

An Infectious Cure

by

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Part I – A Suspicious Treatment for Cholera

*India, 1926 **

It was cholera season again. Cholera struck twice a year; once after the springtime run-off from the high Himalayas, and once after the monsoons. Dinesh was walking home from the fields when his friend, a merchant at the lassi stand, called him over.

“It’s started,” he said in a mournful tone.

“What?” asked Dinesh.

“Harriet just died of cholera.”

And so this year’s outbreak had begun. Cholera was a fact of life in this small village. Several people died of this disease every year. Dinesh would know all of them. Perhaps he would even be one of the victims this year. Cholera was a horrible disease that was dreaded by all. The afflicted endured severe abdominal pain, vomiting, and diarrhea, and some unfortunate people died from dehydration after several days.

A few days later, as Dinesh was walking towards the fields, he noticed a group of European men pouring something into the village well. Coming closer, he overheard one of them explaining to the village elder that this was going to prevent and cure cholera. A similar treatment had apparently helped to treat Europeans. Dinesh had no reason to believe these claims and was suspicious.

The elder was similarly unimpressed by the promises made by the Europeans. Later that day, he called all the able-bodied men in the village to drain the village well. They instructed all the villagers to take water from other wells surrounding the town.

A few days later, the European men returned and asked about the state of the cholera outbreak. Cholera had claimed more lives and new people were getting sick. The foreigners seemed surprised. Their surprise turned to outrage when they discovered that the well in which they poured their treatment had been drained.

In the evening, the European men added a purple dye to all of the town’s wells except for one, in which they poured their treatment. The colored water would be avoided by the town’s people, forcing them to drink from the “treated” well.

After a couple of days, Dinesh noticed that affected individuals were improving, and there were no new cases reported. Despite his misgivings and anxiety about the contents of the water, the treatment seemed effective.



* *Note:* While the characters in this case are fictional, the events of this story did take place in India in 1926 (Hausler, 2006, pp. 84–85).

Credit: Scanning electron microscope image of *Vibrio cholerae* bacteria, which infect the digestive system (<http://remf.dartmouth.edu/imagesindex.html>).

Questions

1. Cholera is caused by bacteria that infect the small intestine. These bacteria are typically contracted by drinking contaminated water. The symptoms are brought about by the secretion of a toxin that disrupts the functioning of the cells of the small intestine. Given this information, what might the European men have poured into the wells? Be as specific as you can in explaining how your proposed treatment cured and prevented cholera. Come up with more than one hypothesis.
2. What ethical issues does imposing a treatment on the villagers without their consent raise? Are there factors that make it more or less acceptable?
3. At the time, the Europeans did not fully understand how their well treatment worked. They only had indications that it worked once in Europe. Were they justified in imposing their treatment? Are there steps they could have taken that would have made the situation more acceptable?
4. Is it ever appropriate to force a “cure” on a population? Remember that in North America many municipalities put fluoride in the water supply to prevent tooth decay.
5. What do you make of the validity of the results from this historical experiment? What conclusions can be drawn? Were the results scientifically reliable or should they be regarded as indicative or anecdotal?

Part II – Big Fleas Have Lesser Fleas Upon Their Backs to Bite Them, and So On Infinitum

In nature, every organism is part of an ecosystem. It feeds on prey and is preyed upon by its predators. Bacteria (including the cholera-causing bacteria) are no exception. Their enemies are bacteriophages, tiny viruses that use them as virus-making factories, often killing them in the process.

A bacteriophage is composed of genetic information (RNA or DNA) and proteins. It is shaped like a moon lander and has three distinct regions (Figure 1). The head contains the genetic material. Proteins form a protective coat around it, giving the head a roundish appearance. The neck and helical sheath are also composed of proteins and serve as a conduit for injecting the genetic material into a bacterium – like a syringe. Finally, the base plate and the tail fibers are responsible for recognizing and binding to target bacteria. Since they are composed of proteins, they have unique three dimensional shapes that fit into specific targets on a bacteria's surface, in a lock and key fashion.

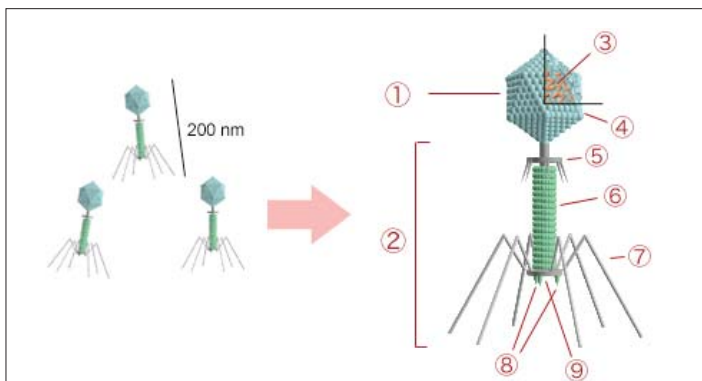


Figure 1. Bacteriophage structure.

(1) Head, (2) Tail, (3) Nucleic acid, (4) Capsid, (5) Collar, (6) Sheath, (7) Tail fiber, (8) Spikes, (9) Baseplate

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Like all viruses, bacteriophages are inert and can only replicate inside a cell, using the cell's energy and machinery. Infection begins when the bacteriophage base plate and tail fibers come into contact with its specific bacterial target (Figure 2). The

bacteriophage attaches to the bacteria, makes a hole in the bacterial cell wall, and injects its genetic material into the cell. The genetic material can then follow one of two fates. It may use the bacterial machinery to replicate its genome and make more of its proteins. In effect, the bacterium becomes a virus-producing factory. Eventually, the bacteriophage directs the production of two proteins that cause the cell wall of the bacterium to rupture, killing the bacterium and releasing the newly synthesized viral particles into the environment to infect other cells. This process is called the *lytic* cycle. Other times, once inside the cell the bacteriophage's genetic material pursues another path called the *lysogenic* cycle. Here, the viral genome integrates into the bacterial genome. Each time the bacterium replicates, its progeny contains a copy of the viral genome. In time, perhaps when the bacteriophage detects that nutrients are depleted, it resumes a lytic life cycle and exits the bacteria.

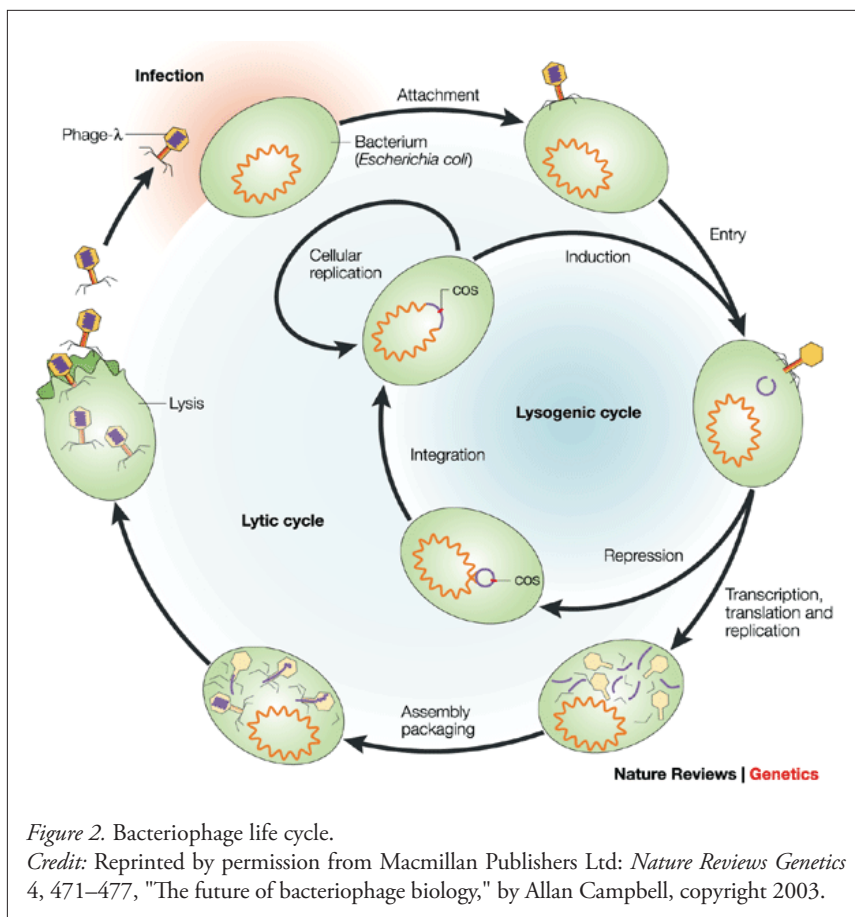


Figure 2. Bacteriophage life cycle.

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Each bacteriophage binds to, infects, and kills only specific target bacteria by virtue of the specificity conveyed by its proteins. Bacteria that suffer mutations that change the proteins displayed on their cell surface will escape infection. Through natural selection, these bacteria will thrive and their genome will become the most prevalent one in the bacterial population. However, these bacteria constitute fertile grounds, and natural selection predicts that mutations in the bacteriophage proteins that allow the virus to recognize and infect these bacteria will develop over time. Through this process of co-evolution, bacteriophage can adapt to bacterial defenses.

Although not completely understood by the Europeans at the time, this was the basis of the treatment poured into the wells of Dinesh's village.

Questions

1. Would the villagers need to drink repeatedly or only once from the treated wells to obtain a sufficient dose to serve as a cure? What is the basis of your answer?
2. Once a person ingests a dose of phages, how long will the treatment remain effective in his or her body? In other words, what is the time course of the treatment? Would you expect the effectiveness of this treatment to increase or decrease over time? Why?
3. Will the process of viral and bacterial co-evolution continue indefinitely?
4. Should the villagers be concerned that this bacterial virus will be harmful to their microbial flora (such as their intestinal bacteria)? Why or why not?
5. What might be a negative health consequence of killing many cholera bacteria with bacteriophage in a short time? Hint: Think about what causes the symptoms of cholera (see Part I, Question 1).
6. The villagers should not be concerned that the bacteriophage will lyse and infect their cells. Bacteriophages are not believed to be able to infect human cells. Propose why this might be. Hint: Consider the structural differences between human and bacterial cells.
7. What would be the effect on treatment if the bacteriophage that was poured into the wells adopted a lysogenic life cycle?
8. What will happen to the bacteriophages once the supply of cholera bacteria in the intestine of an infected person has run out?
9. What do you think are some of the risks of "phage therapy" (using bacteriophage to treat bacterial infections)?
10. Imagine the following scenario. A bacteriophage taken for the treatment of cholera infects a cholera-causing bacterium and temporarily enters the lysogenic stage. When the virus re-enters the lytic cycle, it includes some pieces of the bacterial DNA in its own genome. Specifically, this DNA encodes information for making the cholera toxin. Imagine some of the possible consequences of this scenario. What will happen to the next bacteria that the virus infects? What will happen to the human in which this bacteriophage exists?
11. From this information, would you subject yourself to bacteriophage therapy for the treatment of cholera? How safe and effective is this form of treatment?

Part III – Why Not Use Antibiotics?

The potentials of phage therapy as a treatment for bacterial diseases were overshadowed by the discovery of antibiotics in the early 20th century. Antibiotics are chemicals that can prevent the growth of bacteria or even kill them. Typically, a range of bacterial species are susceptible to the effects of an antibiotic.

Unfortunately, bacteria are evolving in response to the pressures imposed by antibiotics. When an antibiotic is applied to a population of bacteria, some bacteria have inherent mutations that make them less susceptible to the antibiotic. These bacteria survive, multiply, and eventually make up the majority of bacteria in the population. Thus, we are now facing a situation where bacteria are evolving resistance to our antibiotics faster than we can find new antibiotics to challenge them (Levy & Marshall, 2004).

Figure 3 shows the result of an experiment where the growth of a bacterial population was monitored over time. *Staphylococcus aureus* bacteria were grown with all the required nutrients. These common bacteria are the culprit of a range of infections from pimples to pneumonia. Growth was monitored by measuring the turbidity of the culture (the more turbid a culture, the more cells are present). At 1 hour, the bacteria were divided into 7 different flasks. The bacteria in each of these flasks were subjected to different treatment (see below), and the bacteria were incubated and their growth monitored.

- The open circles show the growth of bacteria when nothing was added to the flask.
- The open rhomboids, triangles, and squares show the growth of the culture when rifampicin, vancomycin, and oxacillin, respectively, were added to the culture at time = 1 hour. All three of these chemicals are antibiotics.
- The closed circle, triangle, and square show the growth of the culture after the addition of bacteriophages that target *Staphylococcus aureus*. Different amounts of phage were added in the three flasks: 0.1, 1, and 10 MOI, respectively. An MOI is a Multiplicity of Infection and corresponds to the ratio of infectious agent to bacteria.

Questions

1. Based on the results of this figure, comment on the comparative effectiveness of antibiotics and phage on the growth of *Staphylococcus*.
2. This experiment does not capture some of the complexities of administering treatments in a human body. What are some of the factors that would affect the effectiveness of these treatments in a human body that are not measured in these flasks? How might these factors affect treatment?
3. What are some advantages of antibiotics over phages in the treatment of infections in humans?
4. What are some advantages of phages over antibiotics in the treatment of infections in humans?

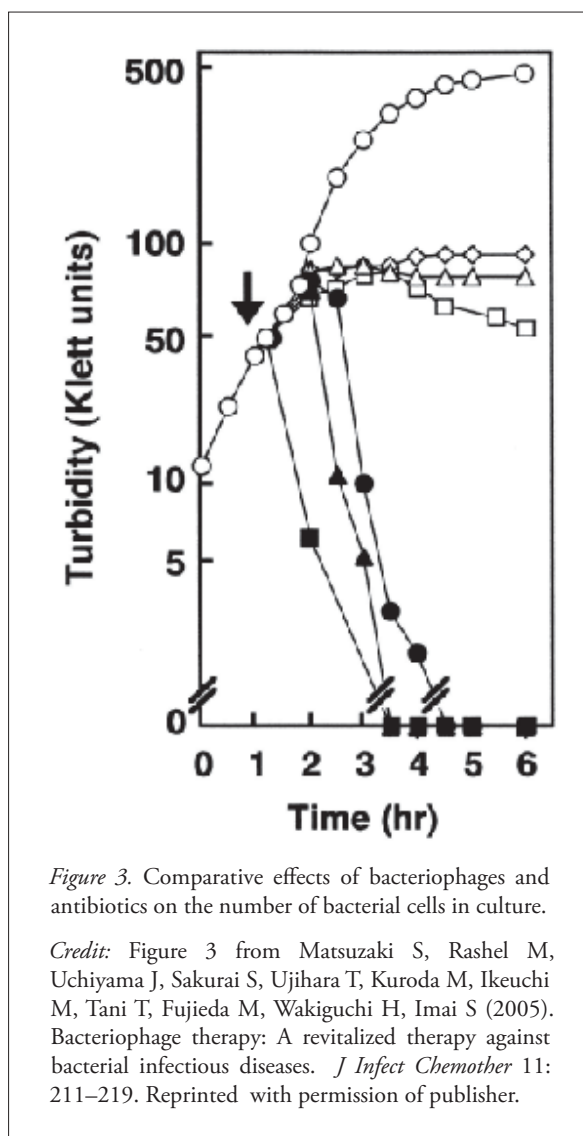


Figure 3. Comparative effects of bacteriophages and antibiotics on the number of bacterial cells in culture.

Credit: Figure 3 from Matsuzaki S, Rashel M, Uchiyama J, Sakurai S, Ujihara T, Kuroda M, Ikeuchi M, Tani T, Fujieda M, Wakiguchi H, Imai S (2005). Bacteriophage therapy: A revitalized therapy against bacterial infectious diseases. *J Infect Chemother* 11: 211–219. Reprinted with permission of publisher.

Part IV – Why Not Use Phage Therapy?

Phage therapy is not an approved treatment for human use in North America. It has yet to pass a rigorous clinical trial to show its effectiveness. Some pharmaceutical companies are showing interest in developing it, but phage therapy offers a few challenges. First, since phages only kill specific bacteria, phage therapy is an extremely individualized treatment. Bacteria must be isolated from the patient and an appropriate virus found. This runs contrary to the current pharmaceutical company model, which is based on the mass production and marketing of products that work for everyone. In addition, given the individualized nature of the cure, it is very difficult to patent each virus used. The patent process ensures that the companies that develop these treatments can recuperate their costs. Finally, such individualized treatment would require a fundamental shift in our medical practices, which is geared towards a one-disease, one-treatment model. Phage therapy requires personalized treatment and customized medicines. It is very laborious, time intensive, and costly. In addition, the time taken to select an appropriate virus may not be possible with certain bacterial diseases that progress rapidly. However, phages are able to access areas of the body that antibiotics cannot, and viruses are produced in those areas where they are most needed (the maximal viral dose is in the area of greatest bacterial infection), ensuring continued interest in this treatment.

Despite the challenges, phage treatment of bacterial infections is routinely used in other applications. Meats are sometimes treated with solutions of phages to kill off potentially harmful bacteria such as *Salmonella* (Hausler, 2006). Certain high-value crops, such as tomatoes, are now sprayed with phage solutions (Obradovic et al., 2004). There is a very good chance that you have already eaten phages.

Questions

1. Given this new information, review the advantages and disadvantages of each form of treatment. Create a table that summarizes your answer.
2. Are there some diseases that lend themselves more to phage therapy than antibiotics? Are there types of infections for which the reverse is true? Explain your reasoning.
3. Do you think restrictions on the use of phage therapy in North America should be loosened? Why or why not?

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Suggested Readings

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