researchers generate phages by growing them inside bacteria and then letting them burst out, explains Merrill. That process creates bacterial debris, which often clings to a phage, impairing its ability to infect. The debris can also include endotoxins, poisonous bacterial substances that cause illness.

"You can kill people with those alone," says Merrill, who notes that with modern equipment, investigators can purify phages easily. Merrill and his colleagues, for example, place their lab-grown phages in tubes containing a salt solution. Then they spin the tubes in a high-speed centrifuge. The bacterial debris and the phages have different densities, so they end up at different levels in the solution, which enables researchers to pluck out the phages.

Early proponents of phage therapy may also have thought that all bacterial infections are equally vulnerable to phages. That's not necessarily true. Because they hide away inside cells, some bacteria may escape encounters with phages, notes Merrill. Other bacteria, he adds, infect parts of the body that would be difficult for bloodborne phages to reach.

Another historical stumbling block for phage therapy, says Merrill, has been the speed with which a treated person or animal gets rid of most administered phages. This swift clearance—within hours—often gives phages too little time to infect bacteria.

More than 20 years ago, Merrill and his colleagues found an explanation for this expeditious removal of phage. A consensus had emerged that preexisting antibodies attach themselves to phages and facilitate clearance, recalls Merrill. His group, however, demonstrated that mice without such antibodies clear phages just as speedily. Furthermore, they established that the elimination of phages actually stems from the reticuloendothelial system, a cell-mediated defense system found largely in the spleen and liver.

Merril didn't follow up on this line of investigation until a few years ago, when he read about the rise of drug-resistant bacteria. He began to wonder if there might be a way to isolate phages that could avoid the reticuloendothelial system.

An idea popped into Merrill's head. What if researchers injected billions of phages into mice, waited a day or so, then isolated any phages left in the rodents' blood? Investigators could allow the few remaining viruses to reproduce in the lab, then inject them into mice in order to repeat the selection strategy. After several repetitions of this procedure, it should be possible to cull a population of phages ignored by the reticuloendothelial system.

Merril's idea has now borne fruit. When the investigators injected more than 100 billion unselected phages into a mouse, fewer than 100 phages were found in a blood sample taken 2 days later—a drop of nine orders of magnitude. After just one selection cycle, phage concentrations generally dropped by only four orders of magnitude within 18 hours of injection. By the end of 10 selection cycles, there emerged a population of phages whose concentrations rarely dropped by more than one order of magnitude in 18 hours, reported Merrill and his colleagues in the April 16 *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (PNAS)*. "We have clearly solved the clearance problem," says Carlton.

The researchers also believe they can explain the evasiveness of their bacteriophages. While

examining one long-circulating phage, Merrill's group found that a protein on the outer shell of the virus differed by a single amino acid from its counterpart on normal phages. A second long-circulating phage, isolated from another selection series, shared the same mutation.

The viral coat mutation substitutes lysine for glutamic acid, says Merrill. He and his colleagues are still studying how this change alters the ability of the reticuloendothelial system to capture the mutant phage.

Nonetheless, the investigators have already obtained evidence that long-circulating phages may offer a more effective therapeutic option than normal phages. When they injected mice with lethal quantities of the *Escherichia coli* bacterium, treatment with normal *E. coli*-specific phages did save the lives of the rodents, but the animals got quite sick before recovering. In contrast, mice treated with long-circulating strains of the *E. coli* phage survived and had few side effects from the phage therapy. "They were just slowed down a bit, as if they were under the weather," says Carlton.

As promising as Merrill believes phage therapy is, he stresses that the new strategy for selecting long-circulating phages is just a small step toward a comeback. If investigators do try to revive d'Hérelle's dream, as Merrill hopes they will, they should be mindful that the phages they choose for therapy might do more harm than good. Diphtheria, notes Merrill, actually stems from a phage gene utilized by the diphtheria bacterium. The gene produces an enzyme that is toxic to humans.

"We know that phages can carry genes that cause disease themselves. With modern techniques, we can cut those genes out," says Merrill.

The message of Merrill and his colleagues—that phage therapy should be systematically reexamined in light of science's vastly improved understanding of phages and bacteria—has so far received a warm reception.

"I read with great enthusiasm this paper. I believe it will induce, maybe a little late, new interest in this area," comments David Schraye of Brown University in Providence, R.I. Schraye, who immigrated to the United States from the former Soviet Union, once worked on phage therapy at a Russian bacteriophage institute that d'Hérelle had helped establish.

The PNAS report was also accompanied by an encouraging commentary from Nobel laureate Joshua Lederberg, an infectious disease specialist at Rockefeller University in New York. "The paper is important as a reopening of an interesting idea," Lederberg told SCIENCE NEWS.

He cautions, however, that far too many questions remain for bacteriophage therapy to be seen today as a viable alternative to antibiotics. Even if the new selection strategy identifies phages that sneak by the reticuloendothelial system, will an eventual antibody response eliminate those long-circulating phages? "That's a legitimate concern," admits Carlton.

In addition, bacteria often mutate to evade the attacks of phages, notes Lederberg. Merrill acknowledges the problem but points out that phages are also continually evolving new tricks in their campaign against bacteria.

"It's a biological warfare that's probably been going on since life evolved on Earth. And there's no reason we shouldn't use it to our benefit. It will work. It's just a matter of investing the time and money to build up the armament of phages to do what we need to do," contends Merrill.

The next morning, on opening the incubator, I experienced one of those rare moments of intense emotion which reward the research worker for all his pains: at the first glance I saw that the broth culture, which the night before had been very turbid, was perfectly clear: all the bacteria had vanished, they had dissolved away like sugar in water. As for the agar spread, it was devoid of all growth, and what caused my emotion was that in a flash I understood: what

caused my clear spots was in fact an invisible microbe, a filtrable virus, but a virus parasitic on bacteria.

Another thought came to me also: "If this is true, the same thing has probably occurred during the night in the sick man, who was in serious condition. In his intestine, as in my test tube, the dysentery bacilli will have dissolved away under the action of their parasite. He should now be cured."

I dashed to the hospital. In fact, during the night, his general condition had greatly improved and convalescence was beginning.

—Felix d'Hérelle, recalling his
discovery of bacteriophages

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