

# Phage Therapy: Bacteriophages as Natural, Self-Limiting Antibiotics

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## CHAPTER CONTENTS

|  |                                   |
|--|-----------------------------------|
| <b>Introduction</b> 1147                           | <b>Clinical Applications</b> 1153 |
| <b>Historic Context</b> 1147                       | Current Research 1153             |
| Discovery 1147                                     | Bacterial Pathogenicity 1157      |
| Early Research 1147                                | Advantages of Phages 1158         |
| Initial Attempts at Commercialization 1148         | <b>Toxicology</b> 1158            |
| Specific Problems of Early Phage Therapy Work 1149 | <b>Drug Interactions</b> 1159     |
| <b>Bacteriophage Physiology</b> 1149               | <b>Conclusion</b> 1159            |
| Properties of Phages 1150                          |                                   |
| <b>Phages and the Immune System</b> 1152           |                                   |

## INTRODUCTION

Phage therapy involves the use of specific viruses—viruses that can attack only bacteria—to kill pathogenic microorganisms. The art was first developed early in the twentieth century, but since the advent of chemical antibiotics in the 1940s, it has been little used in the West. Today, however, the growing incidence of bacteria that are resistant to most or all available antibiotics is leading to widespread renewed research interest in the possibilities of phage therapy.<sup>1-9</sup> Most of the recent articles appearing in the West reflect little knowledge of the extensive Eastern European research and clinical utilization of phage therapy. The good clinical results of Eastern European research provide a substantial basis for optimism and complement the limited animal work in the West. We need to draw as much as possible on the largely unknown body of knowledge that has accumulated in Poland and the former Soviet Union as we again explore phage therapy and to give credit where it is due for the many years of hard, careful work in the field even though it has primarily been done in a clinical rather than controlled-research mode. This chapter has been written to put phage therapy into historical and ecologic context and to explore some of the most interesting and extensive research in Eastern Europe, with the hope that

this modality will soon be available for implementation by physicians of all schools.

## HISTORIC CONTEXT

### Discovery

More than a century ago, Hankin<sup>10</sup> reported that the waters of the Ganges and Jumna rivers in India had a marked antibacterial action that could pass through a porcelain filter, and then could be destroyed by boiling. He particularly studied the water's effects on *Vibrio cholerae* and suggested that the substance responsible was what kept cholera epidemics from being spread by ingestion of the water of these rivers. However, he did not explore the phenomenon further. Edward Twort and Felix d'Herelle independently reported the isolation of filterable entities capable of lysing bacterial cultures and of producing small cleared areas on bacterial cultures, implying that discrete particles were involved.<sup>11</sup> They are jointly given credit for the discovery of bacteriophages.

### Early Research

It was d'Herelle, a Canadian working at the Pasteur Institute in Paris, who gave these newly discovered organisms the name *bacteriophages*—using the suffix *phage*

“not in its strict sense of *to eat*, but in that of *developing at the expense of*.”<sup>12</sup> He carefully characterized them as viruses that multiply in bacteria, and he worked out the details of infection of different bacterial hosts by various phages under a variety of environmental conditions. The 90th Annual Meeting of the British Medical Association in Glasgow featured a very interesting discussion among d’Herelle, Twort, and several other eminent scientists of the day on the nature and properties of bacteriophages. The main question was whether the observed bacteriolytic principle was an enzyme produced by bacterial activity or a form of tiny virus. Gradually, it became clear that the phage is indeed viral in nature, able to reproduce and direct the synthesis of its own enzymes.

D’Herelle summarized the early phage work in a 300-page book, *The Bacteriophage: Its Role in Immunity*.<sup>12</sup> He wrote classic descriptions of plaque formation and composition, infective centers, the lysis process, host specificity of adsorption and multiplication, the dependence of phage production on the precise state of the host, isolation of phages from sources of infectious bacteria, and the factors controlling stability of the free phage. He quickly became fascinated with the apparent role of phages in the natural control of microbial infections. He noted, for example, the frequent specificities of the phages isolated from recuperating patients for disease organisms infecting them and the rather rapid variations over time of the phage populations. Throughout his life, he worked to develop the therapeutic potential of properly selected phages against the most devastating health problems of the day. However, he initially focused on simply understanding phage biology. Thus, the first known report of successful phage therapy came from Bruynoghe and Maisin,<sup>13</sup> who used phage to treat staphylococcal skin infections.

After much travel, including the study of epidemics in Latin America and a year at the Pasteur branch in Saigon, d’Herelle left the Pasteur Institute in 1922. He worked in Holland and then became employed as a health officer by the League of Nations, based in Alexandria, Egypt. Phage therapy and sanitation measures were the primary tools in his arsenal to deal with major outbreaks of infectious disease throughout the Middle East and India. In 1928, he was invited to Stanford to give the prestigious Lane lectures; his discussions were published as the monograph *The Bacteriophage and its Clinical Applications*.<sup>14</sup> He gave many lectures for medical schools and societies as he crossed the country. He accepted a regular faculty position at Yale, where he was supported by George Smith, translator of his first two books into English. D’Herelle continued to spend summers in Paris working with the phage company he had established there and returned permanently to France in 1933, with excursions to Tbilisi, Georgia, to help establish phage work there.

George Eliava, director of the Georgian Institute of Microbiology, saw bacteriocidal action of the water of the Koura River in Tbilisi (Tiflis) that he could not explain until he became familiar with d’Herelle’s work while spending 1920 to 1921 at the Pasteur Institute. He became a very early collaborator of d’Herelle’s; several of his phage papers are cited by d’Herelle.<sup>12</sup> The two developed the dream of founding an Institute of Bacteriophage Research in Tbilisi—to be a world center of phage therapy for infectious disease, including scientific and industrial facilities, and supplied with its own experimental clinics. The dream quickly became a reality through the support of Sergo Orjonikidze, the People’s Commissar of Heavy Industry, despite KGB opposition to this “foreign project.” A large campus on the river Mtkvari was allotted for the project in 1926. D’Herelle sent supplies, equipment, and library materials. In 1934 and 1935, he visited Tbilisi for a total of 6 months and wrote a book, *The Bacteriophage and the Phenomenon of Recovery*,<sup>15</sup> which was translated into Russian by Eliava. D’Herelle intended to move to Georgia; in fact, a cottage built for his use still stands on the institute’s grounds. However, in 1937, Eliava was arrested as a “people’s enemy” by Beria, then head of the KGB in Georgia and soon to direct the Soviet KGB as Stalin’s much-feared henchman. Eliava was soon executed, sharing the tragic fate of many Georgian and Russian progressive intellectuals of the time, and d’Herelle, disillusioned, never returned to Georgia. However, their institute survived and is still functioning at its original site on the Mtkvari (which it now shares with the more modern Institute of Molecular Biology & Biophysics and Institute of Animal Physiology).

In 1938, the Bacteriophage Institute was merged with the Institute of Microbiology & Epidemiology under the direction of the People’s Commissary of Health of Georgia. In 1951, it was formally transferred to the All-Union Ministry of Health set of Institutes of Vaccine and Sera, taking on the leadership role in providing bacteriophages for therapy and bacterial typing throughout the former Soviet Union. Under orders from the Ministry of Health, hundreds of thousands of samples of pathogenic bacteria were sent to the institute from throughout the Soviet Union to isolate more effective phage strains and to better characterize their usefulness. In 1988, an official Scientific Industrial Union “Bacteriophage” was formed, centered in Tbilisi with branches in Ufa, Habarovsk, and Ghoroki. Their later work is further discussed here.

### Initial Attempts at Commercialization

From the beginning, a major commercial use of phages has been for bacterial identification through a process called *phage typing*—the use of patterns of sensitivity to a specific battery of phages to precisely identify microbial strains. This technique takes advantage of the fine

specificity of many phages for their hosts and is still in common use around the world. However, the sophisticated ability of phages to destroy their bacterial hosts can also have a very negative commercial impact; phage contaminants occasionally spread havoc and financial disaster for the various fermentation industries that depend on bacteria, such as cheese production and fermentative synthesis of chemicals and medications.<sup>16</sup>

Phage therapy has been evaluated extensively, with many successes being reported for a variety of diseases, including dysentery, typhoid and paratyphoid fevers, pyogenic and urinary tract infections, and cholera. Phages have been given orally, through colon infusion, and as aerosols as well as poured directly into lesions. They have also been given as injections: intradermal, intravascular, intramuscular, intraduodenal, intraperitoneal, and even into the lung, carotid artery, and pericardium. The early strong interest in phage therapy is reflected in some 800 papers published on the topic between 1917 and 1956. Many of these works have been reviewed in some detail by Ackermann and DuBow.<sup>17</sup> The reported results were quite variable. Many of the physicians and entrepreneurs who initially became excited by the potential clinical implications jumped into applications with very little understanding of phages, microbiology, or basic scientific process. Thus, many of the studies were anecdotal and/or poorly controlled; many of the failures were predictable, and some of the reported successes did not make much scientific sense. Often, uncharacterized phages, at unknown concentrations, were given to patients without specific bacteriologic diagnosis, and there is no mention of follow-up, controls, or placebos.

Much of the understanding gained by d'Herelle was ignored in this early work, and inappropriate methods of preparation, "preservatives," and storage procedures were often used. On one occasion, d'Herelle reported testing 20 preparations from various companies and finding that not one of them contained active phages! On another occasion, a preparation was advertised as containing a number of different phages, but it turned out that the technician responsible had decided it was easier to grow them up in one large batch than in separate batches. Not surprisingly, a check of the product showed that one phage had out-competed all the others, and this was not, in fact, a polyvalent preparation. In general, there was no quality control except in a few research centers. Large clinical studies were rare, and the results of those that were carried out were largely inaccessible outside Eastern Europe.

### Specific Problems of Early Phage Therapy Work

Many still believe (erroneously) that phage therapy was proven not to work; however, it simply was never

adequately researched, and the work that was done well is not known widely enough. It is thus important to carefully consider the reasons for the early problems and the question of efficacy. They were as follows:

- Paucity of understanding of the heterogeneity and ecology of either the phages or the bacteria involved
- Failure to select phages of high virulence against the target bacteria before using them in patients
- Use of single phages in infections that involved mixtures of different bacterial species and strains
- Emergence of resistant bacterial strains, which can occur through selection of resistant mutants (a common occurrence if only one phage strain is used against a particular bacterium) or through lysogenization (if temperate phages are used, as discussed later)
- Failure to appropriately characterize or titer phage preparations, some of which were totally inactive
- Failure to neutralize gastric pH prior to oral phage administration
- Inactivation of phages by both specific and nonspecific factors in bodily fluids
- Liberation of endotoxins as a consequence of widespread lysis of bacteria within the body (the herxheimer reaction), which can lead to toxic shock (which can also be caused by antibiotics)
- Lack of availability or reliability of bacterial laboratories for carefully identifying the pathogens involved (necessitated by the relative specificity of phage therapy)

## BACTERIOPHAGE PHYSIOLOGY

Viruses are like spaceships that are able to carry genetic material between susceptible cells and then reproduce in those cells, just as human immunodeficiency virus (HIV) specifically infects human T lymphocytes that carry the CD4 surface protein. In the case of bacteriophages, the targets are specific kinds of bacterial cells; they cannot infect the cells of more complex organisms. Each virus consists of a piece of genetic information, determining all of the properties of the virus, which is carried around packaged in a protein coat (Figure 113-1). Most phages have tails, the tips of which have the ability to bind to specific molecules on the surfaces of their target bacteria (Figure 113-2). The viral DNA is then ejected through the tail into the host cell, where it directs the production of progeny phages; often more than 100 are produced in just half an hour. Each strain of bacteria has characteristic protein, carbohydrate, and lipopolysaccharide molecules present in large quantities on its surface. These molecules are involved in forming pores, motility, and binding of the bacteria to particular surfaces. Each such molecule can act as a receptor for particular phages. Development of resistance to a particular phage generally reflects mutational loss of its specific receptor; this

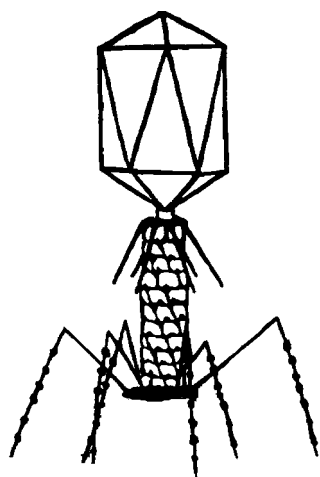


Figure 113-1 Phage diagram (bacteriophage T4).



Figure 113-2 Electron micrograph of phage infecting a bacterium.

loss often has negative effects on the bacterium and does not protect it against the many other phages that use different receptors.

Each kind of bacterium has its own phages, which can be isolated wherever that bacterium grows: from sewage, feces, soil, even ocean depths and hot springs. The process of isolation is easy. The sample is placed in an appropriate salt solution; the supernatant is separated and then is passed through a filter with a pore size small enough to remove the bacteria. The solution is then mixed (at several different dilutions) with a culture of the bacteria in question. A few drops are spread on a block of appropriate nutrient-agar medium. The next day, a dense lawn of bacteria is seen, dotted with round cleared areas called *plaques*. Each plaque contains about a billion phages, all of them progeny of a single initial phage that multiplied at a high rate and destroyed the bacteria there in the process. An individual plaque

is then transferred to a fresh culture of the bacteria in a liquid medium, allowing the culturing of a homogeneous stock of that particular phage, whose properties can then be studied.

### Properties of Phages

One major source of confusion in the early phage work was the perception that all phages were fundamentally similar, though subject to adaptive change according to the recent conditions of growth. One consequence of this belief was that new phages were often isolated for each series of experiments, so there was little continuity or basis for comparison. Phages specific for virtually every studied bacterial species have now been isolated, but few have been well classified.

A second early source of confusion affecting therapeutic use of phages was the question whether the lytic principle termed *bacteriophage* simply reflected an inherent property of the specific bacteria or required regular reinfection by an external agent. During the 1930s and 1940s, it became increasingly clear that in some senses both were true—that there were in fact two quite fundamentally different groups of bacteriophages. Lytic phages always have to infect from outside, reprogram the host cell, and release a burst of phage through breaking open, or lysing, the cell after a relatively fixed interval. Temperate phages, on the other hand, have another option; they can actually integrate their DNA into the host DNA, much as HIV can integrate the DNA copy of its RNA.

Key technical developments that helped clarify the general nature and properties of bacteriophages were (1) the concentration and purification of some large phages by means of very-high-speed centrifugation and the demonstration that they contained equal amounts of DNA and protein<sup>18</sup> and (2) visualization of phages by means of the electron microscope (EM).<sup>19,20</sup>

Soon after these developments, Ruska<sup>21</sup> reported the first attempts to use the electron microscope for phage *systematics*. This has since become a key tool of the field.<sup>17</sup> Each phage was found to have its own specific shape and size, from the “lunar lander”-style complexity of T4 and its relatives to the globular heads with long or short tails of lambda and T7, to the small filamentous phages that looked much like bacterial pili (Figure 113-3).

### Lytic Phages

A much better understanding of the interactions between lytic phages and bacteria began with one-step growth curve experiments.<sup>22,23</sup> These demonstrated an eclipse period, during which the DNA began replicating and there were no free phages in the cell; a period of accumulation of intracellular phages; and a lysis process that released the phage to go in search of new hosts. This phage infection cycle is illustrated in Figure 113-4.

In 1943, an event occurred that was to have a major impact on the orientation of phage research in the United States and much of western Europe, strongly shifting the emphasis from practical applications to basic science. Physicist-turned-phage biologist Max Delbruck

met with Alfred Hershey and Salvador Luria to form the "Phage Group," which eventually expanded largely through the influence of the summer "Phage Course" at Cold Spring Harbor, Long Island, in 1945. The influence of this group on the origins of molecular biology has been well documented.<sup>24,25</sup> A major element of the successes of phages as model systems for working out fundamental biologic principles was that Delbruck persuaded most phage biologists in the United States to focus on one bacterial host (*Escherichia coli* B) and seven of its lytic phages. These were arbitrarily chosen and named types T1 through T7.

As it turned out, T2, T4, and T6 were quite similar to one another, defining a family now called the *T-even phages*. These phages were key in demonstrating that DNA is the genetic material, that viruses can encode enzymes, that gene expression is mediated through special copies in the form of "messenger RNA," that the genetic code is triplet in nature, and many other fundamental concepts. The negative side of this strong focus on a few phages growing under rich laboratory conditions, however, was that there was very little study or awareness of the ranges, roles, and properties of bacteriophages in the natural environment, or of potential applications.

### Lysogenic Phages

The integration of lysogenic phage DNA into the host DNA leads to virtually permanent association of a prophage

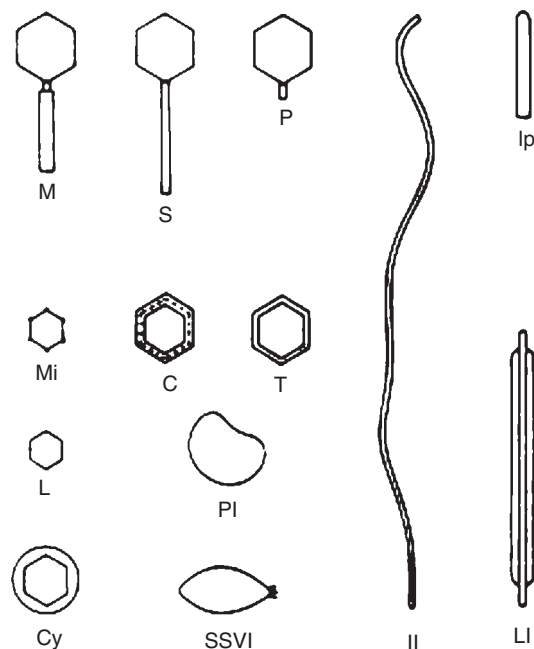


Figure 113-3 Various phages.

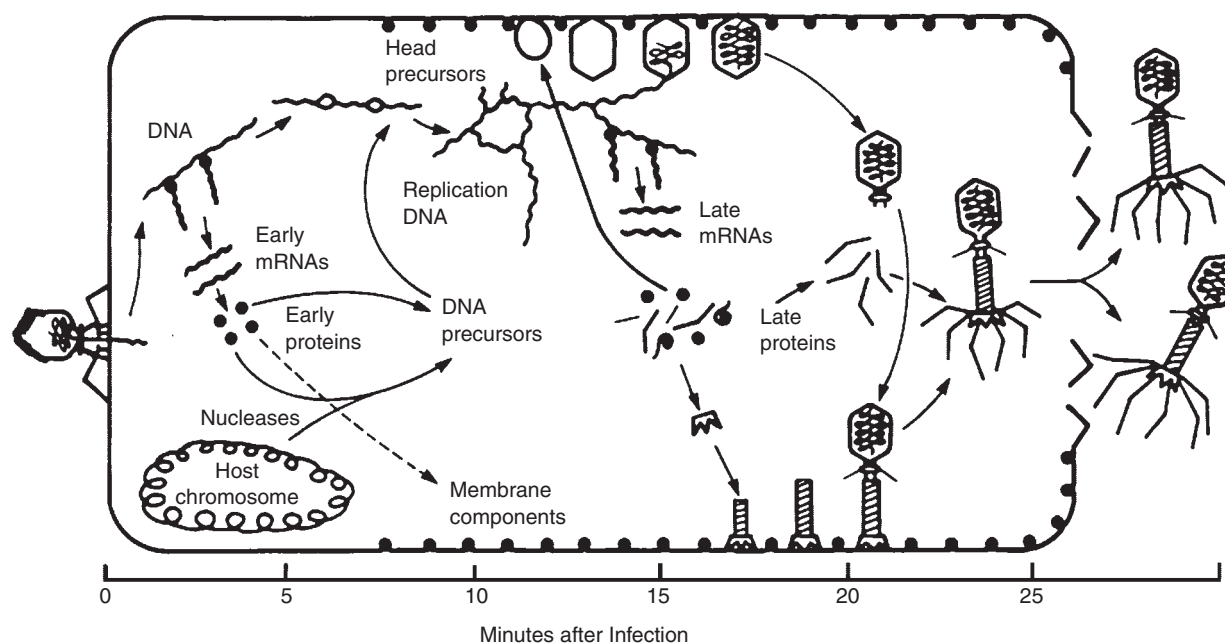


Figure 113-4 Bacteriophage intracellular growth cycle. Noteworthy features: nucleolytic action on host chromosome furnishes DNA precursors; replicating DNA is much longer than virion DNA; several phage-coded proteins become associated with the host membrane; maturation of phage head occurs at a membrane site.

with a specific bacterium and all its progeny. The prophage directs the synthesis of a repressor, which blocks the reading of the rest of its own genes and also those of any closely related lysogenic phages—a major advantage for the bacterial cell, giving it protection from infection by a significant class of phages as well as a potential weapon against many competing bacteria. Occasionally, a prophage escapes from regulation by the repressor, cuts its DNA back out of the genome by a sort of site-specific recombination, and goes ahead to make progeny phage and lyse open the cell. Sometimes the cutting-out process makes mistakes, and a few bacterial genes are carried along with the phage DNA to its new host; this process, called *transduction*, plays a significant role in bacterial genetic exchange. Such lysogenic phages are bad candidates for phage therapy, owing both to their mode of inducing resistance and to the fact that they can potentially lead to transfer of genes involved in bacterial pathogenicity; this issue is discussed in more detail later. However, their specificity often makes them very useful for phage typing in distinguishing between bacterial strains.

## PHAGES AND THE IMMUNE SYSTEM

A number of early experiments involving the injection of phages into animals led to the widespread impression that phage therapy could not in fact succeed because the phages were too rapidly cleared by the immune system; the remarks in this regard by Gunter Stent<sup>26</sup> had a particularly strong impact on the phage community. Two early experiments involving rabbits showed rapid disappearance of the particular phages used from the blood and organs, but long-term survival in the spleen.<sup>27,28</sup> Subsequent experiments in rats and mice also showed rapid loss from the circulation. When Nungester and Watrous<sup>29,30</sup> injected  $10^9$  plaque-forming units (pfu) of a staphylococcal (staph) phage intravenously into albino rats, a blood concentration of only  $10^5$  pfu/ml was seen after 5 minutes, which dropped to 40 pfu/ml by 2 hours.

There is one major concern with these early articles on phage pharmacokinetics in animal models: The experiments were done in the absence of host bacteria in which the phage could multiply and find protection. Furthermore, they were carried out by the very unnatural mode of intravenous injection, exposing the phage almost immediately to the reticuloendothelial system. Results of many later studies have made it clear that phages are often seen in the mammal circulatory system; however, this generally occurs under conditions in which they are entering the circulatory system from some sort of reservoir in other tissues and in which the mammal is dealing with an infection by a bacterium that they can infect—precisely the sort of situation seen in gene therapy as currently practiced in Eastern Europe.

One of the best early sets of experiments was published in 1943 by the noted Harvard bacteriologist René Dubos and colleagues.<sup>31</sup> They injected white mice intracerebrally with a dose of a smooth *Shigella dysenteriae* strain that was sufficient to kill more than 95% of the mice in 2 to 5 days and treated them with intraperitoneal injection of a phage mixture isolated from New York City sewage, grown in the same bacteria, and purified only by sterile filtration. With no treatment or when treated with filtrates of staph cultures or with heat-killed phage, only 3 of 84 mice (3.6%) survived; in contrast, 46 of 64 (72%) of the mice given  $10^7$  to  $10^9$  phages survived.

These researchers also carried out pharmacokinetic studies. When phages were given to uninfected mice, they appeared in the blood stream almost immediately, but the levels started to drop within hours and very few were seen in the brain. In the infected animals, however, brain phage levels quickly greatly exceeded blood levels; around  $10^7$  to  $10^9$  phage/g were often seen between 8 and 114 hours after administration, with the level starting to drop anywhere between 75 and 138 hours. After the first 18 hours, blood levels were far lower than brain levels, but phages were still present in blood at  $10^4$  to  $10^5$  phage/ml in those subjects in which the brain levels were still more than  $10^9$  phage/g. These findings clearly established that (1) the phages themselves were responsible, not something in the lysate that just stimulated normal immune mechanisms, (2) phages could rapidly find and multiply in foci of infection anywhere in the body, and (3) phages could be maintained in the circulation as long as there was a privileged reservoir of infection where phages were continually being produced. Without providing data or pharmacokinetics, the researchers mention that the mice were also rescued by phages administered subcutaneously or intravenously but not by stomach tube or in drinking water.

Carefully controlled experiments carried out in 1943 through 1945 by Henry Morton and Enrique Perez-Otero at the University of Pennsylvania supported those of Dubos et al. These investigators further showed the lack of any protection when lysates of phage with inappropriate host specificities were used. A final review authorized by the Council on Pharmacy and Chemistry discussed the major advantages of phages, such as the ability to replicate into problem areas and treat localized infections that are relatively inaccessible via the circulatory system and the fact that their high specificity greatly aided in reducing later resistance problems.<sup>32</sup> The review also emphasized that almost all of the earlier research had been so poorly conceived and/or carried out that it offered no proof either for or against the promise of phages as antibiotics.

The background of these experiments, as described by Häusler,<sup>33</sup> is very interesting. In 1942, both *The Lancet* and the *British Medical Journal* published editorials about

the apparently successful use of antidysentery phages by the Soviet military in the Middle and Far East. The U.S. National Research Council Committee on Medical Research (NRC/CMR) immediately approached Morris Rakieta, d'Herelle's close associate in his phage work at Yale, to discuss the possibilities offered by phages for dealing with this perpetual scourge of armies. (The German army was already producing massive amounts of phage, without prior efforts at research, because 155,000 German soldiers had been affected by dysentery in World War I, with 8600 deaths). By November of 1942, the NRC was supporting antidysentery phage research in several top U.S. bacteriology laboratories that do not appear to have previously been engaged in phage work; the group at University of Pennsylvania mentions that their work was started in November of 1942 with NRC support, but they were initially required to keep the results secret. Others involved in this NRC-supported work were Arthur Schade and Leona Caroline at the Overly Biochemical Research Foundation, New York.

U.S. work with dysentery phages largely ended in 1944, when the end of World War II made penicillin available to the general public. The military secrecy, the end of the war emergency funding, the rapid rise in antibiotic availability and their broad spectra, and Max Delbruck's success in persuading the phage community to shift its focus to basic mechanistic research involving a few model systems probably all contributed to the fact that there was little U.S. follow-up to these interesting and successful results; few people even knew about them or about two successful subsequent human applications.

Penicillin worked against only some kinds of bacterial infections. Typhus, for example, was not treatable, and some excellent phage work was carried out in the interim. It was known that the strains of *Salmonella typhi* that created the main pathogenicity problems were those carrying one particular antigen, named Vi (for "virulence"). In 1936, a pair of Canadians had identified phages specific against the Vi antigen. In the early 1940s, Walter Ward,<sup>34</sup> of the Los Angeles County Hospital, was trying to deal with repeated serious outbreaks of typhoid that were killing one in five of those afflicted. He tested the Vi-specific phages against mouse typhus and found that the death rate fell to 6%, versus 93% in the controls. Some of his colleagues then used these phages to treat patients with typhoid; only 3 of their 56 treated patients died, compared with the 20% mortality for the other treatments available at the time.<sup>35</sup> Most impressively, the rest of the patients receiving phage therapy rapidly changed from being largely comatose to full of vigor, with renewed appetite, in 24 to 48 hours. In 1948-1949, near Montreal, Desranleau treated nearly 100 patients with dysentery by giving them a *cocktail* of six Vi-specific phages, and the death rate dropped from

20% to 2%. By 1947, however, chloramphenicol had been shown to work well against typhoid, and it was much easier for pharmaceutical companies to deal with, so that seems to have been the end of phage clinical trials in the Western hemisphere.

The high specificity of phages still plays a strong role in the Phage Typing sets used for detecting and following problem strains of such bacteria as *Shigella*, *Salmonella*, and *Cholera*, but phage therapy itself is only beginning to stage a comeback.

## CLINICAL APPLICATIONS

### Current Research

The growing understanding of phage biology has the potential to facilitate more rational thinking about the therapeutic process and the selection of therapeutic phages. However, there was generally little interaction between those who were so effectively using phages as tools to understand molecular biology and those working on phage ecology and therapeutic applications. Many in the latter group were spurred on by a concern about the rising incidence of nosocomial infections and of bacteria resistant against most or all known antibiotics as well as by the fact that phages are far more effective than antibiotics in areas of the body where the circulation is bad and in not disrupting normal flora. This strong sense of the potential importance of phages was particularly seen in Poland, France, Switzerland, and the former Soviet Union, where use of therapeutic phages never fully died out and there has been some ongoing research and clinical experience. In France, Dr. Jean-François Vieu led the therapeutic phage efforts until his retirement some 15 years ago. He worked in the Service des Entiobactéries of the Pasteur Institute in Paris and, for example, prepared *Pseudomonas* phages on a case-by-case basis for patients. His experience there is discussed in two articles.<sup>36,37</sup> In Vevey, Switzerland, the small pharmaceutical firm Saphal made "Coliphagine," "Intestiphagine," "Pyophagine," and "Staphagine" in drinkable and injectable forms, salves, and sprays into the 1960s.<sup>33</sup> The owner, Harrmann Glauser, had been encouraged and trained by d'Herelle's old colleague Paul Hauduroy, who had become a professor of microbiology at the University of Lausanne during the second world war. The preparations were officially approved and were paid for by insurance there.

Phage therapy was used extensively in many parts of eastern Europe as a regular part of clinical practice, and companies in Russia now make phages for this purpose. However, most of the research and much of the phage preparation came under the direction of key centers in Tbilisi, Georgia, and Wroclaw, Poland. In both cases, the close interactions between research scientists and physicians play an important role in the high degree

of success obtained, just as appears to have been the case for d'Herelle's early work.

### **Institute of Immunology and Experimental Medicine, Polish Academy of Sciences**

The most detailed publications documenting phage therapy have come from the group led by Stefan Slopek, director for many years of the Institute of Immunology and Experimental Medicine, Polish Academy of Sciences, Wrocław. They published a series of extensive papers describing work carried out from 1981 to 1986 with 550 patients.<sup>38-40</sup> This set of studies involved 10 Polish medical centers—including the Wrocław Medical Academy Institute of Surgery Cardiosurgery Clinic, Children's Surgery Clinic, and Orthopedic Clinic, the Institute of Internal Diseases Nephrology Clinic, and Clinic of Pulmonary Diseases. The patients ranged in age from 1 week to 86 years. In 518 of the cases, phage use followed unsuccessful treatment with all available other antibiotics. The major categories of infections treated were as follows:

- Long-persisting suppurative fistulas
- Septicemia
- Abscesses
- Respiratory tract suppurative infections and bronchopneumonia
- Purulent peritonitis
- Furunculosis

In a final summary paper, these investigators carefully analyzed the results with regard to such factors as nature and severity of the infection and monoinfection versus infection with multiple bacteria.<sup>40</sup> Rates of success ranged from 75% to 100% (92% overall), as measured by marked improvement, wound healing, and disappearance of titratable bacteria; 84% of subjects demonstrated full elimination of the suppurative process and healing of local wounds. Infants and children did particularly well. Not surprisingly, the poorest results occurred in elderly patients and those in the final stages of extended serious illnesses, two groups with weakened immune systems and generally poor resistance.

The bacteriophages all came from the extensive collection of the Bacteriophage Laboratory of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław. In the later studies, some of the specific phages were named. All were virulent, capable of completely lysing the bacteria being treated. In the first study alone, 259 different phages were tested (116 for *Staphylococcus*, 42 for *Klebsiella*, 11 for *Proteus*, 39 for *Escherichia*, 30 for *Shigella*, 20 for *Pseudomonas*, and 1 for *Salmonella*); 40% of them were selected to be used directly for therapy. All of the treatment was conducted in a research mode, with the phage prepared at the institute by standard methods and tested for sterility.

Treatment generally involved 10 ml of sterile phage lysate given orally half an hour before each meal, with gastric juices neutralized by the taking of (basic) Vichy water, baking soda, or gelatum. In addition, phage-soaked compresses were generally applied three times a day where dictated by localized infection. Treatment ran for 1.5 to 14 weeks, averaging 5.3 weeks. For intestinal problems, short treatment sufficed, whereas long-term use was necessary for such problems as pneumonia with pleural fistula and pyogenic arthritis. Bacterial levels and phage sensitivity were continually monitored, and the phage(s) were changed if the bacteria lost their sensitivity. Therapy was generally continued for 2 weeks beyond the last positive test result for the bacteria.

Few side effects were observed; those that were seen seemed to be directly associated with the therapeutic process. On about days 3 to 5, pain in the liver area lasting several hours was often reported. The investigators suggested that this pain might be related to the extensive liberation of endotoxins as the phage is destroying the bacteria most effectively. In severe cases of sepsis, patients often ran a fever for 24 hours on about days 7 to 8.<sup>38</sup>

Various methods of administration were successfully used, including oral, aerosols, and infusion either rectal or in surgical wounds. Intravenous administration was not recommended for fear of possible toxic shock from bacterial debris in the lysates.<sup>38</sup> However, it was clear that the phages readily entered the body from the digestive tract and multiplied internally wherever appropriate bacteria were present, as measured by their presence in blood and urine as well as by therapeutic effects.<sup>41</sup> This interesting and rather unexpected finding has been replicated in many studies and systems.<sup>42-45</sup>

Detailed notes were kept throughout on each patient. The final evaluating therapist also filled out a special inquiry form that was sent to the Polish Academy of Science research team along with the notes. The Computer Center at Wrocław Technical University carried out extensive analyses of the data. These researchers used the categories established in the World Health Organization (WHO) (1977) International Classification of Diseases in assessing results. They also looked at the effects of age, severity of initial condition, type(s) of bacteria involved, length of treatment, and other concomitant treatments. The reports included many specific details on individual patients that helped to give some insight into the ways phage therapy was used as well as an in-depth analysis of difficult cases.

After Slopek's retirement, Dr. Beatta Weber-Dabrowska carried on with the treatment work, publishing a summary in English of the results for the next 16 patients.<sup>46</sup> In 1998, immunologist A. Górski took over as Institute director and revived a strong focus on phage work, with special emphasis on the immunologic

consequences of phage treatment.<sup>47</sup> These researchers are also now working with the basic phage group of Dr. M. Lobočka in Warsaw to sequence and further characterize key phages—an important step in eventually making them available to the outside world.

### **Bacteriophage Institute, Tbilisi**

The most extensive and least widely known work on phage therapy was carried out under the auspices of the Bacteriophage Institute at Tbilisi, in the former Soviet republic of Georgia. According to various physicians there, phage therapy is part of the general standard of care, used especially extensively in pediatric, burn, and surgical hospital settings. Phage preparation was carried out on an industrial scale, employing 700 people in the factory and several hundred more in the research arm of the Institute just before the breakup of the Soviet Union, and many tons of a variety of products were regularly shipped throughout the former Soviet Union. They were available both over the counter and through physicians. The largest use was in hospitals, to treat both primary and nosocomial infections, alone or in conjunction with other antibiotics and particularly when antibiotic-resistant organisms were found. The military is still one of the strongest supporters of phage therapy research and development, because phages have proved so useful for wound and burn infections as well as for preventing debilitating gastrointestinal epidemics among the troops. The International Science and Technology Centers program, set up jointly by the United States, Europe, and Japan to give constructive opportunities to scientists formerly working with Soviet military projects, is now one of the strongest supporters of basic and applied research in this area in Tbilisi.

From the Bacteriophage Institute's inception, the industrial part was run on a self-supporting basis, and its scientific branch was government supported. The latter included the electron microscope facility, permanent strain collection, laboratories studying phages of the enterobacteria, staphylococci, and pseudomonads, and formulating new phage cocktails, and groups involved in immunology, vaccine production, *Lactobacillus* work, and other therapeutic approaches. The Institute also carried out the very extensive studies needed for approval by the Ministry of Health in Moscow of each new strain, therapeutic cocktail, and means of delivery. This careful study of the host range, lytic spectrum, cross-resistance, and other fundamental properties of the phages being used was a major factor in the reported successes of the phage therapy work carried out through the institute, as was their method for initially selecting highly virulent phages from among the myriad potentially available against any given host. All of the phages used for therapy are lytic, avoiding the problems engendered by lysogeny. The problems of bacterial resistance were

largely solved through the use of well-chosen mixtures of phages with different receptor specificities against each type of bacterium as well as of phages against the various bacteria likely to be causing the problem in multiple infections. The situation was further improved whenever the clinicians typed the pathogenic bacteria and monitored their phage sensitivity. Where necessary, new cocktails were then prepared to which the given bacteria were sensitive. Not infrequently, using a phage in conjunction with carefully chosen other antibiotics was shown to give better results than either the phage or the antibiotic alone.

The depth and extent of the work involved are very impressive. For example, in 1983 through 1985 alone, the Institute's Laboratory of Morphology and Biology of Bacteriophages carried out studies of growth, biochemical features, and phage sensitivity on 2038 strains of *Staphylococcus*, 1128 of *Streptococcus*, 328 of *Proteus*, 373 of *P. aeruginosa*, and 622 of *Clostridium* received from clinics and hospitals in towns across the former Soviet Union. New broader-acting phage strains were isolated using these and other institute cultures and were included in a reformulation of their extensively used Piophage preparation; it now inhibited 71% of their *Staphylococcus* strains instead of 58%; 76% of *Pseudomonas* instead of 55%; 51% of *E. coli* instead of 11%; 30% of *Proteus* instead of 3%; 60% of *Streptococcus* instead of 38%; and 80% of *Enterococcus* instead of 3%.<sup>48</sup> In the years since, the formulation has continued to be improved on the basis of further studies, and phages against *Klebsiella* and *Acinetobacter* have been isolated and developed into therapeutic preparations. The other major product, used very extensively by the military, by pediatric centers, and in regions with extensive diarrheal problems, is *IntestiPhage*, which consists of 23 different phages active against a range of enteric bacteria and was often prepared in tablet form.

A good deal of work has gone into developing and providing the documentation for Ministry of Health approval of specialized new delivery systems, such as a spray for use in respiratory tract infections, in treating the incision area before surgery, and in sanitation of hospital problem areas such as operating rooms. An enteric-coated pill was also developed, using phage strains that could survive the drying process, and accounted for the bulk of the shipments to other parts of the former Soviet Union.

Much of the focus in the last 20 years has been on combating nosocomial infections, in which multidrug-resistant organisms have become a particularly lethal problem and it is also easier to carry out proper long-term research. Clinical studies of the effectiveness of the phage treatment and appropriate protocols were carried out in collaboration with a number of hospitals, but little has been published in accessible form. Zemphira Alavidze

and her colleagues, who are currently doing most of the actual therapeutic development and clinical application, have manuscripts in preparation that describe their work in institutions such as the Leningrad (St. Petersburg) Intensive Burn Therapy Center, the Academy of Military Medicine in Leningrad, the Karan Trauma Center, and the Kemerovo Maternity Hospital. Some of the most intensive studies were carried out in Tbilisi at the Pediatric Hospital, the Burn Center, the Center for Sepsis, and the Institute for Surgery. Special mixtures were developed for dealing with strains causing nosocomial infections in various hospitals, and they were very effectively used in sanitizing operating rooms and equipment, water taps, and other sources of spread of the infections (most of them predominantly involving *Staphylococcus*). The number of sites testing positive for the problem bacteria decreased by orders of magnitude over the several months of the trial at each site.

An exciting new product completed the approval process and was licensed in 2000 by the Georgian Ministry of Health. PhagoBioDerm is a biodegradable, nontoxic polymer composite that is impregnated with the Pyophage cocktail of phages, along with other antimicrobial agents.<sup>49</sup> Markoishvili<sup>50</sup> reported the results of a study of PhagoBioDerm involving 107 patients with ulcers that had failed to respond to conventional therapy—systemic antibiotics, antibiotic-containing ointments, and various phlebotonic and vascular-protecting agents. The ulcers were treated with PhagoBioDerm alone or in combination with other interventions during 1999 and 2000. The wounds or ulcers healed completely in 70% of the 96 patients for whom there was follow-up data. In the 22 cases for which complete microbiologic analyses were available, healing was associated with the concomitant elimination or very marked reduction of the pathogenic bacteria in the ulcers.

### Recent Work in the West

Levin and Bull<sup>1</sup> and Barrow and Soothill<sup>4</sup> have provided good reviews of much of the animal research carried out in Britain and the United States since interest in the possibilities of phage therapy began to resurface in the early 1980s. The results, in general, are in very good agreement with the clinical work just described in terms of efficacy, safety, and importance of appropriate attention to the biology of the host-phage interactions, reinforcing trust in the reported extensive eastern European results.

In Britain, Smith and Huggins<sup>42,43</sup> carried out a series of excellent, well-controlled studies on the use of phages in systemic *E. coli* infections in mice and then in diarrhetic disease in young calves and pigs. For example, they found that injecting  $10^6$  colony-forming units (CFUs) of a particular pathogenic strain intramuscularly killed 10 out of 10 mice, but none died if the researchers simultaneously injected  $10^4$  pfu of a phage selected against the

K1 capsule antigen of that bacterial strain. This phage treatment was more effective than using such antibiotics as tetracycline, streptomycin, ampicillin, and trimethoprim/sulfafurazole. Furthermore, the resistant bacteria that emerged had lost their capsule and were far less virulent. In calves, these researchers found very high and specific levels of protection. They had to isolate different phages for each of their pathogenic bacterial strains, because they did not succeed in isolating phages specific for more general pathogenicity-related surface receptors such as the K88 or K99 adhesive fimbriae, which play key roles in attachment to the small intestine. Still, the phage was able to reduce the number of bacteria bound there by many orders of magnitude and to virtually stop the fluid loss. The results were particularly impressive if (1) the phage was present before or at the time of bacterial presentation and (2) multiple phages with different attachment specificities were used. Furthermore, the phages could be transferred from animal to animal, supporting the possibility of their prophylactic use in a herd. If the phages were given only after the development of diarrhea, the severity of the infection was still substantially reduced, and none of the animals died.<sup>45</sup>

Levin and Bull<sup>1</sup> carried out a detailed analysis of the population dynamics and tissue phage distribution of the 1982 Smith and Huggins<sup>42</sup> study, which can be helpful in assessing the parameters involved in successful phage therapy and its apparent superiority to antibiotics. They have gone on to perform interesting animal studies of their own and conclude that phage therapy is at least well worth further study.<sup>1</sup>

Barrow and Soothill<sup>4</sup> carried out a series of studies preparatory to using phages for infections in burn patients. Using guinea pigs, they showed that skin graft rejection could be prevented by prior treatment with phages against *Pseudomonas aeruginosa*. They also saw excellent protection of mice against systemic infections with both *Pseudomonas* and *Acinetobacter* when appropriate phages were used.<sup>4</sup> In the latter case, as few as 100 phages protected against infection with  $10^8$  bacteria—several times the LD<sub>50</sub> (dose at which 50% of the subjects died)!

Merrill et al<sup>51</sup> have carried out a series of experiments designed to better the understanding of the interactions of phages with the human immune system, and helped Richard Carlton, MD, start a company called Exponential Therapeutics to explore the possibilities of phage therapy. They initially worked with lytic derivatives of the lysogenic phages lambda and P22—a poor choice for therapeutic use, as discussed earlier and later in this chapter—but they gathered some very interesting data about factors affecting interactions between these phages and the innate immune system and patented a process for isolating longer-circulating phages. They have now published successful animal studies with

phages against vancomycin-resistant *Enterococcus*, and Exponential Biotherapies has completed successful phase 1 clinical trials with these phages.

### Bacterial Pathogenicity

Most bacteria are not pathogenic; in fact, they play crucial roles in the ecologic balance in the digestive system, mucous membranes, and all body surfaces. They often actually help protect against pathogens. This is one reason why broad-spectrum antibiotics have such a broad range of side effects and why more narrowly targeted bacteriocidal agents would be highly advantageous. Interestingly, most of the serious pathogens are close relatives of nonpathogenic strains.

Studies clarifying the mechanisms of pathogenesis at the molecular level have progressed remarkably in recent years, crowned by the determination of the complete sequence of (nonpathogenic) *E. coli* K12 and several other bacterial species, and extensive cloning and sequencing of pathogenicity determinants. Generally, a number of genes are involved, and they are clustered in so-called pathogenicity islands, or *Pais*, which may be 50,000 to 200,000 base pairs long. They generally have some unique properties, indicating that the bacterium itself probably acquired them as a sort of “infectious disease” at some time in the past and then kept them because they helped the bacterium infect new ecologic niches where there was less competition. Many of these *Pais* are carried on small extrachromosomal circles of DNA called *plasmids*, which can also be carriers of drug-resistance genes. Others reside in the chromosome, where they are often found embedded in defective lysogenic prophages that have lost some key genes in the process and cannot be induced to form phage particles. However, the prophages can sometimes recombine with related infecting phages. Therefore it makes sense to avoid using lysogenic phages or their lytic derivatives for phage therapy to avoid any chance of picking up and moving such pathogenicity islands.

For bacteria in the human gut, pathogenicity involves the following two main factors:

- The production of toxin molecules, such as shiga toxin (from *Shigella* and some pathogenic *E. coli*) or cholera toxin; these toxins modify proteins in the target host cells and thereby cause the problem
- The acquisition of cell-surface adhesions that allow the bacterium to bind to specific receptor sites in the small intestine, rather than just moving on through to the colon

They also all contain the components of so-called type III secretion machinery, related to those involved in the assembly of flagella (for motility) and of filamentous phages, and instrumental in many plant pathogens. For all the pathogenic enteric bacteria, the infection process

triggers changes in the neighboring intestinal cells. They include degeneration of the microvilli, formation of individual “pedestals” cupping each bacterium above the cell surface, and, in the case of *Salmonella* and *Shigella*, induction of cell-signaling molecules that trigger engulfment of the bacterium and its subsequent growth inside the cell.

Recently, *E. coli* O157 has been the subject of much concern, its contamination of such products as hamburgers and unpasteurized fruit juices leading to serious problems. Particularly in young children and the elderly, deaths have occurred from hemorrhagic colitis (bloody diarrhea) and from hemolytic-uremic syndrome, in which the kidneys are affected. Antibiotic therapy has shown no benefit; it is actually generally contraindicated because it leads to increased toxin release.<sup>52</sup> My colleagues and I have isolated a phage from sheep resistant to inoculation with *E. coli* O157:H7 at the U.S. Department of Agriculture in College Station, Texas. A major concern of this facility is to eliminate this human pathogen, which is found in the normal flora of one quarter of the cattle in the United States, from which it contaminates water sources and creates massive recalls for industries from meat packers to apple juice producers; this phage turns out to also be T4-like and to infect virtually all tested O157 strains. Studies are suggesting that this and additional phages we have isolated from sheep are good candidates for controlling O157 in the gastrointestinal tracts of ruminants.<sup>53,54</sup>

### T-Even Phages

A substantial fraction of the phages in the therapeutic mixes for gram-negative bacteria are relatives of bacteriophage T4, which has played such a key role in the development of molecular biology. This family is often called the T-even phages, from an historical accident reflecting the fact that T2, T4, and T6 from the original collection of Delbruck’s Phage Group all turned out to be related. Large sets of T-even phages have been isolated for study from all over the world: Long Island sewage treatment plants, animals in the Denver Zoo, and patients with dysentery in eastern Europe (the last using *Shigella* as host). The laboratory of Harald Bruessow at Nestle, Inc., in Lausanne, Switzerland, has become interested in the possibility of using phages to treat infant diarrhea in underdeveloped countries—a longstanding concern of theirs, and no other approaches have worked for the 27% that involve coliform bacteria. These researchers have isolated a number of broad-spectrum phages from patients at the world-famous Diarrheal Center in Bangladesh; all of them are T4-like phages.<sup>55,56</sup> They have now selected a group of therapeutic candidates and are carrying out various in vitro and animal studies in preparation for potential human trials.

T4-like phages are found infecting all of the enteric bacteria and their relatives.<sup>57</sup> Most of the T-even phages use 5-hydroxymethylcytosine instead of cytosine in their DNA, which protects them against most of the restriction enzymes that bacteria make to protect themselves against invaders and gives them a much more effective host range. T4's entire DNA sequence is known, and we know a great deal about its infection process in standard laboratory conditions and about the methods it uses to target bacteria so effectively.<sup>58,59</sup> We can potentially use this knowledge to develop more targeted approaches to phage therapy, particularly as more is learned about the similarities and differences in its extended family.<sup>60,61</sup> We know that different members of the T-even phage family use different outer membrane proteins and oligosaccharides as their receptors, and we understand the tail-fiber structures involved well enough to potentially predict which phages will work on given bacteria and to engineer phages with new specificities.<sup>62,63</sup>

The T-even bacteriophages share a unique ability that contributes significantly to their widespread occurrence in nature and to their competitive advantage. There have still been far too few studies of T4 ecology and its behavior under conditions more closely approaching the natural environment and the circumstances it will encounter in phage therapy, often anaerobic and/or with frequent periods of starvation. The limited available information in that regard has been summarized by elsewhere.<sup>58,59</sup> A variety of studies are shedding light on the ability of these highly virulent phages to coexist in balance with their hosts in nature. For example, they can reproduce in the absence of oxygen as long as their bacterial host has been growing anaerobically for several generations. They are also able to control the timing of lysis in response to the relative availability of bacterial hosts in their environment. When *E. coli* are singly infected with T4, they lyse after 25 to 30 minutes at body temperature in rich media, releasing about 100 to 200 phages/cell. However, when additional T-even phages attack the cell more than 4 minutes after the initial infection, the cell does not lyse at the normal time. Instead, it continues to make phages for as long as 6 hours.<sup>64,65</sup> We have found that the phages can also survive for a period of time in a hibernation-like state inside starved cells, allowing their host to readapt when nutrients are again supplied, and produce a few additional phages. This is particularly interesting and important because bacteria undergo many drastic changes to survive periods of starvation that increase their resistance to a variety of environmental insults.<sup>66</sup>

Thus, for many reasons, the T4-like phages make excellent candidates for therapeutic and prophylactic use against enteric and other gram-negative bacteria, and studies of their ecology and distribution are now being carried out with these goals in mind in Tbilisi,

Bangladesh, Lausanne, and College Station, Texas, and at The Evergreen State College.

## Advantages of Phages

Phages have many potential advantages, as follows:

- They are self-replicating but also self-limiting because they multiply only as long as sensitive bacteria are present.
- They can be targeted far more specifically than most antibiotics to the problem bacteria, causing much less damage to the normal microbial balance in the gut. The bacterial imbalance or "dysbiosis" caused by many antibiotic treatments can lead to serious secondary infections involving relatively resistant bacteria, and often increasing hospitalization time, expense, and mortality (see Chapters 12 and 14). Particular resultant problems are *Pseudomonads*, which are especially difficult to treat, and *Clostridium difficile*, the cause of serious diarrhea and membranous colitis.<sup>67</sup>
- Phages can often be targeted to receptors on the bacterial surface that are involved in pathogenesis, so any resistant mutants are attenuated in virulence.
- Few side effects have been reported for phage therapy.
- Phage therapy would be particularly useful for people with allergies to antibiotics.
- Appropriately selected phages can easily be used prophylactically to help prevent bacterial disease at times of exposure or to sanitize hospitals and help protect against hospital-acquired (nosocomial) infections.
- Especially for external applications, phages can be prepared fairly inexpensively and locally, facilitating their potential applications to underserved populations.
- Phages can be used either independently or in conjunction with other antibiotics to help reduce the development of bacterial resistance.

## TOXICOLOGY

From a clinical standpoint, phages appear to be very safe. This feature is not surprising, given that humans are exposed to phages from birth. Bergh et al<sup>68</sup> reported that nonpolluted water contains about  $2 \times 10^8$  phages/ml. Phages are normally found in the gastrointestinal tract, skin, urine, and mouth, where they are harbored in saliva and dental plaque.<sup>69-71</sup> They also have been shown to be unintentional contaminants of sera and thence of commercially available vaccines,<sup>72-75</sup> which were given dispensation to be sold despite this discovery because of the general consensus that phages are safe for humans.

Extensive preclinical animal testing was required for approving new phage formulations in the former Soviet Union, but few of these studies were published. Bogovazova et al<sup>76,77</sup> evaluated the safety and efficacy of *Klebsiella* phages produced by the Russian company

Immunopreparat. Pharmacokinetic and toxicologic studies using intramuscular, intraperitoneal, or intravenous administration of phages were carried out in mice and guinea pigs. The researchers found no signs of acute toxicity or gross or histologic changes, even using a dose/g 3500-fold higher than the projected human dose. They then evaluated the safety and efficacy of the phages in treating 109 patients infected with *Klebsiella*. The phage preparation was reported to be nontoxic for humans and to be effective in treating *Klebsiella* infections, as manifested by marked clinical improvements and bacterial clearance in the phage-treated patients.

Side effects such as occasional liver pain and fever reported in the early days of Western phage therapy may have been due to bacterial byproducts contaminating phage preparations used intravenously.<sup>78-80</sup> Concern for this possibility is a major reason that the Polish phage therapy group never administers their phages intravenously. The same is true for almost all of the therapeutic work carried out in Tbilisi and probably helps explain the group's virtually total lack of significant problems. Because the phage readily enter the blood stream after infusion in or near wounds and other sites of localized infection and then travel to sites of infection throughout the body, as discussed previously in descriptions of the work by DuBow, there generally seems to be no particular reason for undergoing the extra risks of intravenous administration.

## DRUG INTERACTIONS

No negative effects on the efficacy or safety of other drugs have been reported as a result of phage administration in the long history of work in Eastern Europe. No systematic studies have been carried out in this regard, but phages are so specific in their actions that it is hard to see where such interactions might be predicted to occur. On the other hand, at least some antibiotics would tend to interfere with phage treatment of localized infections in areas with poor circulation, by killing off the most accessible of the bacteria in which the phages need to multiply as they work their way deeper into the lesion; this would be a particular problem in cases in which the phages can still attach and infect but cannot complete their replication cycle. (Many of the Georgian physicians believe that antibiotics should never be used topically for wounds and deep-seated infections, because the decrease in antibiotic concentration below the surface provides a strong selection for antibiotic resistance and this problem does not occur with phages.)

## CONCLUSION

Clearly the time has come to look more carefully at the potential of phage therapy, both by strongly supporting

new research and by scrutinizing the research already available,<sup>5</sup> such as the very interesting human anti-typhoid phage research carried out in this country in the 1940s that has come to light<sup>35</sup> as well as the earlier European work and the very extensive applications in the former Soviet Union.

As Barrow and Soothill<sup>4</sup> conclude:

Phage therapy can be very effective in certain conditions and has some unique advantages over antibiotics. With the increasing incidence of antibiotic-resistant bacteria and a deficit in the development of new classes of antibiotics to counteract them, there is a need to investigate the use of phage in a range of infections. Phages are quite specific as to the bacteria they attack, and the stipulations of Ackermann & DuBow<sup>17</sup> are important here. The specificity of phages means that: Phages have to be tested against the patient's bacteria just as antibiotics [should be], and the indications have to be right, but this holds everywhere in medicine. However, phage therapy requires the creation of phage banks and a close collaboration between the clinician and the laboratory. Phages have at least one advantage. . . . While the concentration of antibiotics decreases from the moment of application, phage numbers should increase. Another advantage is that phages are able to spread and thus prevent disease. Nonetheless, much research remains to be done . . . on the stability of therapeutic preparations; clearance of phages from blood and tissues; their multiplication in the human body; inactivation by antibodies, serum or pus; and the release of bacterial endotoxins by lysis. . . . In addition, therapeutic phages should be characterized at least by electron microscopy.

With the exploding possibilities and decreasing costs of genomic analysis since the last edition of this book, it is now possible to perform at least partial genomic sequencing of phages to be included in general cocktails so as to know more about the phage families involved and exclude phages from temperate families and those likely to carry or acquire genes related to pathogenicity or toxin production; this is now standard done procedure for phages being developed in the West. Such modern techniques are now also being applied to some of the Georgian phage preparations with help from grants from the International Science and Technology Centers (ISTC) and Civilian Research and Development Foundation (CRDF) programs, both of which were set up to support civilian applications of science formerly funded by the Soviet military. This is an important step in considering the importation of such phages for topical use in the Western world.

Although it seems premature to broadly introduce injectable phage preparations in the West without further extensive research, their carefully implemented use in external applications and for a variety of agricultural purposes could potentially help reduce the emergence of antibiotic-resistant strains and deal with problems we have difficulty handling today. Furthermore, compassionate use of appropriate phages seems warranted

in cases in which bacteria resistant to all available antibiotics are causing life-threatening illness. Phages are especially useful in dealing with recalcitrant nosocomial infections, in which large numbers of particularly vulnerable people are being exposed to the same strains of bacteria in a closed hospital setting. In this case, the environment as well as the patients can be effectively treated.

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