Phage therapy – Everything old is new again

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The study of bacterial viruses (bacteriophages or phages) proved pivotal in the nascence of the disciplines of molecular biology and microbial genetics, providing important information on the central processes of the bacterial cell (DNA replication, transcription and translation) and on how DNA can be transferred from one cell to another. As a result of the pioneering genetics studies and modern genomics, it is now known that phages have contributed to the evolution of the microbial cell and to its pathogenic potential. Because of their ability to transmit genes, phages have been exploited to develop cloning vector systems. They also provide a plethora of enzymes for the modern molecular biologist. Until the introduction of antibiotics, phages were used to treat bacterial infections (with variable success). Western science is now having to re-evaluate the application of phage therapy – a therapeutic modality that never went out of vogue in Eastern Europe – because of the emergence of an alarming number of antibiotic-resistant bacteria. The present article introduces the reader to phage biology, and the benefits and pitfalls of phage therapy in humans and animals.

Key Words: *Bacterial virus; Bacteriophage; Diagnostic tools; Human and animal studies; Novel therapies; Phage therapy; Phage typing; Phagotherapy*

Le traitement par les phages : Une cure de jouvence

L'étude des virus bactériens (les bactériophages, ou phages) a été un point charnière de l'émergence de la biologie moléculaire et de la génétique microbienne et a fourni de l'information importante sur les processus centraux de la cellule bactérienne (réplication, transcription et traduction de l'ADN) et sur le mode de transmission de l'ADN d'une cellule à l'autre. Grâce aux études génétiques d'avant-garde et à la génomique moderne, on sait maintenant que les phages participent à l'évolution de la cellule microbienne et à son potentiel génétique. En raison de leur capacité de transmettre des gènes, les phages ont été exploités pour mettre au point des vecteurs de clonage. Ils fournissent également une pléthore d'enzymes pour le biologiste moléculaire moderne. Jusqu'à l'apparition des antibiotiques, les phages étaient utilisés dans le traitement des infections bactériennes (avec un succès variable). La science occidentale doit désormais réévaluer l'application du traitement par les phages, une modalité thérapeutique qui n'a jamais cessé d'être populaire en Europe de l'Est, en raison de l'émergence d'un nombre alarmant de bactéries antibiorésistantes. Le présent article présente au lecteur la biologie des phages, ainsi que les bienfaits et les écueils de la thérapie par les phages chez les humains et les animaux.

The discovery of viruses specific to bacteria (bacteriophages or phages) is credited to the Englishman, Frederick Twort (1), and the French Canadian, Felix d'Herelle (2), but it is the latter scientist who probably more accurately recognized what he was dealing with and who is responsible for naming these agents of bacterial death. He is also responsible for recognizing their potential clinical significance. He noted (3):

> "Another thought came to me also. If this is true, the same thing will have probably occurred in the sick man. In his intestine, as in my test-tube, the dysentery bacilli will have dissolved away under the action of their parasite. He should now be cured".

It is interesting to note that phage therapy ceased to be used in the West with the advent of the antibiotic era but has been rediscovered because of the rise in antimicrobial-resistant bacteria. While the study and exploitation of phages flourished in the West, particularly in the development of molecular tools, its use in the former Soviet Union as a therapeutic tool has remained steady for over 80 years.

Phage therapy has been the subject of numerous recent review articles (4-16). (Regrettably, the present article largely ignores the literature from the former Soviet Union because of language problems and lack of detail provided in the published studies.) To simplify the discussion, the present article focuses almost exclusively on the tailed lytic (also known as virulent) bacteriophages belonging to the viral order *Caudovirales* (17).

Members of the *Caudovirales* order are divided morphologically into three families (based on the length and complexity of their tails) and functionally into two groups (based on their effect on infection of host cells) (Figure 1). Lytic phage infection leads exclusively to cell death, lysis and the release of progeny phage particles. While infection by temperate phages may also lead to propagation and lysis (the lytic pathway), this is not always the case. If the phage lytic functions become repressed, the virus genome coexists in a stable form (ie, the prophage) within its host. In the latter case, the phage genome is either integrated into the bacterial chromosome (most common) or remains separate, as a 'plasmid' (lysogenic pathway).

Temperate phages are seldom used in phage therapy because, first, they do not kill 100% of the infected bacteria, and second, in certain cases, they contain genes that render the bacterium more virulent – a phenomenon known as 'lysogenic conversion'. This has been observed with many human pathogens, including *Vibrio cholerae* (18-20), *Escherichia coli* (21,22), *Salmonella typhimurium* (23-25), *Pseudomonas aeruginosa* (26,27), *Staphylococcus aureus* (28), *Streptococcus pyogenes*

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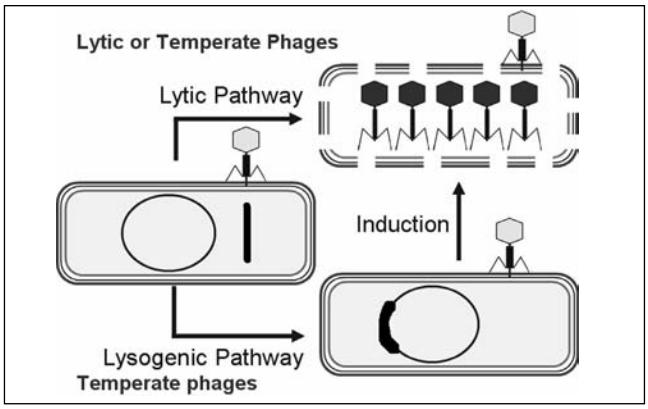

Figure 1) *The lytic and lysogenic pathways of bacteriophages. In the lysogenic pathway, the phage genome (thick line) is shown integrated into the bacterial genome. Bacterial genome projects have revealed that, on average, there are 2.5 phage genomes per bacterium. In the case of* Streptococcus pyogenes *(154-157) and* Escherichia coli *O157:H7 (158,159), the bacterial genomes contain multiple phage genomes. The reversion to the lytic state ('induction') occurs spontaneously but can be enhanced by certain chemical and antibiotics (160,161)*

(29,30), *Clostridium botulinum* (31,32) and *Corynebacterium diphtheriae* (33,34).

LIFE CYCLE OF LYTIC PHAGES

Phages, like other viruses, can be characterized on the basis of their host range. While most phages probably possess a narrow host range – that is, they lyse relatively few bacterial strains – others have a broader spectrum of hosts. Some, like phages Felix O1, lyse almost all *Salmonella* serotypes (35-37), while ϕS1 lyses a broad range of fluorescent pseudomonads (38). From a practical perspective, those possessing a broader host range offer advantages as therapeutic agents.

Diffusion brings the phage and potential host into contact. Adsorption occurs through interaction of the distal ends of the phage tails with (usually one of) a plethora of cell-surface components, including pili, flagella, capsules, proteins, lipopolysaccharides (LPSs) and teichoic acids (39). Strong binding then leads to injection of the phage DNA – contained within the capsid – into the host cell (Figure 2A); the mechanisms by which this happens are just beginning to be understood. Once inside the host, the viruses take over the cell's machinery, subverting it to make new phage particles. This process involves degradation of the host genome and the reutilization of the nucleotides in phage DNA replication (Figures 2B and 2C). Electron microscopy and the analysis of mutants have revealed that, in the subsequent step, phage precursors (such as proheads) appear within the infected cell (Figure 2D). The DNA is packaged into the proheads (Figure 2E), thereby making them competent for tail assembly (Figure 2F). This ultimately leads to the appearance of complete phage particles in the infected cell (Figure 2G).

Release of these intracellular viruses requires two phage products, commonly referred to as 'holins' and 'lysins'. The former are small, pore-forming proteins that permit the cytoplasmic lysins access to the peptidoglycan layer in the periplasm. The degradation of this layer leads to osmotic lysis of the cell and the release of tens to hundreds of progeny virus particles that can, in turn, infect and lyse remaining host cells (Figure 2H).

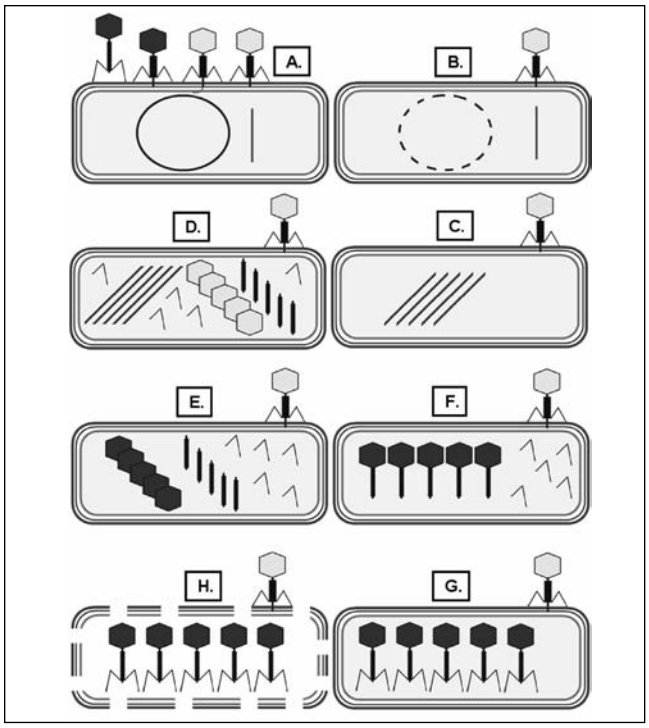

Figure 2) *The life cycle of lytic bacteriophages.* **A** *Phage adsorption and DNA injection;* **B** *Host genome degradation;* **C** *Phage DNA replication;* **D** *Appearance of morphogenesis intermediates, including empty heads (proheads);* **E** *Packaging of phage DNA into capsids;* **F, G** *Phage assembly;* **H** *Lysis and release of progeny phage. The time to lysis varies with the phage host system but can be as little as 10 min*

IMPORTANCE OF PHAGES

Phages are important ecologically as agents in the recycling of organic matter, including cells, and as tools in molecular biology and epidemiology. Almost all natural environments have high concentrations of phage-like particles (40-45). It is conservatively estimated that the prevalence of phages worldwide is in excess of 10^{31} (46) – equivalent to approximately 10^{9} metric tons – making viruses the most abundant life form on earth.

Epidemiological fingerprinting of bacterial isolates (phage typing)

In epidemiological work, it is necessary to track individual bacterial isolates from clinical specimens to their source (47). This is accomplished by fingerprinting the strains using a wide variety of phenotype- or genotype-based typing methods. One of the classical procedures is phage typing, which is still used in Canada at the Laboratory for Foodborne Zoonoses (Guelph, Ontario) and the National Microbiology Laboratory (Winnipeg, Manitoba), for *Salmonella* and *E coli* strains. This procedure involves exposing the bacterial isolate to a battery of 'typing' phages, and recording the pattern and degree of lysis. This approach offers import advantages, including the incredible specificity of phage, and a high degree of typability and reproducibility (48,49). Furthermore, in contrast to serotyping and pulsed field gel electrophoresis analysis, phage typing is relatively inexpensive.

Use of phages as tools in molecular biology

Phage research has had a pivotal impact on molecular biology. In their book *Phage and the Origins of Molecular Biology*, Cairns

et al (50) show how phages have contributed not only to our understanding of vital cellular processes, but also to the development of a considerable number of important genetic and biochemical tools. For example, the realization that viable bacteriophage lambda particles could be constructed with a significant portion of their genome deleted led to the development of insertional and replacement vectors, as well as cosmids and integrative plasmids. Phage serine integrases, particularly those of *Streptomyces* phage ϕC31, have been exploited by Michele P Calos (Stanford University, USA) to integrate foreign DNA into mammalian cells (51-53) and *Drosophila* (54), with the goal of producing transgenic animals or curing biochemical defects (55-57). In addition, phage packaging signals, promoters and terminators, together with a great variety of enzymes, are used in today's molecular biology laboratory – including polynucleotide kinases, DNA ligases, DNA polymerases, RNA polymerases, recombinases, single-stranded DNA binding proteins, endo- and exonucleases, and even methylases and restriction endonucleases (58).

Use of bacteriophages to express peptides and proteins (phage display)

Several systems have been developed to create peptide or protein fusions on capsid proteins of bacteriophages of coliphages lambda (59,60), M13 (61), T7 (62-64) and T4 (65). The M13 and T7 systems have been commercialized. These extremely elegant molecular tools have been used to identify antibody binding epitopes (66-68), amino acid residues involved in protein-protein interactions (69-71), peptides that mimic nonpeptide ligands (72), enzyme substrates and inhibitors, and have even been used to express proteins. One major advantage of this system over standard protein chemistry is that the sequence of the peptide insert can be rapidly and inexpensively determined by DNA sequencing.

A fascinating application of phage display technology is the production of M13 derivatives that express specific antibodies (73). This was pioneered by Cambridge Antibody Technology Ltd (United Kingdom) and is commercially available through GE Healthcare (formerly Amersham Biosciences) as the Recombinant Phage Antibody System. This technology has been the subject of numerous recent reviews (74-77).

New phage diagnostic tools

In addition to the classical uses of phages in molecular biology and diagnostic microbiology, new tools have been developed. The phage amplification assay, for example, is a simple yet elegant way to identify the presence of specific pathogens in food products. The intracellular replication of phage and concomitant lysis of the susceptible bacteria leads to an increase in free phage, which can be easily measured (78,79).

Another new tool involves phage-luciferase fusions. Phages active against a wide variety of bacteria have been tagged with luciferase cassettes (created from either *Vibrio luxAB* genes or *Aequorea* green fluorescent protein), which, when expressed, result in the emission of visible light. The major advantages of this system are that light production is absolutely dependent on phage infection of sensitive cells and can easily be measured with exquisite sensitivity.

Phage-luciferase fusions have been used to identify *E coli* O157 (80-82), *Listeria* (83), *Salmonella* (84) and *Mycobacterium* (85-88). Lastly, bioluminescent phages for the latter bacterium have been used to rapidly determine the antibiotic susceptibility properties of the host cells; that is, in the presence of an effective antimicrobial, light production is inhibited (89-91).

ANIMAL STUDIES

The earliest research on the therapeutic efficacy of phages was conducted by Felix d'Herelle, who carried out field studies on fowl typhoid (*Salmonella enterica* subspecies *enterica* serovar Gallinarum) and laboratory studies on dysentery in rabbits (*Shigella dysenteriae*) (92). Subsequent animal studies have shown that phage therapy works and is, at least in theory, a practical therapeutic modality.

Unfortunately, one has to wade through a number of studies that, while offering tantalizing evidence of the efficacy of phages (see, for example, the chicken studies by WE Huff and colleagues [93-98]), were either poorly conceived or used impractical treatment regimens with a low probability of being used in agriculture. Examples include treatments that are ineffective once the disease symptoms are expressed, the coadministration of high doses of phage preparations with the pathogen and treatment delivery protocols that would be too costly on a large scale. In addition, a wide variety of animal types and infection strategies have been used, and the phages, which have generally been isolated on a needs basis, are all poorly characterized. This makes comparisons across studies difficult at best.

Nevertheless, there do exist well-controlled animal studies that indicate that phages can be used therapeutically in animals and have some advantages over antibiotics. In 1943, René Dubos and colleagues (99) at Harvard University (USA) demonstrated that anti-*S dysenteriae* phage injected intraperitoneally in mice appeared in the blood stream (and even crossed the brain-blood barrier) but were rapidly cleared (by the reticuloendothetial system, particularly the spleen) (Figure 3, left). When approximately 2×10^7 colony-forming units of the bacterium were injected intracerebrally, more than 95% of the control mice died within four days; this rate was reduced to 28% with the intraperitoneal injection of bacteriophage (10^9 plaque-forming units). In contrast, sterile, uninoculated broth, bacterial filtrates and heated phage preparations (60°C) were ineffectual in rescuing the mice. Of particular note was the observation that in the presence of susceptible bacteria (Figure 3, right), the level of phage actually increased and remained high for longer. In the words of Ryland Young (Texas A&M University, USA; personal communication), phages are "the only medicine that multiplies".

Substantially similar results were obtained in chickens inoculated intramuscularly or intracranially with *E coli* O18:K1 by Paul Barrow and colleagues (Institute for Animal Health, United Kingdom) (100).

For the most promising animal studies, one must turn to those conducted in the 1980s by H Williams Smith and colleagues at the Institute for Animal Disease Research (United Kingdom) (101-104); these still stand up to scientific scrutiny and indicate clearly the potential for phage therapy in animals. Six- to 12-hour-old calves were fed a mixture of enteropathogenic *E coli* strains (at a dose of 10^9), nonpathogenic *E coli* (10^{10}) and *Lactobacillus* species (10^{10}). Ninety-seven per cent of the treated animals developed diarrhea within 12 h to 46 h, and 80% died. Microbiological examinations showed the highest counts of bacteria ($10^{9.3\text{-}9.8}$/g) in the anterior of the small intestinal and rectal area.

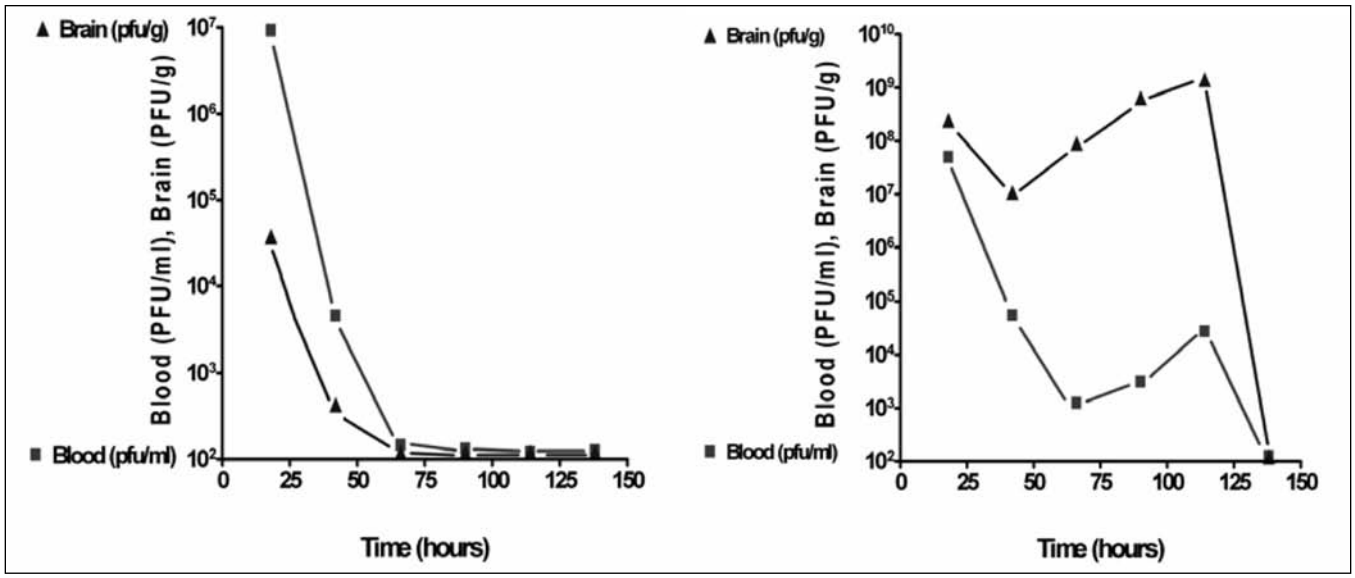

Figure 3) Left panel *Fate of 10^9 plaque-forming units (PFU) of* Shigella dysenteriae*-specific phage injected intraperitoneally into a mouse*. **Right panel** *Change in titre of the phage as a result of simultaneous injection of phage (intraperitoneally) and* S dysenteriae *(approximately 2×10^7 colony-forming units) intracranially*

In studies with *E coli* O101:K30, the addition of 10^5 K-antigen specific phage B41/1 orally at the onset of diarrhea resulted in no deaths. Other studies showed that the in vitro virulence of the phage did not always correlate with its effect in vivo; phage could have a protective effect if introduced up to 6 h before and 12 h postinfection; and phage could be administered in milk, or picked up from sprayed stall litter or even from contaminated stool material.

Earlier mouse studies by Smith and Huggins (101) using *E coli* demonstrated that single doses of phage were just as effective as a single administration of streptomycin in eliminating infections, but delaying the treatment regimens negatively affected the outcome for both bacterial viruses and antibiotics. This was re-examined by JJ Bull and coworkers (105), who showed that delaying the treatment in mice by 8 h had little effect on the efficacy of the phage, but markedly reduced the percentage of survivors in the antibiotic-treated group.

Lastly, James Soothill and colleagues (Great Ormond Street Hospital, United Kingdom) (106) demonstrated the potential of using phages against otitis externa caused by *P aeruginosa*. Like humans, dogs are susceptible to *Pseudomonas* ear infections. The animals in the study responded well to a single dose of a polyvalent phage preparation (Biovet-PA).

HUMAN STUDIES

Safety

The first safety trials were conducted by d'Herelle (3), whose methods would not pass bioethics approval today: he tested the safety of phage preparations (taken orally or by injection) on himself, his family and colleagues. Neither they nor subsequent test subjects (patients) experienced any ill effects (107). To these early studies, we can add a wide range of anecdotal evidence, as well as results of actual human experimentation (though not always deliberate), that support the conclusion that exposure to phages poses minimal, if any, health risk. It is impossible to avoid ingesting phages. Both the human oral cavity (108,109) and fecal matter contain phages (41,110), and they are present in municipal drinking water (111), food substances such as yogourt (112) and salami (113), and have even been contaminants in live polio vaccine preparations (114).

Extensive safety trials were undertaken on Staphage Lysate by Delmont Laboratories (USA). This product, which contains high concentrations of antistaphylococcal phages (15,16), was administered to humans intranasally, topically, orally, subcutaneously and intravenously. In over 12 years of use in humans, only minor side effects were observed (16). Lastly, double-blind studies by Anne Bruttin and Harald Brüssow (Nestle Research Center, Switzerland) in 2005 showed that the oral administration of coliphage T4 had no ill effects on human volunteers (115). It is worth reiterating the words of Andrzej Górski et al (116) (Institute of Immunology and Experimental Therapy, Poland), whose institute has 60 years of experience with phage therapy:

> "While our past studies did not formally meet current strict criteria of controlled clinical research, they still strongly suggest a high efficacy of phage treatment, its safety, and virtual lack of side effects. Our more recent studies also suggest that phages can migrate to organs that are usually not readily accessible to drugs (prostate gland, bone)".

Successes

As with the earliest animal experiments, the first studies in humans were conducted successfully by d'Herelle, who used them on patients suffering with dysentery and bubonic plague (3,92). This work led d'Herelle and the Georgian scientist, Giorgi Eliava, to establish an institute, which now bears his name, in Tbilisi, Georgia (1923), for the investigation of practical uses of phage in therapy. In one study (of many) conducted by the Institute in their 83-year history, over 31,000 children younger than seven years of age participated in a 16-week clinical trial of the efficacy of anti-*Shigella* phage tablets. One-half of the children (from one side of the streets) received weekly

tablets containing the phages, while those on the opposite side of the streets received a placebo. The incidence of clinically verified shigellosis in the subjects receiving the treatment was reduced 2.6-fold (5).

The most clearly documented phage therapeutic research – and the most relevant to the global problem of antimicrobial resistance – was conducted at the Institute of Immunology and Experimental Therapy (also known as the 'Hirszfeld Institute') in Wroclaw, Poland. Their experience with phages over the past thirty years has been the subject of numerous publications (many of which are accessible from their Web site at http://www.iitd.pan.wroc.pl/about_en.html). In all, almost 2000 patients infected with a variety of life-threatening (predominantly antibiotic-resistant) infections have been treated with phages. The overall success rate is from 60% to 90% (117,118). These studies have been described as the "most detailed studies published in English on the use of phages in clinical settings" (14) and "probably the most important data published in the English literature" (119).

One study that deserves mention did not involve active intervention by the physician, nor was it a clinical study of a therapeutic agent. Rather, Shah M Faruque and colleagues from the International Centre for Diarrhoeal Disease Research, Bangladesh, examined the self-limiting nature of seasonal cholera epidemics and the role of host-mediated amplification of phage (110,120). The team found that the number of cholera patients spikes in mid-September, just after the level of *V cholerae* in water reaches its maximum, and then decreases in subsequent months (Figure 4). This was found to be a natural consequence of enrichment for lytic phages in the intestinal tracts of infected patients. These phages then enter ('contaminate') the community water sources, leading to further decreases in the levels of this pathogen. These studies suggest that the early phage studies by d'Herelle on the ecological control of cholera in India bear re-examination with a less skeptical eye (16).

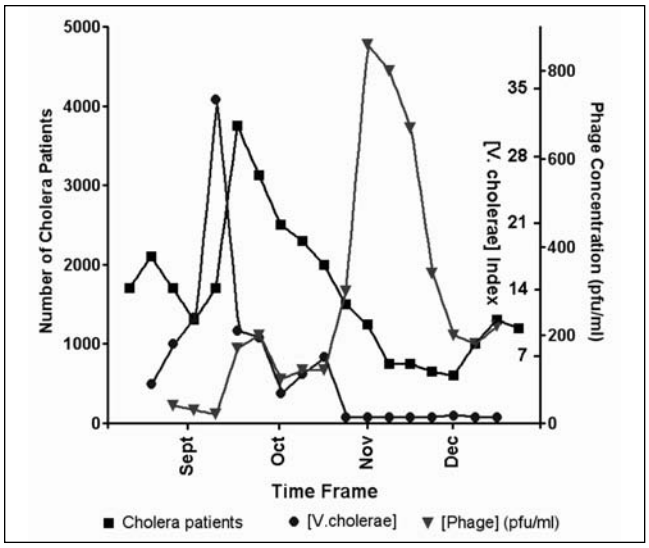

Figure 4) *Changes in the concentration of* Vibrio cholerae *in water over the summer and autumn months in Bangladesh, as well as its impact on the number of cholera patients. The decrease in phage patients and the pathogen (measured in plaque-forming units [pfu]) are due to enrichment in specific cholera phages within the patients and in water. Data from reference 120*

Problems

There are some major problems with the reintroduction of phage therapy into North America, but the alarming increase in antibiotic-resistant bacteria and the move against the use of antimicrobials in food production in Europe is forcing us to look more favourably at phages, either for treating infections in humans or (more likely) to reduce the spread of zoonotic bacterial diseases. The problems arise from phage biology, from proteomics and from difficulties in obtaining regulatory approval. Some of these challenges have been addressed experimentally as outlined below.

The behaviour of phages under anaerobic conditions (ie, in the gut) or in starved cells has been addressed by relatively few studies (121-126) and requires further study. Sandra Chibani-Chennoufi and colleagues (6) showed that *E coli* isolated from normal mice was generally susceptible to a cocktail of four broad host range coliphages in the laboratory, but oral ingestion of these phages had little impact on the resident flora, suggesting that the bacteria were somehow protected or that the phages behaved differently under anaerobic conditions. Phage Rb33, for example, is a broad host range, Teven-like virus that displays oxygen-dependent growth on *ECOR4* (127). The scientists also noted extensive lysis inhibition under anaerobic conditions. They hypothesized that, under anaerobic conditions, different phage receptors were expressed, a theory that echoes the observations of Wegrzyn and Thomas (125). It is also possible that gut carbohydrates and bile salts chelate divalent ions, which are required for the adsorption and replication of many phages.

Mutation of bacteria to phage resistance is often listed among the problems with phage therapy. Indeed, in the laboratory, the famous Luria and Delbrück fluctuation test (128) showed that the rate of mutation of *E coli* to phage T1 resistance was 1.4×10^{-8} to 4.1×10^{-8}. In phage-treated animals, bacteria resistant to the therapeutic phage have been observed to arise. This has been modelled in the laboratory in studies on predator-prey successions. But in vitro predator-prey studies do not accurately reflect in vivo conditions. If one is dealing with K1 or LPS-specific phages, the most likely resistant mutants are capsule or LPS defective. In both cases, the mutant bacteria are less virulent. In a recent in vitro study of *E coli*, *S typhimurium* and coliphages T5 and T7, William R Harmcombe and James J Bull (University of Texas at Austin, USA) (129) showed that resistant mutant outgrowth occurred in pure *E coli* cultures, but in mixed culture conditions, the phage-resistant coliforms were outcompeted by *Salmonella*. It is also worthy noting that therapeutic phage cocktails may contain viruses with different receptor specificities; this would reduce the likelihood of the outgrowth of virulent, phage-resistant mutants.

Bacteriophages are antigenic and are rapidly cleared by the reticuloendothelial system. The former problem can only be dealt with by substituting another phage, if proven necessary. In their mice studies at the National Institutes of Health (USA), Carl Merril et al (130) were able to select "long-circulating" or "Argo" mutants from virulent derivatives of the well-studied coliphage lambda and *Salmonella* phage P22 (*λvir* and P22*vir*). These possess changes in their capsid proteins (131).

Relatively little is known about the proteomes of the large, virulent phages. In silico analysis has failed to reveal 'toxin'

genes in virulent coliphages, and the studies by Bruttin and Brüssow (115) revealed no human toxicity, but we have identified the functions of at most 50% of proteins of coliphage T4 – one of the best-studied viruses. We know far less about many of the other potential therapeutic phages; indeed, most have not been sequenced.

Regulatory approval is the major hurdle for standard mono- or polyvalent phage therapy. In the words of Harald Brüssow, "phagetherapy … challenges current pharmacokinetic concepts", in large part because this is "the only medicine that multiplies". The Georgian approach – to isolate new phages when the existing cocktail does not work – is unacceptable to North American regulators.

In addition, phage therapy is decidedly 'low technology'. It is questionable whether drug companies would be willing to invest in the development of phage-based products without considerable confidence that they could make a profit. And the patenting of viral cocktails would be venturing into new legal territory.

NEW ALTERNATIVE THERAPIES

The newest therapies involving phages can be arbitrarily divided in two, based on whether the approach is simply an outgrowth of traditional phage therapy or is truly unique. In the former group, we have the immobilization of phages in or on membranes, as in PhagoBioDerm (Phage International, USA), a biodegradable, polymer-based membrane that contains phages, ciprofloxacin, benzocaine and alpha-chymotrypsin, which was developed at the Eliava Institute (132-134). The phages (PyoPhage, BioPharm-L, Georgia) target a variety of Gram-positive and Gram-negative bacteria. The preparation has been successfully used (70%) to treat recurrent leg ulcers and infections in burn victims. Janice Spencer and colleagues (University of Strathclyde, United Kingdom) (135) developed a variant of this procedure. They immobilized phages on nylon strips and found that the preparation was "effective against most of the major epidemic methicillin-resistant *Staphylococcus aureus* strains". They have proposed that this could be used "in different forms, including strips, sutures and beads" (see http://www.news-medical.net/?id=8938).

The potential exists for at least two types of novel phage therapies: recombinant therapeutic phages and phage-derived lysins. In the first therapy, highly lytic phages could be selected or engineered for different receptor specificities (136,137). This has yet to be attempted, but its proof of concept is the isolation of spontaneous phage host range mutants and the existence of recombinant phages with different receptor specificities (138). Diane L Schaak (Rowland Institute, Harvard University, USA) (139) has suggested developing what she calls "toxin-phage bacteriocides" or "trojan silver bullets" – that is, phages whose genomes are supplemented with host lethal toxins to enhance cell killing.

As proofs of concept, both temperate and several nonlytic phages have been loaded with toxic protein genes. Steven Hagens et al (Max F Perutz Laboratories, Austria) (140) created a nonreplicating mutant of *P aeruginosa* phage Pf3 that carries the restriction endonuclease *Bgl*II. This virus was more effective than the pilus-specific virulent phage Pt1 in animal protection studies and had the added benefit of abrogating the release of LPS as a result of cell lysis, thus decreasing the danger of endotoxic shock. Similar studies have been carried out in *E coli* by Caroline Westwater et al using phage M13 carrying the addiction toxins Gef and ChpBK (141). Very recently, Michelle L Embleton (Eastman Dental Institute, University College, United Kingdom) (142) labelled the *S aureus* serogroup F typing phage 75 with the photosensitizer tin (IV) chlorin e6 and noted its enhanced activity against this bacterium. This targeted photosensitizer was even effective in significantly reducing the viability of strains that were apparently not susceptible to this bacteriophage, suggesting that phages may still bind to bacteria that they cannot productively infect.

The construction of lysis-deficient phage mutants and their use therapeutically is the subject of patents issued to Gangagen (USA) (143,144). If effective, one can envisage their immediate application to bacteria containing intracellular toxins, such as *Clostridium difficile*.

One of the most exciting new technologies is based on the activity of phage lysins and is derived from the pioneering work of WM Mullan and RJ Crawford (145). This approach has been used by Vincent Fischetti (Rockefeller University, USA) (146) and others (147,148). The advantages of lysin-based therapy are numerous: they can be prepared with high purity and possess high specific activity; they exhibit rapid lethal action; they are nontoxic; and apparently, antibodies that form against these proteins do not neutralize their lytic activity. Lastly, no bacterial resistance develops to these proteins, probably because they possess multiple domains for cell wall binding and hydrolysis.

Experimental studies have indicated that lysins possess considerable potential for decontaminating foods (149) or treating infections. For example, lysin PlyGBS was found to be effective in preventing group B streptococcal colonization of the mouse vagina and oropharynx (150), the C(1) lysin prevents group A streptococcal colonization of the mouse upper respiratory tract (151); and Cpl-1 lysin prevented *Streptococcus pneumoniae* endocarditis in a rat model (152). Likewise, LysK cloned from broad host range staphylococcal phage K may have therapeutic efficacy against methicillin-resistant *S aureus* (153). (This therapeutic modality is only effective against Gram-positive bacteria because of the outer membrane permeability barrier in Gram-negative cells.)

WHERE NEXT IN CANADA?

The Public Health Agency of Canada's Laboratory for Foodborne Zoonoses and its director, Mohammed Karmali, are intent on it becoming *the* research centre in Canada investigating the use of phages to limit the animal carriage and transmission of zoonotic bacteria (*Salmonella*, *E coli* O157 and *Campylobacter*).

They propose becoming the organizational home of a network of Canadian scientists and clinicians interested in all aspects of phage therapy. Interested clinicians should contact Mohamed Karmali (Mohamed_Karmali@phac-aspc.gc.ca), while basic scientists should contact the author (Andrew_Kropinski@phac-aspc.gc.ca).

RESOURCES: Two basic resources on phage therapy are the Web sites of Elizabeth Kutter (Evergreen State College, USA) at <http://www.evergreen.edu/phage/> and Stephen Abedon (Ohio State University, USA) at <http://www.mansfield.ohio.edu/~sabedon/>. The Appendix lists some of the companies involved in phage research or phage therapy. The Intralytix Web site contains Alexander Sulakvelidze's detailed history of phagotherapy.

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APPENDIX
Companies involved in phage and phage therapy research

Company	Location	Web site address
Biophage Pharma Inc	Canada	http://www.biophage.com/
Exponential Biotherapies, Inc	USA	http://www.expobio.com/
Gangagen Inc	USA	http://www.gangagen
Hexal Genentech	Germany	http://www.hexal-gentech.de/
InnoPhage	Portugal	http://www.innophage.com/
Intralytix, Inc	USA	http://www.intralytix.com/
New Horizons Diagnostics Inc	USA	http://www.nhdiag.com/index.htm
Novolytics Ltd	United Kingdom	http://www.novolytics.co.uk/about_us.html
Phage Biotech Ltd	Israel	http://www.phage-biotech.com/
Phage International, Inc	USA	http://www.phageinternational.com/
Phage Therapy	Georgia	http://www.phagetherapycenter.com/
Targanta Therapeutics Inc	Canada	http://www.targanta.com/
Biochimpharm	Georgia	http://www.biochimpharm.ge/

USA United States of America

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