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Phage treatment of human infections

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Phages as bactericidal agents have been employed for 90 years as a means of treating bacterial infections in humans as well as other species, a process known as phage therapy. In this review we explore both the early historical and more modern use of phages to treat human infections. We discuss in particular the little-reviewed French early work, along with the Polish, US, Georgian and Russian historical experiences. We also cover other, more modern examples of phage therapy of humans as differentiated in terms of disease. In addition, we provide discussions of phage safety, other aspects of phage therapy pharmacology, and the idea of phage use as probiotics.

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Introduction

Phage therapy involves the targeted application of bacteriophages that, upon encounter with specific pathogenic bacteria, can infect and kill them. As typically practiced, phages then lyse those bacteria, releasing virion progeny that can continue the cycle, including migrating to other sites of infection anywhere in the body. The actual phage-mediated bacterial killing, however, occurs well prior to the lysis step—e.g., such as in the first minutes of infection for a phage such as phage T4 [1]—as the phage converts the cell into a factory for making new phages. Phages are unique among antibacterial agents in their ability to increase their numbers when in the presence of bacterial targets. Of similar importance, phages only minimally impact non-target bacteria or body tissues. A more complete list of advantages associated with phage therapy, relative particularly to chemical antibacterials, is presented in this issue[2] and elsewhere.[3] Here we review the potential for phages to treat bacterial infections afflicting humans. Other therapeutic applications, such as in veterinary medicine, have been reviewed in reference 4, and will be also covered in future issues of this journal. Other reviews focusing on various aspects of human phage therapy are also available.[3,5-11]

History of Phage Therapy

The viruses of bacteria were discovered in 1915 by Frederick Twort.[12] The "bacteriophage" era, however, did not begin until the seminal publication demonstrating "un bactériophage obligatoire" by Félix d'Hérelle in 1917.[13] Microbiologists subsequently began to incorporate the idea of phages into their world view, with phage therapy almost immediately coming to play a central role in the development of the field. Indeed, one can readily trace the progression of phage biology as starting with an early, enthusiastic period during which claims were excessive and often unrealistic, while at the same time little was understood of the viral nature of phages or their strengths and limitations (early 1920s into the 1930s). An important exception to these concerns, most closely associated with the concept of phage therapy as practiced during these early years, and as formulated in impressive detail, is the work of Felix d'Hérelle (see "France," below).

This time of excessive expectations was followed by a period of declining enthusiasm for phage therapy in much of the western world, subsequent displacement of its use after World War II by antibiotics, and a shift in focus to using phages as model genetic systems. This second stage started with the quite critical 1934 Eaton-Bayne-Jones report[14-16] reviewing the available literature on phage therapy[3] and continued through the late 1940s. At the same time, development of phage therapy and its active application continued to increase within the Soviet Union and eastern Europe, where it was well supported until the fall of the Soviet Union. In the West, the golden age of phage-based development of molecular biology involved intense work with just a few phages infecting one avirulent lab host (*E. coli* B) rather than broad exploration of phages targeting a range of key pathogens.

Subsequently, phage therapy was "rediscovered" by the English-language literature starting with the work of Smith and Huggins in the 1980s.[17-20] This western phage therapy renaissance gained momentum only in the 1990s, however, as access was increasingly gained to the rich trove of Soviet and Polish work. The field finally began maturing from those heady "wild west" days of the 1990s starting approximately in the year 2000, a progression that was coupled with an explosion of genomics and broad ecology-based phage research, with this latest era of phage therapy research as well as application continuing to this day. Over the rest of this section we provide an outline of phage therapy development in different parts of the world with special emphasis on France, which we cover over three sections; this extensive and well-documented French work has been largely ignored in previous reviews, presumably due to the language barrier. For additional reading, the history of phage therapy has been recounted in some depth and from various perspectives in a number of reviews[3,10,21-23] and the history of phage biology too has also been extensively reviewed in reference 24–28.

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France. Human phage therapy has been practiced in France since 1919, when d'Hérelle first successfully treated several children at the Hospital des Enfants Malades in Paris who were suffering from severe dysentery, using the phage he had first isolated from the stools of soldiers he had observed at the Pasteur Institute.[3,22,29] He delayed both publication and further clinical work, though, until he had carried out very extensive studies of the properties of phages, particularly those relevant to clinical applications, with work especially in fowl typhoid and in cholera. His study of the role of phages in combating infections are laid out in a series of books,[30-38] including five that have been translated from French into English.[36,39-42] His methods for the preparation of therapeutic phages were particularly well laid out in an appendix accompanying one of his later works, the first English translation of which is found elsewhere in this issue.[43] However, although d'Hérelle carried out the first human therapeutic phage trial, the first article documenting phage therapy was on research conducted in Belgium by Bruynoghe and Maisin in 1921.[44] They reported that injecting phages targeting Staphylococcus near the base of cutaneous boils (furuncles and carbuncles), in six patients, led to improvement within 48 hours: reduction in pain and swelling and some reduction in fever.

Subsequent phage therapy work in humans is reviewed in 1931 by d'Hérelle[45,46] who also describes the first use of intravenous bacteriophage, which was used in the treatment of cholera by Asheshov in India. In addition, at his suggestion, Dr. Davioud in France was able to cure a "hopeless" case of staphylococcal bacteremia. Five mL of a suspension of staphylococcal phages was diluted in 500 mL of physiological saline and infused over one hour. d'Hérelle also described the intravenous use of phages for Streptococcus by Gratia. Further use of intravenous phage in France is outlined below.

Much other work in the field rapidly followed in France. D'Hérelle, for instance, went on to establish his own Laboratoire du Bactériophage, run by his son in law, Theodore Mazure, which produced the first commercial phage cocktails—Bacté-Coli-Phage, Bacté-Intesti-Phage, Bacté-Dysentérie-Phage, Bacté-Pyo-Phage and Bacté-Rhino-Phage. Although France is clearly a western country, most of the reviews of phage therapy have not mentioned the continuation of phage therapy in France, which had been ongoing with some vigor until the early 90s, with the commercial phages from d'Hérelle's previous company available until 1978. The following detailed discussion of phage therapy as historically practiced in France draws both on the very interesting recent monograph by Dublanchet[47] and translations made by Kuhl of the original French literature.

Lang et al.[48] reported the use of bacteriophage in seven patients with chronic orthopedic infections with resistant organisms. They were able to cure two cases of hip prostheses infected with gram-negative bacteria (after removal of the prostheses), one case of tibial osteomyelitis due to Proteus, *Staphylococcus aureus* and Klebsiella; one case of septic arthritis of the knee due to Enterobacter and *Staphylococcus aureus*, and one case of septic non-union of the femur due to pan-resistant Providencia. A surgical site infection associated with Harrington rods also showed improvement: The staphylococcal infection was eradicated, but the Pseudomonas infection persisted. One case of a post-traumatic septic knee, with variable flora associated with a chronic pseudomonal infection, however, relapsed after apparent improvement and was counted as a failure.

There continued to be regular literature reports on phage therapy in France until at least 1979 (cf. 49 and 50). Henri de Montclos spent the majority of his career at the Pasteur Institute of Lyon where he was Chief of Clinical Microbiology for 10 years and he and his research team produced antistaphyloccal vaccines and therapeutic phages until the early 1990s. In his 2002 review,[51] he described how several European laboratories maintained an individualized, essentially artisan-like production of phages by classical methods until the 1980s. He stated that phages appear to be safe for human cells though potentially there could be problems associated with modes of preparation. Among his recommendations is purification from pyrogen released by the lysing of bacteria, for example by cesium chloride gradient centrifugation. He also recommended against propagation on media produced from animal tissues. Successful treatment was typically achieved in a few weeks and there was a general impression of a real service rendered, although this individualized approach did not lend itself to double-blind study establishing proof. After the AIDS crisis in the blood supply, regulations within the public health system became less conducive to continued production of pharmaceuticals, including phages, in this individualized manner.

A few French physicians have continued to use phages therapeutically even after the Pasteur Institute stopped making therapeutic cocktails in the mid 1990's, now generally obtaining their phages from Russia or Georgia.[47] Staphylococcus infections seem to be the most common target of these more-recent efforts. Dublanchet and colleagues have recently reported successful phage therapy of two patients from France and one from Australia who had failed other therapies, including all available antibiotics.[52]

***La Médicine* (1936).** A full 1936 monograph issue of the journal *La Médicine* in 1936 was devoted to phage therapy. Its individual reviews give detailed data on the treatment of such conditions as typhoid fever, acute colitis, peritonitis, prostate and urinary tract infections, furunculosis, sepsis and otolaryngology. For example:

Mikeladze[53] described the treatment of 21 patients with typhoid fever with per os bacteriophage, using 10 mL of lysate for three to five consecutive days. Compared to 64 controls treated by the usual methods, they noted a reduction in mortality from 15.6% to 4.8% and a reduction in complications from 56.2% to 13%. There was a doubling of the recurrence rate from 4.5% to 9.5%, however, when phage treatment was delayed until between the tenth and fifteenth day of illness. The duration of illness was essentially the same, though. Subsequently, they used intravenous phages for patients whose blood cultures yielded the typhoid bacillus, infusing one mL of phages daily for three consecutive days. The duration of illness was decreased, but "violent reactions" were seen after the injection. Following d'Hérelle's advice, they then began using phage cultured in the absence of peptone, slowly infusing 3 to 5 mL in 200 mL of physiological saline. They still observed severe reactions before a rapid fall

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in temperature, and decided to reserve this treatment for young patients with a healthy cardiovascular system, preferably treated early, before the typhoid had weakened the patient. In general, they also decided to decrease each dose, but repeat the injections.

Mikeladze[53] also described the treatment of "acute colitis" due to Shigella or Salmonella in Georgia. One ampoule (5 mL) of bacti-intesti-phage diluted in boiled and cooled water was administered orally every 2 hours. The patients ingested a total of 8–10 ampoules while taking a liquid diet. In general, they noted that phages caused a decrease in temperature and an improvement in the patient's feeble and rapid pulse, intestinal pain and tenesmus. In unfavorable cases, treated late in infection, the improvement was still considerable, although the colitis tended to persist. They found that a second series of ampoules, given after a day of rest, always yielded good results. In 47 cases of dysentery, 3 died (6.4%), which is half the usual mortality. Of 43 patients with colitis, all were cured.

Tsouloukidze[54] discussed the phage treatment of twenty patients with peritonitis caused by intestinal perforation in typhoid fever. After surgical laparotomy and the repair of the perforation, they administered 8 to 20 mL of a mixture of equal parts of Bacté-Pyo-Phage and Bacté-Intesti-Phage, undiluted. The peritoneal cavity was then completely closed surgically, without drains or dressing. The mortality was reduced from 85% to 20–35%, depending on how they counted two patients who died four and five days after surgery without peritoneal signs. He felt they had enough data to energetically recommend phage therapy for all cases of peritonitis, and particularly for perforations caused by typhoid.

Gougerot and Peyre[55] described the treatment of skin infections using phages, with particular emphasis on recurrent furunculosis. Local treatment was best, but each pustule needed to be opened with a phage-containing syringe. Then a pad moistened with phage was rubbed over the area and a compress dampened with phage was used as the dressing. The next day they observed that each pustule had increased in size and was surrounded by an indurated, red zone of inflammation. But after 48 hours, the lesions would dry up and disappear. They repeated this every two days for new or overlooked pustules, with improvement in nearly all cases. After eight or ten applications, most of the patients were cured. In cases of bacterial infection of the dermis and epidermis, such as impetigo, they recommended unroofing the lesions, removing the crusts, opening bullous lesions and rubbing a bit roughly in order to introduce the phages into the skin, then applying a large compress moistened with the same bacteriophages. As with abscesses, it was helpful to advise the patient to expect a worsening of inflammation in the first 24 hours, prior to improvement.

Sauvé[56] described the treatment of surgical infections with bacteriophages. He noted the availability of extremely polyvalent phages which lysed approximately 90% of strains of Staphylococcus from both Gratia and d'Hérelle, and recommended using stock bacteriophages. On the anal margin, he had obtained surprising cures of voluminous abscesses, in two or three days, without resulting fistulas and without surgical intervention. He noted that breast abscesses, dental abscesses and many others could be cured by phage therapy without the expense of anesthesia nor the long recovery necessitated by surgery. He was surprised that a method so simple, so brilliant, and so effective had not become more common, but felt it would with time. For chronic staphylococcal infections, he recommended not injecting subcutaneously to avoid stimulating the production of antibodies to phages. For minor, non-life-threatening infections, he recommended drainage of the abscess followed by injection of phages into the abscess cavity. Sauvé further studied streptococcal infections with Sertic and Boulgakov from d'Hérelle's laboratory, who prepared a polyvalent streptophage active against 50% of streptococcal strains. As streptococcal infections are less prevalent than staphylococcal infections, he recommended treatment with a mixture of staphylococcal and streptococcal phages until the organism could be identified. He also discussed the effectiveness of phages for the treatment of coliform infections, noting that the available phage cocktails were becoming more and more polyvalent. However, in many urinary tract infections there were difficult-to-treat strains, and it had been necessary to adapt a coliphage for the resistant strains. He concluded that phage therapy was more efficacious and less dangerous than other methods, particularly vaccination.

Sauvé[56] continued with the use of phages in septicemia, and described the cure of 9 cases of staphylococcal infection. They diluted 4 to 6 mL of phage cultivated in broth without peptone (to avoid the shock induced by peptone) into 200 mL of nontoxic serum, which was slowly infused over 40 to 50 minutes. "The temperature is taken every 2 hours to record the lysis. When it occurs it is massive and accompanied by shock. The temperature falls within 3–5 hours to 37–40 degrees and below. From the time of the decrease in temperature, the patient feels truly reborn; his face brightens, and the observer sees a true resurrection. The theoretical objections to the intravenous treatment with phage and the possibility of introducing unlysed filtered pathogens do not hold up against the fact of definitive cure." He had observed no fatalities with treatment, but noted that this treatment does not work in terminal cases associated with rapid decline. He used a second and third injection at two-day intervals in cases of incomplete lysis. No other method, including serotherapy, had achieved these results. He had also published a case of treatment of a severe case of coliform septicemia which was cured within 24 hours of intravenous bacteriophage infusion. He emphasized that phage therapy should always be preceded by surgical treatment including incision and drainage, which is then followed by debridement if necrotic tissue is present.

Michon[57] described the treatment of urinary tract infections. He stated that it was necessary to first alkalinize the urine. After evacuation of the bladder, they infused phage for three consecutive days. Twenty mL were instilled into the bladder on the first and second days and 10 mL on the third day. The patient continued urinary antiseptic treatments for the entire period, including three to four days following the infusion of phage. In cases of uretero-prostatitis, they instilled the phage to the area of the posterior urethra. For pyelonephritis, he recommended alkalinization of the urine, renal lavage with 20 to 30 mL of phages followed by urethral lavage, and then phage instillation into the

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bladder. They noted much less relapse than they had seen with silver nitrate treatment.

Halphen[58] described the use of phage therapy in otolaryngology. He noted the treatment of furunculosis of external otitis, but noted that general anesthesia or great bravery on the part of the patient was necessary for the injection of phage into the already-inflamed cartilage. A second treatment was often needed two days later, but they concluded that if these first two treatments were not effective, then the phages used were not effectively lysing the bacterial strain. They had also used phages in dressings applied to the nasal furuncles with success. They treated furuncles of the upper lip (known in the pre-antibiotic era as the danger triangle) with several mL of lysate without anesthesia. "Immediately the lip swells greatly and very painfully, but after 5–6 hours, the drainage from the incision becomes serous (i.e., yellow, transparent and benign), and within 24 hours, the patient is cured."

Summary of Pasteur Institute reviews. A 1961 review in French by J.F. Vieu[59] of the Bacteriophages Service of the Pasteur Institute summarized the fundamentals of phage therapy in France at that time, noting that bacteriophage were particularly useful in the treatment of Staphylococcus, Pseudomonas, Proteus and coliforms. His key points about their approach included statements that:

• Only virulent phages that completely lyse the bacterial culture in vitro were used in therapy. The liquid lysates, typically containing 10^8 to 10^9 virions per mL, were filtered through a Chamberland filter.

• The media in which the lysates were prepared was of utmost importance when administered intravenously, and even more so when administered intrathecally. The media that were best tolerated contained the least amounts of large protein molecules. Well-tolerated molecules included broth, peptone water and synthetic as well as semi-synthetic media.

• Two categories of therapeutic bacteriophages were utilized: bacteriophage stocks (cocktails) and adapted bacteriophage. The phage stocks had a broad spectrum of activity, while the adapted phages were prepared for specific bacterial strains.

• The efficacy of a minimally virulent phage was improved by repeated passage through the same bacterial strain. When the preparation of an adapted staphylococcal phage was necessary, the delay of several days needed for their propagation was typically compensated by their remarkable efficacy.

• There were multiple modalities of administration: Local application and subcutaneous injection were used in the first staphylococcal trials, and required no particular precautions. They remained the methods of choice when the bacteriophage could be placed in contact with the infectious site. In the particular cases of intrapleural or pericardial injection, it was preferable to use lysates prepared as for intravenous injection. Per os delivery also was possible, with the introduction of phages into the GI tract of mice resulting in virions circulating into the blood.[60] See reference 61 for a recent review and discussion of phage transfer from the human gut to the blood.

• Vieu discussed the potential for interference by phage-neutralizing antibodies, a common concern, which might appear after repeated injections of therapeutic phage. He concluded that extending phage treatment for long enough for antibodies to become a problem shows an error in judgment, as typically phage therapy should at least begin to be effective within a few days or one should consider adding another phage that does not cross-react serologically.

In 1975, Vieu[49] tabulated the 476 phages isolated and prepared by the Bacteriophage Service at the Pasteur Institute from 1969 through June 1974, most of which targeted Staphylococcus, Pseudomonas, *E. coli* and Serratia. Staphylococcal infection was the major indication for phage therapy. Among 90 phage requests to the Pasteur Bacteriophage Service in 1959–60, more than half concerned staphylococcal infections. These included septicemia with endocarditis, chronic osteomyelitis, suppurative thrombophlebitis, pulmonary and sinus infections, pyelonephritis, skin infections and furunculosis, and represented situations in which clinical and bacteriological cure had not been achieved by extensive antibiotic treatment. Indeed, most of the staphylococcal strains sent to the laboratory with requests for adapted bacteriophages were resistant in vitro to multiple antibiotics.[59] Overall the Pasteur Institute of Lyon appears to have produced over 60 therapeutic phages in 1976[51]: about 20 phages for enterobacteria, about 30 for Pseudomonas, and over 10 for Staphylococcus.

Many of the details of phage administration and its effects as described in the 1936 *La Médicine* special monograph and as discussed by Vieu[59] still appear valid in light of today's knowledge and information from other sources. The data and conclusions presented there are in stark contrast to the phage-therapy-critical 1934 [14-16] and 1941 [62,63] JAMA reviews (see below, and as discussed in some detail in ref. 3). A reasonable inference is that phage therapy worked when the phages were manufactured and administered correctly and, not surprisingly, far less so when they were not. The latter was all too often true in much of Europe and the US in the early days of phage therapy, as considered below; see also reference 3, 9, 16 and 19.

Poland. Thousands of patients have been treated with phages in Poland, particularly in association with the Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław, which was founded in 1954. This work has been more thoroughly documented than any other in the English-language literature, mainly in the Institute's own journal in the earlier years and much of the work is available at their web site, www.aite.wroclaw.pl, and/or at www.evergreen.edu/phage. Treatment was performed by physicians from throughout the region, using phages specifically selected and prepared for each patient from the large Institute collection, and with detailed records kept. Every one of their 550 patients from 1981–1986 were included in a series of overview articles and specific discussions of particular conditions.[64-70] Reported cure rates for specific infection types ranged from 75 to 100%.[64]

As with much of the ongoing clinical use of phages, these results represent phage use more as a standard of care than an experimental application, with a typical approach as discussed by Slopek et al. (see ref. 64, p. 570) which we quote:

In phage therapy the use was made of virulent bacteriophages, i.e., inducing a complete lysis of bacterial strains isolated from patients. Bacteriophages were administered orally 3 times daily in the dose of

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10 ml before the meal, after previous neutralization of gastric juice. Phages were also used locally as moist applications to pleural, peritoneal, cavities, urinary bladder and as eye, ear and nose drops. In the course of treatment, sensitivity of isolated bacteria to phages applied was under control; in the case of confirmed resistance, bacteriophages were changed.

Often these were treatments of last resort for chronic bacterial infections that had not responded to standard antibiotic treatment. Such infections are among the most difficult to successfully treat. Indeed, as noted on p. 582 of the same publication, "Unfavorable treatment results may be accounted, to a great extent, to too late initiation of the treatment and also great cachexy of patients with long course of disease." In this vein, however carefully phages were chosen and whatever the nature of the problem, the fundamental health of the patient was also a key factor in the effectiveness of phage therapy, implying utility to not excessively delaying phage application. Thus, for example, Slopek et al.[68] report 84.2% positive results given "severe" disease versus 92.8% for "medium-severe" and 96.9% for "generally good".

Because most of these infections had neither spontaneously resolved nor yielded to antibiotic therapy over long periods prior to phage application, positive results observed after phage treatment have been taken as a strong indication of phage-associated efficacy. However, since these treatments (1) were not blinded, (2) are not presented for all cases in great detail, and (3) since antibiotics were occasionally used in parallel, many have questioned to what extent these apparently impressive results can be considered as definitive proof of phage therapy efficacy from the perspective of western medicine.

Since 2005, the institute itself has had a phage therapy center dedicated especially to treating antibiotic-resistance infections. As Poland is now a member of the European Union, this clinical phage application is being conducted officially within the purview of a western medical regulatory system. The Polish phage therapy experience has been the subject of numerous reviews, particularly as generated by the practitioners themselves, including Beata Weber-Dabrowska, Andrzej Górski, and the late Stefan Slopek;[6,8,71] see also in reference 11 and below for further discussion.

United States. Interest in phages and the use of phage therapy spread quickly to the United States during the above-noted "enthusiastic period", that is, the 1920s and 1930s, which has been described in some detail in reference 3, 16 and 19. One of the first studies of subcutaneous phage administration was carried out at the Michigan Department of Health, where Larkum[72] reported treating 208 patients with chronic furunculosis; 78% of them had no recurring infections for at least 6 months after treatment and only 3% showed no improvement. Schultz[73] and Schless[74] reported remarkable success with staphylococcal septicemia and meningitis, respectively.

Several well-known pharmaceutical companies became actively involved in efforts to produce therapeutic phage preparations in the 1930's. Eli Lilly, for example, produced sterile-filtered phage-lysates (Staphylo-lysate, Colo-lysate, Ento-lysate, Neiso-lysate) and the same preparations in a water-soluble jelly base (e.g., Staphylo-jel) for treating abscesses, purulent wounds, vaginitis, mastoiditis and respiratory infections. As reported by Straub and Applebaum,[75] E.R. Squibb and Sons and a division of Abbott Labs were also involved in commercial therapeutic phage production. Unfortunately, they found that all of them had problems with quality control, stability and establishment of efficacy. For example, they analyzed several lots of commercial phage preparations made by each of the 3 manufacturers and found that several had very low activity titers, presumably due to some of the additives they contained. D'Hérelle also reported that some commercial preparations contained no detectable biologically active phage.[26]

In a year-long American Medical Association review of phage therapy, Eaton and Bayne-Jones[14-16] thoroughly explored over 100 studies of therapeutic phage applications and raised many serious questions, as discussed in some detail by Sulakvelidze and Kutter.[2] Their discouraging JAMA report found consistent, convincing data only for the treatment of localized staphylococcal infections and for cystitis, and the review generally had a dramatically negative impact on the opinions of the medical and scientific communities. As in France, Staphylococcus nevertheless continued to be a major focus of phage therapeutic efforts. Working in New York, MacNeal and Frisbee[76] described their relatively successful treatment of staphylococcal bacteremia in 100 patients. MacNeal et al.[77] subsequently reported very positive results in the cumulative treatment of 500 patients with staphylococcal bacteremia, using cocktails of phages that were lytic in vitro. More details of the early work on Staphylococcus are included in the sections on MRSA and on purulent infections under "Treatment of Specific Diseases", below.

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While the 1934 review made a real effort to deal with the complexities of the then-available English-language literature, a subsequent 1941 JAMA review by Krueger and Scribner[62,63] reflected a singular lack of care in researching the available data, if not outright personal bias in the conclusions. Unfortunately, both had profoundly negative and long-lasting effects on general US attitudes about the potential of phage therapy.

In 1942, editorials in the Lancet and British Medical Journal on Soviet military use of phages against dysentery and gangrene led the US National Research Council/CMR to sponsor a variety of successful and interesting animal studies with phages targeting *Shigella dysenteriae.* René Dubos, who had been very impressed with what he had seen of d'Hérelle's work when they were at Rockefeller and Yale, respectively, was the best known of those who took on the NRC challenge, and obtained important results that still are among the best illustrations of why phages can be so effective. Dubos et al.[78] reported in vivo lysis of bacteria, with multiplication of bacteriophages as protective against experimental infection of mice with *S. dysenteriae*. The bacteria were injected intracerebrally while the phages were injected intraperitoneally, meaning that phages had to get into the bloodstream and then cross the blood-brain barrier to reach their bacterial host. He reported 72% survival if the mice were treated with 10^7 to 10^9 phages, versus only 3.6% with no treatment, and went on to study the phage distribution in the blood and brain with and without the infecting bacteria (see **Fig. 1**). Injection of phages

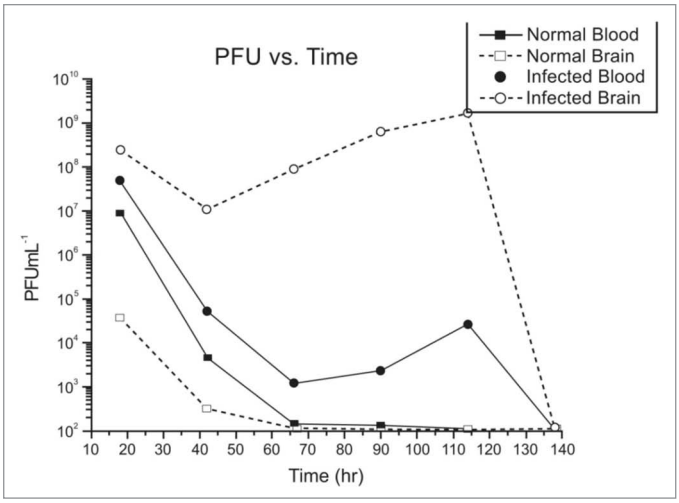

Figure 1. This figure, based on the data in the 1943 mouse studies of Rene Dubos,[78] provides significant insight into why phage therapy works well even in treating infections that antibiotics can't reach. When he injected the mice intraperitoneally with 10^9 phages, they quickly appeared in the blood stream, entering the brain, but they were rapidly cleared. However, if the mice were also injected intracerebrally with *Shigella dysenteriae*, the host for these phages, then 46/64 of the mice survived (as compared with 3/84 in the absence of appropriate viable phage) and the brain level of phage climbed to over 10^9 per gram. Once the bacteria were cleared, phage levels dropped below detection limits.

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that were heat-inactivated or that did not target the infecting bacteria afforded no protection.

Further data refuting many of the conclusions of the JAMA reviews[14-16,62,63] were published by Morton and Perez-Otero[79] who noted an increase in bacteriophages in vivo during experimental infections with *Shigella paradysenteriae*. Even after the widespread introduction of antibiotics in the early 1940's, phages were still used successfully to treat typhoid fever, which was refractory to the antibiotics available at that time. Human typhoid work went on in Los Angeles with increasing success from 1936 to 1949.

At a January, 2001, AstraZeneca Research Foundation conference on drug discovery and development in Bangalore and later on line, Gary Schoolnik, then Chief of Infectious Diseases at Stanford, described what led him to enter the field of microbiology (see also ref. 80). Quoting Schoolnik:

My mother, in 1948, was dying of typhoid fever, before they had antibiotics. My father… read in J. Bacteriology about a Los Angeles scientist who had discovered a phage that killed Salmonella typhi. *He called up this guy, and it was flown up to us in Seattle on a DC-3. My father injected my mother in the hospital with this phage, and the next day she was perfectly well… That's real infectious disease experimentation: a mix of science, and daring and desperation.*

He only later learned that there was much data that laid a solid foundation for this life-saving decision, not just the mouse work he was aware of. Beginning in 1936, for example, many human typhoid patients had been treated with phages in Los Angeles, with increasing success. As reported by Knouf et al. "One of the most spectacular accomplishments… was the rapidity with which the patient returned to his normal mental outlook. In 24–46 hours, patients who had been comatose and in the 'typhoid state' amazed everyone by cheerful, grateful attitude… asked for food vociferously". Similar work was going on in the province of Quebec, as reported by Desranleau.[82] Various formulations of Vi-specific phages were used to treat nearly 100 typhoid patients, at least some of them intravenously with no serious side effects. One of their most effective formulations, containing 6 phages, reduced the mortality from 20% to 2%. However, once chloramphenicol was available for the treatment of typhoid, reports of work with phages seem to have stopped.

As mentioned at the beginning of this section on the history of phage therapy, the renewed realization in the US that phages represented a possible if partial solution to the problem of antibiotic resistance began in the 1990s, prompting the formation of several new companies. Among these companies were Intralytix and Exponential Biotherapies; both initially targeted Vancomycin-resistant Enterococcus (VRE).

Georgia. The Georgian experience with phage therapy centers around what is now the Eliava Institute in Tbilisi, which was founded in the 1930s by George Eliava, in association with Félix d'Hérelle. The pattern followed was one of receiving strains of pathogenic bacteria from throughout the Soviet Union, against which phages were isolated, tested and adapted. Testing included checking for virulence against target bacteria, along with host range, using appropriate panels of what currently were the most problematic strains of the bacteria in question. The result ultimately was a large operation employing 1,200 people, most of whom were involved in phage production, with a production capacity of approximately 2 tons per week. The bulk of their output was shipped to the Soviet military—for treatment of diarrhea and wounds, predominantly—while the rest was available in various forms to the general public. Georgia declared independence from the states of the Soviet Union just before the latter's collapse in 1991. The Georgian military employed phage formulations, with soldiers in 1991 and 1992 carrying canisters of phages during battles in the disputed territory of Abkhazia, and developed special formulations for the key battlefield strains. Phage treatment was again used extensively during the battles following the 2008 dispute in the region between Georgia and Russia (Gvasalia G, personal communication).

"Intestiphage" is among the phage products that are directly available to the public without prescription in Georgia and Russia. This cocktail targets about 20 different pathogenic gastrointestinal bacteria. A second cocktail, "Pyophage", containing phages targeting Staphylococcus, Streptococcus, Pseudomonas, Proteus and *E. coli* is routinely employed in the treatment of various purulent skin or wound infections. We place quotes around the names of the Georgian phage products upon first mention, such as "Intestiphage" and "Pyophage," because Georgia employs a different alphabet, with transliterations therefore varying in spelling, where ინტესტიფაგი and პიოფაგი correspond to the names of these products, respectively. These are actually generic product names, as additional versions are produced by a second Georgian company, Biochimpharm, and in several cities in Russia, such as Ufa and Perm, where factories also flourished in

Table 1. Some human phage therapy studies performed in the former Soviet Union

Authors	Year	Ref	Target organisms	Disease	n	Route	Success	Details
Markoishvili et al.	2002	83	*E. coli* Proteus Pseudomonas Staphylococcus	Ulcers and wounds	96	Phage BioDerm	70%	Healing associated with reduction or elimination of target organisms in 22 patients with ulcers
Lazareva et al.	2001	84	Proteus Staphylococcus Streptococcus	Burn wounds	54	Tablets		Pyophage; Reduced septic complications, better temperature normalization, two-fold reduction of staphylococci and streptococci, and a 1.5-fold Proteus with phage use
Perepanova et al.	1995	85	*E. coli* Proteus Staphylococcus	Acute and chronic urogenital inflammation	46		92%, 84%	92% for marked clinical improvement; 84% for bacteriological clearance
Miliutina and Vorotyntseva	1993	86	Salmonella Shigella	Salmonellosis				Phages versus combined phages and antibiotics was examined with combination effective but not antibiotics alone
Bogovazova et al.	1992	87	*K. ozaenae* *K. pneumoniae* *K. rhinoscleromatis*		109			Adapted phages used; treatment reportedly effective; see also references 88 and 89
Sakandelidze et al.	1991	90	Enterococcus *E. coli* *P. aeruginosa* Proteus Staphylococcus Streptococcus	Infectious allergoses	936		86%	Phages only, n = 360, 86% success; antibiotics only, n = 404, 48% success; antibiotics plus phages, n = 576, 83% success
Kochetkova et al.	1989	91	Pseudomonas Staphylococcus	Post-surgical wounds	65		82%	Cancer patients; treatment was successful in 61% of antibiotic-only treatment
Anpilov and Prokudin	1984	92	Shigella	Dysentery (prophylaxis)				Double-blinded; ca. 10-fold lower incidence of dysentery in phage-treated group

Soviet times. These preparations are not static phage mixtures but are continuously updated in response to changes in pathogen targets. Indeed, they represent formulations that were originally derived from phage products developed by d'Hérelle. Thus, they are not identical through time, or from place to place across the former Soviet Union.[11] In Tbilisi, they are updated every 6 months by addition of phages particularly targeting newly emerging problem strains. Each batch is tested by the manufacturer and then by their Medication Certification Lab against batteries of each of the target strains; they are required to hit a certain specified fraction of the strains for each type before state approval is received for them to be marketed.

The major medical school in the Georgian capital, Tbilisi, now also hosts a Surgical Infections and Phage Therapy program, which is aimed particularly towards thoroughly training a few Georgian surgeons per year in the management of severe surgical and wound infections using phages as key therapeutic elements. Pyophage is the primary phage formulation employed. This cocktail has also been incorporated into biodegradable products such as PhagoBioDerm,[3,11,83,101] a polymeric bandage into which the phages along with other active ingredients are added during manufacture so that phages can be released slowly and continuously over a period of time after application. PhagoBioDerm can be applied to wounds or infections as sheets; it can also be cut into small pieces or ground into powder and placed directly into wounds. In all cases, a basic component of phage treatment of wounds and infections is thorough wound cleaning as well as mechanical removal of infected and dead tissues (debridement) so that the wound can heal properly and phages can easily reach actively replicating bacteria.

For quite detailed discussions of the Georgian phage therapy experience, see in reference 3, 10 and 11. Also, in their 2009

Table 1. Some human phage therapy studies performed in the former Soviet Union (Continued)

Authors	Year	Ref	Target organisms	Disease	n*	Route	Success	Details
Martynova et al.	1984	93	*P. aeruginosa* *S. aureus*	Prophylactic	27 (10)	Mouth rinse		2 times/day for 3-5 days in 27 patients; normalization of microflora in infected sites with IgA production stimulated
Meladze et al.	1982	94	Staphylococcus	Infections of the lung parenchyma and pleura	223		82%	Full recovery seen with phages versus 64% with antibiotics only (n = 117)
Tolkacheva et al.	1981	95	*E. coli* Proteus Dysentery		59			Immunosuppressed leukemia patients treated with improved results in combination with bifidobacteria
Ioseliani et al.	1980	95	*E. coli* Proteus Staphylococcus Streptococcus	Lung and pleural infections	45			Successful phage use in combination with antibiotics
Litvinova et al.	1978	97	*E. coli* Proteus	Antibiotic-associated dysbacteriosis	500		successful	Premature/low-birth-rate infants; phages used in combination with bifidobacteria
Zhukov-Verezhnikov et al.	1978	98	*E. coli* Proteus Staphylococcus Streptococcus	S.I.	60			Improved efficacy using phages selected against bacterial strains isolated from individual patients versus commercial phage preparations
Pipiia et al.	1976	99		Abscessing pneumonia		Parenteral		Multiple treatment approaches including use of phages
Sakandeldze et al.	1974	100	Proteus Staphylococcus Streptococcus		236	Subcutaneous or through surgical drainage	92%	Success = elimination of infections

*Parenthetically are healthy volunteers.

English-language review of the older Georgian phage therapy literature[7] (some of which is in Georgian, some in Russian), Chanishvili and Sharp extensively discuss phage treatment of a range of human diseases. This material is variously cited in the below sections on specific applications and provides an invaluable overview of otherwise hard-to-obtain, non-English language phage therapy studies. Though not generally double-blinded, many of the studies reviewed by Chanishvili and Sharp[7] nonetheless employ negative-treatment controls. This resource also provides extensive insight into the practice and understanding of phage therapy as a regular part of medical practice. The book can be purchased directly from the Eliava Institute, with contact information available through eliava-institute.org.

Despite a long and apparently successful history of phage therapy in Georgia and other parts of the Soviet Union, there has been little primary publication in English-language journals. This was in part due to the intense secrecy behind the Iron Curtain surrounding militarily applicable sciences. Additionally, as phages were often used to deal with otherwise-intractable medical problems and represented the standard of care long before development of double-blinded clinical trials, little of this western "Gold standard" of medical documentation has been performed. So far, there has been little transfer of phages or phage therapy expertise, or indeed enthusiasm for phages to western medical practice. One counter-example is the successful treatment of many Lubbock Wound Center patients with Pyophage from Georgia that led Randy Wolcott to conduct his FDA-approved phase I trial of phage against leg ulcers.[102] The trial itself used 8 fully sequenced phages prepared for him by Intralytix: 2 targeting *S. aureus*, 5 for *P. aeruginosa*, and 1 for *E. coli.*

Russia. The story of Russia's role in the development of phage therapy is long, inaccessible to the non-Russian speaker, and has not been subject to the same kind of scrutiny as has the Georgian experience. Due to limitations in both space and access to resources, we only briefly outline the Russian story here and include a table (**Table 1**) of important papers from the former Soviet Union (FSU). Sulakvelidze and Kutter[3] and Häusler[28] provide substantially more detail of the older Soviet-era work. A review by Letarov et al. on rational phage therapy[103] gives insight into some of the more recent thought and work. See also various English-language reviews by Viktor Krylov.[104,105]

Focus in the USSR was on treatment of diarrheal infections, with anti-dysentery treatment a top priority; infections associated with battle and trauma, particularly targeting gas gangrene; and

also infections of infants and small children. Phages were applied as liquids, tablets, creams, in association with tampons as well as enemas, as aerosols, parenterally via injection, etc., and the technology for making stable, effective tablet forms of phage was first developed there. Phage formulations were produced in large quantities at, for example, the Alma-Ata branch of the Central Institute of Epidemiology and Microbiology and so too were phages produced in Moscow, Stalingrad, Sverdlovsk, Tashkent, etc. Research institutes included the Gorsky Research Institute of Epidemiology and Mirobiology. As in Georgia, in addition to language barriers, phage research funded by the military was deemed to be a state secret, thereby hindering publication. An additional issue that interfered with wide consideration of this work stemmed similarly from phages representing a standard of care, resulting in a tendency in many (but not all) publications to not compare phage treatments with phage-less controls but instead, for example, to compare the efficacy of one phage formulation with each other. A major Soviet pharmacological giant, Microgen, has now taken over all of the commercial phage production in Russia, and a wide variety of their phage preparations are available in pharmacies as well as on line,[3,103] and, reportedly, are used extensively in many parts of Russia. However, they seem to have published little in terms of phage characterization or clinical trials.

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Phages as Antibacterial "Drugs"

Before discussing additional documentation of phage therapy of human patients—as has been used in the treatment of specific categories of infection—we first explore basic principles of phage use as antibacterial "medicinals". In this section in particular we consider issues of phage isolation, purification and choice for therapy; phage therapy pharmacokinetics; phage safety; and phage use as probiotics. Note that the *safety* of phage therapy can be described as a *secondary pharmacodynamic* issue. Efficacy, which for phage therapy can be measured in terms of whether or not bacteria are reduced in numbers or improvement occurs in other clinical signs and symptoms, is the *primary* issue of pharmacodynamics, which we return to in the above-noted last section of this review.

Phage isolation, choice and purification. The first steps of all phage therapy protocols involve some combination of phage isolation and phage choice.[106,107] As practiced, phage therapy typically relies on one of two models of phage choice. The first involves cocktails of multiple phages that display a wider spectrum of activity than their individual phage components, such as Intestiphage and Pyophage, which allows for use against a wider array of bacterial targets and virtually eliminates any resistance developing in the short term. The first modern, commercially available therapeutic phage preparations in the West will probably be based on this approach. In the second approach, pathogenic bacteria are isolated from infections and tested against a large, generally well-characterized collection of previously isolated phages. Some studies from Russia, e.g., Zhukov-Verezhnikov et al.[98] showed that the resulting custom-designed phage preparations often worked better than mainstream-production phage preparations. This is also the approach generally used in Poland.

The procedures by which phages are isolated are similar regardless of the phage-choice model used and generally involve some form of phage enrichment. Though enrichment tends to preclude an even sampling of a phage-containing community, this serves two basic useful functions with regard to phage therapy. The first is that enrichment tends to bias isolation towards those phages that display greater antibacterial virulence, at least in the sense of being able to propagate at the expense of target bacteria under the specific environmental conditions within which enrichment takes place. The second is that phage isolation is biased towards those phages that are readily propagated in the laboratory, which is a very helpful property when it comes to amplifying phages to prepare stocks for application to patients.

Some degree of purification is generally required following laboratory amplification.[106] The simplest of phage purification protocols involve clarification of lysed cultures via either centrifugation or filtration. Phage precipitation, employing polyethylene glycol or, for the larger phages, high-speed centrifugation, also can be employed. These approaches largely remove remaining uninfected cells, post-lysis bacterial ghosts and some other bacterial lysis products. More stringent purification—generally used for more invasive applications of phages—involves either ultracentrifugation, a series of filtration and washing/buffer-exchange steps, or various forms of chromatography.

Phage therapy pharmacokinetics. The pharmacology of phage therapy has been subjected to a handful of reviews that emphasize various aspects of the subject[61,108-110] along with a few additional articles that touch upon it.[111-113] Pharmacology, as generally defined, is the study of a drug's impact on the body as well as the body's impact on drugs. These two perspectives are differentiated, respectively, into what are known as pharmacodynamics and pharmacokinetics. Note that the concept of body, when considering antimicrobials as drugs, includes both normal body tissues and the numerous symbiotic microorganisms. In this section we focus on considerations of pharmacokinetic aspects of phage therapy pharmacology.

Pharmacokinetics is a description of a drug's potential to reach densities in the vicinity of target tissues that are sufficient to achieve primary pharmacodynamic effects. This description typically is distinguished into absorption, distribution, metabolism and excretion components. Absorption is drug movement into the blood, distribution is drug movement into other body tissues, metabolism is the modification of drugs within the body, and excretion is the movement of drugs out of the body. All four of these pharmacokinetic aspects have the effect of both *reducing* and *increasing* drug densities. Both absorption and distribution, for example, result in declines in drug densities due to drug dilution, which at the same time increases drug density in specific body compartments. For phages, metabolism can represent phage inactivation, as due to phage interaction with immune systems, or "activation", such as phage in situ replication. Lastly, while excretion can certainly play a role in the reduction of drug densities in the body, so too it can also serve as a route towards increased drug densities in places such as the bladder, with therapeutic benefits.[6,114]

In any case, phage therapy success depends on the generation of sufficient phage densities in the vicinity of the target bacteria to

cause bacterial clearance from the body at some adequate rate or to some adequate degree. Phages will increase to sufficient densities due either to in situ replication, so-called active treatment, or as a consequence of what can be described as pharmacologically conventional dosing, so-called passive treatment. These means of increasing phage density must be sufficiently robust that they are able to counter mechanisms of phage loss. The goal, pharmacokinetically, is thus to attain and then sustain in the vicinity of target bacteria whatever minimum phage densities are necessary to achieve desired levels of bacterial eradication.

Safety of phage therapy. Secondary pharmacodynamics are descriptions of a drug's toxicity as well as its degree of impact on non-target tissues. In this section we briefly discuss the issues of phage impact on body tissues (as opposed to impact on normal microbiota), the potential for phage particles to stimulate immunological reactions, phage impact on non-target microbiota, and the ability of phages to modify bacterial targets, all as can result in side effects. Notwithstanding our presentation strategy, emphasizing what can go wrong, in fact phage therapy as currently practiced rarely if ever results in more than minor side effects. As phage applications broaden and escalate, care in phage selection and therapeutic implementation may well be important in assuring a continuation of this enviable track record. A number of reviews address issues of phage therapy safety in some detail.[3,22,105,115,116]

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Non-antimicrobial chemical drugs are generally intended to interact with and modify body tissues in some manner over the shorter or longer term. Drugs that interact with multiple tissues, however, run the risk of interacting with non-target tissues, which can result in changes that would not be considered clinically favorable. Phages clearly interact with non-target tissue to some extent. For example, at least some phages are taken up from the gastrointestinal tract into the blood[61] and there is reason to believe that such uptake can be a consequence of specific phage-to-epithelium interactions,[117] as also appears to be the case given phage interaction with the reticulo-endothelial system.[118] These interactions with body tissues, however, do not appear to result in side effects.

The immunology of phages has been a subject of study for well over a half a century, both in terms of the generation of humoral immune responses and the potential of immune responses beyond just humoral immunity to result in the inactivation of phage virion particles.[3] In the course of these studies, no phage potential to initiate substantial anaphylaxis has been reported. Indeed, a group led by Górski have provided evidence of a positive impact of phages on immune system functioning[6] and have explored potential phage anti-tumor properties mediated through observed shifts in levels of various cytokines as a consequence of interactions between extra decorative *head* proteins with surface proteins of certain immune-system cells.[119] Note that this does not involve nor imply an ability of phages to *infect* mammalian cells.

Part of the apparent mildness of phage particles in their interaction with human tissue is that animals have been exposed to large numbers of phage virions presumably over the entire course of animal evolution, perhaps resulting in greater levels of tolerance than one observes with the application of novel—to us—chemotherapeutic drugs. Furthermore, activation of substantial immune response to, for example, bacteria or foreign cells generally involves extremely large numbers of copies of the same cell-surface component. Getting high-level production of phage antibodies from rabbits and other animals, however, involves the use of strong adjuvants and carefully-controlled, wide-spaced timing of multiple injections (Gachechiladze L, personal communication). It should be noted, though, that Ochs and colleagues (in ref. 120) have repeatedly shown production of antibodies to IV-administered phage ϕX174 without adjuvants, including in patients with a variety of immunodeficiencies.

An important aspect of many chemical antibacterials is their breadth of action. This is a positive aspect because it can allow antibiotic application prior to determination of the antibiotic susceptibility of a pathogen. This property, however, is a double-edged sword, since non-target as well as target bacteria are impacted by such broad-spectrum antibiotics. The result can be *dysbiosis*, that is, a negative impact on important normal bacterial flora. One result of antibiotic usage consequently can be antibiotic-associated superinfections, such as vaginal yeast infections or *Clostridium difficile*-associated colitis. Most phages, by contrast, possess only narrow spectra of activity: Even when phages are mixed into cocktails, their overall activity spectrum remains relatively narrow. The result is a lower potential for side effects associated with dysbiosis, a phenomenon that does not appear to be a concern associated with phage therapy.

The one potentially serious concern that has been expressed of phage therapy safety is the ability of at least some phages to modify host bacteria in ways that could make them more pathogenic. In particular, it is generally important to avoid using temperate phages for phage therapy purposes. A temperate phage is able to display a property known as *lysogeny,* where phages incorporate their genomes into the bacteria they are infecting rather than immediately killing the host and producing phage progeny. Once there, a long-term relationship ensues where the phage exists as a component of the bacterium, forming a lysogen. The problem with lysogens is at least four-fold. First, bacteria that are lysogenically rather than lytically infected do not die as a consequence of infection. Second, bacterial lysogens tend to be resistant to the phage types that have lysogenically infected them, resulting in no bacterial killing even if subsequent phages of the same type succeed in infecting. Third, temperate phages often display lysogenic conversion, meaning that they modify the bacterial phenotype, sometimes in ways that result in increased bacterial virulence.[121-123] Lastly, temperate phages are associated with certain forms of transduction, meaning that they can fairly readily pick up new genes from the bacteria they are infecting and then transfer those genes to subsequently infected bacteria—without killing those new bacteria. For each of these reasons one should avoid temperate phages as therapeutic agents, if that is possible.

Non-temperate phages can also display transduction and perhaps could also carry bacterial virulence factor genes, either constitutively or inadvertently. However, those phages either kill the new host if they carry only a few non-phage genes, avoiding longer term problems, or if they carry either primarily or only

bacterial genes then at least the phage cannot propagate further. These concerns can be mitigated in the course of phage characterization, both genomic and phenotypic. However, a substantial amount of phage therapy has been conducted on humans as well as animals, and to date there does not appear to be evidence that these factors are concerns in the course of phage therapy where non-temperate, "obligatorily lytic" phages are used. These mostly theoretical concerns can be further addressed by analyzing genomes of all phages that are considered for inclusion in therapeutic phage cocktails, to ensure that they do not carry any toxin-encoding or other undesirable genes (for example those specified by the FDA in 40 CFR §725.421).

Also related to the phage potential to enhance the toxicity or virulence associated with bacteria is phage-mediated bacterial lysis, resulting in the release of *bacteria*-encoded toxins. In the case of endotoxin, this release can be substantially greater than in the absence of such lysis,[124,125] though keep in mind that many antibiotics also possess this property of lysing target bacteria. In the case of exotoxins it is an open question whether phage application will result in any more than an acceleration of toxin release, particularly to the extent that phage infection results in a shut down in bacterial gene expression and thereby a cessation at least of the transcription of bacterial exotoxin genes.

The release of endotoxin places limits on the phage treatment of gram-negative systemic infections,[22] just as phages employed systemically also require additional purification steps to avoid carrying over endotoxin generated during phage production. Slopek et al.[65] for example, though otherwise observing few side effects, did report that some patients experienced several hours of pain in the liver area 3 to 5 days into treatments. This, as the authors suggest, might conceivably reflect phage-mediated liberation of endotoxins and other bacterial pathogenicity factors in the course of bacterial lysis (see also ref. 22), though also it could have been treatment-unrelated symptoms of the ongoing illness in the patients. In other patients some fever was observed approximately one week into treatments. Lysates from Gram-positive organisms when injected also can lead to "side effects ranging from mild to severe", as discussed by Sulakvelidze and Kutter[3] (and more generally may explain the "violent reactions" following phage injection described by Mikeladze[53] and as discussed above). These issues are less of a concern when treating bacterial infections locally. It is also possible to modify phages to prevent lysis so that toxin release is less of a concern,[107] but that obviates one of the basic advantages of phage therapy—the ability to move throughout the organism and multiply where needed.[107]

Phages as probiotics. One concept of probiotic intervention that has received very little attention in the past is the use of phages to specifically target "problem" bacterial species in the GI tract. Although not typically thought of as probiotics, bacteriophages actually fit well the definition by the Food and Agriculture Organization (FAO) of the United Nations (UN) and World Health Organization (WHO) of probiotics: "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host".[126] Because of the specificity of bacteriophages, bacteriophage-based "probiotic products" may provide a novel, safe and effective approach for favorably manipulating the GI tract's microflora. In particular, (1) phages may reduce or eliminate their targeted pathogenic bacteria in the gut, with no deleterious effects on the beneficial microflora and (2) regular consumption of phage-based probiotics (alone, or in combination with bacteria-based probiotics and prebiotics) may gently and favorably alter the GI tract's microflora and thereby provide added protection against the specifically-targeted diarrhea-producing bacterial pathogens.

Phage-based probiotics would be used prophylactically rather than therapeutically, although it is clear that prophylactically supplied phages also could act therapeutically if administered early in bacterial infections. While probiotic bacterial formulations introduce nonpathogenic bacteria to interfere with the ability of pathogenic bacteria to colonize the GI tract, phage-based probiotics aid the GI-tract balance by targeting specific pathogenic bacteria. They are likely to be most successful for managing pathogens such as *Salmonella* spp., *Clostridium difficile,* diarrheagenic *E. coli* and other bacteria that have an oral portal of entry and require short- or long-term colonization of the GI tract in order to cause disease. At least one recently-published paper suggests that the approach has merit,[127] while additional research is currently underway (Sulakvelidze A, personal communication). Because of their high specificity, phages are likely to be unique tools for manipulating the microflora composition of the GI tract in a much more specific way than has been possible with other probiotic organisms or other antibacterial agents. Hence, a phage-based approach is likely to open new and exciting avenues for subsequent research in many areas related to basic and applied probiotic research, the GI tract's microflora and nutrition.

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Phage Approaches to Treatment of Specific Diseases

In a world in which antibiotics represent the standard first-line therapy against bacterial infections, the envisaged use of phages tends to focus on three main indications: to combat infections involving antibiotic-resistant bacteria, to combat infections that are antibiotic resistant despite the sensitivity in culture of their etiologies (e.g., as due the poor circulation of osteomyelitis and diabetic ulcers or to biofilm formation), and to target bacteria under circumstances where antibiotics are counter-indicated due, for example, to patient allergies, irritable bowel problems or fear of *Clostridium difficile*, as well as in food and agricultural applications due to concerns with excess human and environmental exposure to antibiotics. Though we might imagine that phage studies therefore would tend to involve direct comparisons between phage and antibiotic application, the bulk of the literature documenting phage treatment of humans involves case studies rather than blinded experiments and is therefore subject to experimenter's bias. Though treatments typically are of infections that have resisted antibiotics—sometimes over many years—one must still ask whether something other than the phages employed might in fact be responsible for these documented patient recoveries or whether the infections might have spontaneously resolved during therapy due to factors independent of the phage treatment process. These are questions to keep in mind as we explore the

published phage therapy literature, with a focus on work from the antibiotic era, particularly that has taken place since the molecular nature of bacteriophages became well understood,[128,129] the tools of molecular biology have become available as applied to phages,[130] and phages have been better appreciated as ecological[131] as well as pharmacological[109] entities.

Skin ulcers. We begin with skin ulcers because their treatment has recently been subject to active experimentation within the US. In addition, the treatment of skin ulcers can be used as a good example of the benefits as well as concerns associated with phage therapy, with concerns tending to be associated predominantly with how procedure efficacy is determined (previous paragraph). Overall, the employment of phages to treat infected skin ulcerations within the context of western medical practice would appear to be quite promising. Infected skin ulcerations can be chronic and resistant to antibiotic treatment. Phages can be topically applied with impressive success, though rarely if ever within the context of rigorous, double-blinded and peer-reviewed studies.

Markoishvili et al. report on the use of PhagoBioDerm, the phage impregnated polymer, to treat infected venous stasis skin ulcers. To patients that had failed to respond to other treatment approaches, PhagoBioDerm was applied to ulcers both alone and, where appropriate, in combination with other treatment strategies. Complete healing of ulcers was observed in 70% of nearly 100 patients. Slopek et al.[64] report 75% positive results for phage treatment of 36 cases of "Varicose veins with ulcer and inflammation" and 95.0% positive cases (our calculation) for phage treatment of 162 "Diseases of the skin and subcutaneous tissue", which includes furunculosis, "inflammation of the connective tissue and lymphatic vessels" and decubitus ulcer.

In the Chanishvili and Sharp[7] book, the chapter on "Phage therapy in dermatology" reports on the successful treatment of deep forms of dermatitis, such as furunculosis, abscesses and hidradenitis, as described in several articles. Treatment of acute furunculosis and hidradenitis was highly successful and treatment of chronic furunculosis was successful in the majority of cases. After disinfecting the lesion and surrounding area, pus was drained and cultured, tested for phage sensitivity, and phages were injected with an initial dose of 0.5 mL into the lesion and surrounding areas, and with increasing daily doses depending on the response. Vartepetov (1957), as cited, noted that a total of 6,000 patients had been studied: healing occurred within 4–8 days, and 70–100% of patients were cured. New lesions were healed with a reduction in scarring. Pain typically disappeared after 1–2 injections. Intradermal injections were more efficient than subcutaneous injections. A series of intradermal injections was typically given every other day at increasing doses (0.1, 0.2, 0.3, 0.4 and 0.5 mL, repeated for a total of 7–10 injections). A later article by Shvelidze in 1970 reported the treatment of staphylococcal boils, furuncles, carbuncles and hidradenitis in patients who had failed antibiotic treatment and noted 94.4% successfully treated, 4.3% with significant improvement and 1.3% with no improvement.

A successful physician-initiated FDA-approved phase I safety trial of phage therapy against skin ulcerations and other wounds was completed in 2008 at the Wound Care Center in Lubbock, Texas, following a series of positive results with Pyophage brought from the Eliava Institute over a period of several years under "compassionate use" provisions. For the phase I trial[102] a special formulation of fully sequenced phages prepared by the company Intralytix, containing only two phages active against *S. aureus*, five against *P. aeruginosa* and one against *E. coli*, was applied to chronic infections without observation of significant side effects. Phage phase I safety trials have also been administered by the companies Exponential Biotherapies[11] and Biocontrol, though only the latter is published.[132] See also in reference 133 for an additional phage safety trial and reference 11 for review and discussion of these trials.

Cislo et al.[134] review the treatment of 31 patients displaying suppurative (a.k.a., purulent) chronic infections of the skin; this work is of particular interest due to photographic documentation. They reported "outstanding", "marked" and "transitory" improvement in 16, 7 and 2 cases, respectively; phages were delivered both locally and per os. These positive results were observed between 2 and 16 weeks after the initiation of treatment and followed all of the bacteria isolated from the infections, implementing replacement of phages when resistance to the initially-used phages was noted. No antibiotic co-treatment was performed but patients with leg ulcers were given drugs that resulted in dilation of peripheral blood vessels. Side effects included intensification of pain (2 cases) and eczema near the point (4 cases). Oral application was associated with nausea in about 10% of the cases and vomiting in one.

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Purulent infections. Purulent infections are those from which exudate, that is, pus, is actively released. An early example of such treatment (1927) took place in Poland as conducted by Jaseinski, reportedly with particular success against furuncles as well as other purulent wounds and few side effects.[10,135] In Georgia, the pyophage formulation is routinely employed to treat purulent infections with high rates of success even given severe infection.[10]

Generally, treatment of infections close to the surface of the body involves a number of steps. The first is cleaning the wound quite radically, including removal of necrotic tissue (debridement), which is crucial in an ongoing fashion with all wounds. Often, the typical procedure of incision and drainage is done, so that the wound is open and still-living tissue is highly accessible. The next step is to assure adequate drainage of exudate from the wound. As usual, premature closure of the wound before the infection is adequately treated such that the wound has been rendered sterile can lead to many complications. Georgian surgeons find that use of a Pyophage cocktail—including as infusions and in the form of PhagoBioDerm, etc.—during early wound treatment facilitates far earlier wound closure and faster healing.

Weber-Dabrowska et al.[135] indicate that phage therapy has been effective in the treatment of purulent cerebro-spinal meningitis, otitis media and postoperative infections.[65] Slopek et al.[64] reported 100% positive results for phage treatment of 7 cases of "Purulent pericarditis" and high success rates for a variety of other infections described as suppurative. Similarly, Slopek et al.[68] reported 92.4% positive results in the treatment of 370 cases of suppurative infection of which 241 cases did not involve

parallel antibiotic therapy (96.2% positive results). In 18 cases, phages were used in patients who had not been previously treated with antibiotics. As a novel twist, phages in this study to some degree were "propagated on the strains isolated from patients". Six additional papers by Slopek et al. consider "Results of bacteriophage treatment of suppurative bacterial infections"[65-70] and generally indicate high rates of positive results.

***Staphylococcus aureus*: methicillin-resistant (MRSA).** MRSA is a particular concern given its inherently reduced susceptibility to antibiotic treatment, wide prevalence in hospital-acquired infections and in the community, and potentially lethal and otherwise serious consequences. These pathogens are targeted by the anti-*S. aureus* activity of phage preparations such as Pyophage (which include potent anti-Staphylococcus phages of the broad-spectrum Sb1-staph phage K family; Kutter EM, unpublished results). Here as elsewhere, there is no cross-resistance between phages and antibiotics. Furthermore, very little development of resistance to this family of phages is observed, presumably implying that their still-unidentified primary receptor is a molecule of significant importance to the cell. Thus, so far as phages are concerned, MRSA is simply another strain of Staphylococcus.

Treatment of MRSA using phages can be accomplished by local application for local infections or, if necessary, and with substantially more caution, more systemic dosing such as intraperitoneally for systemic infections.[76] The use of phage treatment for local infections, including particularly those due to Staphylococcus, has the distinction of being one of only two phage therapy strategies that were deemed to be convincingly efficacious by the 1934 Eaton and Bayne-Jones report,[14-16] an otherwise phage-therapy skeptical publication; see also reference 77. Indeed, the first human phage therapy publication reported on treatment of *S. aureus* skin infections.[3,22,44] Phage preparations for systemic application were developed at the Eliava Institute during the 1980s, including safety studies in human volunteers without adverse effect. They were particularly effective in infants, in immune-compromised patients and for infusion into the urethra in cases of pelvic inflammatory disease. The preparation subsequently was used to treat 653 patients.[3]

Historically, questions have been raised as to whether the efficacy documented in these classic articles was due to the phage itself giving rise to bacterial lysis in situ. It has been suggested that the debris in the phage lysate, stimulating the host immune system, could be a major factor in bacterial clearance.[3,62] See, for reference 4 and 11 for discussion of what is known variously as Lincoln Bacteriophage Lysate, Staphylococcus Phage Lysate or simply SPL, produced by Delmont laboratories. This product is marketed as a veterinary vaccine conferring resistance to staph through immune stimulation by its staphylococcal-phage induced bacterial lysis products, which is advertised as the major active ingredient. It does also contain viable phages, often at ~10^8 PFU/mL (Kutter EM, unpublished data; Kuhl SJ, unpublished data), a level as high as the total phage in Pyophage as determined by direct fluorescent microscopic count (Brown N, personal communication). These staph phage lysates initially were produced for human as well as animal use against chronic infections.[3] However, in 1994 they were limited by the FDA to animal use pending further human efficacy trials, for which no funding has yet been found; no questions have been raised as to their safety.

Phage use to *prevent* Staphylococcus infections has been both proposed and employed. Use of phages for disinfection has been carried out in Georgia to sanitize operating rooms and medical equipment and prevent nosocomial infections.[22] A complementary approach proposed by the company Novoltyics is to use "a gel containing a cocktail of phages targeted at MRSA to treat nasal carriage of MRSA, thus significantly reducing the incidence of MRSA transmission" (see www.novolytics.co.uk/technology.html; see also ref. 136). O'Flaherty et al. describe removal of *S. aureus* via experimental hand washing with a phage-containing Ringers solution. Approximately 100-fold reductions in bacterial densities were observed after washing with a solution containing 10^8 phages/mL versus the phage-less control solution.

Leszczynski et al. describe the use of oral phage therapy for targeting MRSA in a nurse who was a carrier. This individual was MRSA colonized in her gastrointestinal tract and also had a urinary tract infection. The result of phage therapy was complete elimination of culturable MRSA. In an earlier publication the same group argued that MRSA treatment using phages can be economically preferable to MRSA treatment using antibiotics;[139] by contrast, though, see in reference 140. Jikia et al. describe phage treatment of MRSA infecting radiation burns (below).

Slopek et al.[64] report 92.4% positive cases for phage treatment of 550 single- and mixed-etiology infections involving *Staphylococcus aureus*. Slopek et al.[70] addresses specifically phage treatment of suppurative staphylococcal infections, with a reported 93% "effective" rate "based on case history and data contained in a special questionnaire" (p. 262), while Slopek et al.[69] consider the treatment of Staphylococcus infection of children (95.5% positive results, our calculation, of 90 children treated).

Wound prophylaxis. Wounds can be due to surgery, accident or burns. The term wound does not imply infection, and of course one object in wound care is to avoid infection. Thus phages can be used for prophylaxis to prevent infection, including for the prophylaxis of surgical site infections. Phage treatment is particularly indicated in Georgia under circumstances where wounds are large in area and where therapeutically effective concentrations of systemically administered antibiotics are not easily attained.[11] In this section we concentrate specifically on such wounds and on the prevention of infection, drawing especially on the "Phage therapy in surgery and wound treatment" chapter in the Chanishvili and Sharp[7] book and the detailed discussion of the current Georgian situation by Kutter et al.[9] Phages can be supplied in the course of wound irrigation, can be soaked into bandages, and/or can be provided in a time-release manner (as discussed above in terms of PhageBioderm). Resulting phage densities are a product of the numbers provided and those generated in the course of in situ phage replication.

Wounds can become infected with a variety of pathogens, including those that can give rise to gangrene. The Russian army during World War II successfully treated wounds using phages. In one study, for example, of those receiving anti-Clostridium phages following battlefield wounds, ~80% survived versus only

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~60% survival among those not receiving phage treatment.[10] In another study, prophylactic treatment saw three-fold greater gangrene incidence among non-phage-treated soldiers versus those who were phage treated.[141] Pyophage spraying of wounds has been employed by Georgian soldiers on the battlefield, reducing infection, prolonging the time over which treatment is optimally performed, and increasing rates of recovery.[11] Slopek et al.[64] report 90.8% positive cases for phage treatment of 305 "injuries".

Burns. An ongoing major concern in burn wounds is infection, which also often deters skin grafting. In a promising animal model, Soothill[142] showed that phage application to wounds prior to grafting could block challenges with *Pseudomonas aeruginosa* bacteria. This is an impressive result given that successful grafting requires not just prevention of bacterial growth but also tissue healing. More generally, phages have not been found to interfere with wound healing and, as some propose, may even contribute positively to healing.

Jikia et al. describe the use of phages to treat radiation burns suffered by three Georgian lumberjacks. These individuals had the misfortune of encountering Soviet radiothermal generators in the woods (^{90}Sr), which they used to warm themselves during the night. The burns subsequently became infected with MRSA. Following unsuccessful antibiotic treatment, phage therapy was attempted using PhagoBioDerm preparations in sheet form. Due to the large size of the resulting ulcerations, multiple PhagoBioDerm sheets were applied to fully cover the burn area. Purulent drainage, which had not been impacted by antibiotic treatment, decreased "to almost none" after two days of PhagoBioDerm treatment. Testing failed to detect MRSA on day seven. As no phage-less controls were employed, this study does not constitute proof of phage-mediated efficacy. Nonetheless it is strongly indicative that further study is warranted. Lazareva et al. similarly have reported positive results of phage treatment of infected burns.

Abdul-Hassan et al. reported on the treatment of 30 cases of burn-wound associated antibiotic-resistant *P. aeruginosa* sepsis. Bandages soaked with 10^{10} phages/mL were applied three times daily. Half of the cases they describe as "improved" (such as in terms of granulation criteria). In 18 of 30 cases there was good (12) or excellent (6) skin graft take. Discharge was eliminated in 12 cases and diminished in another 12. Furthermore, in 8 of the 30 cases, infection was eliminated ("sterile cultures").

These results are fairly typical of modern, documented phage therapy applications such as those presented in the Slopek et al. studies, that is, achievement of sterility and/or complete healing in a moderate fraction of cases, substantially positive clinical results in a much greater fraction and at least some improvement in many or most. Following this precedent, Marza et al. applied filter disks to which phages were applied to the *P. aeruginosa* infected burns of a 27-year-old male. After 48 hours the phages were shown to increase their presence in the disks by three orders of magnitude. Phages were then applied more generally but no reports of microbiological impact are provided and whether phages contributed to subsequent patient healing is inconclusive.

Recently, a group of Belgian surgeons and scientists have developed an extensive collaboration with phage biologists in both Moscow and Tbilisi to explore the possibilities of using phages in burn applications. In preparation, they carried out a year-long study of *P. aeruginosa* colonization and infection during which a total of 441 patients were treated at the 32-bed Burn Wound Centre of the Queen Astrid Military Hospital in Brussels. Of these, 70 were colonized with *P. aeruginosa*, 57 of whom acquired the organism during their stay. Eight patients infected with *P. aeruginosa* died. For three of them, no other bacteria were detected and death was directly attributed to the *P. aeruginosa* infection. They have now carried out a small phase I study involving nine patients, approved by a medical ethics committee, in which a section of a large burn on each patient was exposed to a single spray application. A distant portion of the same wound was used as a control, with no phage included in the treatment applied there. Both regions were monitored with tissue biopsies before application and between two and five hours after treatment application by bacterial quantitative culture. The patients were carefully monitored for a period of 3 weeks after the treatment. No adverse events, clinical abnormalities, or changes in laboratory test results that could be related to the application of phages were observed.

Merabishvili et al.[144] describe in extensive detail the quality-controlled production of the BFC-1 phage cocktail used for the above Belgian human clinical trials. This cocktail consists of three phages, a Myovirus and a Podovirus against *P. aeruginosa* and a Myovirus against *S. aureus*. These obligately lytic phages were selected from a pool of 82 *P. aeruginosa* and eight *S. aureus* phages using a batch of *P. aeruginosa* and *S. aureus* strains that are representative of the most prevalent isolates in the BWC of the QAMH. The cocktail was purified of endotoxin. The elaborate quality control included stability (shelf life), determination of pyrogenicity, sterility and cytotoxicity, confirmation of the absence of temperate phages, and transmission electron microscopy-based confirmation of the presence of the expected virion morphologic particles as well as of their specific interaction with the target bacteria. Phage genome and proteome analysis were consistent with the conclusions that the chosen phages were not temperate and that there was an absence of toxin-coding genes. Their efforts are discussed also by Kutter et al.[11]

Poorly accessible infections. A number of infections for various reasons are not highly susceptible to either systemically administered or topically applied antibiotics. With systemic circulation the problems can be two fold, with either poor penetration to peripheral tissues starting from general circulation (which is *distribution* in pharmacokinetic terms) or a requirement for higher plasma antibiotic densities than can be safely realized (which is a secondary pharmacodynamic concern). The concerns with topical application are similar but with a slightly different emphasis, one of limitations to diffusion—from the point of application to sufficiently deeply into infections—and again possible toxicity given antibiotic application in sufficient amounts that indeed satisfactory densities are attained in the vicinity of target bacteria. Antibiotics also may be diluted or indeed inactivated within the complex conditions found directly within infections,[11] or can be ineffective even given reasonable penetration such as into bacterial biofilms.[145] Examples of such situations

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where antibiotics typically display poor or inadequate penetration properties to target infections include osteomyelitis, diabetic infections of the feet, burns and infections of the central nervous system, which can be protected from antibiotics due to the presence of the blood-brain barrier (but which does not necessarily prevent adequate phage penetration to combat infections as shown by Dubos et al.—see **Fig. 1**). These are all circumstances in which phage therapy may be more efficacious than antibiotic chemotherapy despite infection with fully antibiotic-susceptible bacteria.

By way of example of the phage potential for treating poorly accessible infections, Lang et al.[48] reported treating five out of seven orthopedic cases successfully and one case partially successfully, out of a total of seven. Slopek et al.[64] report 89.5% positive cases for phage treatment of 19 "Pyogenic [arthritis] and myositis" and 90% positive cases for phage treatment of 40 "Pyogenic ostitis". Furthermore, in Georgia, chronic osteomyelitis serves as a primary indication for phage treatment; in most cases this requires debridement of necrotic tissue along with the phage treatment, but is much faster and more likely to be effective than treatments not involving phage. Slopek et al.[64] also report 90% positive cases for phage treatment of 10 cases of "Meningitis". For additional discussion of phage use against infections to which antibiotics can display poor penetration, see Kutter et al.[11]

Eye infections. In summarizing the literature available to them on phage treatment of eye infections, Górski et al.[146] write (p. 164):

Current experience with phage applications in [ophthalmology], judging from available literature, is very limited and involves some 28 patients treated with antistaphylococcal phages for conjunctivitis and blepharitis. Proskurov[147] applied phages as eye drops (2 drops 3 times daily) in conjunctivitis, while in blepharitis this was supplemented with topical application of a phage suspension onto the eyelids. The authors claimed good effects in all 17 patients, although no details were provided.[147] In 7 cases of conjunctivitis, 1 of dacryocystitis and 3 of hordeolum described by a group from our institute, eye drops and moist applications caused full recovery.[64] No side effects were reported by either group.

The book reviewing the Eliava experience also details the experience with phage therapy in ophthalmology.[7] Dautova and colleagues used "Pio" bacteriophage to successfully treat 30 patients with traumatic bacterial keratitis and 16 patients with purulent corneal ulcers. Equal numbers of patients in control groups were treated with gentamicin eye drops. The patients treated with bacteriophage showed a more rapid improvement in inflammation, pain and eye watering, and were discharged on average at 11 days instead of 15. The successful treatment of 32 children with acute bacterial conjunctivitis was also described by Kilasonia and Karanadze (2001), as cited. The bacteria were antibiotic resistant and the patients were allergic to antibiotics, making antibiotic treatment impossible. All cases improved by the third day and were cured by the seventh day; there were no relapses during the next month of observation.

Gastrointestinal ailments. D'Hérelle discovered bacteriophages in association with the examination of dysentery in humans[148] and quickly developed an interest in the purposeful treatment of dysentery using phages.[22,29] Experimental anti-dysentery trials also were extensively conducted over multiple decades in eastern Europe and Georgia, though often with insufficient publication of documentation.[10,32] Much of what we know of them relies on abstracts presented at meetings held around the former Soviet Union—particularly at the Eliava Institute—as well as dissertations. One of the principle advantages of phage treatment of gastrointestinal ailments over treatment with antibiotics appears to be a reduced disruption of gut flora.[11]

There are reports of prophylactic phage therapy success in treating Russian soldiers suffering from dysentery both during and after World War II, and even extensive trials in the southeastern Muslim Republics that showed ten-fold decreases in incidence, though generally with either poor or difficult to penetrate documentation.[10] One well-controlled anti-dysentery trial[149] was conducted in Tbilisi on 30,769 children. Neighborhoods were split up with one side of each street treated prophylactically with a phage cocktail and the other a placebo. The result was a 3.8-fold decrease in dysentery incidence with phage treatment. Similar follow up trials were undertaken with 20,000 and 5,000 children respectively as well as another against Salmonella-associated disease. Additionally, the Slopek et al.[64] report of trials between 1981 and 1986 shows 91–100% positive cases for phage treatment of 42 "diseases of the digestive system" and six "infection diseases of the alimentary tract". In Georgia the Intestiphage formulation is routinely employed prophylactically to prevent nosocomial infections, especially in pediatric hospitals, where such gastrointestinal infections are particularly prevalent. Dosing has traditionally been done with phages prepared in tablets, though since the break-up of the Soviet Union the tablet form has been seldom available, so the liquid form is used instead.

Relatively early in the development of phage therapy its power was directed towards the treatment of infections by *Vibrio cholerae* as well as treatment of wells in India with anti-cholerae phages, both with some success, as reviewed very extensively by Summers.[25,42] The cholera prevention trials in particular were found to have such a beneficial effect that the government demanded the experiments be terminated early so that the control populations could be treated, though with the ironic consequence that results were not taken sufficiently seriously for subsequent institutionalization of this phage-preventative procedure to have become established.

Chanishvili et al.[150] provide a 25-page, 23-reference chapter reviewing the Georgian literature on "Phage therapy against intestinal infections". In the treatment of dysentery, highlights include phage production to densities up to 10^{12} phages/mL, the application of many mL per dose, the use of multiple doses, buffering to prevent phage loss during passage through the GI tract, and reductions in disease symptoms. Substantial reductions in mortality were reported. In addition to oral delivery, deep rectal delivery was employed in 1952 by Litsinik (as cited), who concluded that phage therapy was as effective as antibiotic therapy. Not all treatments resulted in positive results, however, perhaps in some cases because treatment was started too late.

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Treatment of Salmonella infections as reviewed by Chanishvili et al.[150] involved numerous routes of delivery, in contrast to the primarily oral delivery for anti-dysentery therapy. These included intravenous, intra-duodenal, intramuscular, rectal and oral. They note that 1 to 2°C increases in patient temperature were commonly reported prior to the lowering of temperatures. A common observation also is a shortening of the duration of illness following phage administration, though not all treatments and studies were successful in impacting the course of disease. Phage treatment of gastroenteral *E. coli* and Proteus infections are also described.

The most exciting and furthest-advanced current clinical trials of phage therapy using modern protocols are being carried out under the leadership of Harald Brüssow of the Nestlé Corporation, Lausanne, Switzerland (clinicaltrials.gov/ct2/show/NCT00937274). Nestlé presently is sponsoring a study currently taking place in Dhaka, Bangladesh, that is designed to study the safety and efficacy of phage therapy in treating ETEC and EPEC induced diarrhea in children. The therapy is being applied to the standard oral rehydration solution and a novel cocktail of T4-like phages used in earlier safety trials as well as a commercially available Russian anti-*E. coli* phage cocktail (Microgen), and is being compared with a randomly and double-blind applied placebo. This work in particular demonstrates all of the key elements of modern clinical trials. In vivo replication of the family of phages being used has been studied in several mouse experiments.[151,152] The phages being used for the key experimental arm of the trial were isolated from the stools of pediatric diarrhea patients in Bangladesh,[136] with the potentially-useful broad-spectrum ones all turning out to be members of the highly-studied T4 family of phages.[137] They have also reported the details of their very extensive genomics and gut-related infection studies of their several groups of T4-like phages in their set.[133]

Respiratory tract infections. Respiratory infections can be differentiated into numerous types, though of course with phage therapy limited in efficacy to those which have a bacterial etiology. Here we briefly consider phage treatment of respiratory infections differentiating primarily between those that either don't or do involve cystic fibrosis (CF). For the former, Weber-Dabrowska et al.[135] reported success in treating pneumonia in six cancer patients. Similarly, Slopek et al.[64] report 86.7% positive cases (our calculation) for phage treatment of 180 "Diseases of the respiratory system"; see also reference 65. In terms of cystic fibrosis, a paper has been recently published describing successful treatment of *P. aeruginosa* infection of the lungs of a seven-year-old patient (using Pyophage) along with treatment of a *S. aureus* co-infection in the same patient using phage Sb-1, also successfully.[153] The company previously known as Biocontrol (below) has received a grant from the US CF foundation to expand its anti-Pseudomonas phage therapy efforts to include treatment of children with CF.[11] Success in treating infections in animal models of CF-associated infection has also been reported in references 154 and 155, as too has exploration of the utility of nebulization as a phage delivery strategy.[156] Phage treatments of lung infections, however, can also be reached effectively in at least some circumstances from systemic circulation, as animal models have shown.[155]

Chronic otitis. Chronic otitis, known less formerly as swimmer's ear, is a *P. aeruginosa* ear infection that in many cases can resist antibiotic treatment. The company, Biocontrol (recently acquired by Targeted Genetics of Seattle to form a new joint company, AmpliPhi Biosciences), has been developing anti-*P. aeruginosa* phages targeting this condition, having published similar animal studies using dogs.[143,157] In 2009 they published the results of their double-blinded phase 1/2a (safety and small-number efficacy) trial in human patients also suffering from the condition.[132] Increases in phage numbers in situ, microbiological improvements (reductions in bacterial presence), and reduction in disease symptoms in the phage-treated cohort but not the phage-negative controls were observed. No side effects were seen. Complete bacterial eradication was not observed, though this could be because only a single phage dose was administered. Note that Weber-Dabrowska et al.[135] also report phage therapy success in treated purulent otitis media and Slopek et al.[64] report 93.8% positive cases for phage treatment of 16 cases of "Conjunctivitis, blepharoconjunctivitis, otitis media".

Urogenital tract infections. Phages can be applied to treat various infections of the urogenetical systems either systemically, via direct injection such as into the bladder, or topical application. Eaton and Bayne-Jones in their 1934 [14-16] report were convinced of the efficacious use of phage therapy against cystitis. Letwiewicz et al.[158] describe phage application rectally to target *Enterococcus faecalis* infection of the prostate, with some success. In this case phages are presumed to be taken up through the rectal wall prior to gaining access to the prostate. The result of treatment was elimination of the target bacteria from prostatic fluid. Letarov et al.[103] note that rectal phage suppositories are available on the Russian market. Slopek et al.[64] report 92.9% positive cases for phage treatment of 42 "Diseases of the genito-urinary tract".

In the Chanishvili and Sharp[7] book there is a chapter on "Phage therapy in urology" and a second on "Phage therapy in gynecology". The former chapter[159] indicates, in the case of Tsulukidze (1938), for example, that phage application was made directly into the bladder as well as the pelvis of the kidney: "In cases of acute cystitis a therapeutic effect was observed within 4–5 hours of the first administration and resulted in relief of pain, a decrease in the frequency of urination and a normalization of the composition of the urine. Full recovery was achieved within 1–3 days in all 13 cases (100%) however treatment of chronic forms of cystitis was less successful, with only a moderate improvement observed."

Sepsis. Sepsis refers to systemic infections resulting in a dangerous, whole-body inflammatory state. A related term, septicemia, is used to describe bacterial infections in which pathogens are present in the blood or lymph in substantial numbers, particularly as a consequence of bacterial growth within those tissues. The commonality is a dangerous, typically life-threatening infection that has disseminated systematically rather than remaining locally contained.

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The Polish phage therapists have avoided intravenous phage treatments and this approach also has been largely avoided in Georgia, so there is far less modern, human-treatment experience to draw on than for most forms of phage therapy. The concern with fighting systemic infections using phages systemically is two-fold. First is the danger of the infection itself. This includes the potential to worsen infection symptoms given excessive bacterial lysis upon antibacterial application, but that is actually at least as likely with various other forms of treatment and may be partly mitigated by starting with fairly low levels of phage. The second is the fact that parenteral or systemic phage application requires greater levels of purification so as to avoid systemic exposure to endotoxins formed during phage production. Notwithstanding these concerns, some phage therapy of human systemic infections has been undertaken in modern times, generally using non-intravenous routes, such as intraperitoneally.

Weber-Dabrowska et al.[160] review the impact of phage treatment of 94 patients with septic infections, all of whom had been treated with antibiotics prior to phage application. In 23 patients, antibiotic treatment was discontinued during phage application. 61 of the patients were afflicted with mixed infections and for the remaining 33 only a single etiology was identified. In the latter, 15 were *S. aureus* and the rest Gram negative. Phages were matched to presumed etiologies in all cases rather than applied as cocktails. In 80 cases, complete recovery was achieved while in the remaining 14 cases treatment was unsuccessful except for a drop in patient temperature. Weber-Dabrowska et al.[135] report successfully treating 7 people who suffered similar infections while afflicted with cancer and Slopek et al.[64] report 88.8% positive cases for phage treatment of 98 "Septicemias".

In the Chanishvili and Sharp[7] book there is a chapter on "Phage therapy against septic infections", authored by Teimuraz Chanishvili,[161] which includes the use of highly purified Staphylococcus phages by IV and other more invasive routes. In 1993, Pavlenishvili and Tsertsvadze[162] explored, using a combination of per os phage therapy and enterosorbtion, the multi-day (7 to 12) treatment of sepsis of newborns, infants and other patients caused by gram-negative bacteria. Thus phages could attack the infection while, at the same time, at least some of the toxins produced in the course of infection, treatment, and presumably bacterial lysis could be sequestered within the gastrointestinal lumen by absorptive materials. The authors indicated that acute disease was shortened by about four days, on average, with "relatively fast improvement of the patients' general state compared with that revealed in the case of usual treatment… No patients revealed toxic, allergic, or pyrogenic or accessory phenomena…" Lastly, bacterial endocarditis, the infection of heart valves, has been successfully treated with bacteriophages, in France.[163]

Conclusion

Phage therapy has a long history, though for most of that history this approach has been neglected by the English-speaking western world. We show here, however, that there is much more in the literature than has generally been realized, with many studies demonstrating that phages as natural and self-amplifying antibacterial "drugs" could be used to safely and effectively treat or prevent many common human diseases of bacterial etiology. Especially in light of concerns regarding the serious menace of antibiotic resistance, as has been recently stressed by the World Health Organization (www.who.int/world-health-day/2011/en/), we believe that there is enough of at least semi-anecdotal evidence of efficacy to strongly recommend continued evaluation of this alternative antibacterial approach.

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