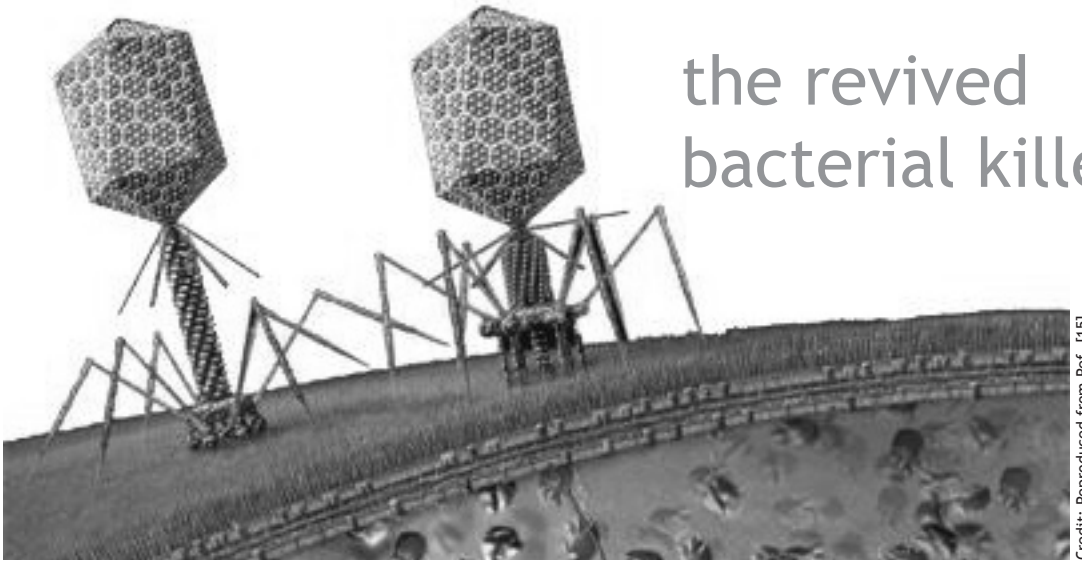
BACTERIOPHAGES

the revived bacterial killers



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By Nicolae Done

The discovery of penicillin by Alexander Fleming in 1928 is considered by many to be one of the greatest accomplishments of modern medicine in the 20th century. It marked a turning point in the historic struggle of mankind against infectious diseases caused by bacteria. However, at the beginning of a new millennium, the battle is still far from over. Another health care crisis has developed: bacterial resistance to antibiotics. An infectious disease like tuberculosis kills around two million people across the globe each year. However, the rate at which new antibiotics reach the market is surpassed by the rate at which bacteria acquire resistance. The eagerly-sought after solution may come from bacteriophages, viruses that target and kill bacteria with phenomenal efficiency and selectivity.

"The eagerly-sought after solution [to the antibiotic crisis] may come from bacteriophages, viruses that target and kill bacteria with phenomenal efficiency and selectivity..."

Reviving a natural assassin of superbugs

"Superbugs", as they are commonly known, are bacteria that have evolved resistance to antibiotics. Resistance to the "miracle drug" was reported in strains of *Staphylococcus aureus* only a year after the first treatment of penicillin was administered to patients in 1942 [1]. Today, Staph infections rapidly make their way out of hospitals into communities of for example, inmates and military recruits [2]. These infections are becoming increasingly more dangerous and difficult to control as multi-drug resistant pathogens causing diseases such as tuberculosis, cholera and typhoid fever have been observed all over the world. In an attempt to treat these "superbugs," the scientific world is turning to bacteriophage therapy – a method that was suggested, but never actually used, even before the discovery of antibiotics.

In 1896, antibacterial activity of an unknown "substance" was found in two rivers in India. Scientists recognized the mysterious substance as viruses that had infected bacteria [3]. By 1930, bacteriophage-based products were licensed for sale in the United States [4]. However, the quality of these products was poor due to the unsophisticated methods of phage purification that existed at the time. Thus in the 1940s, after the introduction of antibiotics, most of the research on bacteriophage-based products was abandoned. A few remote centers in Stalinist Soviet Union continued to research bacteriophage therapy which was used with some success in the Soviet military. Research in the West was not reinitiated until the 1980s.

Classification of bacteriophages

Order	Family	Morphology	Nucleic acid
Caudovirales	*Myoviridae*		Double-stranded DNA
	Siphoviridae		
	Podoviridae		
	Tectiviridae[a]		
	Corticoviridae[a]		
	Lipothrixviridae[b]		
	Plasmaviridae[b]		
	Rudiviridae		
	Fuselloviridae		
	Inoviridae		Single-stranded DNA
	Microviridae		
	Leviviridae		Single-stranded RNA
	Cytoviridae[b]		Segmented, double-stranded RNA

[a] Lipid containing
[b] Enveloped

Credit: Reproduced from ref. [6].

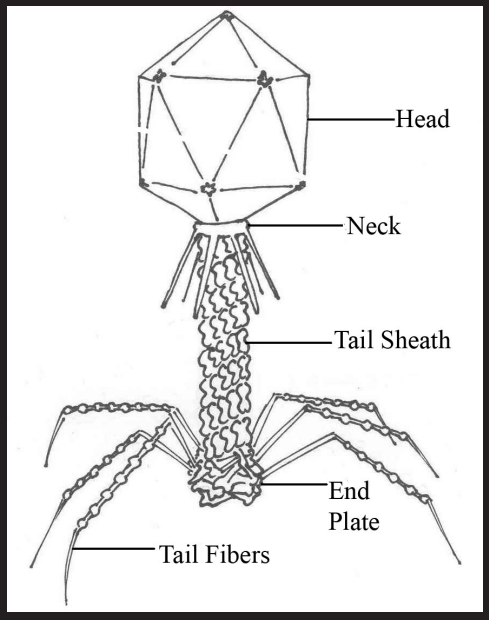
Table 1. The 13 classes of phages known, classified by the nature of their genetic material (DNA or RNA, double or single stranded) and their morphology. the lytic phages are useful for therapy.

Understanding phages and how they kill

Phages are the most abundant and diverse forms of life on Earth [5]. There are 13 families of phages known to date (table 1), but little detail is known about their biology. The families are classified according to genetic material and morphology [6]. More importantly however, phages are divided into two categories based on their life cycles. In the lytic form, the virus replicates about 100 times inside the cell, causing it to burst open and releasing the newly-formed viruses. Conversely, in the lysogenic form, the viruses remain dormant in the host cell DNA and are passed on to the daughter cells upon cell division. Studies done on coliphage λ have revealed that the life cycles can be interconnected [7](figure 1).

Complex mechanisms control the adoption of one lifestyle or the other. Conditions that threaten the cell's continued existence, such as nutrient scarcity or DNA-damaging agents, cause lysogenic phages to switch to lytic growth. The switch ensures that the virus can escape a bacterial host that is about to die. Thus, viruses are able to preferentially activate one pathway or the other in their best interest. About half of the known phages prefer to "hibernate" inside their bacteria host cells, only lysing them under life-threatening conditions. This is important in phage therapy because only viruses that are able to lyse cells have therapeutic potential. Moreover, lysogenic viruses have a high probability of carrying genes that confer resistance to antibiotics or genes that encode bacterial toxins. The conditions that allow phages turn on their lytic lifecycles are of great interest to scientists as well as genes that cause lytic viruses in vitro to become lysogenic inside humans [5, 8].

Structure of a "lunar-lander" phage



credit: Vincent Cheng, HRS.

Figure 1. The head contains and protects the essential molecules required for the life of the phage: DNA and processive enzymes that hijack the bacterial cell machinery. The tail fibers allow the virus to attach to the cell membrane, while the endplate perforates it. Then, the DNA and the enzymes are injected through the tail sheath inside the cell.

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The minimalist structure of bacteriophages is perfectly adapted for their parasitic lifestyles. One of the most interesting morphologies of bacteriophages is the "lunar-lander" shape displayed by many common phages (figure 2). The capsid (head) is made of structural proteins and contains the genetic material (DNA or RNA) and proteins (mainly viral enzymes) needed for processing inside the bacterial cell. The tail, responsible for the infection process, is made of a "neck" which links it to the head, a tubular, contractile sheath, and a baseplate surrounded by fibers, which allow the attachment of the virus to the bacterial cytoplasmic membrane. Once attached, the baseplate creates a hole in the cell membrane and then contracts, injecting the genetic material together with the processive enzymes inside the cell.

Advantages and Issues of Phage Therapy

Several reports have demonstrated the efficacy of using phage therapy in animals. In some instances, mice infected with *Staphylococcus aureus* responded better to injections of phage than to multiple shots of antibiotics [6]. Limited data is available

Different life styles of the coliphage λ

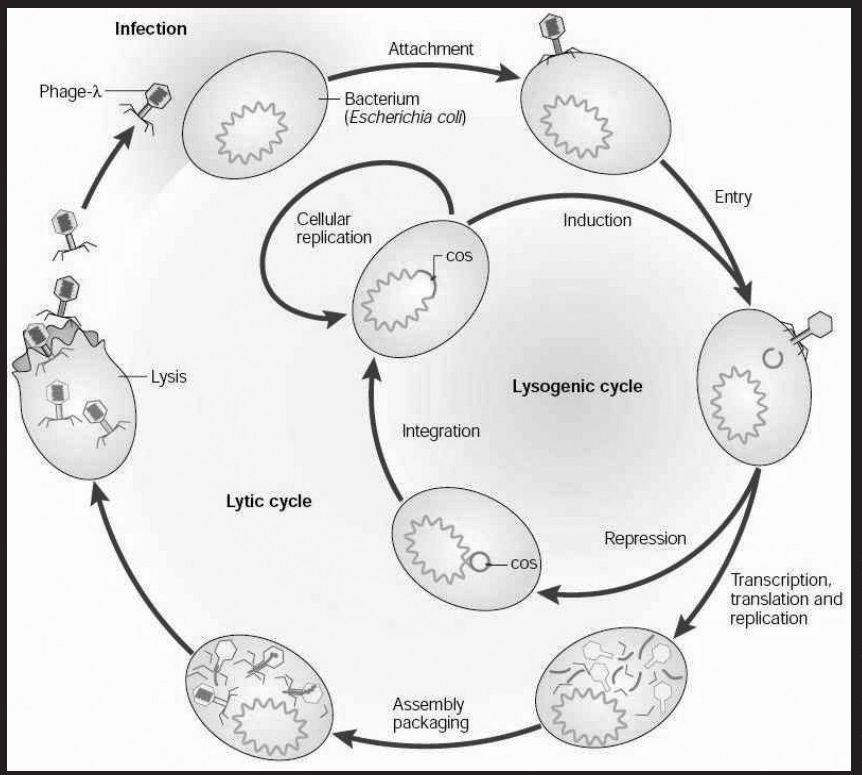

Figure 2. Many phages display two different, interconnected physiologies: lytic and lysogenic. In the lytic form, the virus replicates about 100 times inside the cell, causing it to burst open and release the newly-formed viruses. Conversely, in the lysogenic form, the virus remains dormant and is passed on to the daughter cells upon division. Only phages that can adopt the lytic life cycle are useful for therapy.

regarding the efficacy of phage therapy in humans but available research reinforces the hope that phage therapy will soon be widely available to treat multi-drug resistant pathogens. Although controversial, the most encouraging news regarding phage therapy has come from former Soviet provinces. In 2001, three workers in the country of Georgia suffered from severe skin burns that became infected with antibiotic-resistant Staph. All treatments, including antibiotics, proved ineffective until the doctors applied biodegradable patches impregnated with bacteriophages to their skin. The men recovered soon after receiving the bacteriophage treatment [5, 9].

Despite their apparent effectiveness, treatments like these patches, which are licensed for sale in Georgia, are virtually unknown in Western countries [4]. However, people are beginning to recognize the advantages of using bacteriophage therapy. Chief among these advantages is the ability of bacteriophages to divide and increase exponentially in number over the course of their lifetime. In contrast, antibiotics decrease in concentration over time and several doses are required to maintain the blood concentration above a certain threshold. The ability of phages to replicate themselves makes it possible for doctors to administer minute, singular doses of phage to patients [8]. In addition, because each type of phage targets only specific strains of bacteria, the risk that a patient's other cells or natural bacterial flora will be affected as a result of the treatment is small. Bacteriophages can be administered in a wide range of therapeutic forms, such as pills, injections, nasal sprays, etc. Phage therapy also eliminates the risk of allergic reactions. Allergies to antibiotics are common and potentially lethal. Moreover, bacteriophage therapy is relatively cheap and simple to administer, making it ideal for underdeveloped countries where multi-drug resistant diseases are a major problem [4].

"Despite these benefits, many members of the scientific community are reluctant to acknowledging the therapeutic potential of bacteriophages..."

Despite these benefits, many members of the scientific community are still reluctant to acknowledging the therapeutic potential of bacteriophages. Some argue that too little is known about the biology of phages inside the human body. Most of the clinical trials conducted in the Soviet Union are now considered insufficient because they were not conducted according to Western standards [5]. Other difficulties also arise because a precise diagnosis is required before bacteriophages can be effectively administered to patients. The specificity of bacteriophages means that not only do doctors have to accurately diagnose the bacterial strain causing the infection, they also need to have isolated, purified and tested the phage that kills that strain [4]. In the same way that bacteria develop resistance to antibiotic, bacteria can also develop resistance to phages. However, phages have an advantage because they too are able to mutate to undermine the resistance mechanisms of the bacteria. Normally, doctors will select for phages that are able to maintain their effectiveness in culture for more than a week's time [5]. Another challenge facing the use of bacteriophages

is the threat of septic shock induced by the rapid release of endotoxins from dying bacterial cells [10]. Yet beyond scientific concerns regarding therapeutic efficacy and side effects, there is also the issue of the public's perception of bacteriophages. The popular image of viruses as unwanted pathogens may make some patients or even doctors reluctant to embrace phage therapy [4]. Thus currently, bacteriophages are only considered viable treatment options after all other traditional drugs have failed to have an effect.

In the meantime, phages can also help in other ways

Although the therapeutic use of live phages is not widespread, phages are being used to develop enzyme-based, as opposed to traditional "small molecule", drugs. The isolation of various lytic enzymes, lysins, by the viruses to cause bacterial lysis in vitro suggests the possibility that they can be modified into antibiotic-like medications [11, 12]. These polypeptides would be more efficient than traditional antibiotics such as penicillin. Penicillin works by inhibiting bacterial cell wall synthesis during bacterial replication while lysins work to destroy cell walls directly to kill the bacteria within seconds [6]. Even more elegant experiments have been designed that use the efficient machinery that allows phages to infect the cells: the normal enzymes that are contained in the virus are replaced with endonucleases, enzymes that can cut DNA strands at specific sequences called restriction points [10]. Although these engineered viruses are non-replicating, they can inject the endonucleases inside the cell. These enzymes then effectively cut the host DNA into pieces, causing quick cell death. At the same time, the cells die slower than during the lytic action, thus avoiding the mentioned problem of septic shock caused by a fast release of bacterial toxins inside the host organism.

Not only are phages potential cures for bacterial infections, but they already show great promise as rapid and simple tools for pre-diagnosis of multiple-drug resistant infections. The diagnosis of tuberculosis, for example, can be made using two new bacteriophage-based methods, which hope to provide, upon further development, a more sensitive way of detecting Mycobacterium tuberculosis in patients [13, 14]. In one method, bacteriophages are amplified and detected upon infection of the pathogen. In the other method, viruses are genetically engineered to carry the luciferase gene (a gene that encodes luciferase, a bioluminescent protein). Upon infection of the bacterial cell, this gene is expressed by the cells, causing them to scintillate. The level of bioluminescence is then measured and thus the level of infection deduced [14].

Conclusion

Bacteriophage therapy shows great promises as a tool to fight bacterial infections. However, much research still needs to be done at this level in both the academic and clinic research settings in order to transform these promises into a reliable method of treatment. The very complexity and dynamism of bacteriophages that make them formidable bacterial killers also render them difficult to control. A broader, more comprehensive effort will be needed from the scientific and medical communities to turn phages into our dependable allies in the fight against superbugs. **H**

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