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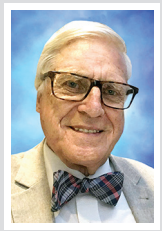

CRITICAL FOCUS

Brian J. Ford

Germ Versus Germ

Bacteriophages — viruses that destroy bacteria — were introduced 100 years ago and used in communist countries, yet the West has not realized their potential to cure infections.

It's July 2022, and there's much excitement in the newsroom at CNN. An astonishing new miracle cure is being announced. Steffanie Strathdee was gently cradling the hand of her husband Tom Patterson in the hospital. He was close to death from a bacterial infection contracted on a cruise to Egypt. Antibiotics had proved useless, when she dramatically decided to try something revolutionary. She contacted Texas A&M University, whom she knew had been working on the treatment of recalcitrant infections using viruses. The viruses that attack and destroy bacteria are bacteriophages — phages in daily parlance, pronounced to rhyme with "Taj" in Taj Mahal. (It's from the Greek *φάγος* [fagos], to eat; but it is a term coined in French, so that's how it's customarily pronounced. Saying it to rhyme with "page" is an unwanted Anglicism.)

Tom Patterson's life was saved only when his wife, Steffanie Strathdee, intervened by proposing that bacteriophage therapy should be used to treat his antibiotic-resistant bacterial infection. Her contacts at Texas Agricultural and Mechanical University were able to provide the right phage virus and, after three weeks, Patterson had been cured.

It was with phage viruses that the Texas team proposed treatment, instead of drugs. Within three weeks her husband was cured. A devoted wife had saved her husband's life. "What she accomplished," said the CNN reporter, "could easily be called miraculous." The news went global, and the story was on the front page of newspapers overseas.

Medical News Today was soon reporting that scientists at Yale had "unexpectedly found a potential weapon against bacteria" in viruses. The U.S. Department of Veterans Affairs announced: "Viruses that target bacteria are showing promise as treatment for some intestinal diseases, according to a review article authored by VA researchers." *EurekAlert!* told their readers: "For the first time, researchers have successfully used bacteriophages — viruses that kill bacteria — to treat an antibiotic-resistant mycobacterial lung infection." This could be the answer we sought, in an era when antibiotic resistance was sweeping

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across the world (see Crisis Point: the Rise and Fall of Penicillin, *The Microscope*, 62:3, pp 123–135, 2014).

This "new discovery" was actually made over 100 years ago and had been used in communist countries since World War I. And, in their eagerness to point up the role of the devoted housewife in healing her helpless husband, the media skated round the fact that Strathdee is Associate Dean of Global Health Sciences, Harold Simon Distinguished Professor in the Department of Medicine at the University of California San Diego School of Medicine, and an Adjunct Professor at both Johns Hopkins and Simon Fraser Universities. Professor Strathdee is a leading medical scholar, who was recognizing the extraordinary discoveries pioneered in 1917 by an unqualified amateur enthusiast named Félix Hubert d'Hérelle. He is the person who discovered what phages could do and who meticulously documented the miracles they could work. This pioneering microbiologist was no university dropout — he never even dropped in. The meticulous research carried out by d'Hérelle laid out one of the most exciting developments of the modern medical world, yet was done as a hobby. He stopped his education after high school and never went to university, yet his output of innovations would put many modern researchers to shame — and he went on to be nominated for a Nobel Prize on several occasions.

We know that in 1884 Robert Koch proved cholera was caused by drinking water contaminated with bacteria. But the Indians knew something far more remarkable: contaminated water could cure cholera. This remarkable revelation intrigued Koch's co-worker, a distinguished English bacteriologist named Ernest Hanbury Hankin. He knew that muddy, contaminated water from the Ganges or the Jumna River had therapeutic properties. He couldn't explain it, and nor could Koch. Hankin even found that you could not culture the cholera bacteria in natural river water. The bacteria were present right enough, but instead of growing in culture, their cells broke down and disappeared. However, if the cholera germs were inoculated into river water that had previously been boiled, they grew fine. There was some anti-bacterial agent in the river that could kill cholera bacteria, but which heat would destroy.

Hankin's observations were published in 1896, as *L'action bactericide des eaux de la Jumna et du Gange sur le vibrion du cholera*, by the Institut Pasteur in Paris. It was a remarkable result, and news of Hankin's discovery spread far and wide. It was even mentioned in Mark Twain's travelogue *More Tramps Abroad* published in 1897. He wrote:

Arrogant, inflexible, and opinionated, Félix Hubert d'Hérelle was untrained in microbiology since leaving high school (contrary to what many sources claim), yet his meticulous and exhaustive research gave us a new way to treat bacterial infections with bacteriophages. He was a professor at Yale University and had been nominated for a Nobel Prize several times.

> "Mr Hankin, the scientist in the employ of the Government at Agra concluded to examine the water. He went to Benares and made his tests. He got water at the mouths of the sewers where they empty into the river at the bathing ghats; a cubic centimeter of it contained millions of cholera germs; at the end they were *all dead* … he added swarm after swarm of cholera germs to this water; within six hours *they always died*, to the last sample."

This is the first popular description of a bacteriophage in literature. Few people remembered it since.

But this was not the first time the action of viruses had been observed. Step back to 1879, when Adolf Mayer, an agricultural chemist, was studying tobacco mosaic virus (TMV) in Wageningen, Netherlands. Dutch tobacco farmers were reporting a serious outbreak of an infection that manifested itself as pale discolored patches on the leaves. Mayer could not find a bacterium responsible. The problem then emerged in Ukraine when TMV was studied by Dmitri Iosifovich Ivanovsky. He also found that no bacteria could be cultured from the infected plants. Ivanovsky showed that, if you passed infective sap through a filter that held back bacteria, the clear sap retained its infective principle. He set down his findings in a paper for the Imperial Academy of Sciences of Saint Petersburg, published in 1892. The community of microbiologists were convinced he had made a mistake, and Ivanovsky felt he could go no further, so he abandoned his quest and turned to studying yeast. At least this gave him an organism to observe.

The next major development came in 1898. Friedrich August Johannes Löffler was a German microbi-

ologist, who we know from Loeffler stain for bacteria, and his egg medium for culturing tubercle. Löffler had been a student of Koch and later, researching foot and mouth disease with Paul Frosch, he also found that infected liquids passed through a fine clay filter — with pores fine enough to hold back all bacteria — and remained infectious.

Adolf Mayer's methodical young colleague Martinus Beijerinck, meanwhile, recognized several important principles. First, that these infections were "not caused by microbes, but by a *contagium vivum fluidum*." Secondly, he realized: "Only those organs of the plant that are growing and whose cells are dividing are capable of being infected." This was a key point; viruses rely on their host cell to replicate more virus. They cannot themselves reproduce. His third conclusion was that the infection could travel through fluid in the host plant, and that the liquid containing it could be dried, yet remain infective. Unlike the other workers, who were either baffled or convinced the pathogen was an ultra-small bacterium, in 1898 (the same year that Löffler and Frosch were publishing their investigations of foot and mouth disease), Beijerinck wrote: "Drops of the extracted juice of diseased leaves … were put on the surface of thick agar plates and left to diffuse with water for several days. I hoped in this way to separate the virus from the raw leaf substance, as well as from all bacteria." Much of this is misrepresented by the standard sources. For example, it is widely claimed that Thomas Milton Rivers announced to the Society of American Bacteriology for the first time in 1926 that: "Viruses appear to be obligate parasites in the sense that their reproduction is dependent on living cells," even though Beijerinck had published this precise conclusion almost two decades earlier.

As World War I broke out in 1914, two brothers, Frederick and Charles Twort, were pursuing a different approach. Beijerinck had methodically concluded that viruses must have living cells in which to replicate, but the Twort brothers were sure that (just as most bacteria are harmless) there must be large numbers of non-pathogenic viruses. They were also convinced that it must be possible to culture viruses in a petri dish, just like bacteria. Working at the now-defunct Brown Institution for Animal Research in London, they made repeated attempts to grow vaccinia virus on agar but found that there was never any sign of the virus, and they could only culture bacteria. But something odd was happening. Some of the bacterial colonies developed small circular zones where the organisms had disappeared. Frederick Twort had studied under the noted bacteriologist William Bulloch, whose step-

In Saint Petersburg, Russia, Dmitri Iosifovich Ivanovsky was the first microscopist to realize that there were invisible pathogens. He studied the tobacco mosaic virus and proved that it was capable of transmission by a clear filtrate that contained no bacteria. Some 60 years ago, the Soviets commemorated his work with this four-kopek postage stamp.

daughter Monica I later knew. She married Clifford Dobell, the Leeuwenhoek scholar, and reminisced with affection about her father ("Bully, everyone called him," she used to say).

The eager young Twort published an account of his thoughts in *The Lancet* in 1915: "The colonies often showed watery areas … if kept they became glassy and transparent." If samples of these glassy areas were plated out on agar, nothing would grow. Something had destroyed the bacteria, though Twort couldn't work out what was happening, and *The Lancet* paper listed some strange speculation: "In the first place, we do not know for certain the nature of an ultra-microscopic virus. It may be a minute bacterium that will only grow on living material, or it may be a tiny amoeba which, like ordinary amoebae, thrives on living microorganisms." Twort added: "It might be some contaminating non-pathogenic ultra-microscopic virus, for it is conceivable that whereas a non-pathogenic variety might grow on micrococci or bacilli, a pathogenic variety might grow only on the animal it infects." He came close, when he wrote that: "It might almost be considered as an acute infectious disease of micrococci," and added: "It seems probable though by no means certain, that the active transparent material is produced by the micrococcus." Twort repeatedly referred to a "dissolving material," but what was really occurring was that bacteriophage viruses were replicating in the bacterial colonies and destroying their hosts.

The coming of war meant that the Twort brothers were unable to continue their research. Charles had abandoned the project as the war began, and Frederick was dispatched to the Royal Army Medical Corps

After publishing on the curious clear plaques that had appeared in his bacterial cultures, Frederick Twort joined the Royal Army Medical Corps during World War I, and in 1915, he was posted to run a bacteriology laboratory in Thessaloniki, Greece. He frequently became embroiled in disputes with subordinates over the nature of dysenteric infections.

in Greece. He bemoaned his lack both of academic facilities and funding, so after the war, he returned to the cash-strapped Brown Institution and continued trying to culture "primitive viruses" on agar plates.

KEY TO A CURE?

Félix d'Hérelle was facing the same phenomenon in France. While the Tworts were trying to culture viruses, he was studying an outbreak of dysentery among soldiers at barracks in Maisons-Laffitte near Paris. He had cultured the *Shigella* bacteria that were responsible for the disease on agar plates and, just as the Tworts were observing in London, he saw that many of the colonies of the bacteria he cultured were developing glass-like, transparent areas where the bacteria had dissolved away. Whereas the Tworts had been plodding along, pursuing their project in the traditions of academia, the free-thinking d'Hérelle was at once diverted by what he saw. Something was killing bacteria? If this was happening on an agar plate, surely it could also happen in an infected patient. To the Tworts, this phenomenon had been a distraction. To d'Hérelle, it might offer the key to a cure.

Few have heard of d'Hérelle, though his discoveries and his body of work should make him as renowned as those other great names, like Robert Koch and Alexander Fleming, though — unlike them — d'Hérelle was a renegade. Throughout his life he claimed he was born in Montreal, Quebec. The standard reference sources, including his obituary in *Nature* and the entry in *Encyclopaedia Britannica* (*EB*) say so to this day, though his birth certificate shows he was born in France. He left school at 17, disillusioned by academia, and traveled extensively with money given by his mother, cycling throughout Europe and sailing to Central America. Later, his 15-year-old girlfriend in Turkey fell pregnant, so they married and he signed up for four years of French military service. It proved not to be to his liking, so he deserted a year later and they fled to Canada. In 1899, he set up a short-lived chocolate works with his brother Daniel, on land they acquired in Montreal, but he wanted to study microbiology and — since he found no scholars from whom he might learn — he set up his own laboratory at home.

Many sources pretend he followed an orthodox career (*Nature* states that he studied medicine, while *EB* goes so far as to grant him an M.D. from Montreal), but he remained entirely self-taught. He was given laboratory bench space at the Institut Pasteur, but was not paid. Being free from the strictures imposed by academia and the conformity of peer-review was clearly appealing, and d'Hérelle relished the freedom that his wealthy mother could guarantee. When there was a glut of maple syrup, he realized that it might be fermented to produce a form of whiskey that could be exported to the United States, and he obtained a Canadian government grant to investigate further. This was his first funded research. He also published his first scientific paper, curiously arguing that carbon was not an element, but a compound.

D'Hérelle then obtained a position as bacteriologist in Guatemala City, studied fungal infections of the coffee plantations, and experimented with producing schnapps from fermented bananas. A swarm of the American locust *Schistocerca americana* in Mexico in 1910 had him searching for ways to control the insects. He isolated *Coccobacillus acridiorum* as the causative organism of the infection and was able to culture the bacteria on agar. Inoculation in healthy locusts proved that the coccobacilli were contagious, and he recommended using sprays containing the bacteria to spread the infection, and so to infect the insects, and curtail the invasion.

His base continued to be in Paris, where Émile Roux was the director at Institut Pasteur. The young d'Hérelle was given the official title of laboratory assistant to a noted bacteriologist, an enthusiast for vaccines, Alessandro Taurelli Salimbeni. For much of the time, d'Hérelle was in charge of vaccine production for the war effort, and he once said that they had been responsible for supplying 20 million doses of vaccines

for the troops in World War I. He was able to pursue his own research only in his spare time, as he later wrote: "I carried out my investigations during rare moments of leisure, between two procedures, or while timing sterilization."

So d'Hérelle cultured *Shigella* from the bloody stool of a sick soldier and obtained a turbid broth culture heavily overgrown with the bacteria. He then added clear, filtered liquid with this curious antibacterial property and reported his astonishment next morning at seeing the cloudy culture liquid become crystal clear. The same preparation was administered to the soldier, who immediately began to recover. To d'Hérelle, this was a crucial discovery, and in 1917, he published his conclusions, announcing his discovery of a new invisible agent that could destroy bacteria. He decided to call it a bacteria-eater (*bacteriophage* in French). His announcement was formally communicated to the Académie des Sciences in Paris by Roux, one of Louis Pasteur's closest associates. D'Hérelle had no idea what he had discovered. He thought of it as a "protobe," some kind of ultramicroscopic infectious entity. What he needed next was to demonstrate how it could be routinely harnessed.

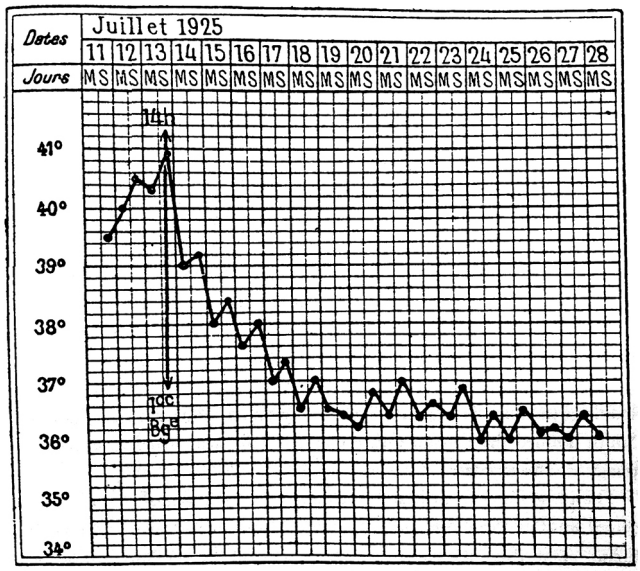
Even then, ethical considerations made it difficult to continue with human experiments, but d'Hérelle was presented with the perfect opportunity to test his theories when there was an outbreak of typhoid — in chickens. The infection was caused by *Salmonella gallinarum*, bacteria that had been imported with fowl from America. It had never been seen in Europe before. This offered a unique situation: a dangerous bacterial disease of animals caused by an organism found nowhere else in the country. Fowl typhoid was the perfect model — highly infectious, fatal, incurable, and previously unknown. To d'Hérelle this was the ideal animal model of the dysentery he had studied in humans. He repeatedly cultured stool samples from the infected chickens and found copious growths of the *S. gallinarum* bacteria.

Then, one single chicken survived. The stool sample gave a heavy growth of *Salmonella*, and d'Hérelle was excited to see that many of the creamy-colored colonies developed the clear patches he had hoped to see. At last, he had a preparation that was destroying the bacteria in culture. Would it cure the disease? Within a few days, all the other birds in the same hen house were surviving, and he found the mysterious phage in every sample he collected from the recovering birds. In 1920, there was an outbreak of cholera in Southeast Asia, and d'Hérelle sailed out to investigate. Just as with the hens, he found that — if a number of cultures were put up — you would observe an occasional tube in which the bacteria had all been destroyed. And the crystal-clear liquid that remained, containing his mysterious phage, could be administered to secure recovery from the infection. The next year, back in Paris, d'Hérelle was asked to investigate dysentery at the Hôpital des Enfants Malades, and he showed that, if the clear liquid containing his bacteriophage was administered, the patients recovered. This was unprecedented and miraculous.

A NEW SCIENCE EMERGES

D'Hérelle launched a series of meticulous experiments to see if his discovery could be routinely harnessed to cure infections. There were no techniques available to prosecute these investigations, so he had to devise his own. He even designed unique little glass flasks specifically for his phage cultures. He soon had a series of protocols and, as well as the preparation of phages that destroyed *Shigella dysenteriae*, he had collected phage solutions that were effective against bacterial meningitis, *Salmonella typhi*, *Escherichia coli*, staphylococci, and the plague. Patients were treated by being given liquid phage preparations to drink, ointments for the skin, and even phages administered by injection. Successful treatment of infections of the eye, the skin and bone were reported, a range of intestinal infections from cholera to typhoid were cured, and even septicemia was reportedly treated by phage therapy. Phages were even added to the water supply in areas where intestinal diseases were common, to help remove bacteria. In Belgium, Richard Bruynoghe and his student Joseph Maisin used bacteriophages to treat staphylococcal skin infections, which resolved within a few days. The report by Bruynoghe and Maisin became the first publication on phage therapy when it appeared in 1921.

D'Hérelle had opened the floodgates to a new science. He wrote up his findings under the title of *Le bactériophage; son rôle dans l'immunité* and presented it to Roux. He endorsed d'Hérelle's conclusions and agreed to sponsor its publication as a monograph of the Institut Pasteur. The book was published in 1922 with a print run of 1,500 copies. There had never been such a torrent of revolutionary discoveries in the fight to cure bacterial infection. Since Pasteur and Koch, new bacteria had been discovered, and many prophylactic vaccines had emerged — but nobody had found a cure. For the first time in history, it seemed we were on the brink of discovering how bacterial infections could be conquered, without harming the patient.



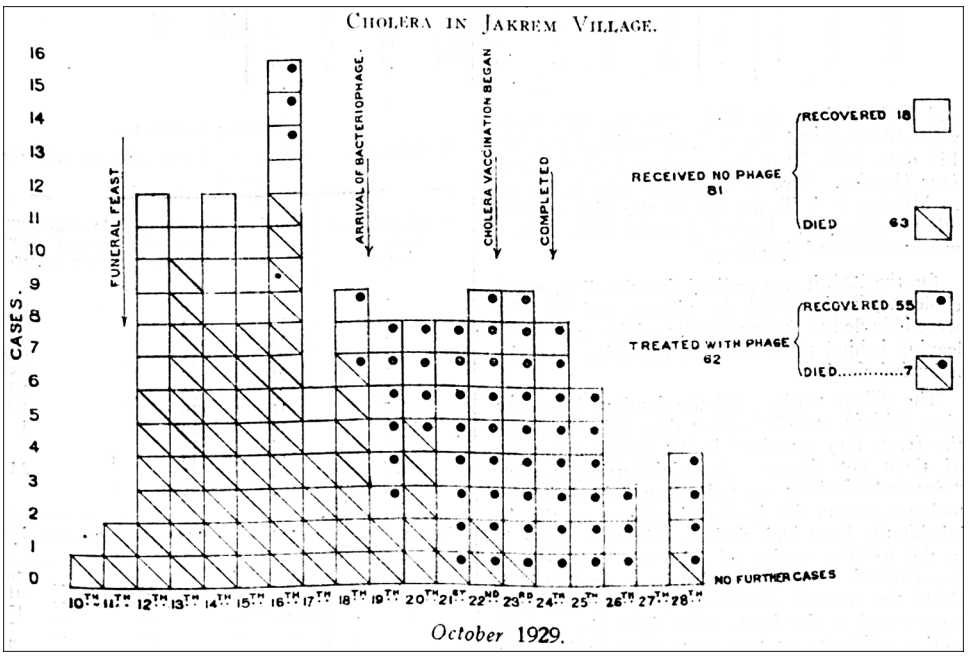
The remarkable results of phage therapy were documented in 1925 by the case of Georges Cap, suffering from bacterial dysentery. By July 13, his temperature had topped 40° C (104° F), and he was no longer responding to treatment. Phages were administered at 2:00 pm that day, and five days later his temperature was normal.

For several years in the 1920s, d'Hérelle lived in Noordwijk, a coastal village in the Netherlands. He had some knowledge of the language because his mother was Dutch, and he was given the post of Conservator at the Institute for Tropical Medicine in nearby Leiden. He worked on a curious idea that the "micellar" theory of living organisms was an improvement on the cell theory and published an obscure monogram entitled *Les Défenses de l'Organisme*. Among the visitors to Leiden, he met Albert Einstein and Konrad Lorenz.

Back in France, his discoveries were attracting attention. The pharmaceutical company Laboratoires Robert & Carrière launched a range of phage treatments, while Parke-Davis, Squibb, and Eli Lilly began marketing phages in the U.S. D'Hérelle's discoveries were widely endorsed, and in 1925, Sinclair Lewis published his novel *Arrowsmith*, which prominently featured phage research. The principal character of his tale is a young doctor named Martin Arrowsmith who faces the trials and tribulations of a medical student, culminating in his developing bacteriophage treatment, when an outbreak of bubonic plague strikes a Caribbean island. The book exemplified the difficulties facing a young pioneer in medical research, while also marking the new era of phage therapy. D'Hérelle also published a book in Paris, 1926 titled *Le Bactériophage et son Comportement*. It appeared in Baltimore that same year as *The Bacteriophage and Its Behavior*.

Phages were riding high. They could be sprayed around a contaminated ward or used to sanitize the walls of an operating theater. D'Hérelle's new discoveries were internationally renowned. For the first time, outbreaks of dysentery were successfully treated in Arabia and Africa. Greece and Italy launched trials with phage therapy to cure typhoid, and China was quick to follow. Phage preparations were used in Michigan and at Stanford University. In Paris, d'Hérelle set up his own Laboratoire du Bacteriophage in 1933. A Russian bacteriologist, Igor Nicholas Asheshov, was appointed research officer at the Medical Research Council in London, and confirmed that phage treatment could curtail outbreaks of dysentery in a few days, whereas conventional epidemiology could take a month to show results. Statistics from Calcutta (Kolkata) further proved the point. The Campbell Hospital was dedicated to treating the lower castes, where hygiene was poor and so the chance of a phage virus spreading was raised. The rate of mortality during a cholera outbreak was 27% at this cash-strapped hospital. By comparison, cholera patients were being treated far better at the nearby European Hospital, established in 1926 for rich, upper-crust patients, and run with the highest aseptic precautions. While these reduced bacterial cross-infection, they also limited the chances of phage viruses spreading from one patient to another. For all the wealth and state-of-the-art hygienic precautions, their rate of mortality was 86%. Wherever you looked, there were facts and figures that showed bacteriophages offered a key to recovery.

Just as d'Hérelle's revolutionary ideas were on the cusp of universal acceptance, there was a growing tide of resentment against this outsider, this outspoken upstart. Much of it stemmed from the research into immunity by Jules Bordet, the Belgian physician who pioneered the complement fixation test since used to characterize a range of diseases. Bordet studied under Élie Metchnikoff, the microscopist who had discovered the phagocytosis of bacteria in 1882 and graduated as Doctor of Medicine in Brussels 10 years later. Unlike d'Hérelle, he was showered with academic distinctions: Foreign Membership of the Royal Society, Membership of the Royal Academy of Science, Letters and Fine Arts of Belgium, winner of the prestigious Cameron Prize for Therapeutics of the University of Edinburgh … he even had a railway station named after him. When he spoke in London in 1930, he explained at length why bacteriophages could not exist. He explained that d'Hérelle was clearly a charlatan who



The detailed records maintained by John Morison in Assam, India, demonstrated the value of phage therapy in rural communities. In his graphic, patients dying were indicated by a diagonal line, and patients where phages were administered were indicated by a dot. The curative effects can be seen with dramatic clarity.

confused autolysis in bacteria with a virus that was no more than imagination. Other Belgian microbiologists were persuaded by Bordet's beliefs, and a group working to undermine d'Hérelle soon began to grow.

D'HÉRELLE VINDICATED

As a result of this attitude, support for phage therapy began to wane. Nothing would change d'Hérelle's mind; he was confident that he understood the nature of the phage. He had shown that it was filterable and would pass through an unglazed porcelain filter that was fine enough to hold back bacteria. He had demonstrated that phages could replicate indefinitely and showed that they destroyed bacteria, even if the preparation containing them was heavily diluted. And he was confident that viruses existed in the form of corpuscles, or particles. Most of these characteristics he could demonstrate — but not the last one.

In 1939, the virus was finally observed for the first time, using the recently invented electron microscope. Helmut Ruska — brother of Nobel Prize winner Ernst Ruska, who had pioneered the design of the first electron microscope — worked with Gustav Kausche and Edgar Pfankuch at the Siemens & Halske Company in Berlin, and they were the first actually to observe a virus (of TMV). One year later, the same microscopists revealed the appearance of bacteriophages for the first time. D'Hérelle had been right all along. Phages were clearly visualized as small particles clustering round an infected *Escherichia coli* cell. Later developments would reveal many of these viruses as extraordinary structures, looking more like a spacecraft than an agent of infection.

D'Hérelle was appointed Director of Bacteriological Services for the League of Nations, and was given a professorship at Yale University. His supporters never wavered; he was repeatedly nominated for a Nobel Prize. But by 1934 d'Hérelle was becoming disillusioned with Western science and sought support elsewhere. He paid two visits to Georgia, to visit his old friend Giorgi Eliava, a Soviet microbiologist who had met d'Hérelle in Paris and who, in 1923, had established the Eliava Institute in the capital, Tbilisi. The USSR believed d'Hérelle's discoveries were immensely valuable, so the Stalin government invited him to carry on his work under communism. He moved to Georgia where he set up phage production. He also established two phage production laboratories in Kharkov and Kiev, Ukraine. The communist authorities were eager to harness this proven approach to curing infections and previously incurable conditions, ranging from peritonitis to urinary tract infections, were successfully treated with d'Hérelle's phage therapy. For d'Hérelle, this was a time of unparalleled acceptance, and he published a book, *The Bacteriophage and the Phenomenon of Cure*. It was translated into Russian (Tbilisi University Press) and was dedicated to Joseph Stalin, who had been born in Georgia in 1878.

However, just as the great collaboration was gathering momentum, d'Hérelle's great friend Eliava became a target of the communist authorities. In 1937, he

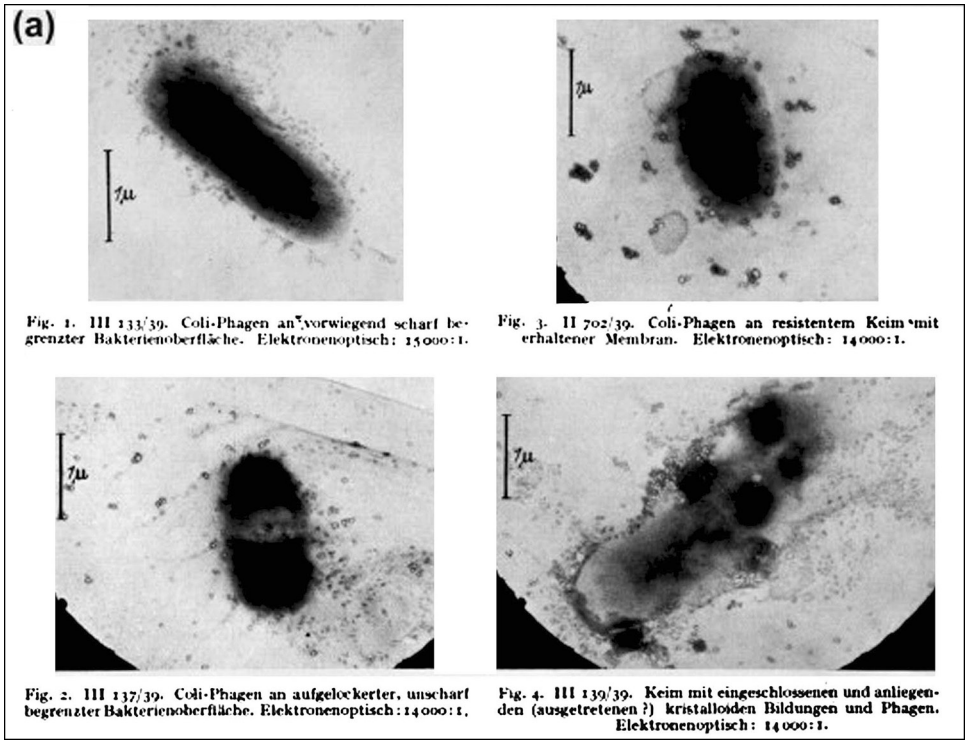


Fig. 1. III 133/39. Coli-Phagen an vorwiegend scharf begrenzter Bakterienoberfläche. Elektronenoptisch: 15000:1.

Fig. 3. II 702/39. Coli-Phagen an resistentem Keim mit erhaltener Membran. Elektronenoptisch: 14000:1.

Fig. 2. III 137/39. Coli-Phagen an aufgelockerter, unscharf begrenzter Bakterienoberfläche. Elektronenoptisch: 14000:1.

Fig. 4. III 139/39. Keim mit eingeschlossenen und anliegenden (ausgetretenen?) kristalloiden Bildungen und Phagen. Elektronenoptisch: 14000:1.

Helmut Ruska obtained these images of bacteriophages clustering around *E. coli* bacteria in 1939. They appeared as "kleine, schwarze Körperchen" (tiny black bodies) — a perfect description that vindicated d'Hérelle's conclusions. He had always insisted that they were not some sort of soluble enzyme, but submicroscopic particles.

allegedly fell in love with a woman who was coveted by Lavrentiy Beria, Stalin's notorious chief of secret police. Beria, who was personally responsible for the murder of tens of thousands of "enemies of the state," concocted a series of security offenses, which led to Eliava's conviction on charges of espionage. Beria had Eliava executed, all photographs destroyed, and records of his illustrious career expunged. D'Hérelle's book had been hailed by the Soviets as a bible to this exciting new branch of medicine, but now it was banned, and he was obliged to return to Paris with his family. The Russians continued to use phage therapy to treat troops suffering from bacterial infections during World War II. The German army planned to occupy Georgia solely to ensure that they could obtain supplies of phages for their own troops.

In America in 1940, a team of eager young scientists at Caltech became known as the "phage group," when Salvador Luria and Alfred Hershey began to study the mechanisms of infection between bacteria and phages. Luria proved that phages, inactivated by ultraviolet, could be coaxed back to activity ("brought back to life") if implanted into a bacterial cell. Their work led to the modern science of molecular biology, and James Watson has said that his work under Luria launched his career in genetics. Watson worked with Renato Dulbecco, one of the last members to join the Phage Group, for it wound down in the 1960s. I came to know Dulbecco when he later worked at the Imperial Cancer Research Fund in London, and he was always certain there remained much untapped potential in phage therapy.

This was the era of the Cold War, and the scientific priorities between the two sides diverged. The well-established protocols perfected by the now-discredited Eliava at the Phage Institute in Tbilisi were used to produce phage preparations in large amounts, and recalcitrant cases of bacterial infections from all over the USSR were sent to Tbilisi for treatment. Endless reports and papers were published, but they were always in Russian and Georgian, languages that were not just unfamiliar to Western science, but expressed in alphabets we did not understand. The language of science everywhere is English, and science must be published in the major scientific journals — *Science*, *Nature*, *New England Journal of Medicine*, *The Lancet*, and *Proceedings of the National Academy of Sciences*. Submitting a paper to one of these was prohibited in the USSR, so Western microbiologists never realized what was going on.

Phages are not a replacement for antibiotics, but represent a radically different approach to anti-bacterial therapy. Antibiotics often cause side effects (including severe allergies, and the opportunistic overgrowth of bacteria like *Clostridium difficile*, or yeasts including *Candida*), whereas there are none with phage prepa-

rations. Phages target a specific organism and cannot compromise the resident microbiome. Once they have been administered, antibiotics start to fade — the half-life of many common types is an hour or two — while phages are replicated and increase in their power to act. New antibiotics can take decades to bring to commercial fruition; phages a matter of days. Resistance to antibiotics is a recalcitrant problem; though there are always alternative phages. Antibiotics, however, are regularly produced, are packed as pills that are easy to transport, and are long-lasting and simple to administer. Phages require specialist units to be produced, few of which are available in the Western world, and they are provided in liquid preparations that can be more complex to store and administer. The pills win for convenience.

The Western world continued to celebrate antibiotics throughout the 1980s, emphasizing the pre-eminence of pills and potions produced by Big Pharma, while the Communist Bloc turned increasingly to biological therapies. To them, phage was phenomenal. The Research Institute and Industrial Department in Tbilisi employed over 700 staff and could produce more than 2 tons of phage-rich liquid in 500 L fermentation vats every week. I have seen phages sold in ointments, lotions, eye drops, and tablets throughout the old Warsaw Pact countries. You can still search for them online, using the Russian term for bacteriophage — бактериофаг. Many of the products are useless. For instance, you can find phage treatments recommended for urticaria (which is actually due to an allergy) and herpes (which is itself caused by a virus), conditions unaffected by bacteriophages.

Everything changed in 1991 when the Soviet Union collapsed, and Georgia resolved to have nothing to do with the new Russian Federation. Ethnic divisions within Georgia erupted into civil war. There was no funding available for the institute, and many of the leading microbiologists deserted the cause. The Russians continued to capitalize on d'Hérelle's legacy, but in Tbilisi much of the extensive reference libraries of different phages that stemmed from his research were lost, partly through a lack of technical staff, and also because the regular power outages compromised incubators and cold-storage facilities. Work at the Eliava Institute ground to a halt, and the premises were left in a damaged and deteriorated state. Teimuraz Chanishvili, who had joined the institute in 1941, held together a small group, and during the 1980s, Amiran Meipariani joined Chanishvili to keep the institute running, supported by occasional grants and the enthusiasm of a few devoted supporters.

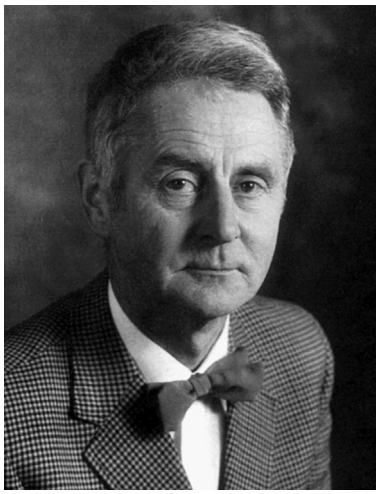
Félix d'Hérelle (right) felt his work was not appreciated in Western Europe and was invited to the USSR by Joseph Stalin in 1934. In Georgia, he was welcomed by Giorgi Eliava (left), and the two soon established phage therapy as an alternative to antibiotics. Eliava was framed by the Soviet security and executed in 1937.

As the Eliava Institute it struggled on, maintaining phage research and commemorating the name of its previously disgraced founder.

WESTERN INTEREST

Phage therapy in America was meanwhile in the doldrums. The association between d'Hérelle's research and the Stalin regime made the subject politically unfavorable in the U.S., and nobody could imagine that Americans would want to be treated with viruses straight from the USSR. The only application of bacteriophages in the Western world that remained in regular use to identify specific strains of bacteria. The idea of characterizing pathogens by the phage to which they were susceptible had first been proposed by Oskar Bail, head of Hygiene and Bacteriology at the German University in Prague, in 1921. The technique was adopted for the identification of the organisms causing typhoid and paratyphoid, and these days it is most widely used to characterize staphylococci, a system I used at the Medical Research Council in the 1960s. It is in this application that the bacteriophage remained a feature of microbiology laboratories, even when it was forgotten as an agent of therapy.

For all those decades, the only person outside the Soviet bloc showing any interest in using phages to cure infections was Herbert Williams Smith. Born in the mining valleys of South Wales, he was a dis-



Research into bacteriophages was maintained in the West by Herbert Williams Smith, a Welshman who worked at the Houghton Poultry Research Station in Cambridgeshire. While other researchers lost interest, Smith showed how phage therapy could be successfully applied in veterinary medicine.

tinguished veterinarian and microbiologist, who was elected a Fellow of the Royal Society for his research on animal diseases. Williams was the pioneer of the bacteriophage who brought the research up-to-date in the Western world. Known to everyone as Willie, he always wore a bow tie. Many of us do in microbiology. People sometimes think it's an affectation, but it is a sensible choice: a straight tie can dangle in your petri dish, and we don't want that. Beginning in 1948, Smith worked on staphylococcal infections, using phages to identify the strains he encountered, but he then went one stage further. In his later years he was appointed Head of the Microbiology Department at the former Houghton Poultry Research Station in Cambridgeshire (close to where I live), and by 1980, he was experimentally using phages to treat animal infections, rather than merely to identify strains of bacteria. In the USSR, phage preparations were often impure, and controlled experiments were ignored. Many of the protocols inherited from d'Hérelle would be unacceptable to Western scientists, and there was no independent regulation. But Smith was meticulous. He ensured that his cultures were carefully maintained using strict aseptic techniques, he always set up controls to verify his results, and his research was methodically recorded. In 1981, he published a 25-page paper in the *Journal of General Microbiology* on using phage therapy to treat *E. coli* infections of calves, piglets, and lambs. During his career he published some 200 peer-reviewed papers, and he attracted admiration for his dedication to the task. His meticulous research laid the groundwork for phage therapy in the Western world — yet he has been forgotten.

When he died in 1987 he had a collection of 7,000 cultures, which were taken over by a young bacteriologist in the next-door laboratory, Paul Barrow. He and Smith's assistant, Michael Huggins, remained the only two people in the Western world who could carry on the campaign, but they found it impossible to obtain approval for funding. It seemed that the scientific establishment was firmly opposed to the uses of bacteriophages as agents of therapy. Then, in 1994, two American researchers, Jim Bull at the University of Texas, Austin, and Bruce Levin at Emory University, Atlanta, came across a paper published in 1982 by Smith and his assistant Huggins. They repeated one of Smith's experiments, injecting 30 mice with a virulent strain of *E. coli*. In 15 of the mice, they then injected the bacteriophage specific to the bacterium. The results were unambiguous: the phage-treated mice lived on as if nothing had happened, whereas the controls all succumbed to *E. coli* within 32 hours. At the same time, at the Evergreen State College in Olympia, Washington, Elizabeth Kutter was studying the molecular biology of phages she had brought back from the institute in Tbilisi. She was certain that finding approval from the Food and Drug Administration (FDA) was going to be difficult, if not impossible, though she too could see the potential benefits of phage therapy. The problem they all faced was simple: biological control of bacteria was unthinkable in an era where antibiotics could so easily cure infections. Wherever they looked, nobody was interested.

That was to change dramatically on Nov. 1, 1996, when a writer named Peter Radetsky published an article in *Discover* magazine, "The Good Virus," — and yes, it was all about phage therapy. *Discover* is one of those magazines you find in waiting rooms and aboard airplanes. When businessman Caisey Harlingten boarded a flight from Toronto as Christmas approached, he picked up a copy from the shelf and idly turned the pages. He was a Canadian multimillionaire stockbroker, now turned venture capitalist, with no scientific background but was an avid reader of popular science magazines. He was riveted by Radetsky's article. Harlingten had read so many newspaper reports about multiple antibiotic resistance in bacteria, and here must be the answer. He set off to Tbilisi for a meeting with Chanishvili, by that time the longest-serving researcher at the institute. Said the Georgian microbiologist: "Phage is now my hobby; but I would like to make some money out of my hobby." Harlingten wasted no time, and within nine months had registered a new company in America, Georgia Research Inc., with Richard Honour as company president. They organized the first international conference on phage therapy in the former communist party headquarters overlooking Tbilisi. Delegates from England, Poland,

Germany, and Canada joined the entrepreneurs from the U.S. Chanishvili was an old hand at dealing with the Soviets, and he knew that, in negotiations, you had to play the game. He resolved to patent their products, then they could discuss licensing the phages and arranging for production. Within months, Harlingten had signed a 15-year lease for real estate on the outskirts of Seattle and set about building a phage therapeutics development laboratory. The Georgian virologists supplied them with the cultures of their phages that Honour's team requested, and the future looked promising. For $75,000 a year, Harlingten would be granted rights to the institute's know-how. The Americans would tour hospitals, looking for patients with incurable infections and would mail over cultures of the bacteria responsible. The Georgians would then identify the matching phage, send it over, and the patient would be cured. At least, that was the theory.

Meanwhile, phage therapy was first brought to a popular audience in Britain in 1997, when the BBC's Horizon television series featured a documentary, *The Virus That Cures*. It was written and produced by Judith Bunting, who had 10 years experience of making science programs for TV. She later became a Member of the European Parliament, and was exuberant, confident, and keen. Her program took viewers to Georgia and showed the institute laboratories, those huge vats of bacteria cultures, and the murky jars of bacteria that turned miraculously crystal clear when those wondrous phages were added. Technicians were shown fishing cloudy jars of contaminated water from a sewage outfall, while excitedly explaining that this would be full of previously unknown phages. And we saw Harlingten turning the pages of that issue of *Discover* magazine, talking with bacteriologists in Georgia, and explaining his expansive plans for the future of the cooperation between the Georgian microbiologists and the American entrepreneurs. It left viewers convinced that we were on the verge of a new era in medicine.

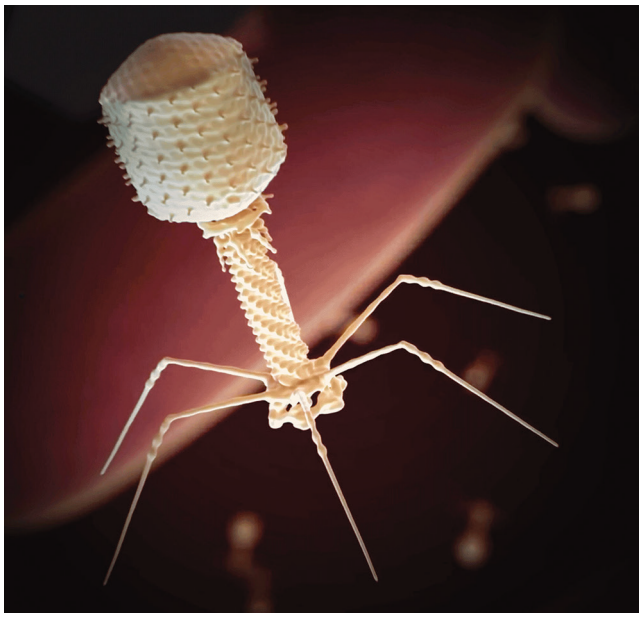
Bunting tells me that her experiences filming with the scientists in Georgia in 1992 were extraordinary. "Three years after the Berlin Wall had come down, the Eliava Institute had effectively collapsed," she states. "Scientists worked unpaid, many showing true dedication, coming in day by day nonetheless to ensure their samples stayed viable and the Saturday phage clinic could continue to operate." I asked if she was skeptical about it all. "After 10 years of producing science documentaries, I was not easy to convince," she told me. "But this was something else."

Within months, the American deal had collapsed. The Georgians had always been excited about the future of their phages; they saw them as saviors of humanity, and almost as their pets. They wanted to liberate their discoveries to the rest of the world. To the Americans, the phages held a key to wealth creation; it didn't matter what they were. Those men were hardheaded realists, who know that such innovations needed a regulator and strict standards with which to comply. The FDA was a major obstacle, and there was nobody who understood the field, nor was there any mechanism for approval. To the Georgian bacteriologists, results were what mattered. They knew their phages could cure infections almost overnight, that they could wipe out drug-resistant bacteria, that they were abundantly available and easy to produce. They were happy to know their half-ton vats could churn out as much of the phage as you wanted, but the Americans were aghast to see the lack of controls and the absence of regulation. Those fermentation vats might do for a microbrewery in Wisconsin, but not for a medical product of guaranteed purity. And they all knew that you could never get away with administering viable viruses to sick people without a load of questions. Antibiotics were tried and tested; phages were unknown. Honour became head of the Viridax Corporation, but not until September 2010 did Viridax announce that their first candidate bacteriophage to cure *Staphylococcus aureus* was ready for appraisal. The first unit devoted to phage therapy in America was set up at the University of California, San Diego, in 2018.

BACTERIOPHAGES TODAY

There is much research going on in Georgia to this day. Mzia Kutateladze currently heads the Eliava Institute, and she is the president of the Eliava Foundation, which supports a group of spin-off companies. At the Georgian National Academy of Sciences, Zina Jorbenadze, who is head of the foreign relations division, tells me that their member Tinatin Sadunishvili is leading research on phages of plant infections. Their primary research publications are in Georgian journals, though. It is related topics, including the characterization of bacterial pathogens, that find themselves into Western publications, and those do not concern phage therapy. So the geographical range of interest in the Georgian research on bacteriophages is restricted. Even so, the Eliava Institute has been approached by Western military intelligence, seeking answers to bioterrorist weapons that might use anthrax bacteria. The institute's online store at myphages.com offers a range of phages for online purchase; this may be new

Micro Gen is a large, successful commercial phage therapy company in Moscow, established 20 years ago. With a little patience and ingenuity, you may pick out the words "bacteriophage pseudomonas aeruginosa" printed in Cyrillic on the carton. They also mass produce vaccines, blood products, and culture media.



The Canadian virologists at Cytophage feature computer graphic images exemplifying the extraordinary structure of a typical bacteriophage. Their aim is for phage therapy to be promoted as a science of Canada, though they make no mention of their compatriot Félix d'Hérelle, without whom their discipline would not exist.

to us, but it's been routine in Georgia and the old Warsaw Pact countries for decades.

In Poland, where phages were first used in the 1920s, a new phage therapy unit was established at the Hirszfeld Institute of Immunology and Experimental Therapy, Wrocław, in 2005. Brazil saw phage research in the 1920s, when the subject was new and fashionable, but those pioneering efforts were lost. There is active research into phage therapy in Southeast Asia, notably in Thailand, Malaysia, and Singapore, while in South Korea there is the Bacteriophage Bank in Hankuk. Phage Australia brings together teams involved in phage research, and in Uganda, a phage hub has been set up at Makerere University to coordinate African research. Two people in the U.K. have worked on phages since the 1980s. Paul Barrow, once at the Institute for Animal Health, is now at Nottingham University, and James Soothill is chief bacteriologist for Great Ormond Street Hospital in London, where a few patients have recently received experimental phage therapy. Not until 2006 did the FDA finally approved the application of phages in the American food industry, and they were quietly introduced to eliminate Listeria monocytogenes bacteria in products ranging from poultry to cheese. We've just seen the launch of a trial using phages to cure Pseudomonas aeruginosa, announced by the National Institutes for Health in October 2022.

There was no interest in phage therapy in Canada until the Canadian Phage Therapy Network emerged in 2019 and Cytophage was set up to produce phages for the treatment of farm animals. The Canadian government now insists that bacteriophage research should be seen as "a science of Canada" even though their compatriot Félix d'Hérelle isn't mentioned anywhere. Journalist Joe O'Connor in the Financial Post (Dec. 30, 2019) even said how fortunate it was that their phage scientists were more than "some amateur with a home chemistry kit and a dream." Yet d'Hérelle was an amateur with a dream, working at home, even though he founded one of the most important disciplines in the whole of medicine.

Professor Strathdee had encountered phage viruses as a student, as we all did — after all, they have been used to characterize bacteria for generations. But she had also read a book by William Summers, Félix d'Hérelle and the Origins of Molecular Biology published by Yale in 1999. And then, as a stroke of good fortune, she tells me that she found an original publication by d'Hérelle himself: The Bacteriophage, its Role in Immunity, published a century ago in 1922. She found her copy on Amazon. If we are ever going to conquer the global spread of infections caused by bacteria resistant to our antibiotics, we should all recognize the astonishing discoveries of Félix d'Hérelle. He, and he alone, gave us the key, and our survival may depend on the application of phage viruses precisely as he proposed a hundred years ago. It has so often been the devoted and single-minded amateur who provides the great strides in science, and everyone — even those brash and boastful Canadians — would do well to celebrate the fact. ■