Phage Therapy in Clinical Practice: Treatment of Human Infections

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Abstract: Phage therapy is the application of bacteria-specific viruses with the goal of reducing or eliminating pathogenic or nuisance bacteria. While phage therapy has become a broadly relevant technology, including veterinary, agricultural, and food microbiology applications, it is for the treatment or prevention of human infections that phage therapy first caught the world's imagination – see, especially, Arrowsmith by Sinclair Lewis (1925) – and which today is the primary motivator of the field. Nonetheless, though the first human phage therapy took place in the 1920s, by the 1940s the field, was in steep decline despite early promise. The causes were at least three-fold: *insufficient understanding* among researchers of basic phage biology; *over exuberance*, which led, along with ignorance, to carelessness; and the advent of *antibiotics*, an easier to handle as well as highly powerful category of antibacterials. The decline in phage therapy was neither uniform nor complete, especially in the former Soviet Republic of Georgia, where phage therapy traditions and practice continue to this day. In this review we strive toward three goals: 1. To provide an overview of the potential of phage therapy as a means of treating or preventing human diseases; 2. To explore the phage therapy state of the art as currently practiced by physicians in various pockets of phage therapy activity around the world, including in terms of potential commercialization; and 3. To avert a recapitulation of phage therapy's early decline by outlining good practices in phage therapy practice, experimentation, and, ultimately, commercialization.

Keywords: Bacteriophages, burn care, Eliava Institute, Hirszfeld Institute, intestiphage, osteomyelitis treatment, phage therapy, purulent infections, pyophage, wound care.

INTRODUCTION

Very soon after the co-discovery of phages by Frederick Twort [1] and Felix d'Hérelle [2], in 1915 and 1917, respectively, the treatment of bacterial infections in humans was initiated [3,4]. More broadly, the history of human phage therapy is just one aspect of the history of the medical treatment of bacterial infections in general. The first commercial antibacterial agents consisted of synthetic chemotherapeutics (salvarsin and, later, sulpha drugs). Chronologically, the second antibacterial agents were phages in the guise of phage therapy. It was nearly a decade after the first phage therapy trial (in 1919) i.e., not until 1930, that treatment of humans using an antibiotic, penicillin, was first attempted [5]. These built on various traditional approaches toward the control of bacterial infections, such as vaccines, honey treatment, goldenseal and other herbs. Thus, phage therapy in humans is far from an exceptional footnote within medicine but instead an integral and, in fact, ongoing aspect of the treatment and control of infectious disease.

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In this article, we explore some of the many phage-based approaches that have been employed to treat or prophylactically prevent human infections by pathogenic bacteria. As a great number of reviews have already been published on this and related subjects (see [6]), what we focus on here are lessons gleaned from the professional experience of practitioners, either as published or as available via personal communication. We differentiate our narratives by group and our larger goal is to not just provide indications of what is possible, but also to describe methods employed.

PHAGE THERAPY IN EASTERN EUROPE

Much of the detailed knowledge we have about the practice of phage therapy comes from two places: the Republic of Georgia, especially as associated with the Eliava Institute of Bacteriophages, Microbiology and Virology, and the Hirszfeld Institute of Immunology and Experimental Therapy (hereon simply Hirszfeld Institute) located in Wrocław, Poland. The Republic of Georgia is the one place in the world where phage therapy is a component of standard medical practice, routinely used in a number of hospitals and clinics for both prophylactic and treatment purposes. Much of the phage availability both presently and historically has been associated with the Eliava Institute. The Eliava's main

1389-2010/10 $55.00+.00

focus has been on therapeutic phage cocktail formulation (with each cocktail containing many phages targeting a particular cohort of pathogenic bacteria [7]), characterization, production, and implementation. Several phage therapy preparations are available over the counter for a variety of applications, used on advice from a doctor or as a component of self care for less serious problems. A broader range of products are available directly to medical personnel for special purposes. The origin of this privileged role for phage therapy in Georgia is an interesting story [3,8], as summarized below, and the extent of phage use is vast. For several reasons, Georgia is a particularly logical place to carry out properly controlled clinical trials, but to date, the available data is disappointingly incomplete. Much phage therapy application was carried out in other parts of the Soviet Union, but it is much more difficult to get any detailed information about work going on there, so consequently that will not be a focus of this article.

The other major source of information is the Hirszfeld Institute, whose approach is very different. They have developed individual therapeutic phages and supported their use by local physicians for a variety of applications where antibiotics have failed. In a series of papers, they have provided some data, and in some cases more extensive information, on all of the several thousand cases that have passed through their system. The overview thus provided has given a tantalizing picture of the possibilities of phage therapy. Now that Poland has come into the European Union, the Institute has set up its own clinic and is moving toward carefully controlled clinical trials. In this section, we explore some of the lessons offered by each of these two sites.

Georgia

The Eliava Institute has made major strides in basic phage related research, enhancing traditional phage therapy cocktails and developing new ones for targets such as prostatitis and cystic fibrosis. They are also tackling environmental and potential bioterrorism problems, including the tracking of cholera and enteric pathogens in regional waters and the rapid detection and typing of anthrax and brucellosis as well as dysentary (eliava-institute.org). Since 1996, the Institute's research has depended strongly on grants from a wide range of international groups including the International Science and Technology Centers (ISTC) and the US Civilian Research and Defense Fund (CRDF). With the recent restructuring of much of the official scientific establishment in Georgia, the Eliava Institute was moved out from under the Georgian Academy of Sciences to become a quasi-independent Institute under the Ministry of Science and Education. It was now permitted to set up a non-profit Foundation that in turn could spin off small companies to commercially develop and distribute their products and services. Grants from the US State Department and DTRA have helped them further develop these plans and resources. Furthermore, there is a strong feeling among most Georgian scientists and physicians that phage therapy has great potential, but that proper clinical trials are desperately needed both to prove its efficacy and to determine the most effective protocols in various kinds of applications. Such research could probably most straightforwardly be carried out in Georgia, with its central pool of physicians and surgeons highly experienced in phage therapy, closely collaborating with appropriate laboratory facilities and basic scientists, and its supportive regulatory climate. However, financing of robust studies and support in developing protocols for studies that will be accepted internationally are major barriers to such clinical trials.

Toward better conveying this Georgian potential, we here provide details on Georgian phage therapy preparations and techniques, many of which are associated in one way or another with the Eliava Institute. In discussing some details of therapeutic phage use in Georgia, we will focus, in two subsequent sections, on two main areas of application: treatment and prophylaxis of enteric infections and the use of phage in wounds and surgical infections.

Early Georgian History

Georgians were celebrated in the writings of the ancient Greeks for their creativity, friendliness, hospitality, and excellent food and wine, and those are still strong characteristics today, despite many challenges that they have encountered. Nestled on the southern slopes of the high Caucasus mountains, along one route of the ancient Silk Road between Asia and Europe, Georgia is an ancient, proud, and independent culture. Though the lowland regions, at least, have often been under the hegemony of one neighbor or another – the Turks, the Persians, the Russians – they have still maintained their own unique alphabet and language, related only to Basque and that of ancient Sumeria, and very distantly at that. (Note that because the Georgian alphabet is totally distinct from all other world alphabets, all English versions of Georgian words are transliterations and may be written in a variety of ways. Thus, for example, among Georgian phage products one often sees such constructs as “piophage”, “pyobacteriophagum”, etc.).

In 1918, the briefly independent Republic of Georgia tackled its problems of infectious disease by developing a new institute of microbiology and sending its first director, George Eliava, to the Pasteur Institute in Paris to learn the most modern techniques and acquire appropriate equipment. There, Eliava soon became a close collaborator and supporter of Felix d’Hérelle, who had just discovered bacteriophages in the course of his work with soldiers with dysentery, and brought the ideas and practices back to Tbilisi when he returned there in 1921. During repeated visits back and forth, the two developed the idea of turning Eliava’s Microbiology Institute into the world center of phage research and phage therapy and got Joseph Stalin – General Secretary of the Communist Party of the Soviet Union but also a native Georgian – interested in the project. The current main Institute building was built in the early 1930s. In the complex political system of the time, Eliava was arrested by Beria’s secret police in 1937 and executed without trial. d’Hérelle, visiting back in Paris at the time, never returned to Georgia.

Phage Production at the Eliava Institute

Notwithstanding the loss of its leader, the Eliava Institute came to thrive and developed into the largest facility in the world dedicated to the creation and production of therapeutic phage preparations, as well as vaccines, antisera, and antiviral compounds (cf. [3,8]). It became a key branch of the So-

viet Ministry of Health, which sent it bacterial samples from all over the Soviet Union for use in developing phage cocktails and carried out the approval and licensing of these products, based on very extensive documentation of testing for safety and efficacy. Regular conferences were held to explore research and advances in phage therapy, but much was treated as military secrets, and little was published in regular journals. In the 1980s, the Institute's 1200 employees made two tons of phage products. These were mainly tablets and liquid against dysentery and other diarrheal diseases, plus other liquid phage preparations targeting gangrene and purulent (i.e. pus-causing) infections as frequently as twice a week, 80% of it for the Soviet Army. There were also production facilities in the Russian cities of Ufa and Gorky. As the Soviet Union broke up in the early 1990s, the Institute fell on very hard times, without the financial support and deep customer base of the Soviet Army and Ministry of Health. In 1995, the commercial production facilities were privatized and largely put to non-phage uses.

Scientists in the research arm of the Institute continued to produce small, 30-liter batches of the major phage cocktails for use in regional hospitals and for sale at their Diagnostic Center, which was also the constant source of current pathogenic bacterial strains to be used in the regular testing and updating of their products. They also have strongly maintained their collaboration with physicians at the major local hospitals, working together on improving treatment outcomes and protocols. One of the primary challenges as well as benefits of phages for therapeutic applications is their specificity. The host receptors that they target often vary widely even within the same species, while multispecies bacterial infections are common (see also [9,10]). Thus, complex cocktails of phages are produced, as first developed by d'Hérelle, to exert a bactericidal effect on most or all of the key pathogens involved in an infection.

Phage Formulations Associated with the Eliava Institute

D'Hérelle's two major cocktail formulations, brought from Paris in the 1930's, are still the primary ones used in Georgia and Russia *pyophage* (Fig. **1**) and *intestiphage*. Note that these names are generic, as befitting their relatively ancient origin. Licensed versions of, for example, pyophage from various producers, and even different batches from the same producer (each of them carefully dated) may have significant differences in host ranges. Indeed, every 6 months, these standard licensed products must by law be tested against a wide range of current problematic strains and, if necessary, upgraded by adding new phages against those strains.

Pyophage targets the bacteria of purulent (pus-causing) infections: *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa,* 2 *Proteus* species, and several species of *Streptococcus. Intestiphage*, by contrast, targets about 23 different enteric bacteria, as well as gut-derived strains of *S. aureus* and *P. aeruginosa.* Intestiphage is used extensively by both Georgians and visitors to deal with traveler's diarrhea and other gastrointestinal upsets. It is indicated for all ages, and generally used without doctor's visits or prescriptions. It is also used extensively in hospitals to prevent nosocomial gastrointestinal infections, which used to be particularly prevalent in the pediatric hospital until regular use of Intestiphage was introduced. One company's intestiphage is advertised as a "Mixture of sterile filtrates of bacterial phage lysates of : Shigella: flexneri 1-6, serogroup B; Sonnei serogroup D; Salmonella paratyphi A,B; t*yphimurium, choleraesuis, oranienburg; enteritidis; E .coli* – serogroups: 0111, 055, 026, 125, 0119, 0128, 018, 044, 025, 020; *Proteus (vulgaris, mirabilis*); *Staphylococcus* spp, *Pseudomonas* spp, *Enterococcus* spp" (the last 3 explicitly of emerging clinical importance). At least in hospital practice, the patient's bacteria are routinely tested against the relevant available cocktail(s). If necessary, other cocktails or individual phages from the Institute's collection may be used or added, or, under extreme circumstances new "auto-phages" may be isolated from environmental sources, using the patient's own bacteria to select them, as also first suggested by d'Hérelle [11].

Fig. (1). A single-dose ampule of Eliava pyophage.

A Private-Public Partnership

In addition to their support of the Eliava Institute, the ISTC has given a series of grants related to phage targeting enteric pathogens to the group that had acquired the tablet-making component of the old Eliava production facility during the 1995 privatization. Later, with substantial private investment (mainly from Georgian medical personnel), they developed a new phage company, JSC Biochimpharm, in that facility, producing its own licensed versions of pyophage and intestiphage. These are already available in pharmacies throughout Georgia and starting to be exported to other countries (see www.biochimpharm.ge). By 2008, they

had renovated the facility to near-GMP standards, capable of large-scale production and related research, and added new products, including tablet forms of phage against *Shigella* for dysentery and phages against a range of *Salmonella*, as well as a specialized preparation of phage against *Salmonella typhi* (for typhoid).

An additional, major positive factor is that phage work in Georgia has substantial basic governmental support (even though that has seldom translated into much financial aid). In 1991, the strong Georgian academic community realized that the only way they could have an acceptable government for their newly-freed country was to get actively involved in politics. For a number of years, for example, the head of their Parliament was a neurobiologist, and many other scientists were in the Parliament. Rezo Adamia, head of the Eliava Institute phage molecular biology lab, became head of the military subcommittee of Parliament, and went on to also be one of the vice chairmen of the Council of Europe and then Georgian Ambassador to the United Nations for 4 years before returning to become Director of the Eliava Institute. There, he has been putting his extensive skills and national and international connections to good use in the further revitalization of the Institute. Furthermore, when the Eliava Institute put on an international phage meeting in 2008, Georgian president Mikhail Saakashvili came to speak, including describing that his grandmother had worked at the Eliava for 30 years in its early days and he had strong evidence for the potential life-saving effects of phage therapy.

Georgian Enteric Applications: Prophylaxis and Treatment

Clinical evidence for the efficacy of phage therapy in enteric infections is largely restricted to extensive trials conducted in India in the 1930s with cholera and in Eastern Europe with dysentery. Ironically, the formal cholera trials were stopped prematurely because their success seemed so evident that the Indian government chose to provide phage to the control villages as well to reduce the ravages of cholera; this left many questions still unanswered. The Eastern European trials are generally poorly documented in scientific journals. Very extensive phage prophylaxis and treatment studies were conducted with soldiers from the Soviet Army, dealing with severe problems of dysentery in their southeastern Muslim republics. It was said, for example, that the incidence of dysentery was 10-fold less in the phage-treated units than in the control groups. However, much got lost through military secrecy and the available reports are mainly in the form of meeting abstracts. Nonetheless, the Soviet military believed strongly enough in the efficacy of phage therapy to generously fund what is now the Eliava Institute in Tbilisi, much of whose military production was in the form of tablets against dysentery and other diarrheal diseases (along with cocktails for wound treatment).

Civilian applications were also important. One well-designed controlled prophylactic trial conducted during the early 1960s in Georgia involved a total of 30,769 children younger than 7. Over the height of the annual dysentery season, the children living on one side of each street regularly received a cocktail of phages targeting *Shigella sonnei, S. boydi, S. flexnerei*, and *S. newcastle*, while children from the other side of the street received a placebo. The children were followed for 109 days by weekly nurse visits. Phage application was associated with a 3.8-fold decrease in dysentery incidence (1.8 versus 6.7 episodes per 1,000 children from treatment and placebo groups, respectively). The *culture-confirmed* incidence of dysentery was decreased 2.6-fold by phage application. Phage exposure also decreased the incidence of *any* form of infant diarrhea (15 vs. 45 episodes per 1,000 children 6 to 12 month-old in treatment and placebo groups, respectively). This observation suggests a protective effect of the anti-*Shigella* phage preparation against pathogenic *E. coli* as well, which is not surprising since *Shigella* and *E. coli* are very closely related and many phages are known to infect strains of both [12]. Protective effects were most pronounced in children younger than three years. Unfortunately, all of these exciting data were reported in a publication just 68 lines long, written in Russian. It turns out that far more data is available on this and related studies. This study was actually carried out in Rustavi, near Tbilisi, as the initial trial of the first Georgian phages in dry tablet form.

These tablets were developed in 1964 by Amiran Meipariani, who still is an active member of the Institute. Preparation of the tablets is described in substantial detail in his doctoral thesis, as is the second trial with these phages, targeting over 20,000 children in one region of Tbilisi. Both this second trial and one of phage tablets against typhoid (involving over 5,000 children in a different Tbilisi district) also used the opposite-sides-of-the-street model, which was further adapted then for *Salmonella*. Ammonium sulfate precipitation was used to concentrate the phages, with calcium carbonate added to make the tablets, and enteric coating was applied, using technology that had been developed by Russian scientists in Vladimir. This tested methodology was used until the end of the 1980's, when tablet and other large-scale phage cocktail production was curtailed as the Soviet Union was breaking up.

It appears likely that some at least of the missing data about other early phage therapy work is available in theses, internal publications, and the voluminous documents required for approval of new cocktails by the Soviet Ministry of Health, and that some of the people involved are still alive, involved in phage work, and able to help track down key data. Projects are also under way to systematically explore and collate the old phage therapy literature in the extensive libraries at the Eliava and elsewhere and make at least the abstracts readily available in English. The results of a major such project, funded by a grant from the UK Global Threat Reduction Programme and managed by the ISTC, Moscow (ISTC project Nr G-1467), has just come out as a book [13]; a summary of the findings was also published in an Australian journal [14]. Analysis of such data, often collected on a vast scale, may well complement modern clinical trials to speed up the evaluation and broader implementation of phage-therapy approaches.

The use of phages to *treat* enteric disease was also the subject of very extensive studies, both human and animal, carried out at the Eliava Institute over the last 50 years, looking at such factors as the relative effectiveness of phage preparations and antibiotics in the treatment of gastrointestinal infections, including dysentery, and optimizing treatment

protocols. The results of studies were often summarized in the proceedings of meetings, many of them held at the Institute and also drawing phage biologists from other parts of the Soviet Union; Bacteriophagy, the Collection of Works of the Interinstitute Scientific Conference, 1955, was a rich source, as were the many volumes of the Transactions of the Eliava Institute.

The general consensus seemed to be that phage therapy was preferable to antibiotics, with one major advantage being that it caused far less disruption of the gut flora. Best results were obtained when complex cocktails such as Intestiphage were administered as early as possible, before complex pathologic changes develop in the intestinal wall. Common doses of phage preparations for the treatment of enteric infections in adults range from 20 to 50 ml of an appropriate phage cocktail, two to three times a day, 30-60 minutes before the meal, giving 50-200 ml of 2-3% Sodium Bicarbonate 20-30 minutes before phage intake; for children, the usual dose is 5-10 ml. Intestiphage is still readily available in pharmacies throughout Tbilisi and is frequently used without prescription when gastrointestinal problems arise. Intestiphage or, where appropriate, more specialized preparations for dysentery or salmonellosis, are applied more systematically by hospital infectious disease specialists in more severe cases.

Scientists from the Eliava Institute conducted very extensive trials to test the efficacy of dysentery bacteriophages, but most of the studies are described only in abstract books and those with more details are not very well analyzed statistically. However, there are some important points that may be useful for future work. For example:

- The method of using actual clinical bacterial strains for passaging bacteriophages to increase the *in vitro* efficacy of phage cocktails was studied, and was claimed to have increased *in vivo* activity as well. The phage cocktail developed with this method had a wider host range and the development of secondary resistant colonies was slower.
- The immune response to bacteriophage administration was studied in a total of 190 experiments in 17 animals. The results indicated that the development of anti-phage antibodies is determined by the route of administration and the duration of the treatment, which was recommended to be considered during clinical applications.
- T. Chanishvili extensively studied diagnostic phages for dysentery, which had a very practical use during those times, considering the incidence and prevalence of the disease in the area and limited availability of alternative diagnostic tests.

Methods were developed for making phage tablets and for making high-titer phage cocktails on a large scale. Phage tablets work very well for both treating and preventing enteric diseases and are particularly good for transporting to distant sites, but their production is more complex, using special equipment, and few batches have been made since 1990. However, Biochimpharm's new products include tablet forms of phage against *Shigella* (for dysentery) and phage against a range of *Salmonella*, as well as a specialized preparation of phage tablets against *Salmonella typhi* (for typhoid).

Combating Surgical and Wound Infections

In the major tertiary care centers as well as wound and burn facilities in Georgia, phages generally play an important role in treatment. Priority indications for phage therapy include:

- Antibiotic penetration difficulties in the infection site, caused by poor circulation or the presence of a fibrogranulate barrier, such as in diabetic foot infections – a key area where phages are very successful when used with circulation stimulation.
- Chronic osteomyelitis.
- Wounds covering a large area, particularly where therapeutic concentration of antibiotic is not possible to achieve during systemic introduction.

Phage therapy is the primary tool in Georgia for successful treatment of multi-resistant infections as there is no correlation between antibiotic and phage resistance. Phages are just one component of successful wound care and treatment of surgical infections. Successful phage therapy requires also a rigorous application of all of the technologies of effective wound care, including: 1. Radical necrectomy and wide opening of the wound, 2. provision of adequate drainage, 3. ongoing provision of a reasonably optimal ratio of phage-to pathogens, and 4. early wound closure.

Phage preparations commonly introduced into the wounds also are polyclonal. Consequently, secondary resistance to phages, that developing during treatment, rarely is seen. Primary resistance of infectious bacteria to the commercial phage preparations, on the other hand, can be close to 20% and should always be checked, but this can often be overcome by selection of new phages from a lab bank or by using phages isolated for specific clinical microbial strains. When the selection of laboratory clones for a particular agent is not possible, over the course of 2-3 weeks, new phage clones can be isolated against the resistant bacteria; see [7] for more on phage isolation. However, this option is usually only used for chronic infections. Close collaboration between hospitals and phage-producing organizations is very important for the optimal production of successful preparations. Epidemiologic conditions in the surgical and ICU wards should be routinely monitored and the phage preparation augmented to deal with any resident pathogenic microflora. Phages are also cost effective enough to be used in larger quantities for sanitation of the hospital environment, personnel, and patient.

Phage preparations can be applied in a variety of ways: by irrigation of wounds with a phage preparation after surgical debridement, ultrasonic debridement of the wound with the phage preparation, soaking of wound bandages in liquid preparations, periodic introduction (4-6 times) of phages through drainage tubes, application of PhageBioderm film and powder on the open wound surface, or as drainage strips, set into a wound incision to facilitate its draining. Preference is generally given to using Eliava's "pyophage" cocktail, at its standard concentration of 10^5-10^6 pfu/ml of each of the phage components. It is used in a variety of fashions (as a lavage, a dressing agent, ear and nose drops) to treat superficial wounds. For deeper wounds, phages embedded in

degradable polymer called PhageBioderm is often used in addition to pyophage wound irrigation. PhageBioderm is a phage-containing anti-microbial polymeric bio-composite material developed by Georgian chemists and microbiologists since 1995 and approved for commercial release in 2000 [3], but not yet under large-scale production. It acts in a sustained controlled-release fashion (Fig. **2**), providing drainage and protection along with therapeutic action, and shows a high therapeutic effect in healing various infected wounds and ulcers. PhageBioderm contains pyophage, the painkiller *Benzocain* (0.9 mg), a biodegradable biocompatible polymer (polyester amide) – 8-9 mg, the proteolytic enzyme *a-chymotrypsin* – 0.05 mg, and sometimes also an antibiotic or other antimicrobial. Liquid phage preparations are usually used locally and, occasionally, also *per os* one to three times a day for 3-7 days, depending on the age and the nature of the problem. The dosage of preparations for wound treatment depends on the extent of the damage. Phage are also used via catheters or tampons for gynecologic and urologic infections – 10-50 ml once a day – or as suppositories twice a day.

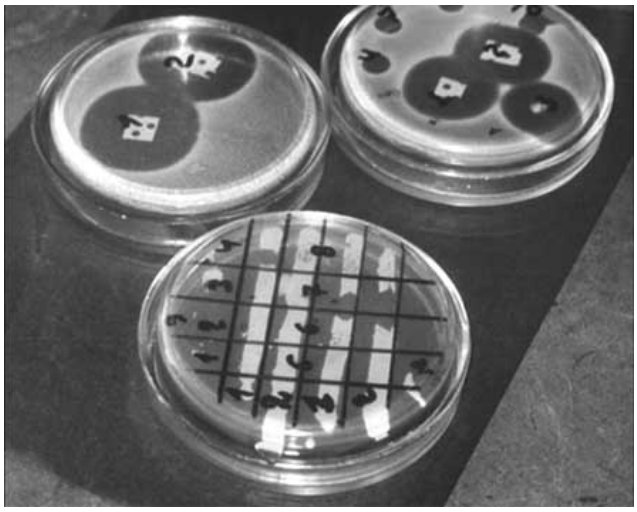

Fig. (2). Sustained release from PhageBioderm squares clearing bacterial lawns (top) and assay for phage susceptibility of bacteria (bottom). Note in the latter the clearing in bacterial streaks indicating phage killing of bacteria (e.g., row 3, column 3). The bacteria on the upper right hand plate are from a patient who had fractured his ankle which then became infected, resulting in *Staphylococcus* draining from both sides of the resulting wound, even after 4 years, including one full year spent on IV antibiotic. The two larger clearings shown in the middle of that plate are due to the action of PhageBioderm subsequently used in the successful reduction in bacterial densities to the point of wound healing. The large clearing in the lower right of the same of the same plate is due to the action of the same antibiotic – which otherwise had been used to control, but not successfully eliminate the infection – clearly indicating the susceptibility of the bacteria to that antibiotic, at least *in vitro*.

Whole-System Approach

The surgeons responsible for the treatment of severe wounds using phage therapy are deeply steeped in the paradigm that the infection is not just a local process but must be dealt with as a disease of the entire organism. They use a mnemonic called PIRO (Predisposition – Infection – Response – Organ failure), which was originally developed for dealing with severe sepsis. Taking this approach to any infectious process often suggests effective treatment regimens which can lead to recovery even in severely injured ill patients.

Predisposition, those factors which make specific infections of specific patients more likely, plays a major role in treating the majority of infectious processes. This includes cases where the infected wound is not the cause but the result of disease, such as diabetic foot ulcers, critical limb ischemia, decompensated venous insufficiency, radiation disease, osteosclerosis due to osteomyelitis, and infections with underlying multitrauma, malignancy or malnutrition. Always, the primary disease must be carefully treated along with the infection. Phage treatments inherently do not address predisposing factors, and therefore represent only one tool, an antibacterial, within a complex array of necessary medical treatments.

Infection is another key part of the PIRO concept. It is important to differentiate between those bacteria that are actually causing the disease process and those that are merely present. For example, research led by Dr. Gvasalia during the fighting in Abkhasia in 1991 determined that only a few bacteria from the complex primary contaminating flora of wound infections play an important role in subsequent infection development. Among common wound etiologies, the most important are the common nosocomial pathogens *S. aureus*, *Streptococcus*, *E. coli*, *Proteus* species, and *P. aeruginosa*, the latter being especially prominent in burn patients. In acute soft-tissue infections, these agents often have well-known antibiotic sensitivity and can be empirically treated with good outcome. However, in nosocomial and chronic infections, bacteria often show multidrug resistance and mature biofilms often impede antibiotic action. Alternative approaches for antibacterial therapy are especially crucial for methicillin-resistant *S. aureus* (MRSA), often found infecting surgical wounds, a notorious example of the new so-called "superbugs". It is especially these commonly encountered and challenging infections that are treated with phage therapies in the context of surgery in Georgia.

The *Response* of the organism to the disease stress and to the treatment involves innate and compensatory mechanisms which can themselves lead to recovery. However, severe trauma often leads to impaired immune responses, so supporting balanced immune function in every way possible is an important component of successful treatment.

Organ failure is common following severe traumatic injuries, malignancies, sepsis and other conditions. Monitoring all systems and taking steps to prevent such failure is a primary factor influencing the control of infections that can have potentially fatal outcomes.

Differences Between Antibiotic and Phage Treatments

It has been well established that phages can kill microorganisms which are resistant to many or all broad spectrum modern antibiotics. This effect has been shown both *in vitro* and *in vivo* and reflects the fact that phage mechanisms of bacterial killing differ radically from those of antibiotics. Resistance is usually easily overcome by employing a differ-

ent phage isolate, a cocktail of different isolates, or an isolate that has been modified in terms of its host range [7,9].

The antibacterial effects of phages in purulent wounds are more manageable than those of antibiotics. This is because the penetration of antibiotics into infected tissue, as well as their concentration there, is directly related to their systemic concentration, with increase of this concentration having very obvious limits. These limits are defined by combinations of antibiotic toxicity along with their rates of uptake and clearance, plus in certain circumstances antibiotic cost. Phages, by contrast and in particular, generally have low toxicities [6] plus can have relatively low per-unit costs, meaning that large phage quantities can be employed in circumstances where increasing antibiotic concentrations (such as to overcome uptake, clearance, and penetrability to target bacteria concerns) is not an option for reasons of toxicity or cost. The cost issue is especially relevant in developing nations where per capita health expense is relatively low, such as in Georgia. In Georgia, in fact, one practice is to employ phages in concert with the more expensive antibiotics, with antibiotics applied systemically in standard relatively low densities while the phages are applied in high densities locally using continuous irrigation to hit the heart of the problem.

Much of the low toxicity of phages can be attributed to their high specificity, where target bacteria can be singled out for killing whereas both human tissues and non-host bacteria making up the human normal flora are either not affected or not impacted negatively (with the caveat that proper phage choice can be important in achieving those ends, particular in terms of avoiding employing phages which encode bacterial exotoxins [7]). Phage therapy in this context corresponds to the Ehrlich "magic bullet" postulate better than do antibiotics or most other chemical antimicrobials – an antiseptic remedy of infectious microorganisms with maximal effect at a concentration which is either minimally or not at all harmful to the individual being treated.

Also contributing to low phage toxicity, in comparison to antibiotics, is that phage concentrations are self-regulatory: They are quickly flushed from the body and/or inactivated by the immune system when their host is no longer present. This latter aspect is linked with challenges in administering phage therapy systemically, however, where a large bolus of antigen enters the circulatory system at once. Even if applied locally, or *per os*, phage particles often enter into systemic circulation, which can be viewed as advantageous in terms of phage penetration to localized or more systemic infections, though this effect is still not well understood [6]. Phage preparations thus are more conservatively and readily applied more locally – intraperitoneally, intrapleurally, inserted directly into a wound, etc. – as opposed to explicitly systemically. This either topical or less-directly systemic use of phages is less likely to be a problem because the phage move gradually from a local reservoir into the circulatory system and reproduce rapidly when they reach another collection of susceptible bacteria, as seen in René DuBos's classic 1943 mouse experiment ([15]; Fig. **3**).

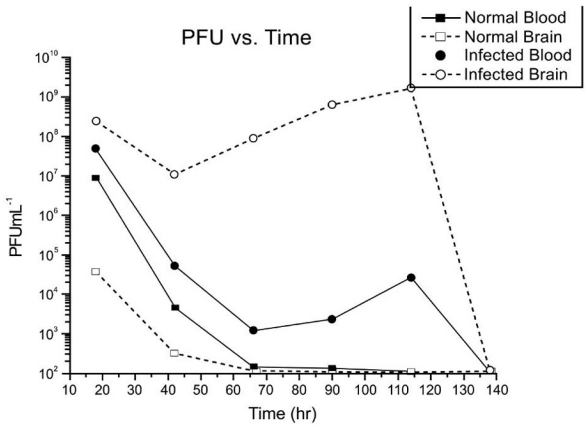

Fig. (3). This 1943 experiment of René Dubos' helps us understand why phage work so well in dealing with infections antibiotics can't reach. When he injected mice intraperitoneally with 10^9 phage, they fairly rapidly got into the blood stream and a significant number even crossed the blood-brain barrier, but they were also rapidly cleared. However, if he also injected the mice intracerebrally with *Shigella dysenteriae*, 46/64 of those given $10^7 – 10^9$ phage intraperitoneally survived and the level of phage in the brain climbed to over 10^9 per gram, dropping below detection levels when the bacteria were gone. With no treatment, or treatment with heat-killed phage or staph-culture filtrate, only 3/84 (3.6%) survived.

Advantages for Local Use and Biofilms: "Active Penetration"

Though low phage toxicity makes them advantageous as antibacterials in general [6], thereby allowing systemic application if need be, phages may be distinguished from antibiotics especially when used locally. Systemic antibiotics may not penetrate sufficiently into the infectious site due to tissue hypoperfusion during vascular occlusive diseases or fibrotic and granulative barriers and tissue necrosis; as a consequence, the concentration of antibiotics is insufficient to eliminate the infection, especially in extensive wounds affecting a large area. Optimal wound concentration and efficacy of antibiotics is also often difficult or impossible to achieve by means of local antibiotic therapy due to their dilution by inflammatory exudates, neutralization by enzymes and other inflammatory mediators, and inability to penetrate adequately into the tissues. The reproductive ability of bacteriophage, in contrast, avoids this problem since they continue to replicate and penetrate into tissue in the presence of susceptible bacteria; see [6] for further discussion. This makes phages ideal for wound treatment, in contrast to antibiotics, whose concentration decays rapidly with distance from the source or, when used systemically, the blood vessel.

Phage therapy is not a substitute for antibiotic therapy and the simultaneous use of localized phage and systemic antibiotics can have additive or synergistic effects. However, local administration of some antibiotics can interfere with phage therapy by killing the more accessible target bacteria in ways that block their ability to serve as phage "factories" but still permit phage adsorption and injection, which is thus suicidal to the applied phages. If no generalized infection or its danger is present, the treatment of purulent wounds can be carried out as a monotherapy, that is, without antibiotic augmentation.

Maxillofacial Studies

Phages have been found to be especially useful for prevention and treatment of maxillofacial infections, as was explored in some depth by Dr. Teona Danelia in her doctoral dissertation. Specific anatomic characteristics of the head and neck region create unfavorable conditions for the localization of infection in this area and favor rapid spreading of the infection in different directions. Despite the rich vascular supply, the multiple anastomoses between different vessels create a special threat for dissemination of infection locally and regionally as well as generally. Maxillofacial infections are easily spread in the cerebral circulation, often creating fatal CNS infections. Because of these challenges, the proper treatment and prevention of maxillofacial infections is especially important in clinical settings.

A necessary condition before phage therapy is the removal of necrotic tissues, opening of blind wound pouches, lavage, and washing with 4% sodium bicarbonate solution. PhageBioderm powder can be sprinkled and left in the wound in severe maxillofacial injuries before the radical surgery can decrease the chances of infection. For more complicated deep wounds, after the same procedure the cavities are drained and phage introduced via thin catheters fractionally three to four times a day. Phage therapy is performed in the same manner for chronic patients. An important aid to the prevention of subsequent infections is the phage sanitation of the oral cavities in ICU patients. In this group of patients, the medical devices in the oral cavity provide excellent conditions for propagation of oral pathogenic flora, which plays a very important role in dissemination of infection in the traumatized oral cavity.

Additional Surgical and Wound Phage Use

Phages have been employed for sanitation of the hospital environment, including the operation and critical care rooms. A special regimen has been developed, which includes daily washing and cleansing of walls, floors and furnishings with phages during the first week following patient admission, then every other day for the second week and twice a week from that point on. It has been demonstrated that this significantly decreases the incidence of nosocomial infections. A trial was carried out in three different hospitals in Tbilisi for evaluating the sanitation potentials of the phage over six months. The results were evaluated at one, two, and six months. 732 samples revealed that the isolation frequencies of *Pseudomonas aeruginosa*, *Proteus* and *Staphylococcus* nosocomial strains were initially 7.2%, 11.2% and 13.6% respectively. After phage sanitation, the frequency decreased to 3.6%, 6.3% and 8.2% after one month; 1.2%, 3.2%, 3.3% after two months; and 0.3%, 1.8% and 0.9% after six months, respectively.

Phages have been successfully used in battlefield trials, when paramedics and soldiers were spraying fresh wounds with liquid pyophage. To increase the effectiveness of the phage therapy there, Eliava Institute scientists continually renewed the pyophage with new phages against primary or nosocomial bacterial strains. Fresh wound swabs from the war zone and also infectious wound microflora from nearby hospitals were delivered to the Eliava bacteriophage Institute within the first day and bacteriophage preparations against the most frequent and virulent strains were constructed and differentiated for infection prevention and treatment, including against nosocomial infections in this region. As a result, very broad-range and effective bacteriophage preparation were obtained and the phage sensitivity of the infections was more than 85%. These preparations were used immediately for empiric phage therapy even before the bacterial sensitivity of the phage had been tested. The results of this trial led to the following conclusions: 1. Prophylactic use of phages in gunshot wounds cannot substitute for primary wound care, but it does effectively prolong the "golden period" for wound debridement; 2. Phage application after appropriate wound care significantly decreases subsequent suppuration of the gunshot wounds; 3. Phage therapy of gunshot wounds substantially shortens the recovery period.

Poland

The Hirszfeld Institute of Immunology and Experimental Therapy is located in Wrocław, Poland. The Hirszfeld Institute has been supplying phages to local physicians dealing with antibiotic-resistant infections and otherwise performing phage therapy-related work for many years and has regularly published detailed summaries of the results since the early 1980s, coauthored by Beata Weber-Dąbrowska with Stefan Ślopek (director of the institute until 1986) or Andrzej Górski (director of the institute, 1999-2007). In 2005, the institute established its own phage therapy clinic, and they are now able to develop more formal trials, under European Union guidelines. In addition to a number of articles describing first-hand their phage therapy experience and related issues (below), the group has also published, in English, general phage therapy reviews [16,17] plus have explored issues of phage purification [18], phage therapy economics [19], phage translocation within bodies [16,20-22], the role of endogenous phages in bacterial control [23], phage interaction with the animal immune system [24-36], and the phage therapy of children [37] and cancer patients [38]. In short, the clinicians involved in phage therapy at the Hirszfeld Institute are the group most experienced with phage therapy and studying phage physiological effects that is found outside of the former Soviet Union.

Experience of the Hirszfeld Institute, Overview

The Hirszfeld Institute has employed phages against a variety of target organisms responsible for a number of diseases. In general they have employed the "phage bank" approach, which is to say that they choose one or more phages from their collection which are active against a given bacterial isolate. From Fortuna *et al.* [37, p. RA128].

> Only lytic phage preparations which are prepared for each individual patient are used therapeutically; therefore the process involves individual matching of the offending bacterium with the respective phage followed by its multiplication and the preparation of a final phage preparation (which contains one or a mixture of the most efficient phages). We are currently expanding our phage collection by searching for new phages from the environmental and clinical isolates. However, we are also preparing a phage preparation repository, where different phage prepa-

> rations are stored and could be applied as soon as their efficacy is confirmed. In this way, beginning phage administration and modifying the kind of phage used in response to changing phage sensitivity during therapy could be significantly upgraded.

A description for a specific case can found in Leszczynski *et al.* [39, p. 236].

> Eleven polyvalent *S. aureus* bacteriophages from the *L. Hirszfeld Institute* collection provided a phage panel with a wide spectrum of activity. The phages were examined for their lytic activity against the MRSA strain isolated from the patient. A phage preparation containing the three most efficient anti-MRSA phage strains was produced… The phage preparation containing the three phages… causing complete lysis of the MRSA strains.

This phage bank method is in contrast to the more presumptive "cocktail" approach in which a collection of phages are employed that together have the potential to be active against bacteria associated with a variety of patients [7]. Reportedly the institute's phage bank "possesses over 300 specific bacteriophage strains active against staphylococci, enterococci, *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*, *Enterobacter*, *Proteus*, *Serratia*, *Acinetobacter*, and *Pseudomonas*" [37, p. RA127].

The phage bank strategy is indicative of the philosophical approach to phage therapy taken by the institute which first and foremost is a local and humanitarian one, rather than an operation dedicated to the development and production of specific products destined for broad distribution. This local work takes place in association with their recently established clinic which, especially due to legal limitations imposed nationally within Poland (and, increasingly, standards in medical care imposed by the European Union), deals predominantly with well-established, antibiotic-resistant chronic infections. From Górski *et al.* [16, p. 131].

> According to Polish law, phage therapy is considered an experimental treatment which is carried out on the basis of the respective legislation (pharmacological law, regulations of the Minister of Health). Experimental treatment (or, translated literally, a therapeutic experiment) occurs when a physician introduces new or only partially tested diagnostic, therapeutic, or prophylactic methods for the direct benefit of the person being treated. In contrast, an investigational experiment has the primary purpose of broadening medical science (and is tantamount to clinical research). To satisfy the existing requirements, two basic items are prerequisites for experimental therapy: (a) the written informed consent of the patient and (b) approval by an institutional review board (bioethics commission). Furthermore, it may be implemented only by a qualified doctor and when available treatment has failed (arts. 29/1, 21/2, and 21/3 of the law on the physician's profession). Therefore, our current therapy involves cases in which prior antibiotic treatment did not lead to the eradication of infection.

Earlier clinical work was all performed outside of the institute and consequently may have been less rigorously monitored by the institute than more recent work.

A very reasonable argument, advanced by the Hirszfeld physicians, is that the chronic infections that they treat using phages are inherently more difficult to cure than would be less advanced, presumably less biofilm-associated, earlier stage infections. Hopefully they can soon go beyond this current limitation and again legally use phage treatment earlier. Phages, as an experimental treatment are employed by the clinic predominantly towards addressing infections which have not adequately responded to conventional treatment approaches (i.e., antibiotic treatment). Thus, the potential for phage therapy efficacy in fact is presumably lower than were phages applied more generally to treat bacterial infections, such as prophylactically against potentially contaminated wounds or as a first-line treatment against newly acquired bacterial infections. It is also important to note that the phage therapy efforts at the Hirszfeld Institute are being performed not, at least in the short term, for the sake of economic gain by the physicians involved but instead for the sake of addressing patient suffering. This point is important because there exists a cohort of biomedical workers who are phage therapy "doubters", that is, who apparently have rejected phage therapy on the assumption that most or all phage therapy efforts are being done for economic rather than humanitarian reasons.

The Hirszfeld Institute has also been active in exploring the impact of phages, as during phage therapy, along with phage lysates of bacteria, as immunomodulators. Phage stimulate humoral immunity against the specific phage administered, and are removed from systemic circulation by the reticuloendothelial system based on their display of specific motifs recognized as foreign by the immune system [6]. Phages can also confer both immunostimulatory and immunosuppressive effects relevant to phage therapy, such as stimulating immune responses to bacterial pathogens and reducing side effects of therapy. It is possible to confuse these efforts towards characterizing phage-mediated immune system modulation with efforts at the Hirszfeld Institute in exploiting phage therapy as more traditionally defined, i.e., the application of phages specifically to kill bacterial pathogens via infection and subsequent lysis. Thus, see [24-36] as references of work aimed primarily toward characterization or application of phage-mediated immunomodulation, as well as [16] which as part of its scope reviews the phage immunomodulation literature. By contrast, in the following section we focus primarily on phage therapy *sensu stricto* as experienced at the Hirszfeld Institute.

Specific Approaches Employed at the Hirszfeld Institute

Phage therapy of humans in Poland is reported to date back, in this case as an anti-staphylococcal treatment, at least to 1925 [16]. Also according to Górski *et al.* [16], a tradition of phage therapy developed by the 1940s which, in 1954, became centered in Wrocław at the center founded by Professor Ludwick Hirszfeld. Using phages specifically selected from its collection by institute scientists for each patient, over 2,000 patients have been treated with phages since the 1970's. English summaries of the results of all treatments

employing institute phages during this period were published in the 1980s [40-46]. These were not controlled clinical trials. However, since reportedly every patient treated during that period of time is included and in almost all cases the patients were brought into the Institute program only after all antibiotics and other approaches had failed, this is in many ways the most significant set of data in the phage therapy literature to date. Substantial detail as to specific results and probable reasons for the occasional failures were included in the more specific articles on particular kinds of applications, with integrative review articles summarizing the overall results at several points. The reported results indicate a strong potential for phage therapy efficacy. However, it is important to keep in mind that the treatments themselves were not done at the Institute and therefore were not performed under full Institute control, were not standardized, and may not have been reported to the Institute by full detail.

In 2001 a reported 1,400 patients had been treated under the auspices of the Hirszfeld Institute since the 1987 summaries, with an "overall cure rate" of 90%; "therapy has proved to be most effective in purulent otitis media, purulent cerebro-spinal meningitis and furunculosis" [138, p. 132]. As noted, in 2005 these efforts were transferred to an out-patient phage therapy center established in the Institute and the work became more standardized under the guidelines of the European Union, of which Poland was now a member. In addition to descriptions provided in individual reports, an overview of the modern Hirszfeld approach as employed in their phage therapy center is provided by Górski *et al.* [16, p. 131].

> According to our protocol, 10 ml of phages are administered orally three times daily before eating and after neutralization of the gastric juices. Phages have also been applied directly to wounds, as ear and nose drops, infusions to fistulas, washing of the nasal cavity, intraperitoneally during washing of the peritoneal cavity, and topically in cases of multiple skin abscesses. Phage treatment has been highly effective in infections caused by different species of bacteria: *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas*, and *Staphylococcus aureus*, with an average success rate of 85%. Importantly, our sets of phages have been highly efficient against such dangerous pathogens as *S. aureus*, methicillin-resistant strains (MRSA), and *Pseudomonas aeruginosa*.

They also report temporary "minor side effects" in 2% of treated patients. Description of non-oral approaches include application of phages to treat an antibiotic-resistant, post-operative *Staphylococcus* infection by applying phages "using a cannula communicating with the site of infection" [16, p. 132]. In addition to treatment of infections, decolonization of otherwise normal flora (in this case gastrointestinal MRSA) has also been performed (with success determined via rectal swabbing).

As one example, Weber-Dabrowska *et al.* [47] provide a description of the treatment of septicemias using phages (which they abbreviate as "BP") at the Hirszfeld Institute:

> All patients had been treated previously with antibiotics without success. In 71 subjects the treatment was continued in addition to BP, whereas in 23 only BP were used. BP directed to a specific pathogen were given orally three times daily at a dose of 10 mL (children 5 mL), 30 minutes before meals, after neutralization of gastric juices. In the cases where blood cultures were positive, BP were matched for bacteria grown from blood; in the remaining cases (blood culture negative), BP were matched for bacterial isolates from other sites (wound, urine). The median time of therapy was 29 days.

In the study, treatment success ("complete recovery") occurred in 85.1% of the cases, with no statistical difference between outcomes for bacteriophages alone versus bacteriophage treatment in combination with antibiotics. Treatment of intransigent infections presented by a group of cancer patients, as reported in another study [38], was 100% successful following 2- to 9-week protocols with phages administered three times daily either orally or locally. Overall, these results are highly suggestive that the Hirszfeld Institute approach to phage therapy can provide substantial efficacy, though as yet these protocols have not been subject to rigorous, doubled-blinded clinical testing.

RENEWED LAUNCH OF CLINICAL PHAGE THERAPY IN THE WEST

A small number of Western physicians have been making occasional therapeutic use of phages in recent years, in Australia, Canada, France, Germany, and the USA. A major problem has often been the obtaining of suitable phage preparations. Most of the commercially available preparations in Georgia and Russia involve very complex mixtures of phages targeting groups of relevant bacteria, an approach that has been found clinically very effective but, it is at least assumed, would probably not be well accepted by regulators. Although often-similar phage preparations were commercially available at the Institut Pasteur in Paris until the end of the seventies/beginning of the eighties, acquiring appropriate phage preparations to support human phage therapy is challenging today [48]. The other major barrier is a difficulty in getting funding, in part due to the lack of formal clinical trials - which of course require funding. A decade ago, two young US phage companies – Intralytix and Exponential Biotherapies - were both ready to start clinical trials with phages targeting vancomycin-resistant enterococcus (VRE), a major intransigent problem in hospital-acquired infections. Each seemed to have made major progress towards funding trials, and Exponential Biotherapies carried out a small phase I (safety) trial in England. Then the dot.com financial crisis hit, their major funding sources dried up, and the very expensive VRE clinical trials were indefinitely postponed, while they switched their attention to more manageable food-safety targets and much of the momentum toward human phage therapy was lost in the US. At last, momentum in a clinical direction seems to be building again. In this section, we discuss several relevant earlier small Western studies and three small recent clinical safety trials.

Historical Overview

Phage therapy has been practiced in France since 1919, when d'Hérelle's preparations were given to patients with dysentery at the Hopital des Enfants-malades (cf. [49]). His Laboratoire du Bacteriophage produced the first commercial

phage cocktails: Bacté-Coli-Phage, Bacté-Intesti-Phage, Bacté-Dysentérie-Phage, Bacté-Pyo-Phage, and Bacté-Rhino-Phage. These preparations were produced commercially in France until approximately 1978. Through the mid 1990's, the Pasteur Institutes of Paris and of Lyon continued to produce small amounts of bacteriophage preparations on demand [48]. There continued to be reports in the literature of phage therapy there until about 1979 (cf. [50,51]).

A few physicians in France have continued to use phages therapeutically even after the Pasteur Institutes stopped making therapeutic cocktails, in the mid 1990's, but now generally obtaining their phages from Russia or Georgia [48]; *Staphylococcus* infections seem to be the most common target of these more-recent efforts. Dublanchet and colleagues have recently reported phage therapy of two patients from France and one from Australia who had failed other therapies, including all available antibiotics (poster presented by J. Garnier, A. Khawaldeh, O. Patey, S. Morales, J. Iredell, A. Dublanchet, H. Mazure, A. Smithyman, 28th Reunion interdisciplinaire de chimiotherapie anti-infectieuse. Communication affichee).

In the initial JAMA review of phage therapy, Eaton and Bayne-Jones [52] found convincing data in only two fields: the treatment of localized staphylococcal infections and cystitis. Krueger and Scribner's [53] subsequent review noted the data in staphylococcal infections, including the relatively successful treatment of staphylococcal bacteremia by MacNeal and Frisbee ([54]; 100 patients) and others, but concluded that successful treatment with bacteriophages was due to "specific and nonspecific immunizing fractions of the crude lysate", rather than to the lytic action of phages and that "phage possesses no measurable degree of therapeutic superiority over properly prepared vaccines and toxoid." MacNeal *et al.* [55] subsequently reported the cumulative treatment of 500 patients with staphylococcal bacteremia, using cocktails of phage that were lytic *in vitro*. Dubos [15] reported *in vivo* lysis of bacteria with multiplication of bacteriophage as protective against experimental infection with *Shigella dysenteriae*, (see Fig. **3**, above), while injection of heat-inactivated bacteriophage afforded no protection unless injected several days prior to infection. Further refutations of the conclusions of the JAMA review were published by Morton and Perez-Otero [56] who noted an increase in bacteriophage *in vivo* during experimental infections with *Shigella paradysenteriae*. thus disputing many of the conclusions of the JAMA review [52,53]. However, the JAMA review appeared to have a long-lasting negative impact on clinical phage practice in the West, with the major use of bacteriophage in the United States over the next 5-6 decades as a vaccine [57].

The major phage preparation marketed in the US from at least the 1950s was initially called Lincoln Bacteriophage Lysate, and subsequently known as Staphylococcus phage lysate, staphage lysate, or SPL. SPL was made from the phage Gratia B 985 [58] used by the early bacteriophage researcher Andre Gratia. Mills [59,60] used this phage both as a vaccine, and as a spray application to the sinus cavities. Smith and Mudd [61] later noted that "the Gratia polyvalent staphylococcal bacteriophage" in staphage lysate was the same phage that had been prepared and distributed by the Michigan Department of health for the treatment of staphylococcal infections from 1926 through much of the 1930s [11,62,63].

Mudd studied the immune response to staphage lysate in animals and humans. According to the documents submitted by Delmont laboratories to the FDA, SPL was used in over 3,000 patients by Mudd and coworkers. They reported that SPL is a nonirritant, non-toxic, and that no hypersensitivity reactions were observed in man. There appeared to be no problems with safety, only with lack of data for efficacy as a vaccine (see www.fda.gov/ohrms/DOCKETS/dailys/03/Apr03/043003/80066cb9.pdf). In 1978, the FDA announced their intent to revoke the licenses and reclassify bacterial vaccines and bacterial antigens with "no U.S. standard of potency". Delmont stopped making SPL for human use in 1994, but continues to market SPL for the treatment of staphylococcal pyoderma in canines; several older physicians have reported having effectively used it and disappointment at its no longer being available.

Progress toward properly-approved reintroduction of phage therapy in the West continues to be slow. A few physicians in places like Germany, Australia, and the United States have reported using phage preparations, either from Tbilisi or ones they have prepared themselves. This has been done on individual patients under "compassionate use" provisions where they felt it was dictated by strong patient needs (personal communication). The California-based company Phage International has taken many patients from Europe, Australia, and the United States to Tbilisi for treatment at the Phage Therapy Center there (cf. http://www.phagetherapycenter.com/), and also has secured rights to import phage preparations from Tbilisi to the US for specific patients when they are prescribed by physicians.

Several phase I clinical trials have been carried out on healthy volunteers (cf. [64]), with companies such as Néstle (Switzerland) actively involved. Three small phase I (as well as IIa) clinical trials involving actual patients have now been reported in the Western literature. Their challenges and results are worth exploring as we look toward more widespread application of phage therapy to help deal with the ever-widening problems of severe antibiotic resistance, including such issues as MRSA.

British Phase I/II Study: *Pseudomonas aeruginosa* Ear Infections

Chronic otitis, a very common and hard-to-treat condition, has been a primary initial target of the British phage therapy firm, Biocontrol Limited. Here, the bacteria are often largely organized into biofilms and relatively protected from both antibiotics and immune cells, with *Pseudomonas aeruginosa* infections being particularly hard to eradicate. Most strains have become refractory to antibiotic treatment, and aminoglycoside use has to be curtailed due to ototoxic effects if the tympanic membrane is perforated. The Biocontrol scientists carried out a reportedly successful trial of phage against *Pseudomonas* dog ear infections [65]. They then used the results of that trial to obtain regulatory approval for a phase I/II human trial, with a clinical outcome measure as the primary indicator and bacterial counts as secondary out-

comes, as specified by the major regulatory agencies of the US and Europe.

Both the canine and human trials involved the same small group of phages (five podoviruses and a myovirus) specifically selected against a target population of ear infections and prepared by Biocontrol Limited. The study population was explicitly chosen from people whose *Pseudomonas* was sensitive to at least one of those six phages. In this case, nothing is said in the published paper about the specific method of preparation and characterization of the phage cocktail, but it must have been sufficiently rigorous to pass the British regulatory requirements. Interestingly, the level used is very low – less than a million phage, and in a single dose, as done in their dog-ear study. Like the Georgians, they have found in various *in vitro* and *in vivo* studies that using relatively low amounts of phage is effective in clinical terms, indicating that active therapy is occurring [6].

Wright *et al.* [66] describe the results of this randomized double-blind placebo-controlled clinical trial, which was approved through the UK Medicines and HealthCare Products Regulatory Agency (MHRA) and the Central Office for Research Ethics Committees (COREC) ethical review processes. The study was carried out at the Royal National Throat, Nose and Ear Hospital in London, under the direction of Prof. Anthony Wright. Exclusion criteria included a recent history of local surgery, current antibiotic use, and unusual ear flora such as hemolytic group A, B, C or G strep; women of childbearing potential were also excluded. They selected 24 patients whose long-term chronic ear infections (2-58 years) involved an antibiotic-resistant *P. aeruginosa* strain sensitive to one or more of the 6 phages in Biophage-PA, the preparation that they used in their canine study. (Of the potential candidates, 86% were sensitive to at least one of the 6 phages.) Pre-weighed, numbered swabs were used to collect the purulent discharge from the ear under study. The sample which was analyzed the same day by CentraLabs, Cambridge, in terms of bacterial concentration (on a variety of different selective plates) and, after treatment, in terms of phage concentrations.

The patients were randomized into 2 groups of 12, with median ages of 56.7 and 56.6, respectively, for the test and placebo groups, and the trials were run within 2 weeks of assessment. They were treated with a single dose containing 10^5 phages of each of the 6 phage types or a 10% glycerol-phosphate-buffered-saline diluent used as placebo, delivered in 0.2 ml volume via syringe. No other treatment was used; specifically, no systemic or topical antibiotics were used and no aural cleansing was performed except at follow-up sessions, performed by the same otologist at 7, 21 and 42 days. Significant clinical improvements from baseline and significant reductions in *P. aeruginosa* counts were seen in the phage-treated but not in the placebo group, independent of the cause of the discharge. Mastoid cavities, perforations and chronic otitis externas were equally distributed between the groups. There were no reportable side effects or evidence of local or systemic toxicity. Encouragingly, there was substantial replication of all 6 test phages, each tested on its own assay strain resistant to the other 5 phages. The mean prior the mean bacterial recovery from all swabs was 1.27×10^8 CFU/g exudate. The mean duration of phage replication was 23.1 days (median, 21 days), and clearance of all phages was observed after resolution of the infection in all cases where resolution occurred. No serious adverse events were reported; both groups had similar numbers of mild to moderate treatment-emergent events associated with the process.

By the end of the trial, reduction to < 10% of the original VAS value was seen for 3 of the 12 even from this single-application trial. In all 3, both *P. aeruginosa* and phages were below the limit of detectability on day 42. None of the placebo patients showed such a reduction. Overall, the scores were 0-101% of the day-0 value for the 12 phage-treated patients and 26-294% for the placebo-treated group. Biocontrol reports that plans are progressing for large-scale, Phase III otitis trials as well as application of their phage cocktails in other infections, such as infected burn sites and the lung infections of children with cystic fibrosis, where *P. aeruginosa* is the major causative agent.

The Belgian Experience: Focus on Burn Infections

A group of Belgian surgeons and scientists have long been interested in the challenges of antibiotic resistance and the possibilities of using phages, particularly in burn applications, and have developed an extensive collaboration with phage biologists in both Moscow and Tbilisi to move this work ahead. During this process an international organization named Phages for Human Applications Group Europe (P.H.A.G.E.), was created by this 'phage community' (www.p-h-a-g-e.org/Home.html). It is an international non-profit organization for the promotion of research and clinical trials in a regulated framework.

The group has focused particularly on carrying out clinical trials with burn patients. There have been huge advances in burn medicine in recent years, but infections remain a major cause of morbidity and mortality; often, these burn-wound infections are virtually untreatable. *Staphylococcus aureus* remains a common early colonizing pathogen in burn wounds, but *Pseudomonas aeruginosa* is increasingly known as the most common cause of life-threatening infection in burn patients [67,68]. Both *S. aureus* and *P. aeruginosa* are among the most thoroughly studied and demonstrably effective targets in terms of the Georgian experience, including in burn and other wound work, and infected burns are both accessible and very problematic targets for antimicrobials. To illustrate the magnitude of the burn wound infection problem at the Burn Wound Centre (BWC) of the Queen Astrid Military Hospital in Brussels, Pirnay *et al.* [69] carried out a one-year-study of *P. aeruginosa* colonizations and infections, during which a total of 441 patients were treated at the 32-bed BWC. Of these, 70 were colonized with *P. aeruginosa*, 57 (13%) 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.

The few burn wound-related phage papers in the scientific literature [70-72] suggest that phages have the potential to control burn wound infection. Soothill [70] applied 6×10^5 CFU/ml of *P. aeruginosa* to excised burn wounds in guinea pigs and then grafted on replacement tissue; when 1.2×10^7 *P. aeruginosa* BS24 phages were applied simultaneously with the bacteria, 6 out of 7 of the grafts took success-

fully, whereas all 7 control grafts with no phage treatment failed [70]. Weber-Dabrowska *et al.* [73] reported on the treatment of 49 recalcitrant burn wounds in human patients infected with *P. aeruginosa*, *S. aureus*, *E. coli*, *Klebsiella*, and/or *Proteus* – infections which were not responding to any of the standard treatments there in Poland. Forty-two of these patients fully recovered, and the condition of the remaining seven patients improved markedly. Abul-Hassan *et al.* [74] described the treatment of 30 Egyptian burn patients with between 15 and 45 phage-lysate-saturated dressings over 5-17 days. The clinical success ratio was difficult to assess because of the lack of validated controls, but the mere fact that not-endotoxin-purified phages [7] had been applied massively to burn wounds was indicative for their intrinsic harmlessness. A recent publication addressed the treatment of local radiation injuries in two individuals, using a novel biodegradable preparation capable of sustained release of phages and ciprofloxacin [71]. The same product was applied in Georgia on 22 patients with infected venous static ulcers and other poorly healing wounds, after standard therapy had failed [75]. Seventy percent of the patients showed full recovery after a period ranging from 6 days to 15 months. Finally, in the UK, the group of Soothill reported the case of a 27-year-old male with 50% TBSA (Total Body Surface Area) burned and excised burn wounds covered with skin grafts, which became infected with *P. aeruginosa* after several months [72]. Grafted areas broke down rapidly despite appropriate antibiotic treatment. Therefore, treatment with 'purified' phages was started. Phages multiplied in the wound and a 43 to 1200-fold increase of phages was observed. Three days after phage application, *P. aeruginosa* could no longer be isolated from swabs and subsequent extensive grafting was successful.

As a key step towards full clinical trials of phage therapy at the Brussels Burn Wound Centre, a small clinical safety study in burn patients infected with *P. aeruginosa* and/or *S. aureus* was launched after approval by a leading medical ethics committee. This small study on nine patients consisting of ten bacteriophage applications was launched after a process which has been published step-by-step [76,77]. In brief, a well-defined and quality-controlled cocktail of three phages, two targeting *P. aeruginosa* and one against *S. aureus*, was applied on colonized burn wounds (nine patients, ten applications).

Merabishvili *et al.* [77] 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 exclusively 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 confirmed that the chosen phages were not temperate as well as the absence of toxin-coding genes.

The general trial setup involved a comparison of the standard treatment for *P. aeruginosa* and *S. aureus* burn wound colonization with a single spray application of this BFC-1 phage cocktail on one part of each colonized burn wound. 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.

Some technical problems were encountered using the initial protocol dictated or suggested by the burn-center clinicians and the safety committee. For example, biopsy samples were used to monitor the bacterial load of the burn wounds because they are considered to be the gold standard by the majority of researchers [68,78,79]. However, this technique was found to be excessively cumbersome, impeding the clinical trial, since it necessitated local anesthesia and complex sample processing, and patient and/or nurse aversion to biopsies was encountered. Also, this way of pretesting, with delays in receiving antibiograms and in being given informed consent, often led to long periods between detection of a candidate with MDR *P. aeruginosa* and/or *S. aureus* burn wound colonization and the inclusion of this patient into the study. In the meantime, the patients were treated, often empirically, with potent topical antimicrobials, dressings, and systemic antibiotics, probably explaining why the microbial level was often quite low by the first time point of the actual clinical trial. Under the circumstances, the results could say little about efficacy, but at least the medical and nursing staff of the BWC grew familiar with phages and now deem them safe for topical use on burn wounds. The next phase of the trial will use larger quantities of BFC-1, in a cream or gel instead of a spray (which runs out of the wound), and this on at least a daily basis. Visual observation of burn wound infection by an experienced clinician will be the main initial selection criterion for patients, instead of bacteriological results such as time-consuming antibiograms. This will mean the inclusion of all burn wound infections, not only those with MDR *P. aeruginosa* and *S. aureus* (which do, however, make up for the vast majority of burn wound infections in the BWC). The monitoring of burn wound colonization will use swabs instead of biopsies.

This very active international consortium has now introduced a new study protocol, "Nasal decolonisation of methicillin-resitant *Stapylococcus aureus* with mupirocin or phage ISP: a prospective randomised double blind comparison of both treatments". They are also exploring the kinds of matrices best suited for phage applications on wounds and such sites as ears and eyes through ongoing *in vitro* experiments also involving pharmacists. Another protocol is in preparation for continuing burn studies in 2010 and the refinement of phage preparations and development of analogous ones against other pathogens is ongoing.

Lubbock, Texas, Physician-Initiated Phase I Trial

In Lubbock, the Wound Care Center had extensive experience in treating individual patients with otherwise-recalcitrant wounds with pyophage brought from the Eliava Institute before making the decision to initiate its own physician-instigated trial of phage therapy to deal with leg ulcers. The FDA-approved phase I, prospective, randomized, double-blind study was performed to treat patients with venous leg ulcers, to evaluate the safety of bacteriophage preparation "WPP-201" developed by Intralytix. The study cocktail contained eight individual phages isolated originally from the environment and selected from a very large number of isolates on the basis of such properties as breadth of host range and efficacy of lysis. They included representatives of both the Podoviridae and Myoviridae families; 5 were lytic for *P. aeruginosa*, 2 for *S. aureus* and 1 for *E. coli*. Each phage was characterized by plaque morphology, taxonomy and genome size (by PFGE), protein fingerprint profile (by SDS-PAGE), genomic fingerprint profile (by RFLP) and by full genomic sequencing. For therapeutic application studies, they were kept in phosphate-buffered saline solution at a concentration of 1×10^9 PFU/ml of each of the component monophages.

Patients were selected using the exclusion and inclusion criteria according to the study protocol. Forty two patients with full thickness venous leg ulcers of over 30 days duration, with or without clinical signs of infection, were included in the study. Of the 42, 39 completed the treatment: 21 in the control and 18 in the treatment group. Patients received up to 50 ml of either 1:12.5X diluted phage preparation or of sterile saline on each visit via an ultrasonic debridement device, using a drip rate of 200ml/h at 15-30 sec/ cm^2. The pushes phage through superficial layers as the raw tissue is exposed in the course of the debridement.

As part of the clinic's standard wound management practices, Promogran (Systagenix) and Acticoat were used in all patients as the primary wound dressing; Allevyn was used as the secondary dressing along with a custom-compounded topical gel containing bovine lactoferrin and xylitol. Compression dressing was used on all patients beginning one week prior to study. Dressings were changed three times per week and the study preparation was applied during each weekly office visit for twelve weeks. Primary endpoint evaluation of all enrolled subjects was performed at week twelve with follow up evaluations at weeks 16 and 24. Results of the study revealed no significant differences in adverse effects between treatment and control groups and no serious problems arising in either. This is not surprising, given that humans are exposed to bacteriophages from birth (and, possibly, even *in utero*). They have been commonly found in the human gastrointestinal tract, skin, urine, and mouth, where they are harbored in saliva and dental plaque, and the safety of bacteriophages has been described in various reports, with no serious complications associated with the use of them.

Although the study was not designed as an efficacy study, wound healing frequency and rate were evaluated. They revealed no statistically significant differences between the control and treatment groups. There are several factors that might have been impacting the efficacy in the trial, beyond the small sample size. The wound bacterial flora were not tested for sensitivity to the study preparation prior to the treatment. Unpublished data from the Georgian clinical phage therapy work indicate the critical significance of sensitivity studies prior to the treatment to ensure highest efficacy, but that did not seem to be a necessary criterion in a trial designed primarily to determine safety. Another issue raised by the low efficacy was the possibility that some of the components included for all patients as part of the Wound Center's standard therapy might be interfering with the bacteriophage activity. Lactoferrin has been described as possessing antiviral activity, and an *in vitro* test indicated that it could indeed also inactivate phages at high concentration. The sonication-based phage delivery, the time between the phage and lactoferrin-layer application, and the intervening layer of Promogran used as the primary wound dressing under the lactoferrin-containing custom compound decrease the likelihood for phage inhibition resulting from interaction with the lactoferrin.

Promogran contains collagen, and unpublished Georgian reports indicate an incompatibility of incorporating phage into collagen-containing dressings. However, that problem seems to be in terms of phage release from the dressing, so that should not introduce any problems here. The authors also mention a possible impact of ultrasound on bacteriophage viability. Very extensive prior studies and clinical experience in Tbilisi indicate no significant reduction in phage titer when it is applied through sonication – the routine method in wound treatment there – but it would be prudent to confirm this with the specific equipment and conditions to be used in any future clinical trials.

In conclusion, as expected, this phase I study raised no concerns about the clinical safety of the "WPP-201" bacteriophage preparation. These trials were undertaken in the first place due to positive but not controlled results associated with phage treatment of infected wounds in this setting. Though such limited initial trials can very rarely demonstrate efficacy, it laid the groundwork for appropriately designed phase II trials to test efficacy and further establish dosing, safety and appropriate trial conditions.

REGULATORY CHALLENGES

Among the challenges hampering the clinical application of phages in Western medicine, a major one is adapting our regulatory framework to appropriately fit this very different sort of self-replicating and self-limiting, natural pharmaceuticals/antimicrobials. There is much uncertainty as to the regulatory status of phage therapy in much of the Western world. Pending the eventual creation of an adapted European regulation tailored to phage therapy, the current European regulatory setting only allows for eventual sporadic clinical trials under the responsibility and supervision of Medical Ethical Committees and/or border-line applications under the umbrella of the Declaration of Helsinki [76]. In this section, we discuss specific regulatory experiences associated with the above-considered Western phage-therapy studies and expand on some of the issues.

British Ear Infections

The human *Pseudomonas* ear infection trial built directly on the successful dog-ear infection work, and the company

worked closely with the British regulatory bodies throughout [66]. The trial was approved through the UK Medicines and HealthCare Products Regulatory Agency (MHRA) and the Central Office for Research Ethics Committees (COREC) ethical review processes. Clinical outcome measure as the primary indicator and bacterial counts as secondary outcomes now seem to be what is specified by the major regulatory agencies of the US and Europe. Biocontrol now has very valuable advanced trial input from both the EMEA and the FDA on which to base plans for future trials.

Belgian Burn Patients

The Belgian clinical burn trials were carried out by an international group of academic collaborators (from Belgium, Georgia and Russia) rather than by a company, and built largely on volunteer labor assisted by some small grants. This approach has both advantages and difficulties. A major part of the challenges came from dealing with paucity of general understanding as well as misconceptions at all levels – in the process at least laying groundwork carefully for future trials. The lack of basic general knowledge concerning the nature of bacteriophages as viruses was illustrated when they were asked to submit their phage cocktail to the National Approval System for Genetically Modified Organisms (GMO), through which the safety for humans, animals and the environment is assessed. Then, during the administrative process, the experts of the insurance company grouped phages with viruses and with delivery vectors as gene therapeutic vehicles, and consequently assigned their modest experiment to risk class 5 (on a scale from 1 to 7), which led to a relatively high premium for the insurance that is mandatory for clinical trials there.

Texas Leg Ulcers

To aid getting FDA approval, the Lubbock wound care center used a cocktail of 8 phages thoroughly characterized, sequenced and prepared for them by Sandro Sulakvelidze of Intralytix, Inc. Intralytix used its very extensive (and at times painful) experience in dealing with regulatory issues regarding the FDA approval of phage cocktails to deal with *Listeria* on ready-to-eat meats and cheeses to gain approval for use of this cocktail. At least for this trial, the FDA classified phages as drugs and specified the sequencing and characterization of each phage in the cocktail. They did, however, include the right to substitute other similar, well-characterized phages into the cocktail – a key concession in being able to respond to complex and evolving bacterial populations. Intralytix also recently won a U.S. Army Phase I STTR contract to support Phase II development of a bacteriophage-based probiotic dietary supplement that could help reduce the incidence and severity of *Shigella* infections – much the prophylactic way in which phage cocktails are often used – and they also have similar products in the pipeline for dealing with the *Salmonella* strains that cause dysentery. Probiotics can be regulated by the FDA as dietary supplements, foods, or drugs, so it will be interesting to see how this product will be regulated. Since the Intralytix phage applied to food is generally recognized as safe (GRAS), perhaps the probiotic phage will be regulated as a dietary supplement.

Historically, our regulatory framework is largely based on the development of "chemical" drugs, including antibiotics. Our usual legal/regulatory way of working and general thinking in the development of new medicines is essentially based on the experience and development of chemical pharmaceutical agents. The FDA classifies products as food, drugs, medical devices, vaccines, blood and biologics, etc. This is very different from the behavior of two entities (a bacteriophage and a bacterium) that evolve continuously as an interactive system. The introduction into medicine of this so-called Darwinian medicinal approach requires a non-linear and continuously dynamic point of view.

Phages may fit more appropriately in to the "natural product" mode, for which there is a very different, more flexible framework but for which no specific health claims can generally be made. There have also been proposals that the FDA treat phages in traditional phage cocktails such as pyophage more like the components of influenza vaccine, which is reevaluated and changed yearly, but does not require clinical trials for each revision. As antibiotic resistance increases, the FDA may well want to classify the phages for at least some types of human therapy as a type of biologic – a very justifiable choice. In fact, it may be appropriate to classify phages in different ways for different applications, as a drug, for example, for intravenous applications, where that seems very appropriate, but as a biologic for more superficial applications, reflecting the ways phage and people are in constant contact in nature.

CONCLUSION

The concept of a self-replicating, self-regulating natural antimicrobial that can penetrate into the most sequestered corners of the body and selectively combat pathogens is very exciting. Phage therapy clearly has many special advantages – the ability to target specific pathogens with minimal destruction of normal body flora, the ability to cross physiological barriers such as the blood-brain barrier and get into the furthest depths of osteomyelitis in a bone, the ability to disappear with little or no trace when the pathogen is no longer present – making phages logical partners of our natural bodily defenses and potential pillars when they break down. The field of phage therapy, including human phage therapy, has been making progress as novel phages, technologies, and techniques are introduced, along with a greater modern understanding of phage biology, phage ecology, and the roles of phages in maintaining microbial balance in general has emerged. Further development of phage therapy as a common alternative to strictly chemical-based treatment of bacterial infections in humans, however, will require far greater and sustained investment than has so far been the case, particularly in basic research. While the need for this alternative to antibiotics is very pressing, it is important to evolve the basic scientific understanding along with the new regulatory frameworks that are necessary and important to avoid repeating the mistakes of the past, and to develop first the areas of phage therapy that are most proven to be effective, such as its use against MRSA and other forms of *Staphylococcus*, which have been recognized as successful targets since the 1930s.

ACKNOWLEDGEMENTS

We would like to thank the many colleagues who have shared their experience, unpublished results and editorial aid -- especially Ketevan Gachechiladze, Amiran Meipariani, Nino Chanishvili and Mzia Kutateladze at the Eliava Institute and its Director, Rezo Adamia; Georgian maxiofacial surgeon Teona Danelia; Alexander Golidjashvili of Biochimpharm; Beata Weber-Dabrowska and Andrzej Górski of the Hirszfeld Institute; Paris physician Alain Dublanchet; David Harper and Alastair Monk of Biocontrol; the Belgian phage community, Jean-Paul Pirnay, Gilbert Verbeken, Thomas Rose, Serge Jennes, Martin Zizi, Mario Vaneechoutte (UGent), Maya Merabishvili (Eliava), Geert Laire (Queen Astrid Military Hospital), Viktor Krylov (Moscow), Rob Lavigne (KUL, FWO WOG grant) and the members of P.H.A.G.E (www.p-h-a-g-e.org); W.022.09.N FWO Vlaanderen; Randy Wolcott and Dan Rhoads of the Lubock Wound Care Center; Harald Brüssow of Néstle; Naomi Hoyle, Grace Filby, Ketie Gabitashvili, and Robert Jeffris; Thomas Häusler, author of *Viruses vs. Superbugs;* d'Herelle's great grandson Hubert Mazure and Sandra Morales of Special Phage Services; Evergreen colleague Andrew Brabban and the Phage Lab students there; and the board and supporters of the PhageBiotics Foundation.

ABBREVIATIONS

BP	=	Bacteriophage
BWC	=	Burn Wound Centre (Queen Astrid Military Hospital, Brussels, Belgium)
CFU	=	Colony-forming units
DTRA	=	Defence Threat Reduction Agency
FDA	=	Food and Drug Administration
GMP	=	Good manufacturing practices
GRAS	=	Generally Regarded as Safe
ICU	=	Intensive care unit
ISTC	=	International Science and Technology Centers
JAMA	=	Journal of the American Medical Association
MDR	=	Multiple drug resistance
MRSA	=	Methecillin or multiple drug resistant *Staphylococcus aureus*
PFGE	=	Pulsed field gel electrophoresis
PFU	=	Plaque-forming units
RFLP	=	Restriction fragment length poylmorphism
SDS-PAGE	=	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SPL	=	Staphage lysate (*Staphylococcus* phage lysate)
STTR	=	Small Business Technology Transfer Program
TBSA	=	Total body surface area
VAS	=	Visual analogue scale

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Received: December 08, 2009 **Accepted: December 10, 2009**