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BACTERIOPHAGE THERAPY

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■ **Abstract** In 1917, bacteriophages were recognized as epizootic infections of bacteria and were almost immediately deployed for antibacterial therapy and prophylaxis. The early trials of bacteriophage therapy for infectious diseases were confounded, however, because the biological nature of bacteriophage was poorly understood. The early literature reviewed here indicates that there are good reasons to believe that phage therapy can be effective in some circumstances. The advent of antibiotics, together with the "Soviet taint" acquired by phage therapy in the postwar period, resulted in the absence of rigorous evaluations of phage therapy until very recently. Recent laboratory and animal studies, exploiting current understandings of phage biology, suggest that phages may be useful as antibacterial agents in certain conditions.

CONTENTS

BACKGROUND AND AIMS

Bacteriophages by their very nature would seem to be good candidates for antibacterial therapy: They are often highly specific to one or another bacterial species; they are nontoxic to animals and plants; and they usually increase in titer as they infect, multiply in, and kill their target microbes. Why then are phages not found routinely in the toolkits of infectious disease specialists, public health workers, and hospital infection control officers? Even though phages were discovered and their infectious cycles understood by the early 1920s, the literature of the past half-century is almost silent on the possible therapeutic role of bacteriophages

in infectious diseases. The reasons for this silence are complex and just recently being re-examined. Several recent reviews on phage therapy have appeared (1, 1a, 3, 5, 19, 40), some by authors with commercial interests in this field[1].

This review discusses the early attempts at phage therapy, the reasons it seemed to be abandoned, the unresolved problems and questions surrounding phage therapy, and recent studies that have revisited this topic. The review of recent literature focuses on work available in English, French, and German; there is, in addition, a substantial literature in Russian and Polish, which is not covered because of linguistic shortcomings of this reviewer. This omission should not be interpreted as evaluative in any way.

EARLY TRIALS

The discovery of bacteriophage is controversial (20, 58), but the first clear description of "the bacteriophage phenomenon" and the description of plaque assays was given by Félix d'Herelle, a French-Canadian microbiologist working at the Pasteur Institute in Paris in 1917 (8). D'Herelle observed what he called an "invisible microbe" that was present in the bacteria-free filtrates of stool samples from dysentery patients. His initial motivation for his investigation was that he suspected some filtrable virus as a cofactor in the pathogenicity of dysentery, but as he investigated this idea, he found that the phage titers usually were low or absent at the beginning of the illness, increased dramatically as the illness progressed, and were highest as recovery was occurring. From this temporal sequence of events, he surmised that the development of phage, specific for the pathogenic bacteria, was the cause of the recovery of the patient from the infectious disease. This inference was not without precedence because d'Herelle had earlier introduced the use of bacteria as a biological control for locust plagues in South America and North Africa. He had what has been called an ecological view of infectious disease and conceived of phage as "an exogenous agent of immunity" that promoted recovery.

A natural extension of d'Herelle's concept of phage as the agent of recovery in natural infectious disease was his attempt to introduce phages as therapeutic agents. This approach, the rapid translation of laboratory findings to the sickbed, was a hallmark of the Pasteurian approach, and it was supported, apparently enthusiastically, by Emile Roux, the director of the Pasteur Institute. D'Herelle's first tests of his belief in the therapeutic utility of phage were carried out in the field with avian typhosis (*Salmonella gallinarum*) (10) and in the laboratory with *Shigella dysenteriae* infection of rabbits (11).

The early studies on the use of phages to control epidemics of avian typhosis seem rather reasonable, even by current standards. Chickens in certain pens

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were treated with phage prior to inoculation with the *S. gallinarum*, other were untreated; groups of chickens, some phage-treated and some not, were exposed in the chicken pen to infected animals so that the infection would be spread under natural conditions. Phage treatment was by the oral route, which minimized the possibility that other material in the phage lysate, e.g., bacterial debris, acted as an active immunogen (as it might, had it been injected parenterally). In these experiments, phage offered a high degree of protection. Extending this approach to rural areas of France where the epidemic was severe, d'Herelle inoculated (either by the oral route or by injection) numerous flocks on farms in several widely separated regions. The overall results suggested that phage-treated flocks had many fewer deaths, the duration of the epidemic was shorter, and second rounds of the infection were prevented (12). D'Herelle's results were confirmed for the same disease in Holland by Kramer (13). The main defect in these early phage experiments is probably the absence of a double-blind design; it should be noted, however, that this level of rigor was very uncommon at the time and that d'Herelle's studies appear to have been conducted according to the best scientific standards of his day.

Phage therapy was also evaluated in field trials against bovine hemorrhagic septicemia (called *barbone* in French) in Indochina. In this disease, too, it appeared that parenteral inoculation of phages specific for this causative bacterium could protect water buffaloes against experimental inoculation with what is now called *Pasteurella multocida*, usually a highly fatal infection (14).

With evidence of therapeutic effectiveness of phage in both gastrointestinal disease (avian typhosis) and septicemic disease (*barbone*), d'Herelle extended his trials to human beings. The procedures for conducting human trials, both scientific and ethical, in the 1920s seem crude and inadequate by current standards, but d'Herelle's approach was typical. He first determined the safety of his phage preparations by self-administration: "Before undertaking experiments on man I had to assure myself that the administration of suspensions of the Shiga-bacteriophage caused no reaction. First, I ingested increasing quantities of such suspensions, aged from six days to a month, from one to thirty cubic centimeters, without detecting the slightest malaise. Three persons in my family next ingested variable quantities several times without showing the least disturbance. I then injected myself subcutaneously with one cubic centimeter of a forty-day old suspension. There was neither a local nor a general reaction" (15). He also injected his coworkers as well as his family. This procedure was considered sufficient to evaluate the safety of this material: "After being assured that no harmful effects attended the ingestion of the Shiga-bacteriophage, this treatment was applied for therapeutic purposes to patients afflicted with [culture-confirmed] bacillary dysentery" (16).

The work that probably attracted the most attention for phage therapy was d'Herelle's report of treating four cases of bubonic plague with antiplague phage. While he was stationed at the League of Nations Quarantine Station in Alexandria, Egypt, d'Herelle observed four patients on a ship passing through the Suez Canal, all of whom had laboratory-diagnosed bubonic plague. D'Herelle treated all four patients with antiplague phage preparations by direct injection of phage into the

buboes (the infected inguinal and axillary lymph nodes). All four patients recovered in what was considered a remarkable fashion, and this result was reported in the widely read French medical periodical, *La Presse médicale* (18). On the basis of this work, d'Herelle was invited by the British government to go to India to work on phage therapy of plague at the Haffkine Institute in Bombay. This short visit led to the later establishment of "The Bacteriophage Inquiry" in India under the patronage of the Indian Research Fund Association. This project studied the application of phage therapy in India, especially for cholera epidemics that occurred regularly in association with religious festivals and pilgrimages (18, 57).

Cholera is in some ways an ideal test case for therapy with phages: The bacteria are initially confined to the gastrointestinal tract; killing of the large numbers of the bacteria reduces the burden of the pathogenic toxin; the mode of transmission and epidemiological characteristics of the disease are well known; and, at least until recently, good vaccines have not been available. Phage therapy for cholera seems to be established as helpful in the treatment of patients with the disease; its use as a preventive measure, as d'Herelle had hoped when he went around India pouring phage stocks into the drinking water supplies, is less clearly established.

From the initial reports from India in the 1920s and 1930s (18, 57), it seems consistently observed that the severity and duration of cholera symptoms and the overall mortality from the disease were reduced in patients given cholera-specific phage by mouth. In several WHO-sponsored studies in Pakistan in the 1970s (33, 36), in which phage were compared with antibiotics (tetracyclines), high-dose phages seemed about equivalent to tetracycline in certain aspects of the clinical control of cholera.

Phage therapy was almost immediately applied to wound infections, again because of the accessibility of the infection and the relatively simple pathogenesis of the conditions. Staphylococcal infections were treated with anti-staph phages for both acute traumatic and surgical wounds, as well as chronic, refractory skin ulcers, and even perforating osteomyelitis. The early literature on this use of phage therapy is extensive, and generally the reports are favorable.

The history of phage therapy can be divided into four periods: early enthusiasm, critical skepticism, abandonment, recent interest and reappraisal. The changing attitudes toward phage therapy reflect both scientific and cultural influences. Although many early phage therapy trials were reported successful, and many of the major pharmaceutical firms sold phage preparations (e.g., Parke-Davis and Lilly in the United States), there were also failures. The Council on Pharmacy and Chemistry, established in 1905 by the American Medical Association to set standards for drugs and lead the battle against nostrums, undertook the evaluation of phage therapy in the late 1930s. The voluminous report of the Council (21), authored by Stanhope Bayne-Jones, a microbiologist, and Monroe Eaton, an infectious disease specialist, concluded with an ambiguous assessment of the literature on phage therapy. They acknowledged that there were both positive and negative results in the literature, but they were concerned that the biological nature of bacteriophage was poorly understood and that the lack of standardization of phage preparations

and the lack of criteria for purity and potency made it impossible to compare most of the studies that had been published. Though in the normal course of events such a report would have generated more research and new and better answers, World War II and the discovery of antibiotics seemed to effectively divert effort away from extensive study of phage therapy in the United States. D'Herelle had returned to France and was held under virtual house arrest in Vichy during the war, and thus the most vigorous advocate for phage therapy was silenced. In Europe, however, there were two major efforts in phage therapy continuing in a decidedly military context; the Soviet Union waged a war against the Finns and there were many battle casualties; phage therapy was extensively used to treat the war-wounded. The German military also used phage therapy; medical kits captured from Rommel's forces in North Africa showed that vials of phages were standard contents of the war medic's supplies (W.C. Summers, unpublished observations).

Antibiotic use increased rapidly in America. The ease of production of antibiotics, the relatively broad spectrum of action of antibiotics, and the stability of the preparations were advantages over phages. In the Soviet Union, however, phages continued to be used, probably for economic reasons, and perhaps even for ideological reasons: The State Serum and Vaccine Institute in Tbilisi, Georgia, was a major source of phages and could be held up as a success of Soviet science against the capitalist West. Conversely, in the postwar period, maintaining a distance from anything Soviet, be it ideas, politics, or even medicine, was important in the United States. Thus, to some extent, phage therapy became politically tainted as well.

Another reason for the eclipse of phage therapy in the postwar period may have been the belief that the concept was fatally flawed because of the problem of phage-resistance in bacteria. Even before antibiotics, the phenomenon of development of bacterial resistance to drugs, dyes, and other lethal agents was recognized, and resistance to antibiotic therapy was soon noted as a clinical problem. The early recognition of the outgrowth of phage-resistant bacteria ("secondary cultures") in lysed cultures was noted, even by d'Herelle, as a potential problem in phage therapy. This phenomenon was the basis for the work on the nature of bacterial mutation by Luria & Delbrück (32) in 1943. Perhaps because of the frequent observation of phage resistance and its exploitation in the young field of molecular biology, the notion that phage therapy was doomed because of frequent mutation to phage resistance became an established part of the unquestioned canon (56).

PROBLEMS AND QUESTIONS

Polyvalency

One of the biological problems faced by the early phage workers was understanding the place of bacteriophages in the evolutionary and classificatory schemes of living organisms. D'Herelle asserted that phage were "microbes," that is, living, organized beings, whereas his main opponents (which included most of the authorities of his day, such as Jules Bordet and John Northrop, both Nobel Prize winners)

favored the interpretation that phages were some sort of self-activating lytic enzyme, more akin to pepsinogen. The debates on the biological nature of phage were protracted, bitter, and acrimonious, at one point even involving legal action (58). The view that phages were bacterial viruses in the modern sense developed after the period of enthusiasm for phage therapy in the 1920s and 1930s (59).

D'Herelle, firmly in the neo-Larmarckian tradition of early twentieth-century French biology, advocated the view that bacteriophage represented one "specie" of microbe (he called it an ultramicrobe to emphasize its invisibility in the light microscope) and that it could readily adapt its tropism for various bacterial hosts. Thus, when a phage stock that had been isolated from a human stool sample was used to infect a culture of *Staphylococcus*, the phage would somehow interact with the host to adapt to virulence for *Staphylococcus*. These ideas had strong precedent in the work of Louis Pasteur and his belief in the modification of microbial properties by specific hosts, e.g., his use of laboratory passages of pathogens to produce "attenuated" vaccine strains. D'Herelle's belief in the "unicity" of bacteriophage necessitated the constant adaptation of phage to the pathogen against which the phage was to be used. This view had particular utility in that d'Herelle insisted that a given phage preparation be checked for virulence against its clinical target, but also it was a liability because it made the commercial production of generally useful phage stocks difficult if not impossible. The aim of those interested in phage therapy then became a search for "polyvalent" phages and for the production of useful mixtures of phages that had been adapted to specific host organisms.

Bacteriophage therapy was evolving at a time when bacterial classification was in turmoil, and even the notion of stable bacterial species was under attack. The theory of "cyclogeny" was seriously accepted by many microbiologist in the 1920s and 1930s; it was a new variant of the old nineteenth-century belief in the interconvertibility of bacterial species (the polymorphism concept). It is not surprising, then, that understanding of the nature of the host specificity of bacteriophage was unclear at best. In retrospect, many early studies of phage therapy were confounded by problems surrounding the phage-host specificity problem. In particular, these problems introduced a bias toward negative results (the failure of phage to kill bacteria).

A related aspect of bacteriophage biology that was poorly understood at the time was the nature, mechanisms, and quantitation of virulence and potency of a phage preparation. D'Herelle had introduced the standard plaque assay and had produced one-step growth curves and estimated burst sizes [later confirmed by Ellis & Delbrück (22)]; however, because his views that phage were viruses rather than enzymes were in the minority, phage preparations were often characterized only as strong or weak depending on how completely a culture was cleared after infection or how rapidly lysis occurred. With no attempt to standardize phage stocks (strains) or titers, comparisons between various therapeutic trials were especially difficult if not impossible.

Some commercial phage preparations that were available for clinical trials were advertized to have as many as 100 different phages; that is, stocks adapted to

many different pathogenic strains. D'Herelle directed a commercial laboratory in Paris for the production of phages, *Laboratoire du Bactériophage*, which offered mixtures of phages specific for one or another group of organisms more or less related to a given part of the body: "Bacté-intesti-phage" for diarrheal diseases, "Bacté-staphy-phage" for superficial infections, "Bacté-rhino-phage" for upper respiratory illnesses, etc. He even toyed with the notion of developing a "Bacté-gazzi-phage" directed at gas-producing intestinal bacteria. In his own academic laboratory work, however, he insisted the organism be isolated from the patient, grown in the microbiological laboratory, and a phage adapted to the individual-patient isolate that was then used to treat the patient. This forerunner of our "culture and sensitivity examination" was a time-consuming process, not readily adaptable to general medical practice.

Commercial polyvalent preparations of bacteriophage were often found to be inactive. This may have been because many of them were "stabilized" with agents, such as phenol and merthiolate, as "preservatives." Because the biological nature of phage was not appreciated and because they were often conceptually associated with serums and vaccines, the major biologicals in the pharmacy, they were handled in the same way. The usual phage preparation was a simple filtrate of a lysed culture. It was only in the mid-to-late 1930s that there was any attempt to concentrate or purify the phages from these crude filtrates. In attempts to study the chemical composition of phages, Max Schlesinger began to concentrate phage by centrifugation (46). This was followed by the work of Lepine et al. (29), using sedimentation and diffusion to estimate the sizes of phages. Only with the visualization of phages in the electron microscope in 1940 was d'Herelle's view of the particulate nature of phage unequivocally confirmed (45). D'Herelle and his colleagues analyzed many commercial phage products in the 1930s, and most, if not all, contained no biologically active phages. One commercial phage laboratory advertised a polyvalent phage preparation as a mixture of many different phages. When one scientist visited the factory and talked to the laboratory staff, he reported that he was told that they no longer grew the stocks separately prior to concocting the required mixture but that, instead, they mixed all the phages together and then carried out only one infection and phage growth as a much more efficient procedure. This scientist (Max Delbrück) reported that upon analysis, this company's polyvalent phage stock contained only one kind of phage, the strain Delbrück designated T7 (M. Delbrück to W.C. Summers, personal communication). Clearly it had overgrown all the others contained in the original mixture during the course of multiple passages.

By the time microbiologists adequately understood the biological nature of phage, the details of the infectious process and the basis of host-range specificity, interest in phage therapy had waned. While the research and clinical experience of the early period of phage therapy can aid in our renewed interest in the possibilities of phage as therapeutic agents, these early studies are handicapped by uncontrolled variables that were unappreciated at the time. One conclusion seems clear: Ignorance of these variables seems to have the effect of diminishing the

chances for positive outcomes in clinical trials of phage therapy. If this is true, we should weigh those trials that did report positive results more than their negative counterparts.

Restriction/Modification

Because many early phage-therapy trials employed phage preparations made in the laboratory with phages grown on hosts that may or may not have been well-characterized, one unknown variable that certainly confounded the outcome of these trials was the phenomenon of host-induced modification of the phage stocks and restriction by the pathogenic target bacteria. Restriction and modification was first recognized in the early 1950s, and thus, could not have entered into the design of the early phage-therapy studies.

For example, if a phage was prepared on a standard laboratory strain of *Escherichia coli*, say *E. coli* K12, it may have its DNA modified at the sites specified by the K-specific modification system. When this phage stock is then administered to a patient infected with *E. coli*, but with a different restriction-modification specificity, say the B-specific modification, the phage DNA will be degraded upon entry into the pathogen, and the phage growth will be halted. Thus, one might conclude that phage therapy failed, whereas the phage, in effect, were not even tested.

This difficulty could be circumvented by preparation of clinical phage stocks on specific patient isolates, as recommended by d'Herelle, because the phage was adapted in the laboratory to grow on, and hence acquire the modification pattern of, the specific isolate from the patient to be treated. In evaluating the early literature, one gets the general impression that there were more successes by investigators who prepared their own phage stocks than by investigators who evaluated the commercial products. While a rigorous meta-analysis is probably not possible, this general impression has a plausible rationale based on the difficulties involved with unknown restriction and modification compatibilities.

Immunogenicity

Surprisingly, the early phage workers did not seem concerned about the immunological reactions to phage therapy. Perhaps this is because phage stocks, although often administered parenterally, were more often given by mouth (for gastrointestinal disease) or topically (for respiratory diseases and cutaneous infections). One reason suggested by Stent for the abandonment of phage therapy was the likelihood of rapid development of antiphage antibodies with prolonged phage treatment (56). This potential complication was recently addressed in experiments by Merrill et al., who showed that by repeated transfer of phage lambda by intravenous injection into mice, variants that escape immune recognition could be selected by the particular strain of mice used (35). This is not entirely unexpected, however, because the immune response to the phage is mainly directed at epitopes on the capsid protein, and mutant capsids may exist for which a given strain of mice cannot mount an immune response.

Immunological reactions to bacterial antigens in the impure phage preparations used in the early phage trials were recognized as a major confounding phenomenon by the late 1930s, but with no agreed-upon characterization of bacteriophage, no standards of purification were possible. Indeed, some early applications of phage were not aimed at bacteria in vivo, but rather the phage was used to lyse bacteria to make killed bacterial vaccines, which had not been subjected to harsh chemical or physical agents in order to prepare the vaccines. Kabeshima explored this use of phage almost as soon as phage had been discovered, and he was able to produce a reasonably effective vaccine against dysentery bacteria (25). D'Herelle's own work on bovine septicemia in Indochina was also interpreted by Kabeshima as showing the immunizing effect of the bacterial antigens in the crude phage lysates (9).

Resistance

From the very early work on bacteriophage, it was recognized that continued incubation of a culture lysed by phage often exhibited reappearance of viable bacteria, a so-called secondary culture. These bacteria were usually resistant to infection by the original phages. The mechanism of the appearance of this resistance was the subject of much debate and experimentation. Of course, such resistance was of primary importance in the clinical use of phage. D'Herelle proposed that the bacteria adapted to the presence of the phage as all organisms adapt to changes in their environment; this was another example of his French neo-Lamarckian, somewhat ecological, approach to biology. In a series of papers in the early 1930s, he interpreted the phage as inducing mutation of the bacteria to phage-resistant forms. These new forms could be antigenically distinguished from the parental strains and such antigenic changes were stable, even in the absence of phage.

An alternative interpretation of phage resistance was provided by the work of Luria & Delbrück (32) and later Lederberg & Lederberg (28), who devised ways to distinguish whether the presence of the phage was needed to induce the appearance of phage resistance. They concluded it was not.

As it has turned out, both interpretations are correct: Phage are not absolutely needed for resistance to appear, as in the case of spontaneous mutations in bacterial genes needed for phage adsorption to the cell surface; yet some phages, under some conditions, can integrate into the host cell chromosome in the form of a lysogen, conferring resistance to further infection as well as endowing the bacterium with new antigenic and toxigenic properties. Lysogeny can be interpreted as a kind of phage-directed mutagenesis.

RECENT RESULTS

Although phage-therapy trials in the United States and most of Western Europe ceased after World War II, probably as a consequence of the widespread success and availability of antibiotics, phage therapy was still actively pursued in the Soviet Union and some other Eastern European countries. The Institute founded

by Georgyi Eliava and d'Herelle in Tbilisi was one of the main centers for such work (43).

Of the few reports in the English language medical literature, several deserve careful consideration. In the late 1960s, the World Health Organization set up an international trial of phage therapy for cholera in Dacca, East Pakistan. This trial was designed according to widely accepted international standards for such studies and was conducted with the support and review of the National Institutes of Health. The first report (36) from this study described the use of high doses of anti-cholera phage (calculated to give a multiplicity of infection of 100–200 phage per vibrio) to test the idea that phage might be able to kill bacteria in vivo but might not be able to complete many cycles of replication and amplification. The study was conducted on acutely ill patients in hospital. Bacteriophage therapy was compared with tetracycline treatment and with fluid replacement alone as a control. All patients received standard fluid replacement therapy and other supportive care. Patients were monitored for stool output volume, duration of diarrhea, vibrio excretion, and phage titers in the stool. The significant finding reported from this work was that very high dose phage therapy was comparable to tetracycline in reducing the excretion of vibrios in the stool; this reduction, however, did not seem to translate into overall clinical improvement, i.e., shorter duration of diarrhea and more rapid recovery. After this initial study, a larger study (33) was carried out with randomization of patients, placebo controls, and comparisons of oral phage, oral and intramuscular injected phage, and tetracycline. Unfortunately as it turned out, this more adequately designed study was conducted with much lower phage doses [multiplicity of infection (m.o.i.) about 0.05–0.1 phage per vibrio]. In this low m.o.i. study, no significant effects of phage treatment were discerned. The authors noted several problems that complicated their evaluations of phage therapy in cholera; first was the diversity of serotypes of vibrios and the varying susceptibility of these bacteria to the phage stocks employed; second was the rapid transit of ingested phage through the gastrointestinal track of cholera patients, a fact that may have precluded second rounds of phage infection essential in low m.o.i. therapy.

Another well-publicized series of studies on phage treatment for *E. coli* diarrhea in calves was conducted by Williams Smith and his colleagues in the United Kingdom in the 1980s (63–65). These studies addressed several of the problems identified in the past. They focused on a particular strain, *E. coli* O18:K1:H7 ColV$^+$, which is a known pathogen in calves, and in a clever exploitation of phage receptor biology, selected phages that required the presence of the K1 antigen for infection, presumably for surface attachment. They reasoned that if rapid in vivo mutation to phage resistance was a major cause of failure of phage therapy and that the major mechanism of such phage resistance was loss of phage receptors, and if the pathogenic bacteria mutated to resistance to the anti-K1 phages, then it would do so by loss of K1 antigen and thus simultaneously mutate to greatly reduced virulence. They isolated nine anti-K1 coliphages and showed that they were effective in vivo, protecting against experimental infection of mice with the pathogenic strain of *E. coli*. In comparisons with several antibiotics, single-dose phage treatment was

more effective than multiple doses of tetracycline, ampicillin, chloramphenicol, or trimethoprim plus sulfafurazole. As expected, phage-resistant bacteria occurred in vivo but lacked the K1 antigen.

The data presented by Williams Smith & Huggins (63) have been analyzed by Levin & Bull using a population biology model for phage and bacterial dynamics in vivo, and they showed that a plausible explanation for the superiority of phage over antibiotics in this work is the intuitive idea that phage have the potential to increase titer as they kill bacteria, whereas antibiotics can only decay by both excretion and metabolism (31).

Recently published laboratory studies on model systems and experimental designs that meet current scientific standards have generally been encouraging. Recently Barrow et al. (2) extended the results of Williams Smith with anti-K1 phage therapy for experimental *E. coli* infections of chickens and confirmed some of the earlier observations on the effectiveness of this treatment in calves. Soothill (55) used a mouse model, and Park et al. (39) and Nakai et al. (38) explored the use of phages to treat or control specific fish pathogens. These three studies concluded that phages were effective as employed. On the other hand, Greer & Dilts (23) attempted to use phages to reduce the bacterial contamination of beef and discovered that while viable bacterial counts were significantly reduced, overall rates of meat spoilage was not. They noted, however, that about half of the bacterial strains contaminating the beef were resistant to the phage pool they used and that the phage-resistant organisms were a significant cause of spoilage. Thus, this study probably should not be overinterpreted. Another trial of phage therapy for rabbit diarrhea indicated long-term persistence of phage in the spleen but no significant effect in preventing disease in rabbits inoculated with the *E. coli* O103 enteropathic strain (44).

The use of phages to control fish diseases and other infections in aqueous environments seems particularly promising from both a practical and a theoretical point of view. The natural bacterial and phage ecology of the organisms of interest is very close to the laboratory culture conditions, where phage-bacterium encounters are in suspension, where the composition of the medium can be either controlled or known, and where the natural phages have evolved to be successful in liquid culture. The host organisms, i.e., fish, mollusks, or crustacea, live in aqueous media and hence the therapeutic phage can have continuous and intimate physiological contact with the pathogens in a natural arrangement.

In addition to these controlled laboratory studies of phage therapy, there is significant clinical literature on phage therapy. Unfortunately, this literature is almost entirely anecdotal or conducted with historical controls. Slopek's group in Wroclaw, at the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, has published several large series of clinical trials of bacteriophage for suppurative bacterial infections (6, 26, 37, 48–54, 61, 62), and less extensive reports from Romania (34, 68, 69), France (24, 27, 30, 60), Czechoslovakia (41), Britain (7, 47) and North America (4, 44, 67) are in the literature. Almost all of these reports are interpreted by the authors as indicating

the efficacy of phage therapy. Although it is tempting to apply a meta-analysis to this collection and conclude that phage therapy is indeed effective, especially because it was usually employed as a last resort only in "hopeless" cases, we will not know if the promise of phage therapy can be fulfilled until more rigorous clinical evaluations have been carried out.

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