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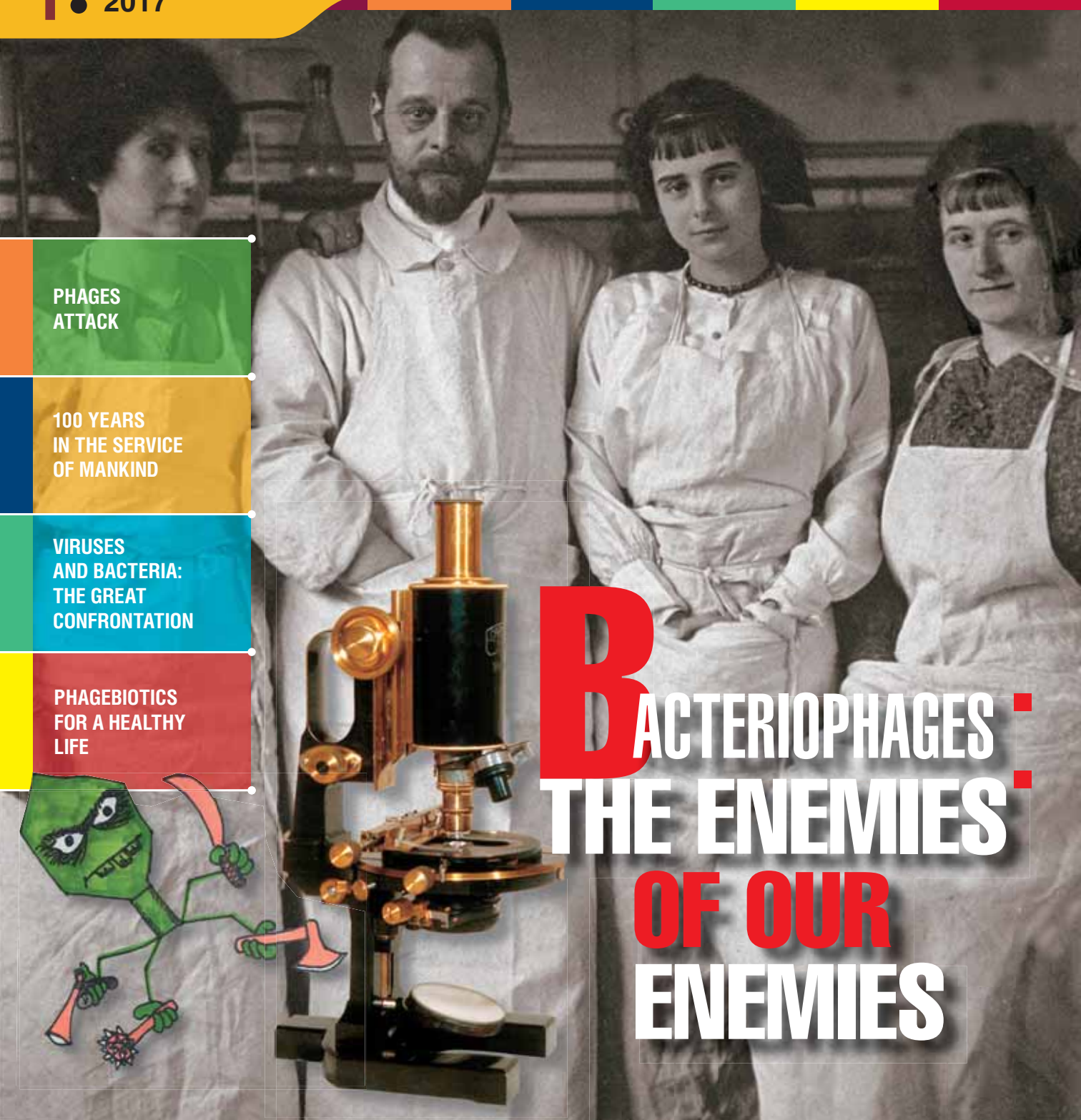

PHAGES ATTACK

100 YEARS IN THE SERVICE OF MANKIND

VIRUSES AND BACTERIA: THE GREAT CONFRONTATION

PHAGEBIOTICS FOR A HEALTHY LIFE

BACTERIOPHAGES: THE ENEMIES OF OUR ENEMIES

1. 2017
popular science journal

SCIENCE First Hand

IN THIS ISSUE:

Bacteriophages "won" the Battle of Stalingrad: they did not let a cholera epidemic spread outside the territory occupied by German troops

If all bacteriophages on Earth line up in a single file, it will stretch as far as the nearest galaxy cluster in the constellation Virgo!

Biologists learned to edit genomes by spotting how bacteria protect themselves from reinfection with bacteriophages

Thanks to filamentous bacteriophages, we will soon have energy-conserving ultrathin displays

Using bacteriophages instead of antibiotics will ensure that we eat organic food

A Journal
for Inquisitive People

The drawings on the cover and inside the journal are by Zhenya Vlassov

“The natural desire of good men is knowledge”

Leonardo da Vinci

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Dear Friends,

This issue of our magazine commemorates the centennial of the discovery of bacteriophages. Bacteriophages are minuscule viruses, but their role in science can hardly be overestimated.

Viruses are known to be the smallest living organisms on Earth; there is even an opinion that calling them “alive” may be wrong as they have too simple a structure. In fact, a virus is a genetic program encoded in DNA or RNA strands packed in a protein shell. Viral genes can work only inside a living cell of another organism by embedding themselves into the host genome and forcing the cellular metabolic machine to produce new copies of viral particles. Like other viruses, bacteriophages (literally, «eaters of bacteria») are intracellular parasites, but they use bacteria and archaea, rather than animals and plants, as their hosts.

In recent years, we have learned that bacteriophages play a huge role in the biosphere: controlling the amount of microbial flora, they are a major factor hindering its unrestrained growth. No wonder that the quantity of bacteriophages is enormous: their total biomass may be as high as 109 tons whereas the total mass of all living organisms is only an estimated 2–3 orders of magnitude higher. Bacteriophages are not just the most common form of life on Earth: being an integral part of trophic cycles, they actively participate in the global cycle of matter and energy.

Since bacteriophages can target specific strains of bacteria, mankind has used them, albeit with varying success, as a very accurate and safe weapon against bacterial infections in humans and animals almost since the time they were discovered in the early twentieth century. The very history of this discovery reads like a fascinating novel, the main characters being two dramatic and tragic personalities—a brilliant self-educated Frenchman Félix d’Hérelle and his closest associate and friend, a Georgian microbiologist George Eliava.

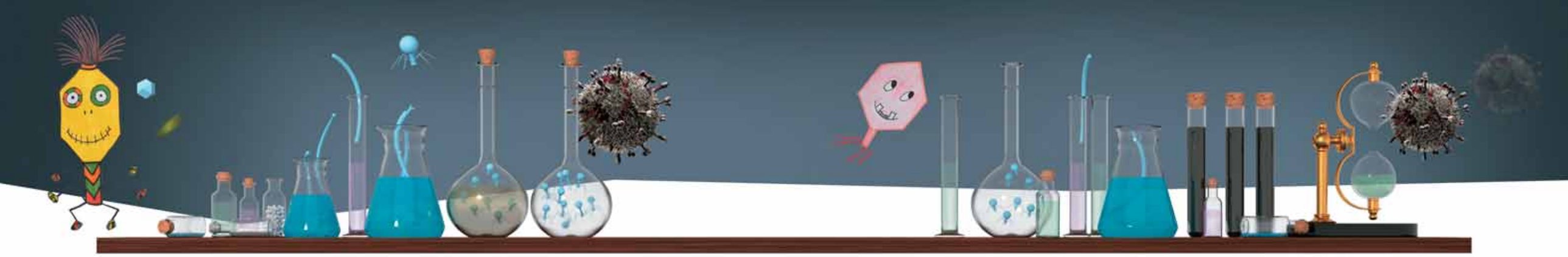
The time when they lived and worked was an era of wars and revolutions, which shattered the very foundations of society, but these two men were not of the kind to hide from reality in the ivory tower. Félix d’Hérelle, the discoverer of bacteriophages, who spent, at the age of seventy, several years under house arrest for refusing to help the German invaders, had to witness at the end of his life how his beloved child was losing ground to the increasing spread of antibiotics. George Eliava, who declined an invitation of the famous Pasteur Institute in Paris with the words “I’m needed in Georgia” and founded the world’s first and only bacteriophage research center in Tbilisi, was shot in his home country as an “enemy of the people.”

However, the ingenious idea to fight bacteria with a living biological weapon has outlived its creators. For many years, the USSR had been the world center of bacteriophage research, and phage therapy passed its first major test during World War II. Suffice it to say that the cholera bacteriophage was one the fighters in the famous Battle of Stalingrad. Produced directly in the besieged city of Stalingrad, this virus helped localize a cholera epidemic that broke out on the territory occupied by German troops.

The subsequent history of phage therapy is an excellent illustration of the philosophical thesis that any development follows a spiral path. In the 1980s, it became clear that the effectiveness of antibiotic treatment decreased dramatically due to the increasing drug resistance to antibiotics. Scientists and physicians around the world turned their eyes, once again, to bacteriophages. After all, the unique advantage of phage drugs over antibiotics is their targeted action against a specific bacterial strain or species without damaging the normal microflora of the organism, which is why they can be used safely for prevention, as well as treatment, of infections.

Of course, no one today argues categorically that, at the present stage in the development of science, bacteriophages can completely replace other antibacterial preparations in medicine and agriculture, but all the articles in this issue clearly show that bacteriophages can be for mankind not only a neighbor with whom we share one biosphere but also a strong and loyal ally.

Academician Nikolay L. Dobretsov,
Editor-in-Chief



TRANSPLANTING healthy fecal microflora with a set of **“GOOD” BACTERIA** and **“PROPER” BACTERIOPHAGES** into the intestine of a patient ensures an almost instantaneous treatment of **DYSBACTERIOSIS**. **P. 58**

Lythic **PHAGES** target **PATHOGENIC** bacteria, keeping safe the normal **MICROFLORA. P. 66**

Using **BACTERIOPHAGES** to combat **PLANT DISEASES** at greenhouse farms will allow farmers to grow large **CROPS** of **ORGANIC** vegetables and greens. **P. 74**

Phage drugs may be a good **ALTERNATIVE** to **ANTIBIOTICS** in industrial **ANIMAL HUSBANDRY**, where there is a limited range of possible pathogens. **P. 82**

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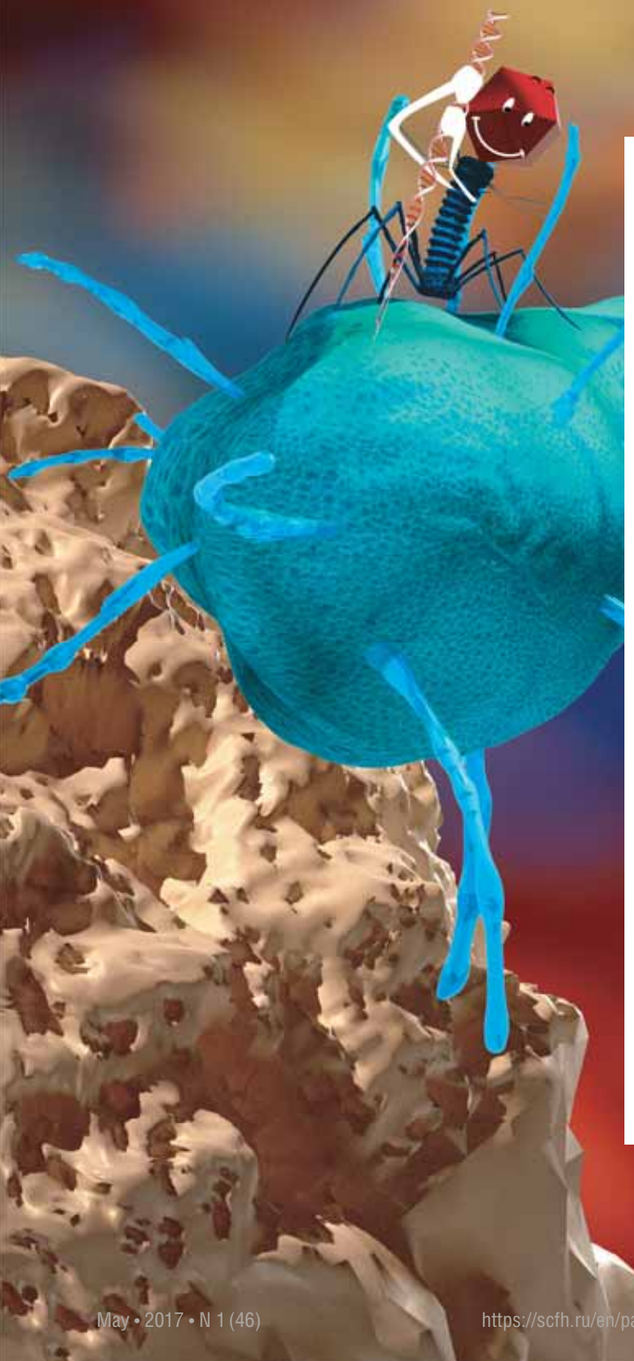
LIFE SCIENCES

Bacteriophages – the enemies of our enemies



by V.V. Vlassov
science editor of the issue,
vice editor-in-chief
SCIENCE First Hand

What is a bacteriophage? Most will say this is a drug doctors told them about. Some will reply that bacteriophages (or just phages) are tiny viruses invading bacteria, and biologists will tell a good many amazing stories about their discovery, the important role they have played in the development of science and the promise held by up-to-date technologies developed on the basis of these tiny viruses.

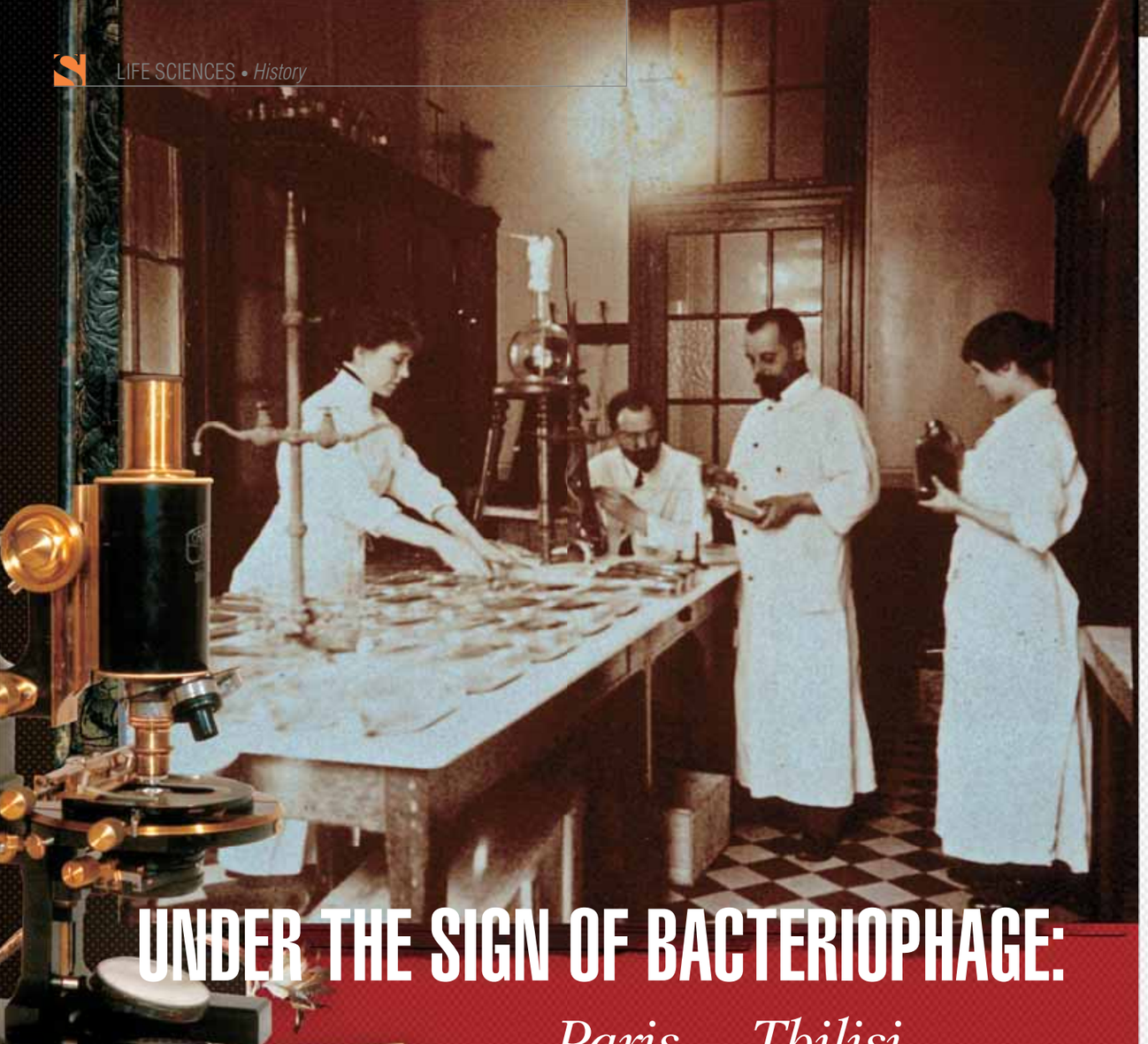
When these bacteria killers were discovered a hundred years ago, they immediately took center stage: enthusiasts believed that the miraculous viruses would completely rid mankind of infectious diseases. The new method – *phage therapy* – became widely known after the publication, in 1925, of *Arrowsmith*, a famous novel by Sinclair Lewis, which told an idealized story of a young microbiologist who had discovered a wonder drug. The book earned the author the Nobel Prize for Literature but in real life no miracle happened: the lack of knowledge on virus biology and absence of key molecular technologies in those distant years prevented the scientists from tapping the full therapeutic potential of bacteriophages and so interest in them, especially in the West, was lost.

In the 1950s, bacteriophages again came to the focus of attention but this time as the most elemental biological organisms having their own genomes. Using them as "laboratory guinea pigs," the scientists presumed they would uncover the basic mechanisms underlying the functioning of living systems. These expectations were met in full: the study of phage genes and proteins gave answers to high-priority problems concerning the basis of life. The "by-product" of this research were basic technologies that gave birth to a new promising area – gene engineering.

In the recent years there has been an upsurge of interest in bacteriophages. A new knowledge of their expansion and diversity has clearly demonstrated the important role these tiny organisms play in the biosphere. Bacteriophages came to be called the "dark matter" of the biosphere; according to some estimates, their number on the earth is about 10^{31} virions and their total mass is 10^9 tons. The human gastrointestinal tract contains about 10^{12} bacteriophages, i. e. almost 0.5 mg.

Today, bacteriophages are an indispensible tool for molecular biologists allowing them to operate with DNA when dealing with the problems of synthetic biology. The study of the defense and attack structures used by phages and bacteria in their eternal war has lead to the discovery of the enzymes and nucleic-protein complexes that have made it possible to create genome editing techniques, which made a revolution in biological research and opened up principally new opportunities for the medicine of tomorrow. Bacteriophage enzymes are used as biologically active substances, and phages serve as a basis for the creation of nanometer objects with a given structure having a wide range of applications, from drug delivery and developing biosensors for diagnostic systems to the production of materials used in nanoelectronics.

And yet the key application area, both at present and in the future, derives from the phages' main property – their ability to destroy bacteria. Bacteriophages are used as disinfectants in the various sectors of agriculture, veterinary medicine and food industry – everywhere where bacteria growth should be prevented and living and organic objects should be protected against them. Due to the abundance of antibiotic-resistant microorganisms, all developed countries have speeded up research aimed at developing new medical technologies for treating and preventing infections, which should make phage therapy a vital ingredient of the personalized medicine of the 21st century.



UNDER THE SIGN OF BACTERIOPHAGE:

Paris – Tbilisi

Many pages in the history of science read like a fascinating novel, and this is exactly the case with the discovery of bacteriophages, or viruses that infect bacteria. The two most significant figures in the history of this discovery—a brilliant self-educated Frenchman, known as Félix d'Hérelle, and a Soviet microbiologist George Eliava, who was part of a well-known Georgian family—were shrouded in mystery and legends even during their lifetime, and some dramatic and tragic episodes in their lives would forever remain behind the scenes, in contrast to their recognized scientific achievements


ВЕЧЕРНИЙ ТИФЛИС

ЦЕНА 10 КОП.

НАУКА НА СТРАЖЕ ЗДОРОВЬЯ

В ПОСЛЕДНИЙ ЧАС

БУРНЫЕ ПРЕНИЯ В КОРТЕСАХ

НАПАДЕНИЕ МАНЬЧЖУРСКИХ ПОВСТАНЦЕВ НА Ж.-Д. СТАНЦИЮ

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СТАРШИЙ МАЙОР ГОСУДАР. БЕЗОПАСНОСТИ:
(ЛИТВИН)

By and large, this story began as early as in the 17th century, when Antonie van Leeuwenhoek, a successful cloth merchant from the Dutch city of Delft, saw in the eyepiece of his self-made microscope the swift-moving tiny "animalcules," little creatures without a head or tail, which looked like no other known animal. That was when the mankind first learned about their powerful, though invisible to the naked eye, neighbors on this planet—the vast world of bacteria, which lived virtually everywhere—in the soil, pond mud, rotten meat, dental plaque...

Surprisingly, people long thought that these microscopic creatures were harmless, i. e., had nothing to do with human diseases. Almost until the end of the 18th century, medicine was dominated by the miasma theory of spontaneous generation of disease, according to which all diseases had only an internal cause. The idea of a live causative agent triumphed in the first half of the 19th century, when experiments with self-infection and simulation of diseases in animals proved the pathogenic role of microorganisms. An important contribution had come from Russian scientists, such as the military doctor Danilo Samoilovich, who proved during a plague epidemics in Moscow in the early 1770s that the infection was transmitted through direct contact with a patient or their belongings. Although Samoilovich could not actually "see" the plague agent, he was the first to propose the idea of preventive vaccination by using an attenuated contagious matter.

As is known, vaccination was officially proved effective for the prevention of smallpox, a dangerous human infection, by an English physician Edward Jenner in 1796. However, it was only a hundred years later that scientists discovered a new type of animalcules causing this and many other diseases; they were so small that one could see them neither through Leeuwenhoek's «magnifying glass» nor through much more powerful optical microscopes. At the end of the 19th century, the Russian plant physiologist Dmitry Ivanovsky, Dutch botanist and microbiologist Martinus Beijerinck and German microbiologists Friedrich Loeffler and Paul Frosch discovered tiny organisms that could easily pass through the pores of porcelain filters, which retained the smallest bacteria. Beijerinck called these organisms viruses (from the Latin word virus meaning 'poison'); this word was also used by the founder of microbiology and immunology Louis Pasteur to designate a contagious matter.

By the way, it took some time for the scientific community to accept the very idea of existence of fundamentally new organisms in the microcosm. In the early 20th century, there were scientists who speculated that viruses were either very small bacteria or toxic substances released inside doomed cells due to unknown factors. An end to this dispute was put by the discovery that those tiny creatures could infect not only plants and animals but also other microorganisms.

The unknown matter with an antibacterial effect was first mentioned in 1896 by an English bacteriologist Ernest Hankin in his study of how the water of individual Indian rivers affected the causative agent of cholera. The water retained its healing properties after passing through a bacterial filter, but lost them after boiling. The scientist suggested that that phenomenon, which was later called Hankin's paradox, held back the spread of cholera among the local population, but gave no explanation. Two years later, the Russian microbiologist Nikolay Gamaleya

Félix d'Hérelle in his laboratory at the Pasteur Institute (Paris) and his main instrument, a Carl Zeiss microscope. *1919. © Institut Pasteur – Musée Pasteur*

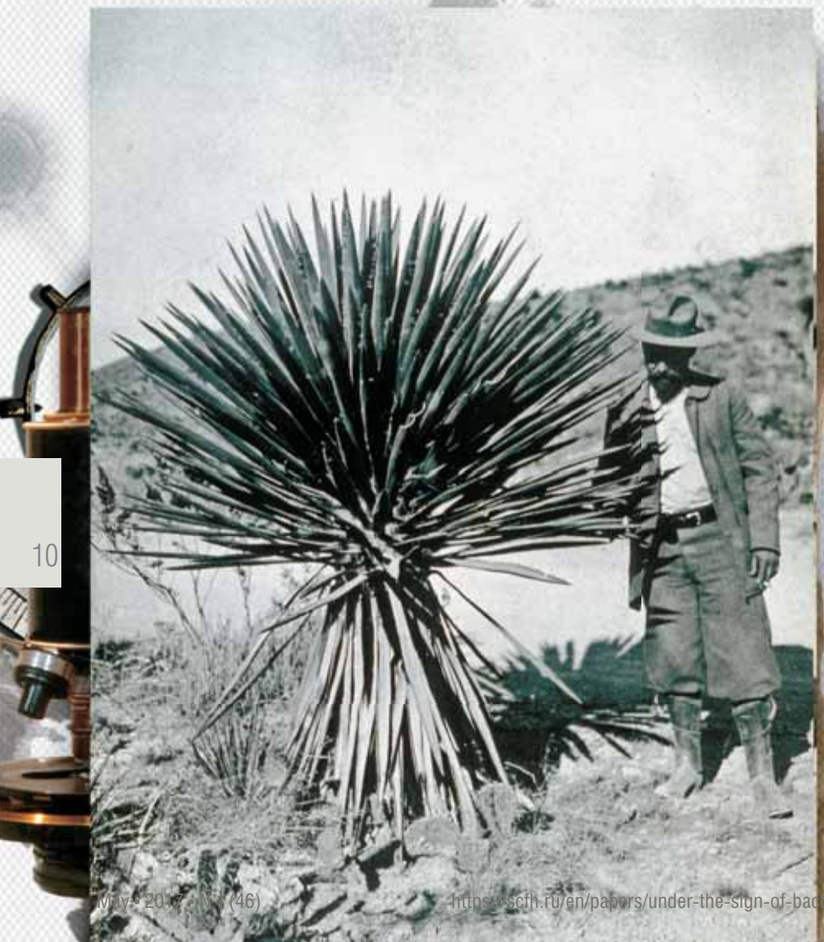
Bottom left: an article in the newspaper *Vecherni Tbilisi* (April 1935) about the release of d'Hérelle's book *Bacteriophage and the Phenomenon of Recovery*, which was translated into Russian by his friend and colleague George Eliava, director of the Georgian Research Institute of Bacteriophages

described the dissolution (lysis) of anthrax bacilli in distilled water under the influence of an unknown agent and the ability of the resulting solution to destroy fresh cultures of the pathogen.

However, the mechanism underlying this phenomenon was scrutinized only decades later by the English microbiologist Frederik Twort (with A. Lond) and Canadian–French scientist Félix d'Hérelle. These scientists described, independently of each other, filter transmissible agents that cause the destruction of bacterial cells. Following the writings by d'Hérelle, they were called bacteriophages (literally, the eaters of bacteria).

Agave aperitif

An amazing fact: the man who is deservedly considered one of the discoverers of bacteriophages and who was nominated eight (!) times for the Nobel Prize had no university degree in biology; what is more—his only education was secondary school! With a striking appearance of a Spanish hidalgo, this brilliant self-educated man had a heart of a true adventurer. This image derives from the descriptions of d'Hérelle's life, especially its early period, of which there is contradictory information in the currently available literature; therefore, we can only roughly outline the path of his life.



Félix d'Hérelle's scientific career began on sisal plantations in Mexico, where he not only used, for the first time in the world, pathogenic bacteria against locusts but also observed, also for the first time, the action of bacteriophages. *Mexico, 1941. © Institut Pasteur – Musée Pasteur*

Thus, some authors claim that Félix was born in Montreal in a family of French immigrants and moved to Paris at an age of six, when his father died. However, the latest version says that d'Hérelle, whose real name was Hubert Augustin Félix Haerens, was born on April 25, 1873 in Paris by an unknown father and 24-year-old Augustine Haerens, a rentier, as indicated in his birth certificate. It is true that he only had secondary education; he went to two secondary schools in Paris, including the Lycée Louis-le-Grand, where he did not show much diligence. Later, he attended lectures on medicine in Europe for a few months, presumably, at the University of Bonn. There is evidence that at an of age 20, he and his younger brother Daniel volunteered to the French army, from which he deserted a year later for unknown reasons.

Félix always had a passion for traveling: as a schoolboy, he rode by bicycle across Western Europe and then traveled to South America, Greece, Belgium... In Turkey, he met his future wife, Marie.

At the age of 24, Félix, who was then a husband and father, emigrated to Canada, where he changed his nationality and adopted new name d'Hérelle (when using English typewriters, he often wrote it as Herelle), probably, for fear of the consequences of his desertion from the army. He was lucky at first: a friend helped d'Hérelle obtain an order of the Canadian government to study the fermentation and distillation of maple syrup in the production of schnapps. In 1899, he even took part in a geological expedition in search of gold on the Labrador Peninsula in eastern Canada as a paramedic, although he had almost no special training. He and his brother invested the money earned in a chocolate factory, which almost immediately went bankrupt.

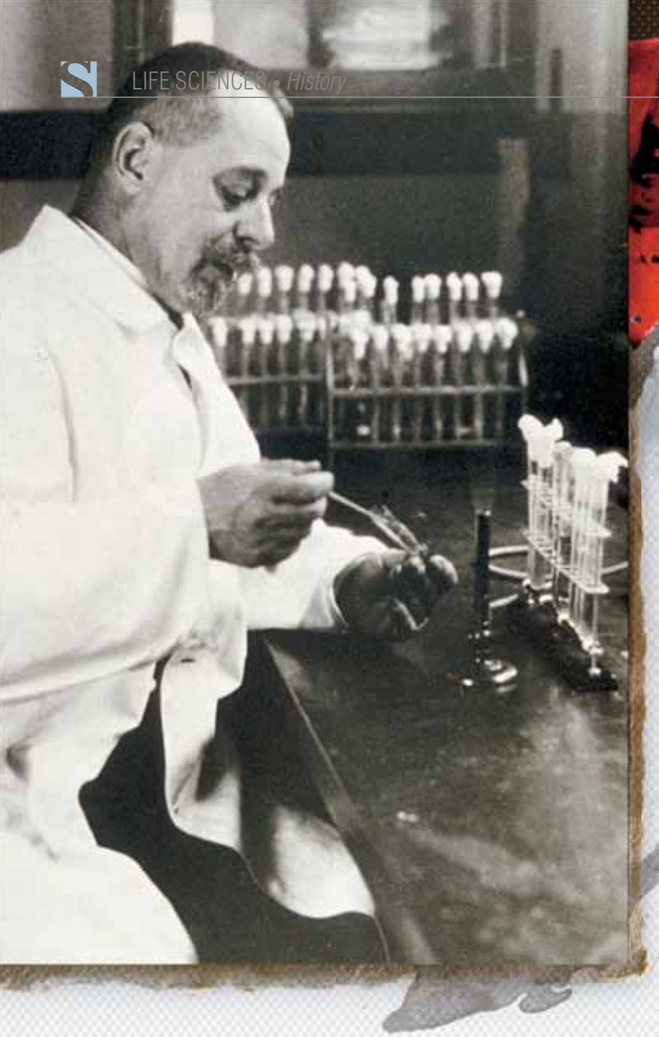
By that time, Félix was already a father of two daughters, and to provide for his family, he went... to the New World, where he began working under a contract with the Government of Guatemala as a bacteriologist in a metropolitan general hospital, where he treated malaria and yellow fever. Obviously, all this time d'Hérelle, who was far from medicine yet keen on microbiology, continued his self-education. At the same time, using his experience in alcohol production, d'Hérelle set himself to develop a process for producing whiskey from bananas. The life in this South American country, which by the end of the 19th century had gone through several civil wars and had become a symbol of chronic instability and civil strife, was far from civilized and simply safe, but it clearly appealed to Félix with his adventurous streak. He said that it was in Guatemala that he set off on his journey to the big science.

D'Hérelle's alcohol career was on the rise: in 1907, at an age of 30, he took an offer of the Mexican government to design a technology to produce a strong alcoholic drink from agaves. This plant of the lily family is known to be a source not only of the rough sisal fiber but also of the famous tequila. D'Hérelle and his family moved to a sisal plantation in Yucatan, where he soon developed a new way to produce schnapps from agaves. The equipment for the mass production of the alcoholic drink was ordered in Paris, and the inventor himself went to the French capital.

This trip marked the beginning of a new era in the life of Félix

Félix d'Hérelle (Hubert Augustin Félix Haerens), an outstanding microbiologist repeatedly nominated for the Nobel Prize, was a self-taught man without higher education. *1905. © Institut Pasteur – Musée Pasteur*

d'Hérelle. Paris was home to the famous Pasteur Institute, where he began spending all of his spare time, working as a voluntary assistant. When d'Hérelle returned to Mexico, he lost interest in his work at the new factory because it became "too boring." Instead, he got fascinated by another, microbiological problem associated with the invasion of migratory locusts, who destroyed sisal plantations on the Yucatan Peninsula. He noticed that vast masses of the insect were dying from an unknown disease accompanied by severe intestinal lesions. He isolated from their dead bodies the Coccobacillus bacterium and proposed to use it as a means against this "plague of Egypt."



Félix d'Hérelle in a laboratory designing vaccine drugs, Pasteur Institute (Paris). *© Institut Pasteur – Musée Pasteur*

This work had a fundamental effect on d'Hérelle's life; in the meantime, we can only say that it was he who first proposed the idea of biological pest control. Although his later attempts to apply this method to combat locust infestation in Guatemala, Argentina and Tunisia were not fully successful, the name of Félix d'Hérelle became known in scientific circles. Until then, as they say in the French Wikipedia, his scientific career resembled that of a charlatan.

Thanks to the locust

In 1911, the d'Hérelle family returned to Paris, and Félix began working at the Pasteur Institute on a method for preparing a vaccine in a model system consisting of Salmonella typhimurium and the house mouse, its natural host. In his spare time, he was examining dysentery patients in a cavalry squadron quartered near Paris.

The 44-year-old d'Hérelle officially announced his discovery, which forever secured him a place on the scientific Olympus, in 1917. Doctor Roux, the director of the Pasteur Institute, presented to the Academy of Sciences d'Hérelle's report on "an invisible antagonistic microbe of the dysentery bacillus." In the text of his report, d'Hérelle called this organism a bacteriophage. According to his observations of bacillary dysentery patients, shortly before the disappearance of blood stool and their recovery, an agent capable of dissolving dysentery bacilli appeared in their intestines. This agent was not found in patients who died of dysentery. This agent, called a bacteriophage, had the ability to replicate itself through bacteria. The bacteriophage phenomenon could be reproduced in experimental conditions as clearly as in organisms. Subsequent experiments showed that the bacteriophage behaved as a creature endowed with life, like a tiniest microorganism parasitizing on bacteria. The bacteriophage was found to have a corpuscular structure and affect bacteria through its enzyme.

Amazingly, d'Hérelle drew all these conclusions from empirical observations, intuition and common sense. It was only 22 years later that Ernst Ruska, the inventor of a transmission electron microscope, could actually see bacteriophages.

However, was d'Hérelle really the first? After all, in 1915 the English scientist Twort described an agent that caused staphylococcus colonies growing on the surface of a culture medium to become transparent. The agent passed through a bacteria-retaining filter, which made it akin to the already known viruses.

Accused of plagiarism, over the years d'Hérelle was publishing detailed accounts of the history of his discovery, which he actually made back in 1910. He was then in Mexico, in the state of Yucatan, when a locust invasion began. Fortunately, there was an epidemic among the locusts. D'Hérelle went to corn fields and collected diseased insects with severe symptoms of deadly diarrhea. He inoculated the faeces of diseased and dead insects to find coccobacilli, the microorganisms that caused the deadly infection of the locusts. After examining the petri dishes with the seeds, he found anomalies in the growth of the microbial culture, i.e., round-shaped transparent areas two or three millimeters in diameter, on a nutrient agar surface. He scraped these transparent plaques from the agar surface and prepared smears. However, he saw nothing under the microscope. Based on that and other experiments, he came to the conclusion that the agent causing the formation of the transparent areas on the microbial culture must have been so small that it could pass freely through bacteria-retaining filters.

In fact, it is not surprising that d'Hérelle, who was an obscure scientist at that time, did not rush to publish his observations until he got another proof of his discovery, i.e., the dysentery bacteriophage. In any case, unlike Twort, he not only gave in his first publication an accurate description of the bacteriophage phenomenon but also predicted the possibility of creating a "live drug." This thought might have also come from his revolutionary idea to use living microorganisms for pest control.

Always fast and resolute in dealing with practical challenges, this man soon showed again that he "practiced what he preached." Only two years

Félix d'Hérelle presented the results of his initial research on bacteriophages in his fundamental work *The Bacteriophage* (1922), which described in detail the lysis of the host bacterium, the isolation of phages from infectious bacteria, and the factors that regulate the stability of extracellular phage. A year before, d'Hérelle's followers Richard Bruynoghe and Joseph Maisin officially reported successful treatment of staphylococcal skin infections with a staphylococcal phage. As public interest in phage therapy was growing, many private European companies began to mass produce commercial bacteriophage preparations. One of the first such manufacturers was the French Harmless Hair Dye Company, founded in 1909, which is now known all over the world as L'Oreal

Félix d'Hérelle with his wife, Marie Claire (*on the left of the scientist*), and his younger daughter Huberta and elder Marcella (*on the right*). *Paris, 1919. © Institut Pasteur – Musée Pasteur*

Surprisingly, despite all the evidence that phages are living beings, the review of 150 most significant works on phage therapy published by the Board of Pharmacy and Chemistry of the American Medical Association in the early 1930s openly stated that experimental studies of the lytic agent called bacteriophage did not disclose its nature. D'Hérelle's theory that this material is a live virus parasitizing in bacteria had not been proved. On the contrary, evidence suggested a non-live material, presumably an enzyme (Eaton and Bayne-Jones, 1931). Such an evaluation could not but have an adverse impact on investment in bacteriophage research and production, at least in the United States

later, d'Hérelle together with Prof. Victor Henri Hutinel conducted in a children's hospital (Hôpital des Enfants-Malades) in Paris the first experiment to treat dysentery with bacteriophages. To ensure the safety of the new drug, d'Hérelle and his staff had also taken a substantial dose, as was a common practice at that time. This clinical trial was preceded by successful experiments on hens with typhus, in which a therapy with phages isolated from hen droppings helped reduce the mortality rate from 95 to 5%!

On the top

The very first paper published by d'Hérelle created a great stir in the scientific community, generating a wave of studies by ever more scientists who were proving him right. Phage therapy began to gain a foothold in the medicine of Western Europe, and d'Hérelle himself strengthened his position at the Pasteur Institute, where he had worked for a few years as an unpaid assistant. However, even then the life of this adventurous man with a hot-tempered and restless disposition often carried him far away from the silence of an academic laboratory: he spent his days in research expeditions organized by the Pasteur Institute in Argentina, Algeria, Turkey, Tunisia and Mexico. Moreover, he made many of his subsequent scientific travels at his own expense.

D'Hérelle resigned from the Pasteur Institute in 1925 for reasons that are still unclear (presumably, due to differences of opinion with the institute's leadership). In the Netherlands, where he held a temporary position of curator at the Institute of Tropical Pathology, he published his first book and received an honorary doctorate from the University of Leiden. In Egypt, he was fighting infectious diseases as a director of the bacteriological laboratory at the quarantine station in Alexandria and as a League of Nations Health Service inspector. In India, he used phage therapy to treat cholera... D'Hérelle treated cholera patients in a simple medical tent in the slums rather than at the hospital organized by European standards. He did so on principle because he believed that bacterial infections should be studied where they arise rather than under sterile conditions. As a result, d'Hérelle and his team were able to achieve a nearly eightfold lower mortality from this serious bacterial disease.

In 1928, d'Hérelle made a triumphal science tour across the United States, where he delivered a series of lectures at Stanford University (his discussion on bacteriophages was published as a separate monograph) and then took a permanent position at Yale, one of the oldest and most famous research and educational institutions in the United States.

At that time in Paris there was a successfully operating private laboratory set up by d'Hérelle for the production of phages and managed by his son-in-law. In 1933, d'Hérelle came back to his Parisian laboratory. At that time he was already a venerable scholar distinguished by prestigious academic titles and awards, such as the Leeuwenhoek Medal, which is awarded only once every ten years. Previously, this award was also given to d'Hérelle's idol—the great Louis Pasteur, who, by the way, had no formal medical or biological education either. In those years, d'Hérelle was, as already mentioned, repeatedly nominated for the Nobel Prize, but he never reached the finish line.

Why would a successful Western scientist at his professional zenith turn his eyes "to the East"? In the 1930s, the French Communist Party was gaining momentum, and the attitude towards the Soviet Union was a litmus test for politicians and governments who came to power. However, apart from his pro-Soviet disposition and restless nature, one of the main motivations for d'Hérelle to go to the Soviet Union was his close relations with the Georgian microbiologist George Eliava.

"I am needed in Georgia!"

This period in the life of Félix d'Hérelle, which he kept quiet about until the end of his days, in contrast to the numerous reminiscences about his scientific expeditions around the world, was briefly yet accurately described by the American microbiologist Donna H. Duckworth: "...everywhere d'Hérelle was, there were fireworks <...> The fireworks materialized rather unfortunately in Russia, where d'Hérelle went several times during the 1930s to found institutes for the study of bacteriophages. During one of his trips his trusted associate, Eliava, was arrested and shot."

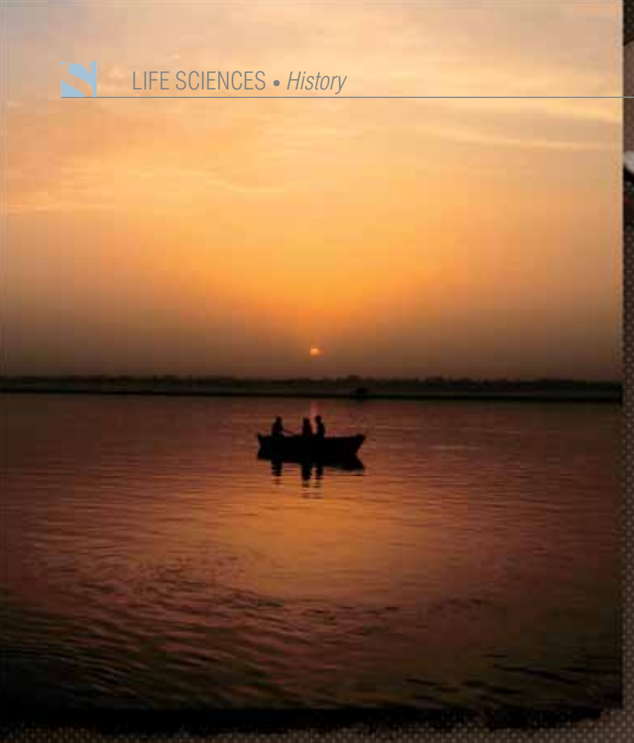
Photo taken during one of d'Hérelle's visits to the Georgian Institute of Bacteriophages, which was led by his long-time friend and colleague George G. Eliava. *Leftmost:* George G. Eliava; next to him: Mrs. d'Hérelle. *Batumi, Georgia, 1934.* *© Institut Pasteur – Musée Pasteur*

Not entirely accurate in facts, Duckworth was right in essence. Félix d'Hérelle was truly devastated by what happened to Eliava also because, being almost twenty years older than the Georgian scientists, he treated him like a son.

The Eliavas were a well-known Georgian family. Born in a wealthy family of a doctor, Gogi was a free-thinker from a young age. Expelled from Odessa University for his revolutionary activities, he entered the Faculty of Medicine at the University of Geneva, but had to finish his education in Moscow because of the First World War. Right after the university, he got to the Caucasus front in Trabzon as the head of a bacteriological laboratory. It was there, in 1917, that Eliava discovered, purely incidentally and independently, while working with bacterial seeds, the bactericidal action of the water in the Kura River. At once he realized the significance of this phenomenon. It was the same year when d'Hérelle went public with his discovery, and it was clear that this phenomenon could be explained by the action of the cholera bacteriophage.

They met in Paris at the Pasteur Institute, where Eliava traveled repeatedly since 1918 to work side by side with the discoverer of bacteriophages. However, when he was offered to stay permanently in Paris, the young scholar and patriot simply said: "I am needed in Georgia."

Supported by d'Hérelle, Eliava organized in Tiflis (from 1936, Tbilisi) the first Soviet laboratory for the study of bacteriophages, which was transformed in 1923 into the Institute of Bacteriophages. The scientists' dream to set up an international center for phage therapy in Georgia with its own production facilities and experimental clinics began to come true with the support from Sergo Ordzhonikidze, who was then a people's commissar



Holy Ganges River. *© Creative Commons*

THE VIRUS OF HOLINESS

Many religions ascribe miraculous properties to water, and the most affluent source of this holy fluid is undoubtedly India's Ganges River, one of the largest lowland rivers in South Asia. Even in the 19th century, Englishmen returning from India brought home some water from this great river, although they did it, first and foremost, for utilitarian reasons: Ganges water did not spoil, mysteriously, during the travel, staying "sweet and fresh" for weeks (Hollick, 2007). At that time, it really looked like a miracle.

The first man to divulge the secret of holy water from the Ganges River was an English scientist Edward Hankin, who published in *Annals of the Pasteur Institute* an article entitled "The bactericidal action of waters of Jumna and Ganges on the cholera microbe" (1896). He showed that autoclaved Ganges water had no effect on *Vibrio cholerae*, but filtered or unfiltered water contained something that could kill cholera. The antimicrobial properties of the Ganges water were confirmed by the fact that cholera did not spread downstream, although in the Hindu tradition, bodies of the dead (including from cholera) were submerged into the sacred river where the dead were to find their last resting place. Hankin examined water samples taken from the Ganges River inside and outside a city. He found that the quantity of *V. cholerae* downstream of the city was higher, but not much higher. The scientist also noted that half-burned corpses thrown into the river were preserved remarkably well: they would have decomposed much faster in the Thames.

The discovery of phages led to the question whether they were the cause of the special properties of the Ganges water. Indeed, by the 1980s the holy river had been found to contain viruses that devour *Klebsiella*, *Salmonella*, *E. coli*, *V. cholerae*, and the causative agent of bacillary dysentery. Moreover, it turned out that different parts of the river had different phage communities (Mukherjee *et al.*, 1984). However, no natural enemy of such a popular pathogen as *Staphylococcus aureus* was found in the Ganges.

Research showed that the Ganges water retains its special properties for much longer than it was thought by British sailors, whose voyages lasted a few months. The Ganges water demonstrated antimicrobial activity against an *E. coli* strain after years of storage: these microbes showed a worse survival rate in the Ganges water that had been stored for eight years than in boiled or filtered water; even after 16 years of storage, the Ganges water was "ahead" of boiled water (Nautiyal *et al.*, 2008).

Nevertheless, the Ganges water must not be taken as medicine: apart from bacteriophages, it contains bacteria, including pathogenic ones, and household and industrial waste. This, however, does not prevent believers from drinking this water and using it for ritual purposes. Internet stores offer to deliver holy water to any point around the world. They sell water both from the Ganges River and from other sacred rivers of India, such as Godavari, Yamuna, Narmada, etc. For example, a 50 ml bottle of holy water costs 1 USD.

Not only Hindus but also Christians make money by selling holy water. There are websites offering water from the Jordan River, or "holy water blessed by the Pope." However, if you want to order water from Zamzam, a holy well of Muslims in Mecca, it is not on sale: Saudi authorities have banned the export of this water. By the way, it was tested years ago by Hankin himself, who found Zamzam water to be powerless against cholera. Subsequently, it was shown that this water could help reduce heartburn, but in 2011 it became clear that this holy water contains a lot of arsenic, nitrates, and potentially dangerous bacteria. The sacred Jordan River, worshiped by Christians, cannot boast of cleanliness either; moreover, it also contains plenty of salts. However, believers all over the world do not worry about these trifles, and the flow of those wishing to connect with holiness does not dry out.

M.S. Kosheleva (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk)

The Eliava family—George, his wife Amelia and his step-daughter Hanna—near Les Invalides (Paris), the burial place of Napoleon Bonaparte, a man greatly admired by Eliava.
Photo from Natalia Devdariani's archive (Tbilisi, Georgia)

of heavy industry. For a few years, d'Hérelle was supplying equipment and library materials to the Institute, mostly at his own expense, and in 1933–1935 he came personally to Tiflis and worked at the institute for two semesters, also free of charge. A true master of all laboratory skills, including glass-blowing works, d'Hérelle was working with his usual fanaticism from morning till night, never showing any signs of fatigue.

The construction and equipment of the institute's new building was in full swing at that time; a two-story double house ("French cottage") for two families—d'Hérelle's and Eliava's—was built in the institute's park. Apparently, at first d'Hérelle had firm plans to move to Georgia and even dedicated to Stalin his new book *The Bacteriophage and the Phenomenon of Recovery*, which was translated into Russian by Eliava and published in 1935. However, by that time d'Hérelle must have grown disenchanted with the country of "universal justice and equality" and taken a grasp of its realities, including the brutal struggle for power within the USSR Communist Party. In any case, after his sudden departure in 1935, he never came back to the banks of the Kura River although he continued to support the institute by sending equipment. One can say he was lucky to escape the subsequent repressions inflicted on foreign specialists who were accused of espionage.

Fate was less kind to d'Hérelle's closest associate: in 1937 Eliava was arrested on the orders of Lavrenty Beria, then the First Secretary of the Communist Party of Georgia, and accused of spying for the French government and trying to spread an epidemic. On July 9, 1937 at a closed session of the Supreme Court of the Georgian SSR, Eliava, along

Amelia Vol-Lewicka-Eliava. Born in Warsaw in 1885, shot in 1937 as the wife of an "enemy of the people." Rehabilitated posthumously.
Photo from Natalia Devdariani's archive (Tbilisi, Georgia). Published for the first time

Amelia Vol-Lewicka-Eliava, diva of Tiflis Opera and Ballet Theater.
Photo from Natalia Devdariani's archive (Tbilisi, Georgia). Published for the first time

Vol-Lewicka-Eliava. George met this beautiful Polish woman, who became the love of his life, long before the described events.

Amelia Vol was born in Warsaw, received her education in London, and later married her professor Mikolai Lewicki. Endowed with a beautiful soprano and excellent technique, she starred as a leading soloist on the stage of the Warsaw Opera in 1910–1912. According to the press, apart from a voice of unusual beauty, enlivened by emotion and a great deal of temperament, she had excellent looks—feminine elegance, an inspiring face and perfect height.

In 1913, Amelia gave birth to a daughter, and two years later her family moved to Russia to avoid the hardships of World War I. She performed successfully first in Kiev and then on the stage of the Tiflis Opera and Ballet Theater, which was the center of the Georgian musical life.

In 1918, the 33-year-old diva met the 26-year-old Gogi Eliava. A great lover of music, he had not missed a single performance by Amelia, but they met accidentally through common friends. According to Natalia Devdariani, it was Eliava's great persistence that made her grandmother divorce from her husband and, two years later, marry the brilliant Georgian bacteriologist.

These events in their lives are like chapters of a novel. First, there were letters: Eliava went to the Pasteur Institute in Paris. Then, the letters stopped coming, which was a usual thing during the Civil War, and his trip lasted longer than expected.

...AND DIED ON THE SAME DAY

There is a lot of speculation and rumors about the causes of death of George Eliava, the director of the Tbilisi Bacteriophage Institute and a scientist of world renown. One of them might by his relationship with Lavrenty Beria, who was also born in West Georgia. Eliava is rumored to have been on friendly terms with Beria (some even thought that they were schoolmates, despite their seven-year age difference). There is a legend that attributes the antagonism between the two men to a love triangle: the "playboy" Eliava fell in love with an opera actress of Polish origin, who toured in Georgia and was courted by Lavrenty Beria himself.

However, all the available evidence, as well as memories of Eliava's granddaughter, Natalia Devdariani, shows that there was only one opera actress in George's life—his wife Amelia (who had a stage name Melania)


Военная Коллегия
Верховного Суда
Союза ССР

Форма № 20

СПРАВКА

14 сентября /86 7г.

№ 4804691/57

Москва, ул. Воровского, д. 13.

Дело по обвинению ЭЛИАВА-ВОЛЬ-ЛЕВИЦКОЙ Амелии Станиславовны, арестованной 22 января 1937 года, пересмотрено Военной коллегией Верховного Суда СССР 22 августа 1957 года.

Приговор Военной коллегии от 11 июля 1937 года в отношении ЭЛИАВА-ВОЛЬ-ЛЕВИЦКОЙ А.С. по вновь открывшимся обстоятельствам отменен и дело за отсутствием состава преступления прекращено.

ЭЛИАВА-ВОЛЬ-ЛЕВИЦКАЯ А.С. реабилитирована посмертно.

ПРЕДСЕДАТЕЛЬСТВУЮЩИЙ СУДЕБНОГО СОСТАВА ВОЕННОЙ КОЛЛЕГИИ ВЕРХОВНОГО СУДА СССР
ПОЛКОВНИК ЮСТИЦИИ

/ШИРИНСКИЙ/

ОС

Amelia decided to return to Poland and went, together with her daughter, to Batumi to board a ship. One morning, they saw a man on the deck of a ship that had just arrived from Marseilles—that was Gogi, who was vigorously waving his hat,—he was sure that Amelia had come to meet him at the port. They would never part again...

In 1937, the Eliavas were arrested. Obviously, Beria did play a big role in this arrest because he became a mortal enemy of Eliava even before the establishment of the Bacteriophage Institute, when Eliava went over Beria's head and sent a memorandum directly to Stalin. In addition, Gogi, a hot and temperamental man, was never one to mince his words when faced with limitations and injustices: the violent disputes between Eliava and Beria were witnessed even by d'Hérelle.

As for the "feminine matter," Beria could have been jealous of Eliava because of his friend's wife, Tinatin Jikia, who worked at the library of the Institute of Bacteriophages. Rumors say that Beria had unsuccessfully courted for years this blue-eyed beauty with chiseled features, a muse of artists and poets in Tiflis. According to Eliava's granddaughter, their paths crossed in hospital: Eliava and Beria came to visit Tinatin on the same day, carrying bouquets of flowers... According to Tinatin's memoirs, her husband received an anonymous letter saying that she was cheating on him with Eliava; the couple were sure that Beria had a finger in the pie.

In the fall of 1936, Vladimir Jikia, a hydraulic engineer, was falsely accused of treason. He was soon executed as an "enemy of people," and a year later Tinatin herself was sent to a detention camp.

George and Amelia Eliava were shot on July 26, 1937. Despite all the odds, they had lived a happy, albeit a short, life and died on the same day...

with other "nationalist deviators" of the "Trotskist center of espionage and sabotage," was sentenced to death.

Eliava was shot on July 26 of the same year. His wife shared the fate of her husband, and her only daughter Hanna, 24 years old, who was adopted by Eliava, was sentenced to a five-year exile in Kazakhstan.

The irony of fate: when Eliava was shot, the French cottage, built to be a happy home for the families of the two outstanding microbiologists, was handed over to the Georgian KGB and surrounded with a high iron fence.

When dreams do not come true

We can only guess how d'Hérelle reacted to the death of his closest and most faithful assistant and friend. His position in Paris had become shaky by that time although he finally managed to prove, in collaboration with the Pasteur Institute and the Radium Institute, that a bacteriophage was a virus to put an end to his long-time dispute with the Nobel prize winner Jules Bordet, who was convinced that bacteriophages had enzymatic nature (Bordet's conviction stemmed from his outstanding discovery of the phenomenon of latent viral infections in bacteria).

Firstly, the founding father of phage therapy was not forgiven for his work for communists; secondly, the practical application of bacteriophage drugs, which were produced in the 1920–1930s by many private firms, often fell short of expectations. The poor repeatability of phage treatment results was largely due to technical issues; moreover, many physicians and entrepreneurs had very little understanding of microbiology and the very basics of biology. The situation was almost ridiculous: when d'Hérelle once tested two dozen commercial bacteriophage drugs, he found that none of them contained any active viruses!

Misdiagnoses, incorrect techniques of preparation, preservation, storage and use of the drugs, the lack of control over the treatments... There was a long list of shortcomings that undermined the authority of phage therapy and diminished investment in this area.

D'Hérelle was in Paris at the onset of World War II. He began, together with his wife and daughters, to produce drugs for the Allied Armies. After the occupation of Paris in 1940, the scientist, who was over seventy then, refused to organize the production of bacteriophage drugs to treat wound infections in German military, for which he was kept under house arrest until the liberation of Paris in 1944.

Five years later, the pioneer of bacteriophagology passed away, virtually in oblivion, from pancreatic cancer and was buried near Paris. Incidentally, a year later another man passed away—Frederick Twort, the coauthor of the discovery of bacteriophages, whose laboratory was blasted during the war.

Eliava was shot; d'Hérelle died... After World War II, the majority of scientists and doctors started to forget about bacteriophages: d'Hérelle's idea of a universal "live" bacteriological weapon was virtually killed by a new legend of the 20th century, antibiotics. However, things were different in the Soviet Union: the Institute of Bacteriophages, which was merged after the death of its founder with the Institute of Microbiology and Epidemiology, indeed became the leading (and the only!) world center of therapeutic phage research. During World War II, bacteriophages were widely used in the Soviet army to treat wounds and prevent outbreaks of intestinal diseases.

George G. Eliava (1892–1937)

In subsequent decades, the Soviet Union was successfully developing industrial production of targeted phages and phage cocktails. Tons of pills, liquid preparations, and aerosol cans containing carefully selected phage mixes for therapy and prevention were delivered every day to different parts of the vast Soviet country. Bacteriophages were used in hospitals and sold in pharmacies both as prescription and non-prescription drugs. There were about 1,200 researchers on staff at the Eliava Institute in Tbilisi, which was called after the name of its founder. The institute's "museum," the world's largest library of bacteriophages, contained more than 3,000 viral clones, including those from d'Hérelle's Parisian collection. In the late 1980s, a research and educational organization Bakteriofag was founded in Tbilisi. The organization had production facilities in Ufa, Khabarovsk, and Gorky (Nizhny Novgorod). The production of bacteriophages was also organized in socialist countries such as Poland and Czechoslovakia.

Nevertheless, the international scientific community had long treated the "eaters of bacteria" as an inconvenient (Soviet!) substitute for antibiotics, almost until the end of the 20th century, when the humankind faced the full force of the drug resistance of bacteria, which unleashed a real arms race. However, this was a different time, different people, and a different story...

NIKOLKA BULGAKOV, A BACTERIOLOGIST

One of d'Hérelle's close associates was none other than Nikolai Bulgakov, one of the brothers of the famous Russian writer Mikhail Bulgakov. Nikolai was the prototype of the cadet Nikolka Turbin in his novel *The White Guard*. After the Crimean evacuation, cadet Bulgakov went to Yugoslavia, where he graduated with excellence from the University of Zagreb. During his studies, he worked as a medical aid-man for patients with smallpox and typhus and as a lead singer in a students' balalaika orchestra. After his graduation, he was left at the Department of Bacteriology, where he got interested, together with Dr. Sertich, in the recently discovered bacteriophage viruses.

Their work was noticed by d'Hérelle, who had established in Paris a laboratory for the study and production of phage drugs, and the young researchers became his coworkers. D'Hérelle recalled that one day he sent from London a streptococci culture with a request to find the corresponding bacteriophage. This task was accomplished in just two weeks, for which, according to d'Hérelle, one had to be Bulgakov, with his abilities and precise technique.

Bulgakov's work involved not only the isolation of new natural bacteriophage races but also the design of equipment for simultaneous automatic sterile filling of several hundreds of vials. In 1936, Bulgakov replaced d'Hérelle in Mexico, where, during a period of six months, he not only organized a bacteriological laboratory and established a training system but also learned to speak Spanish and began to lecture in this language. During the German occupation, he was arrested as a Yugoslav citizen and sent to a camp as a hostage. In the camp, Bulgakov worked as a doctor, helping other inmates. He was awarded with the Order of Yugoslavia for his participation in the resistance

From the preface to the book "The Bacteriophage and the Phenomenon of Recovery" (1935) by Félix d'Hérelle, Honorary Professor, Faculty of Natural Sciences, Tiflis State University (this book was translated into Russian and Georgian by Eliava):

"People of science can be divided into philosophers (who are often forerunners of real knowledge) and scientists in the narrow sense of the word, those who patiently, stone by stone, build the grand edifice of experimental science.

"But 'experimental' does not mean 'infallible': experiment may also lead one astray; it is only the result that is the supreme criterion for the validity or invalidity of a chosen experimental technique.

"For people who have dedicated their life to experimental medicine, this result is to minimize the amount of physical suffering; for a fighter who has devoted his life to the science of social development, the goal is to maximize the welfare and happiness of all mankind <...>

"In both cases, it is only the practical result that can indisputably tell whether one has chosen the right path..."

Félix d'Hérelle.
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Phages Attack

A History of Bacteriophage Production and Therapeutic Use in Russia

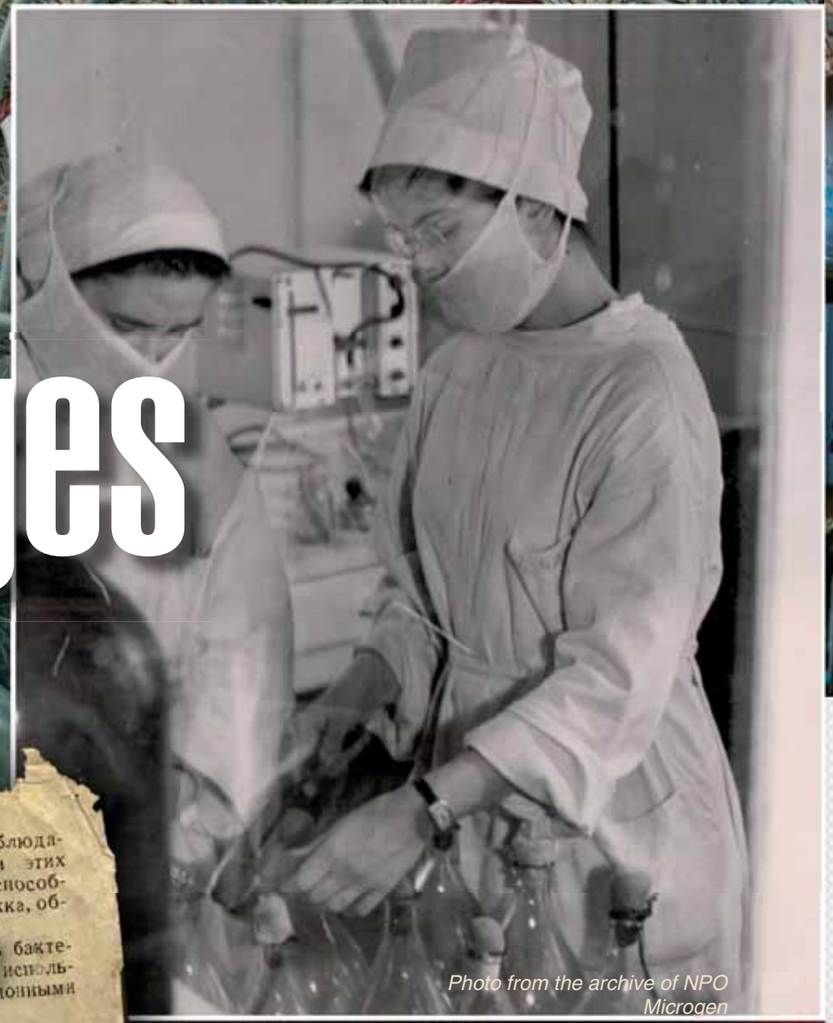


Photo from the archive of NPO Microgen

A. N. DABIZHEVA, N. N. VOROSHILOVA, T. V. PRISADA, M. G. EFIMOVA

Left to right:
Tatyana V. PRISADA, Cand. Sci. (Med.), Head of the Bacteriophage Production Unit at the ImBio company (Nizhni Novgorod), a producer of bacterial preparations and a subsidiary of NPO Microgen;
Marina G. EFIMOVA, Cand. Sci. (Med.), Head of the Bacteriophage Department, NPO Biomed (Perm), a subsidiary of NPO Microgen;
Alexandra N. DABIZHEVA, Cand. Sci. (Med.), Head of the Marketing and Promotion Department, NPO Microgen, Russian Ministry of Health (Moscow);
Natalia N. VOROSHILOVA, Dr. Sci. (Med.), Head of the Bacteriophage Production Unit at the Immunopreparat company (Ufa), a subsidiary of NPO Microgen

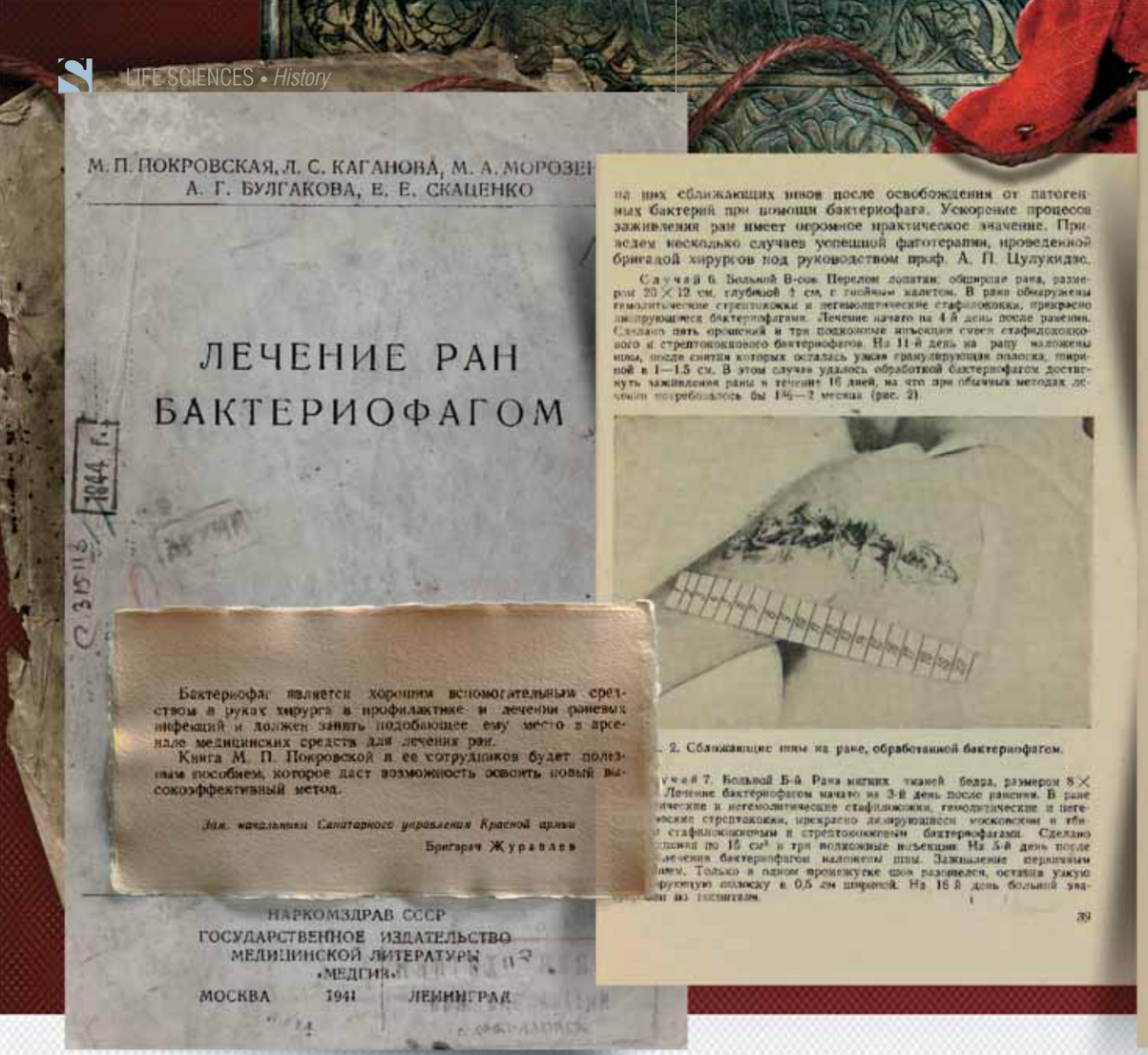
In Russia, bacteriophages have been produced and used for medical purposes for almost 80 years: during World War II, phages saved lives of thousands of wounded soldiers and prevented a cholera epidemic in the besieged Stalingrad before the famous Battle of Stalingrad. The development and widespread use of antibiotics virtually wiped out the production of bacteriophages worldwide; for decades, the Soviet Union had been the only country where bacteriophage technology not only continued to evolve, but a whole industry was built around it. Today, Russia remains the world leader in the production and therapeutic use of these effective and safe antibacterial agents

The cooperation between two outstanding scientists—Félix d'Hérelle from France and George Eliava from Georgia—fruited into the world's first and only research center of bacteriophagology, which was set up in the Soviet Union in the 1920s. Despite political repressions, as a result of which its first director, Eliava, was shot and some of its staff were sent into exile, the Institute of Bacteriophages (Tbilisi, Georgia) survived and persisted to become the world's leading center of therapeutic research and production of these bacterial killers.

Soviet bacteriophages were first used on a mass scale in emergency situations caused by the outbreaks of bacterial infections in the late 1930s. Thus, in 1938 there was a cholera epidemic in several areas of Afghanistan near the Soviet border. To prevent the spread of this serious bacterial disease, it was decided to use the cholera bacteriophage. The phage drug was given to the local population and put into wells and ponds. As a result, not a single case of cholera was recorded on the Soviet territory.

However, a truly serious test for bacteriophages was still ahead, when the Soviet Union was at war with Finland in 1939–1940. Before the discovery of antibiotics, the fate of a wounded soldier during a military campaign often depended on whether the wound was infected. An integrated team of 11 people, including surgeons, bacteriologists and laboratory assistants, began to use bacteriophage drugs developed and produced at the Tbilisi

Key words: phage therapy, choleraic bacteriophage, treat for injuries, battle of Stalingrad, Research and Production Association "Microgen"


М. П. ПОКРОВСКАЯ, Л. С. КАГАНОВА, М. А. МОРОЗЕВ
А. Г. БУЛГАКОВА, Е. Е. СКАЦЕНКО

ЛЕЧЕНИЕ РАН
БАКТЕРИОФАГОМ

1944 г.

Бактериофаг является хорошим вспомогательным средством в руках хирурга в профилактике и лечении раневых инфекций и должен занять подобающее ему место в арсенале медицинских средств для лечения ран.

Книга М. П. Покровской и ее сотрудников будет полезным пособием, которое даст возможность освоить новый высокоэффективный метод.

Зам. начальника Санитарного управления Красной армии
Бригврач Журавлев

НАРКОМЗДРАВ СССР
ГОСУДАРСТВЕННОЕ ИЗДАТЕЛЬСТВО
МЕДИЦИНСКОЙ ЛИТЕРАТУРЫ
«МЕДГИЗ»
МОСКВА 1941 ЛЕНИНГРАД

на них сближающих швов после освобождения от патогенных бактерий при помощи бактериофага. Ускорение процессов заживления ран имеет огромное практическое значение. Приведем несколько случаев успешной фаготерапии, проведенной бригадой хирургов под руководством проф. А. П. Цулукидзе.

Случай 6. Больной В-ов. Перелом голени; обширная рана, размером 20 × 12 см, глубиной 4 см, с гнойным налетом. В ране обнаружены гемолитические стрептококки и негемолитические стафилококки, прекрасно лизирующиеся бактериофагами. Лечение начато на 4-й день после ранения. Сделано пять орошений и три подкожные инъекции смеси стафилококкового и стрептококкового бактериофагов. На 11-й день на рану наложены швы, после снятия которых осталась узкая грануляционная полоска, шириной в 1—1.5 см. В этом случае удалось обработкой бактериофагом достигнуть заживления раны в течение 16 дней, на что при обычных методах лечения потребовалось бы 1½—2 месяца (рис. 2).

2. Сближающие швы на ране, обработанной бактериофагом.

учай 7. Больной Б-й. Рана мягких тканей бедра, размером 8 ×
Лечение бактериофагом начато на 3-й день после ранения. В ране
ические и негемолитические стафилококки, гемолитические и нега-
ические стрептококки, прекрасно лизирующиеся московским и тби-
стафилококковым и стрептококковым бактериофагами. Сделано
шприц по 15 см³ и три подкожные инъекции. На 5-й день после
лечения бактериофагом наложены швы. Заживление первичным
нием. Только в одном промежутке шов разошелся, оставив узкую
рую полоску в 0,5 см шириной. На 18-й день больной вы-
ан из госпиталя.

39

ПРЕДИСЛОВИЕ

В книге изложен опыт применения бактериофага при лечении ран во время войны с белофиннами, представлена методика применения бактериофага и результаты лечения им ран.

Высокая эффективность бактериофага в профилактике и лечении дизентерии, а также опыт ряда хирургов по применению бактериофага при лечении гнойных инфекций дали право д-ру Покровской предложить это средство и для лечения ран в военно-полевой обстановке. Полученные результаты представили убедительные доказательства ценности этого начинания.

С тех пор прошло больше года. За это время накоплен большой опыт применения бактериофага в хирургии. Тысячи больных с помощью бактериофага быстро восстановили свое здоровье, а сотням бактериофаг спас жизнь.

Конференция по бактериофагу, состоявшаяся в декабре 1940 г., записала в своих резолюциях, что бактериофаг «в ряде случаев с успехом применен в качестве активного средства борьбы с гнойными инфекциями, что, несомненно, имеет оборонное значение».

Истекший после конференции период показывает дальней-
шее, все ускор
и его примене
Хирургическ
препарат бакте
фекций после
ний и т. п., а
Понятно, чт
ское антисепти
щего в каждо
В то время
септические ср
тканей раны, б
ствует только
ток и тканей.

9 Лечение р/ф

This unique publication summarizes the experience in the use of bacteriophages to treat wounds and purulent infections in the military field conditions during the Russian–Finnish war of 1939–1940.
Photo: the title page, the preface and an illustration from the book *Treatment of Wounds with Bacteriophage*, *Moscow: USSR People's Commissariat of Public Health (Narkomzdrav), 1941*

"Mass production of bacteriophages for practical purposes requires enormous attention and diligence as well as in-depth theoretical knowledge on the part of the bacteriologist who organizes this production. The isolated bacteriophages must be thoroughly examined before they are put into production. It is only active bacteriophages, which double the number of corpuscles in about 10 minutes – a criterion for the high virulence of a bacteriophage race – that have therapeutic value. The bacteriophage must dissolve the majority of strains of a given bacterial species from most diverse sources and from various locations.

"The bacteriophage must have good viability. The virus must be cultivated on freshly isolated bacterial strains that have undergone as few passages in artificial media as possible. Given that the individual properties of different bacteriophage races are largely different, therapeutic products should use a mix of several virulent races of a given bacteriophage. The bacteriophage must be thoroughly controlled after the production. The control must ensure the high quality of the product, its sterility, and the absence of harmful effects when the product is administered into the body.

"The vials used for bottling the bacteriophage must be made of highest-grade glass that does not emit alkali; otherwise, the pH of the liquid will change over time and the bacteriophage may die. Provided that the bacteriophage is properly prepared at the highest scientific level, health professionals are armed with an extremely valuable weapon to fight various infectious diseases" (Pokrovskaya *et al.*, 1941)

Institute to save the soldiers wounded in the war with the White Finns.

They found that a relatively small amount of bacteria get into the tissue at the time of wounding, and those bacteria could be easily destroyed by bacteriophages during the first hours, when the bacteria were still on the wound surface. In most cases, an early treatment of wounds with bacteriophages prevented suppurative processes in tissues and led to faster healing.

As a result, the use of staphylococcal and streptococcal bacteriophages helped clean wounds from bacteria in more than half of the cases and achieve a complete sterilization of the wound in 30–40% of the patients. The elimination of infection through the use of bacteriophages allowed surgeons to suture the wound a week earlier, thus accelerating the healing process. Bacteriophages also proved effective in treating acute inflammatory processes (phlegmons, tendovaginitis, abscesses, etc.); in most cases, the treatment was conservative, without large incisions.

All these results showed the great importance of the prevention of purulent complications in wounds through the use of bacteriophages, which were not only completely harmless but also widely available, inexpensive and easy-to-manufacture therapeutic agents.

Bacteriophages in the Battle of Stalingrad

The further history of phage therapy is associated with the tragic events of World War II in 1941–1945. In those years, with a total lack of antibacterial drugs (at the beginning of the war, the Soviet Union did not have a technology to produce penicillin), it was decided to organize mass production of bacteriophages to treat infections in the soldiers of the Red Army.

Particular attention was given to phages that destroy bacteria causing intestinal infections such as cholera, typhoid, dysentery, salmonellosis, etc. The reason was the inevitable lack of hygiene in the field conditions. Later,

In the early stages of industrial production of phage drugs, bacteriophages were grown in large glass containers such as three-liter bottles

и поэтому время от времени запасы бактериофага нужно повторно проверять, чтобы для практической работы всегда давать только высококачественный препарат.

ПРЕПАРАТЫ БАКТЕРИОФАГА, ПРИМЕНЯЕМЫЕ В ХИРУРГИЧЕСКОЙ ПРАКТИКЕ

Для лечения хирургических заболеваний в СССР изготовляются различные бактериофаги: стафилококковый, стрептококковый, синегнойный, энтерококковый, бактериофаги против кишечной палочки, против возбудителей газовой гангрены и др. Эти бактериофаги употребляются в тех случаях, когда бактериологическое исследование точно установило вид микроорганизма, обусловливающего данный инфекционный процесс. В тех случаях, когда бактериологическое исследование не произведено или процесс вызывается несколькими видами микроорганизмов, д'Эрелль предложил применять смеси из бактериофагов, не дожидаясь постановки точного бактериологического диагноза. Смеси бактериофагов дают возможность в практической обстановке применить бактериофаг немедленно. Д'Эрелль предлагает в хирургической практике употреблять смесь из стафилококкового, стрептококкового, паракишечного, энтерококкового, протейного и синегнойного бактериофагов. Эту смесь д'Эрелль назвал пиофагом.

При применении пиофага в больном организме «работают» (лизируют) только те бактериофаги, которые встретились с соответствующими их литической специфичности бактериями, обусловливающими данное заболевание. Остальные бактериофаги, имеющиеся в смеси, остаются инактивными; никакого вреда организму они не принесут и через некоторый небольшой срок будут выделены из организма.

При отсутствии готового пиофага можно самим сделать смесь из имеющихся в распоряжении хирурга бактериофагов. Желательно составлять смеси таким образом, чтобы не включать в них лишние бактериофаги. Например, бактериологическими исследованиями установлено, что в первые часы после ранения из аэробных микробов в ране чаще всего обнаруживаются стрептококки и стафилококки. Протей и синегнойная палочка появляются в ранах значительно позже. Поэтому лечение свежих ран можно производить смесью из стрептококкового и стафилококкового бактериофагов, не включая в нее протейного и синегнойного. Нужно иметь в виду, что в очень сложных смесях происходит, по нашим наблюдениям, ослабление отдельных компонентов, что снижает лечебные качества препарата. Подробно об этом сказано ниже, в

18

Soviet microbiologists implemented an inventive idea to produce an integrated phage drug, which was called by its author—Félix d'Hérelle himself—pyophage (from pyo meaning 'purulent'). The goal of combining several bacteriophage species targeting different bacteria was to expand the combat spectrum of the drug. This was particularly important for wounds: the injured area is infected by a variety of bacteria, and emergency prevention and treatment of already festering wounds is best done by different bacteriophages. The same principle was later used in the creation of intesti-bacteriophages, i. e., combined Russian drugs based on intestinal phages, which are still widely used for mass prevention and treatment of intestinal infections

As shown by the experience of the Russian–Finnish War, the use of bacteriophages to treat wounds enabled surgeons to suture the wounds a week earlier, which accelerated the healing process. *Photo:* pages from the book *Guidelines to Surgical Treatment Methods*, *Moscow: USSR People's Commissariat of Public Health (Narkomzdrav), 1942*

hospitals began to use the wound infection bacteriophages that proved effective during the Russian–Finnish war. These drugs helped reduce the stay of a wounded soldier in a field hospital to a week. In total, during the war years, the production units established by Soviet bacteriological institutions manufactured more than 200,000 liters of wound bacteriophages for the Soviet army!

These organizations not only produced tons of phage drugs; they did a huge amount of research. The reason was that nutritional microbiological media were made from meat, which was in deficit during the war. Therefore, concurrent with the production, researchers had to find, as quickly as possible, new ways to prepare nutritional media, and they learned how to make them from placenta, casein and even blood clots.

It was bacteriophages (namely, the cholera bacteriophage) that became a key to success in the famous Battle of Stalingrad, the decisive battle of World War II. Cholera had always been an inevitable companion of armies at war. For instance, during the Sevastopol campaign of 1854–1855, the Anglo–French troops lost 73,000 men in military operations and 18,000 due to cholera! At Stalingrad in the summer of 1942, there was an outbreak of cholera on the territory occupied by German troops. On the one hand, it was an unexpected ally; on the other hand, it was a direct threat because the epidemic would not stop at the front line.

ЛИТИЧЕСКАЯ АКТИВНОСТЬ ПРЕПАРАТОВ БАКТЕРИОФАГА

Мы могли убедиться, что Московский институт имени Мечникова и Тбилисский институт бактериофагии изготовляют препараты высокого качества (табл. 1).

Таблица 1

Лизирующая активность бактериофагов in vitro по отношению к микроорганизмам, выделенным из ран (в %)

Вид бактерий	Лизировано	Не лизировано
Гемолитические стафилококки	68,8	31,2
Негемолитические "	50	50
Гемолитические стрептококки	84,5	15,5
[illegible]кие "	50	50
[illegible]	24	76
[illegible]ка	50	50

[illegible]ческой активности бактериофагов сделан на [illegible] исследований. Это дает материал для [illegible]а о качестве бактериофагов, предназначен[illegible] хирургическими инфекциями. В графе [illegible]мов учтены только случаи полного лизи[illegible]+++; лизис менее отчетливый на [illegible]

This counting and filling machine for the packaging of bacteriophage drugs was designed at the plant in the city of Gorky (now Nizhny Novgorod)

Утверждаю
Начальник Санитарного управления
Красной армии
дивврач *Е. И. Смирнов*
6.VII.1941 г.

ИНСТРУКЦИЯ
ПРИМЕНЕНИЮ БАКТЕРИОФАГА В ХИРУРГИИ

Общие сведения

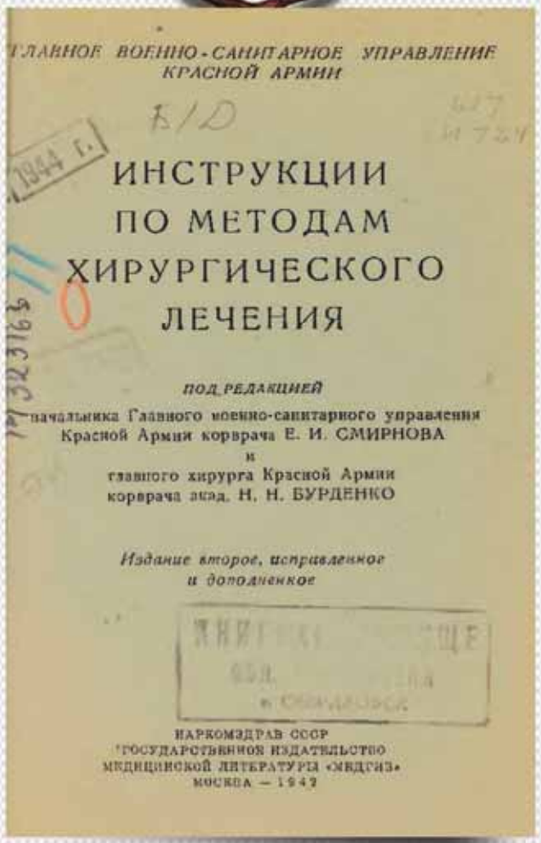
Препарат бактериофага представляет собой прозрачную
сть, содержащую бактериофаг, обладающий способ-
о уничтожать патогенные микробы. Кроме бактерио-
в жидкости содержатся безвредные для организма ко-
е продукты растворения тех микробов, из которых
ален препарат.
Бактериофаг выпускается в ампулах или герметически
тых флаконах. На этикетке приводится название бакте-
га, указывающее, против каких бактерий действует дан-

время для применения в хирургической прак-
следующие препараты: стафилококковый,
протейный, синегнойный, колифаг и бакте-
всех 4 возбудителей анаэробной инфекции
матиеж, вибрион септик, гистолитикус) как
ютив каждого в отдельности.
выпускается также препарат, называемый
ящий из смеси нескольких бактериофагов.
ят преимущественно стафило- и стрептофаги
могут входить также протейный, синегной-
лярный и другие фаги. Фаги против воз-
бной инфекции в состав пиофага не входят.
ного введения может применяться только
ащий специальное обозначение на этикетке.
ляются на специальных средах, содержащих
белка.
актериофагом перед употреблением следует
чего ее содержимое должно оставаться

ампонады; в тяжелых случаях при прогрессирующей инфек-
ции показано также внутривенное введение стрепто- и пер-
фрингенсфагов в количестве 30—50 см³ каждого в смеси
с физиологическим раствором до 300 г в подогретом виде.
12. Лечение бактериофагом не исключает применения
противогангренозных сывороток, так как они действуют анти-
токсически.
13. При тяжелых септических состояниях при метастати-
ческих гнойниках допускается внутривенное введение бакте-
риофага от 30 до 50 см³. Этот способ должен применяться
с большой осторожностью, так как может сопровождаться
тяжелой реакцией как во время инъекции, так и после нее
в ближайшие 2—4 часа. Реакция выражается ознобом, за-
трудненным и учащенным дыханием, появлением цианоза,
падением кровяного давления. Поэтому внутривенное введе-
ние допускается лишь в стационарной обстановке и при воз-
можности последующего тщательного наблюдения за боль-
ным в течение первых суток.
Вводят 30—50 г смеси равных количеств стафило-
и стрептофагов в вену при помощи шприца, медленно (в те-
чение 15—20 минут). Внутривенное введение бактериофага
рекомендуется делать в разведении с физиологическим раство-
ром — 300,0 в подогретом виде. Внутривенное введение по-
вторяется 2—3 раза с промежутком в 1—2 дня.
Для борьбы с тяжелой реакцией применяется лобелин,
кофеин, камфора, кислород, углекислота и согревание тела.

Главный хирург Красной армии
академик Бурденко

As shown by these guidelines for practicing surgeons, which were published at the beginning of World War II, by that time bacteriophages had become a recognized antibacterial agent and were recommended for use in surgical practice to treat wounds and acute infectious and inflammatory processes. *Photo:* the title page and preface to the book *Guidelines to Surgical Treatment Methods*, *Moscow: USSR People's Commissariat of Public Health (Narkomzdrav), 1942*



ГЛАВНОЕ ВОЕННО-САНИТАРНОЕ УПРАВЛЕНИЕ
КРАСНОЙ АРМИИ

1944 г.

ИНСТРУКЦИИ
ПО МЕТОДАМ
ХИРУРГИЧЕСКОГО
ЛЕЧЕНИЯ

ПОД РЕДАКЦИЕЙ
начальника Главного военно-санитарного управления
Красной Армии корврача Е. И. СМИРНОВА
и
главного хирурга Красной Армии
корврача акад. Н. Н. БУРДЕНКО

Издание второе, исправленное
и дополненное

НАРКОМЗДРАВ СССР
ГОСУДАРСТВЕННОЕ ИЗДАТЕЛЬСТВО
МЕДИЦИНСКОЙ ЛИТЕРАТУРЫ «МЕДГИЗ»
МОСКВА — 1942

It was important to assess the risks on site, and this task was assigned to Prof. Zinaida Ermolieva from the Institute of Experimental Medicine (Moscow), who had already had an experience of working with bacteriophages under battlefield conditions. (By the way, it was Ermolieva who obtained in the same year 1942 the first Soviet penicillin (*krustozin VIEM*), which began to be used extensively in military hospitals by the end of World War II.)

To obtain *Vibrios cholerae*, one needed "material," i.e., corpses of those who died from cholera. Berlin began to receive bizarre messages: dead bodies were disappearing from German field hospitals. The corpses were stolen and carried over the front line by Soviet scouts. However, the *Vibrios cholerae* that were sent to a factory in the city of Gorky (Nizhny Novgorod) were too weak to be used for the industrial cultivation of bacteriophages. The factory did a great deal of work to infect rabbits and cultivate the necessary pathogenic bacteria and their bacteriophages. Unfortunately, the train with the phage drugs, which had been so difficult to obtain, was bombed out by German aircraft. Therefore, Ermolieva organized in besieged Stalingrad an underground secret laboratory for the production of the cholera bacteriophage directly. The drug was given to nearly 50,000 people every day.

At the end of 1942, Zinaida Ermolieva received a direct call from the Commander-in-Chief Iosif Stalin, who asked her: "Is it safe to keep more than a million people at Stalingrad? Can the cholera epidemic interfere with the military plans?" The bacteriologist said that she had already won a victory at her front, now it was the time for the Red Army.

These events marked the beginning of the mass use of bacteriophages in the Soviet Union. Notably, this happened at the time when the world switched to antibiotics, which were discovered by a British scientist Alexander Fleming in 1928.

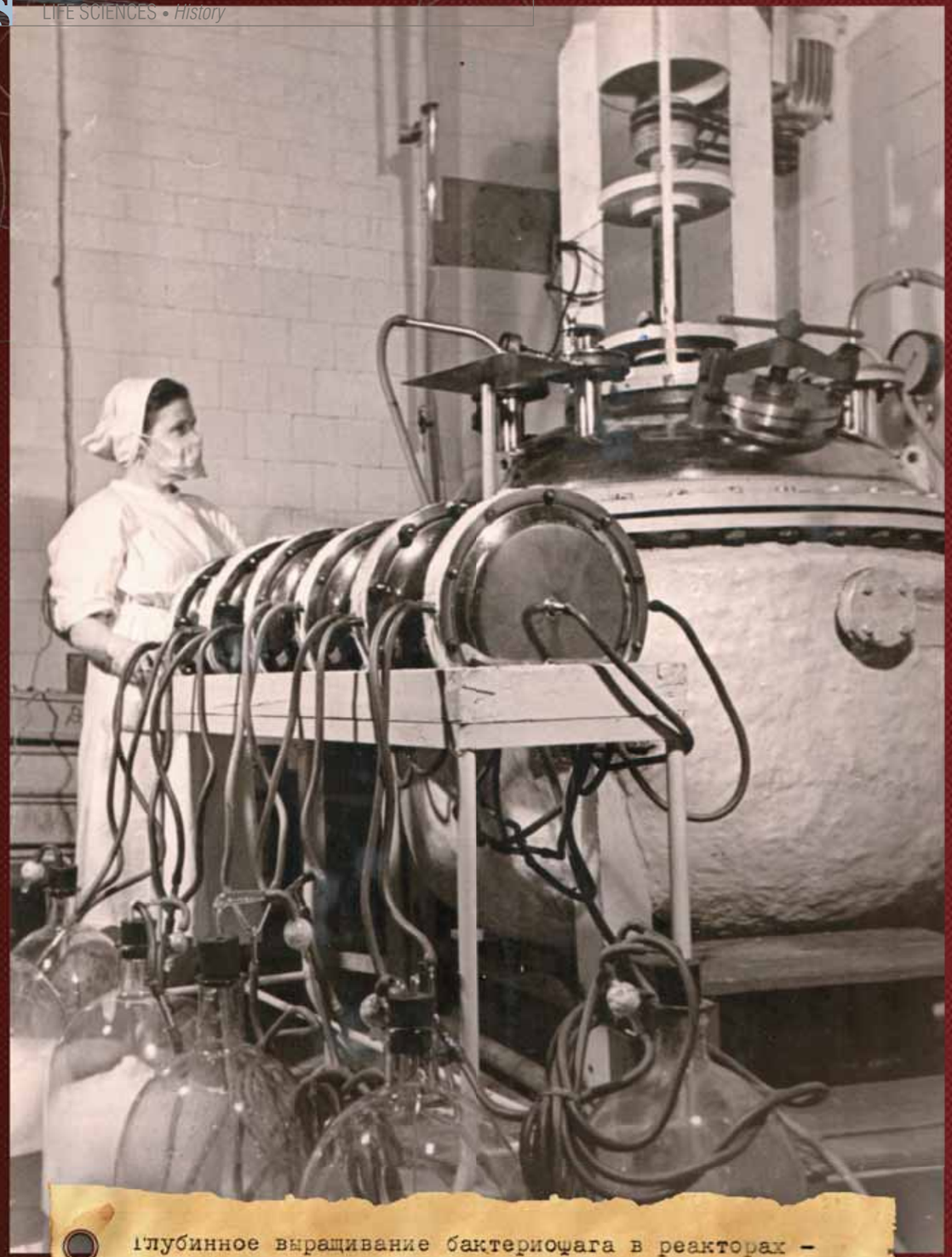
Contemporary history

The history of bacteriophages on the Soviet territory continued after World War II. Up to now, bacteriophage drugs have been successfully produced by Russian companies that make immunobiological products.

Currently, NPO Microgen, Russia's largest producer of such products, a state company under the Russian Ministry of Health, produces 14 drugs containing bacteriophages targeted at the most common pathogens of bacterial infections. They are used for the prevention and treatment of acute intestinal infections (dysentery, typhoid, salmonella, etc.) and for the treatment of purulent-septic and other diseases of various localization: surgical infections, diseases of the ear, nose, throat, lungs and pleura, urogenital pathologies, gastroenterocolitis, intestinal dysbacteriosis, and infectious diseases in newborns and infants. The drugs have been produced at three plants associated with microbiological institutions: in Ufa since 1939; in Gorky since 1941; and in Perm since 1995.

Of course, the bacteriophage cultivation technology has largely improved since the times of World War II. The companies now use a reactor cultivation technology and optimized growth media. Particular attention is paid to the cleaning of bacteriophages from ballast components. To this end, the producers use ultrafiltration, which increases the safety of the drugs.

Bacteriophages are currently produced in Russia on an unprecedented scale, like nowhere else in the world. Bacteriophages are manufactured as high-grade drugs, and the consumption of these antibacterial agents in Russia is more than 1 billion packs per year. However, it was only in 2016—one century after the discovery of bacteriophages!—that these drugs were included into the official Russian pharmacopoeia.



One of the latest examples of the preventive use of Russian bacteriophages on a mass scale flood relief programs.

For instance, in 2013, about 70,000 doses of intesti-bacteriophage, which targets a broad spectrum of bacteria causing gastrointestinal disease, were delivered to the regions of the Russian Far East. In 2014, more than 8,000 packages of this drug were sent, together with dysentery and salmonella bacteriophages, to the Altai Krai and the Republic of Khakassia, which had also suffered from floods.

Thus, phage drugs have been and still are a means of rapid response to bacterial threat at the gravest moments in the Russian history. The culture of producing and using these antimicrobial agents, which has been preserved through the creative and selfless work of Russian scientists, is particularly valuable now, in the light of the rapidly growing bacterial resistance to antibiotics.

An important step in the development of industrial bacteriophage production was to design a technology of deep cultivation in fermenters (*left*). This method, which allows broad variations in the composition of the nutrient medium to achieve the maximum yield of the target product with minimal manual labor, is still successfully used at the plants of NPO Microgen (*above*)

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Instruktsiya po metodam khirurgicheskogo lecheniya (Guidelines to Surgical Treatment Methods). Moscow: USSR People's Commissariat of Public Health (Narkomzdrav), Medgiz. 1942 [in Russian].

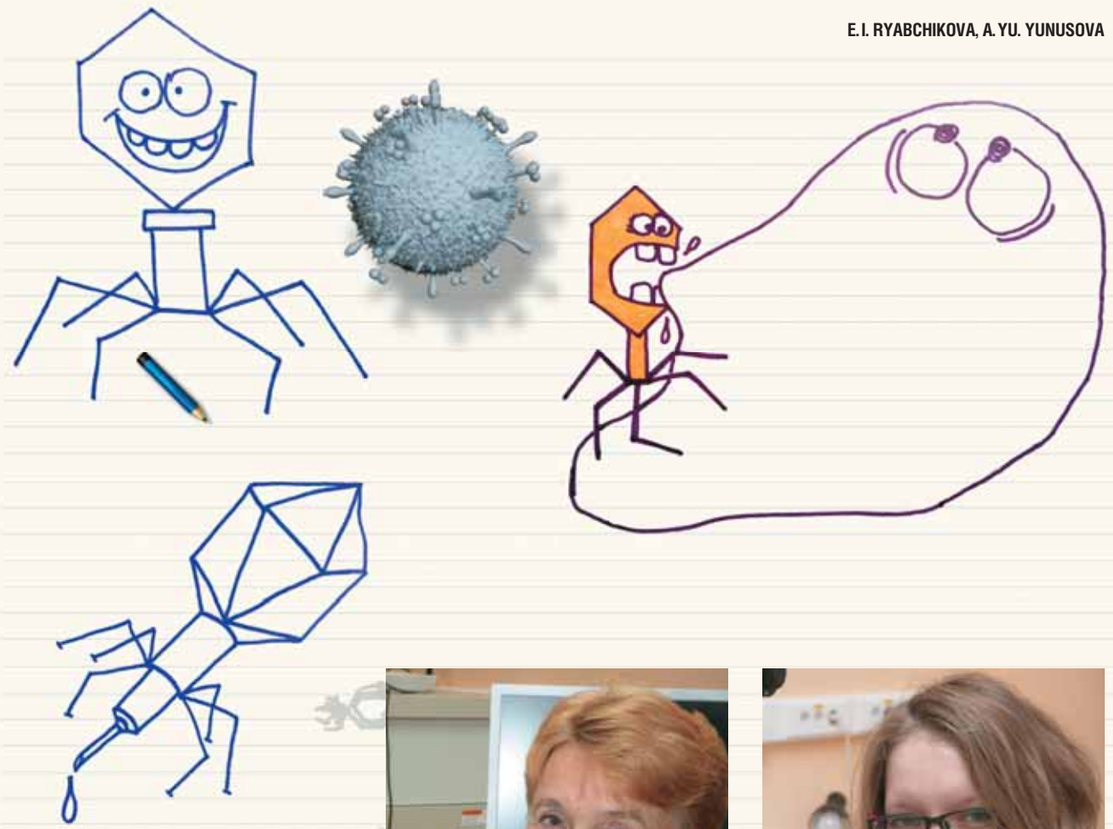
This article uses illustration materials from the archive of NPO Microgen and from the books "Treatment of Wounds with Bacteriophage" (1941) and "Guidelines to Surgical Treatment Methods" (1942)

The editors thank B.A. Ryzhikov (NPO Microgen) for his help in preparing this article

Bacteriophage, We See You!

A century has elapsed since Frederick William Twort, a British microbiologist, noticed transparent glass-like spots formed of dead bacterial cells in colonies of micrococci. For a long time after their discovery, the research into bacteriophages was of a phenomenological character because of the lack of adequate experimental tools. The scientists had no possibility for studying in detail the specific features of an antibacterial impact caused by bacteriophages since they are invisible not only to the naked eye, but also to light microscopy. The advent of electron microscopy brought the study of viruses, including the viruses of bacteria, to a fundamentally new level

E.I. RYABCHIKOVA, A.YU. YUNUSOVA



This is how a filamentous bacteriophage looks like after counterstaining with phosphotungstic acid in the field of vision of a *Jem 1400* (*JEOL*, Japan) electron microscope

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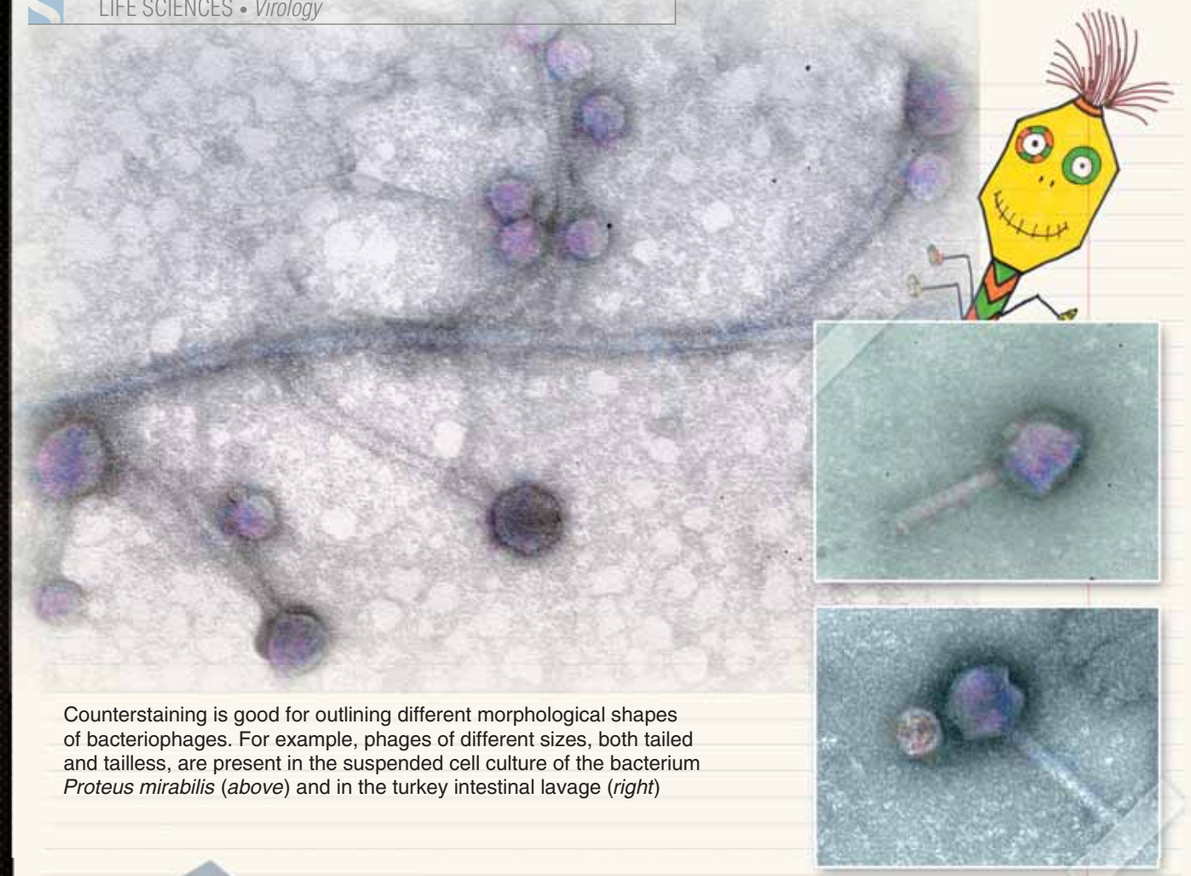
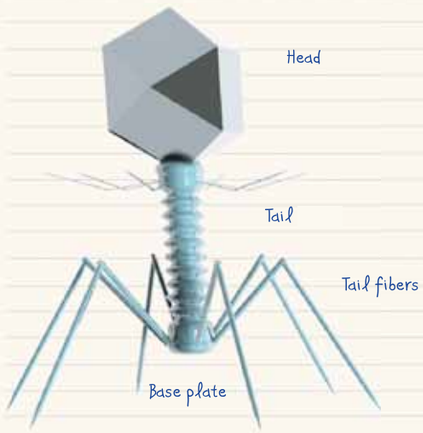
Key words: bacteriophages, bacteria viruses, morphology, electron microscopy



Counterstaining is good for outlining different morphological shapes of bacteriophages. For example, phages of different sizes, both tailed and tailless, are present in the suspended cell culture of the bacterium *Proteus mirabilis* (*above*) and in the turkey intestinal lavage (*right*)

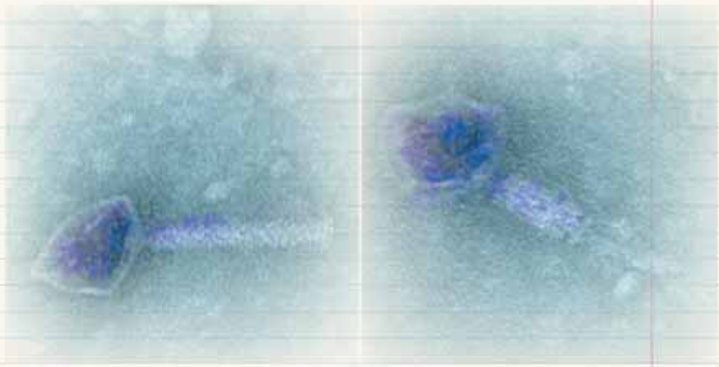


A typical bacteriophage comprises a “head” housing DNA or RNA and surrounded by a protein or lipoprotein envelope (capsid), and a “tail”, a protein tube used by the virus to “inject” its genetic material into the bacterial cell

Electron microscopy studies suggest that bacteriophages are actually nanoorganisms rather than microorganisms, since their size does not exceed 100 nm. Also, it has been shown that bacteriophages are most diverse in their structure. This raises the question of their nomenclature. The very first classification, dating back to 1943, was based on the specific structural features described with the help of electron microscopy. One of its founders, Ernst Ruska, distinguished three types of bacteriophages, using their morphological characteristics (Ackermann, 2009).

The currently used systematics of bacteriophages, developed in 1967, relied on the classification that comprised six morphotypes. However, the discovery of new bacteriophages has added new families, genera, and species to this systematics. The advances in molecular biological tools established additional criteria for their classification which took into account the type of nucleic acid and/or protein composition within the phage.

According to the decision of the International Committee on Taxonomy of Viruses (ICTV), *bacteriophages* are viruses that specifically infect bacterial and archaeal cells. The set of characters used to identify bacteriophages at a species level includes the shape and size of the phage capsid, type of nucleic acid (DNA or RNA) forming its genome, and the presence or absence of an envelope



The “tail” of bacteriophages can be different in length and structure: a long, flexible, and uncontracted tail is a specific feature of the phage family Siphoviridae, and a short tail, of the family Podoviridae. As for the family Myoviridae, its members have a rigid tail that can contract, and a kind of “neck”, separating the tail from its head (*above*)

The use of state-of-the-art molecular techniques in the bacteriophage research has revealed manifold specific features of these interesting organisms. In turn, bacteriophages turned out to be a most useful methodological tool for molecular biologists (Brussow, 2013).

If there is a head, there is a tail

Actually, bacteriophages have a comparatively simple structure: each virus is a complex of a nucleic acid and proteins packaged in a specific manner. They may be curiously shaped but approximately 96 % of all known bacteriophages have a “tailed” phenotype (Matsuzaki *et al.*, 2005). They have a “head” shaped as icosahedron (a protein reservoir housing packaged nucleic acid) and a “tail,” a protein structure carrying the elements that are able to bind stably to receptors (specific proteins or polysaccharides) on the surface of bacteria. Individual species of “tailed” bacteriophages differ in the size of their “head” as well as in the size and fine structure of their “tail.”

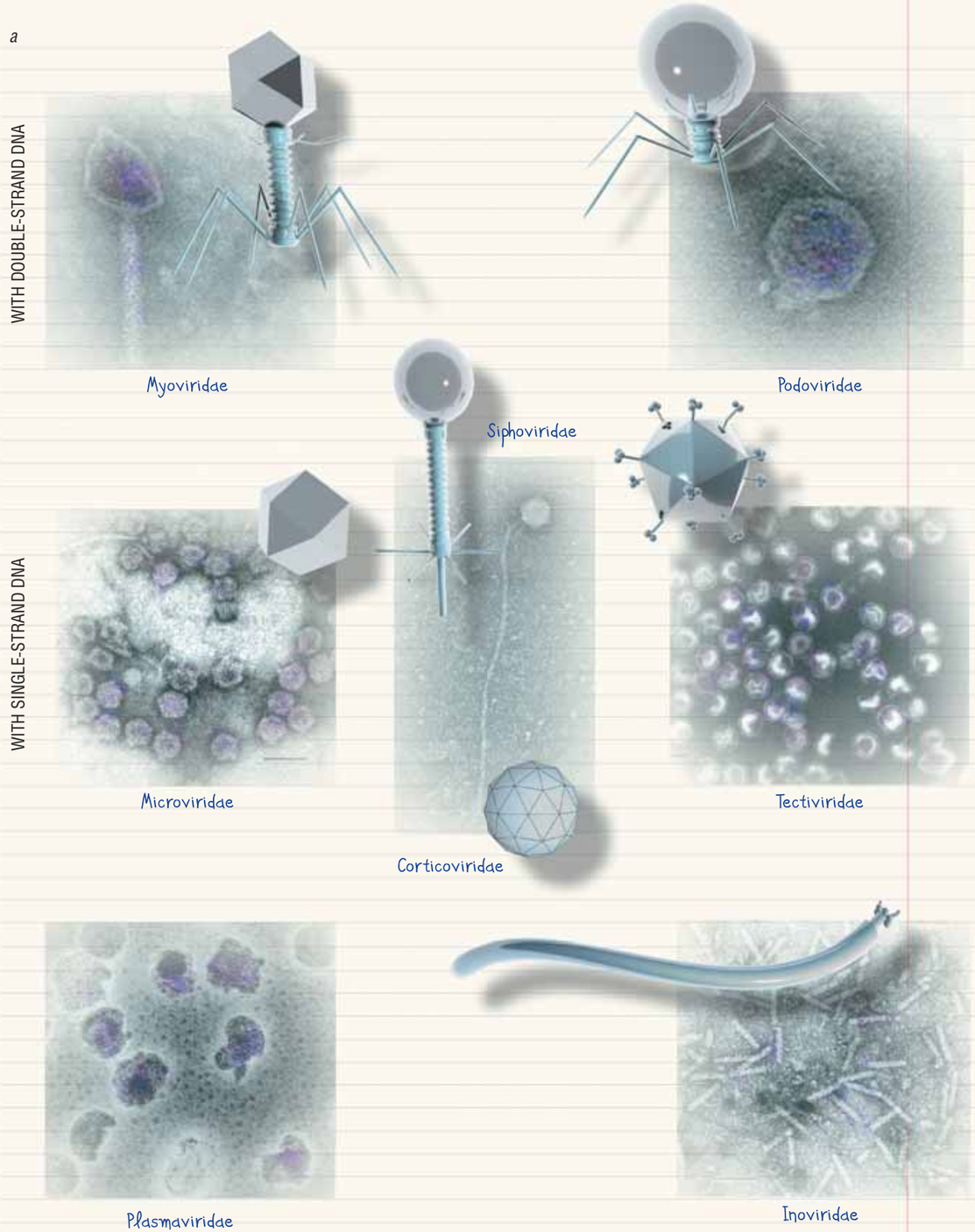
A bacteriophage species is identified according to its ultrastructural characteristics, which are described using the negative contrast technique. Any phage-containing suspension can be used as a sample, be it water from a natural source, animal intestinal lavage specimens, or bacterial cell suspension after incubation with a bacteriophage in a laboratory. A drop of prepared suspension is covered with a special copper grid with a polymer film on it for the bacteriophages to sorb. The grid is then treated with a counterstain (usually uranyl acetate or phosphotungstic acid) that encompasses bacteriophage particles and creates a dark background, rendering bacteriophages, which have low electron density, visible in the electron microscope.

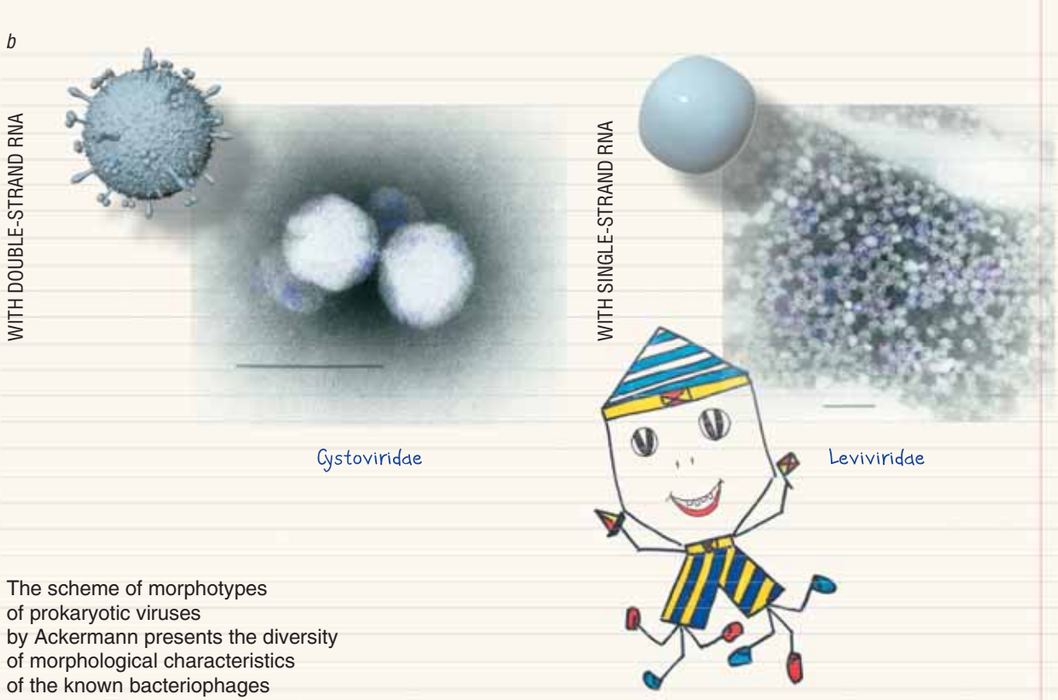
Over 6300 bacteriophages have been described by electron microscopy so far (Ackerman and Tiekotter, 2012; Ackermann and Prangishvili, 2012). It emerges that not all bacteriophages have clearly distinguishable “head” and “tail”; as for their hereditary matter, the phages with double-strand DNA are the most abundant. The systematics of bacteriophages is very dynamic since novel phages are constantly being discovered (Ackermann, 2007).

Hunting for bacteria

Advances in electron microscopy techniques made it possible to visualize not only bacteriophages, but also their reproduction. The penetration of “tailed” bacteriophages into the cell has been studied in most detail, including the molecular mechanisms underlying the “injection” of phage DNA into the cytoplasm of a bacterial cell (Guerrero-Ferreira and Wright, 2013).

The typical behavior of bacteriophages “attacking” a bacterium is demonstrated by a lytic phage. The phage attaches itself to the bacterium surface, using its receptors as an “anchor.” Then its “tail” pierces the bacterial wall with the help of specialized proteins to form a “channel” for the phage nucleic acid to pass into the cell. Within half hour, the protein and nucleic acid components ofthe bacteriophage are synthesized in the infected bacterial cell to assemble new phage particles. Then the cell is destroyed to release mature virions.





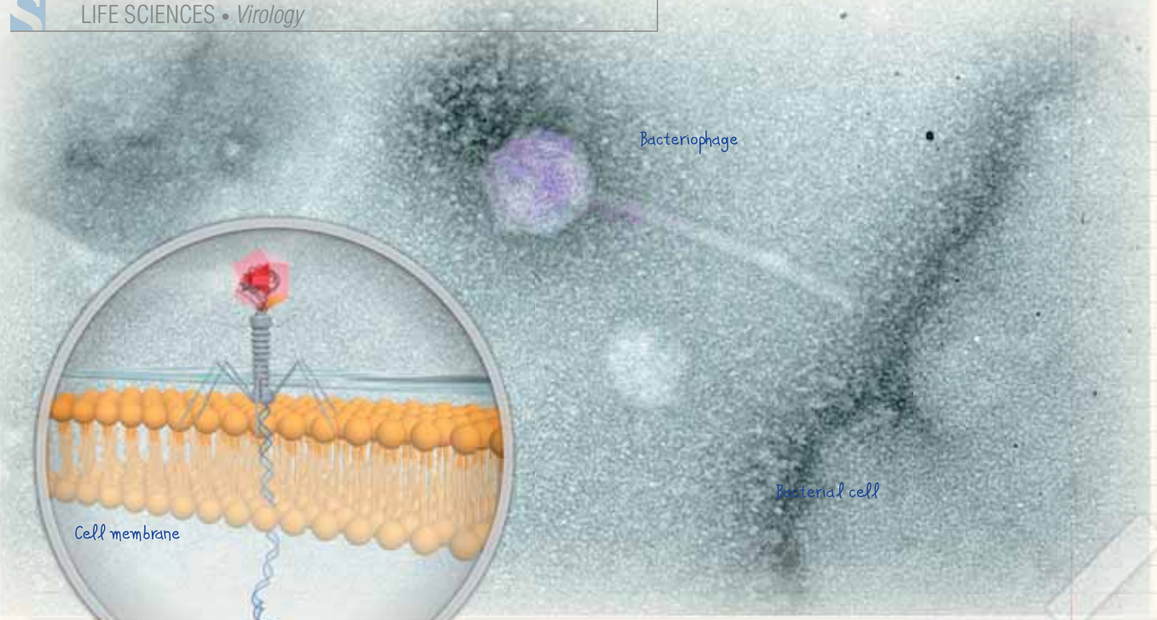
The scheme of morphotypes of prokaryotic viruses by Ackermann presents the diversity of morphological characteristics of the known bacteriophages to the fullest. The scheme contains 10 families of bacterial viruses and 11 families of archaeal viruses; it takes into account the main taxonomic characters of the phages, namely the shape of capsid and type of nucleic acid: (*a*) DNA or (*b*) RNA (Ackermann, 2007). As a rule, the head of a bacteriophage is symmetrical and has a hexagonal outline. Bacteriophages can be tailless but can have an additional envelope (family Corticoviridae), while the absence of the head is a specific feature of the family Inoviridae, which comprises filamentous bacteriophages

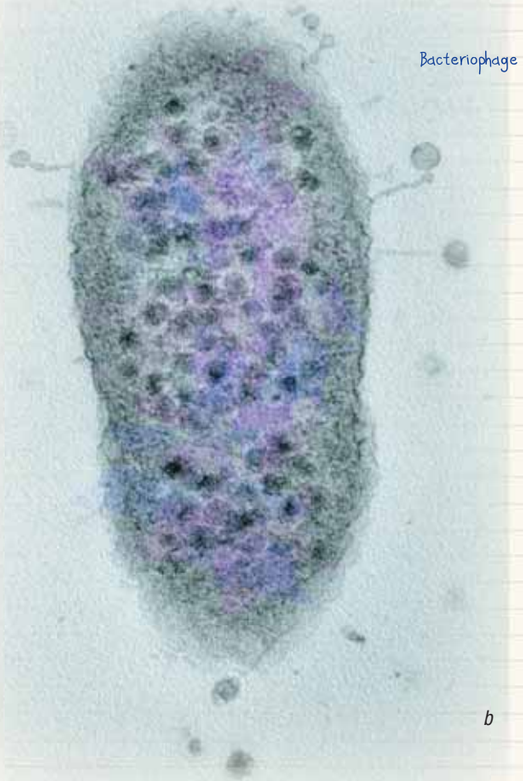
The Prokaryote Kingdom (the organisms lacking the nucleus) comprises Bacteria and Archaea. The members of these subkingdoms differ in the structure of the cell wall, in the specific features of their vital activities, and in the degree of resistance to environmental factors (the majority of archaea live under extreme conditions). Although the archaeal viruses found so far are rather few, their morphological diversity considerably exceeds that of bacteriophages. Some of the archaeal phages are tailed, as is typical of bacteriophages, but the overwhelming majority of archaeaphages have unique morphotypes, including virions shaped as ellipsoids, spindles, drops, and bottles, either tailless or carrying two tails, as well as spherical and rod-like virions. The known morphological diversity of archaeaphages is likely to be only the tip of an iceberg.

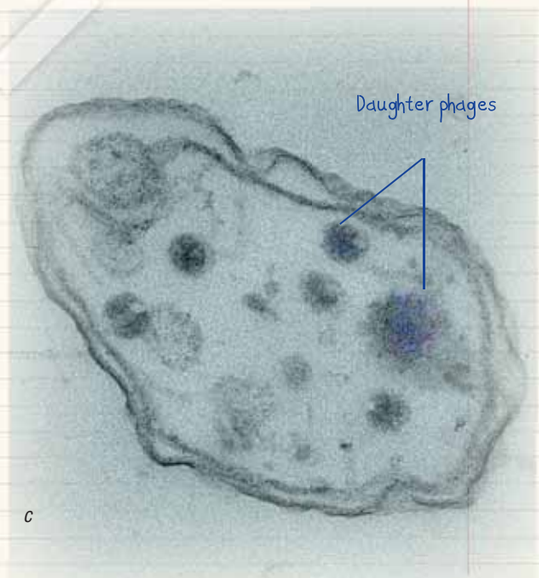
The unique characteristics of archaeophages along with the three cell lineages found on the planet—bacteria, archaea, and eukaryotes (the organisms possessing a cell nucleus)—suggest that there exist three specific virus "domains" that have emerged in the course of a long-term coevolution of viruses and their hosts, although some viruses still retain the traces of their common origin (Pina *et al.*, 2011)

* To obtain ultrathin sections, cells are embedded into a special resin; the resulting hard blocks are cut into sections 60–80 nm thick, using an ultramicrotome equipped with a glass or diamond knife

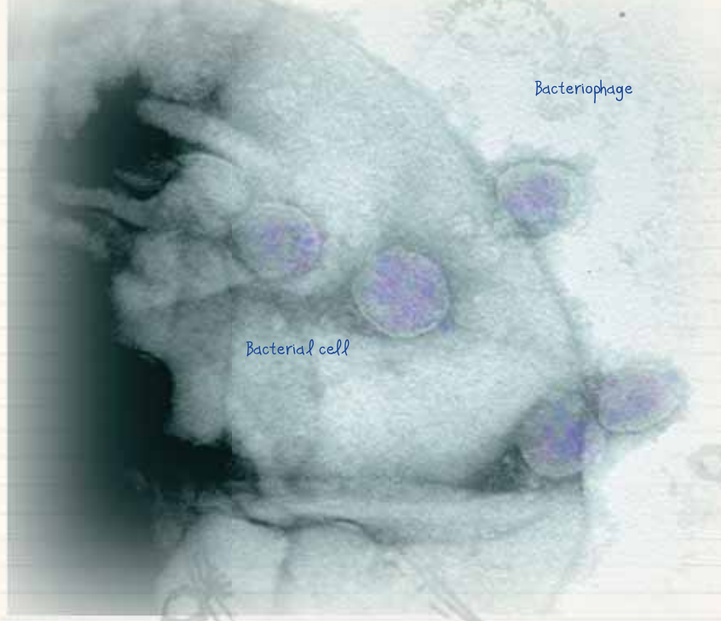
The combination of counterstaining and ultrathin sections * makes it possible to trace all stages in bacteriophage reproduction including the sorption of phage particles on the surface of bacterial cells, their penetration into the cell, and copying. Unfortunately, this approach is much less developed as compared with bacteriophage visualization and identification by counterstaining. And yet, the ultrastructural characteristics of the bacteriophage life cycle are helpful in assessing the efficiency of elaborated phage therapies satisfactorily.







The main stages of Myoviridae bacteriophage reproduction in bacterial cells (*a*). Virus particles both sorbed on the cell surface and in the cytoplasm in the ultrathin section of an infected bacterial cell (*b*). Death of a bacterial cell as a result of phage infection: the cytoplasm is lysed and contains daughter phages (*c*)



"Short-tailed" phage particles of the family Podoviridae sorbed on the surface of a bacterial cell; the phages attach themselves to the cell via small protein subunits

Bacteriophages are undoubtedly a unique phenomenon on the planet: on the one hand, they have a rather simple structure; on the other hand, they are tremendously diverse both in terms of their morphology and potential "victims."

These nanoorganisms are not just safe for us – they are "friendly" because they can kill pathogenic bacterial cells without affecting any cells of higher organisms, including the cells of humans, agricultural animals, and plants. This property allows us to use bacteriophages for treating bacterial infections following the principle that the enemy of our enemy is our friend.

Phage therapy has agreat potential not only because phages can kill bacteria, but also because of a high specificity of the phage–host interaction. Finally, since phages are a natural phenomenon, we can affect pathogenic bacteria avoiding harmful chemical agents.

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V. V. VLASSOV, V. V. MOROZOVA, I. V. BABKIN, N. V. TIKUNOVA

Bacteriophages: 100 years in the service of mankind

In the mid-last century, the science of biology made a leap forward when it established the molecular basis of living systems functioning. A crucial role in the successful research that resulted in determining the chemical nature of genetic molecules, breaking the genetic code and creating the gene manipulation technologies was played by the bacteriophages discovered at the beginning of the last century. Today, these bacterial viruses have learnt many jobs useful for people: they can be used not only as safe anti-infective drugs but also as disinfectants and even as basic elements of electronic nanodevices

One of the best-studied viruses, bacteriophage T4, targeting *Escherichia coli*. *Glass. Luke Jerram Gallery*

When in the 1930s a group of scientists took up the problems of living systems functioning, their search for the simplest models lead them to *bacteriophages* – bacterial viruses extensively studied and applied in clinical practices. Indeed, there are no biological objects simpler than bacteriophages; in addition, they are easy to grow and analyze and have small genetic programs.

A phage is a minimal-sized natural structure that contains a densely packaged DNA or RNA genome and has nothing extra. It is encapsulated in a protein shell with a minimal set of devices designed to plant it into a bacterial cell. Bacteriophages cannot replicate on their own, and in this respect cannot be considered proper living objects. Their genes can only start working inside a bacterium with the help of its biosynthetic systems and stock of molecules necessary for synthesis. However, the genomes of these viruses are not fundamentally different from the genomes of more complicated organisms, therefore experiments on bacteriophages have allowed scientists to establish the guiding principles of the genome structure and work.

Subsequently, this knowledge and techniques developed as part of the study provided the basis for the development of biological and medical sciences as well as a wide range of biotechnological applications.

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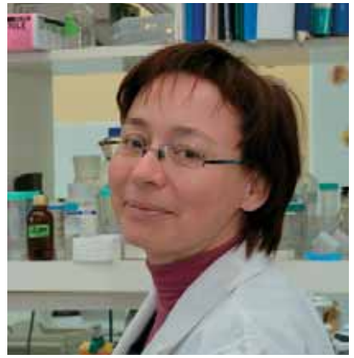

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Key words: bacteriophage, phage therapy, enzymes, phage display, nanomaterials

Bacteriophages are our friends when we deal with bacteria pathogenic for humans. However, there are other, human-friendly bacteria employed by modern biotechnological productions as well as by conventional food processing, such as cheese making. Here, the phages can do a lot of harm since intensively growing large populations of microorganisms create favorable conditions for phage reproduction, which results in the lysis of industrial bacterial cultures. For cheese makers this is not a major concern because, as a rule, they use ferments consisting of many cultures, some of which can withstand the phage attack and maintain lactic fermentation. Serious difficulties emerge when just one specific bacterial strain is employed, for instance, in the production of antibiotics or therapeutic proteins

Fighting pathogens

The first attempts to use bacteriophages for treating bacterial infections were made almost directly after they were discovered, though the lack of knowledge and imperfect biotechnologies of the time prevented unconditional success. Nevertheless, further clinical practice showed that in principle bacteriophages could be successfully applied to treat gastrointestinal, urogenital and surgical infections as well as acute purulent-septic conditions.

In contrast to antibiotics, bacteriophages have a number of advantages: they have no side effects and are strictly bacterium-specific so do not disrupt the normal human microbiome. On the other hand, this high selectivity creates problems: a successful treatment requires an exact identification of the infectious agent and an individual selection of a bacteriophage.

Phages can be used for preventive purposes. For instance, the G.N. Gabrichevsky Moscow Scientific Research Institute for Epidemiology and Microbiology has developed FOODPHAGE, a prophylactic product made of a cocktail of bacteriophages to reduce the risk of acute enteric infections. Clinical research has shown that the administration of FOODPHAGE during a week allows the patients to get rid of the hemolyzing bacterium and other pathogenic and potentially pathogenic bacteria causing intestine dysbacteriosis.

Bacteriophages are suited for treating not only human infectious diseases but also these of domestic and livestock animals: mastitis in cows, colibacteriosis and colibacillosis in calves and pigs, salmonellosis in chicken... Phage drugs are especially easy to use for aquaculture – to treat farmed fish and shrimps – as they keep for a long time in the water. Bacteriophages can help to protect plants, too, though in this case the application of phage technologies is handicapped by exposure to sunlight and rain, which are destructive to the viruses.

Phages can be valuable for maintaining the microbiological safety of food products as antibiotics and chemical agents applied in food industry do not solve this problem. Moreover, they are not environmentally friendly. The scale of the issue can be demonstrated statistically: every year up to 40,000 people infected by salmonellosis are recorded in the USA and in Russia, and 1% out of them die. The spread of this disease is largely connected with the farming, processing and consuming different kinds of poultry, and attempts to apply bacteriophages have produced promising results.

Thus, the American company *Intralytix* makes phage products targeting Listeria, Salmonella and bacterial contamination. The have been licensed as additives preventing bacterial reproduction in foods; phages are sprayed over meat and poultry products, as well as over fruit and vegetables. Experiments have shown that a phage cocktail can be successfully applied in transporting and selling live pond fish to decrease the bacterial contamination of the fish and of the water.

An evident application of bacteriophages is *disinfection*, i.e. destroying bacteria in the places that should be clean of them, like hospitals and food processing facilities. For this purpose, the British company *Fixed-Phage* has developed a technology for fixing phage products on diverse surfaces, which keeps the phage biologically active for up to three years.

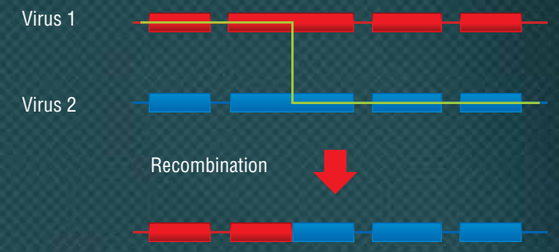
The seven days of Creation

Modern methods of synthetic biology allow not only modifying the phage genomes but also creating completely artificial active phages. This is not a big technological challenge: you only need to synthesize a phage genome and introduce it into a bacterial cell, and the genome on its own will start all the processes necessary to synthesize proteins and assemble new phage particles. In modern laboratories, this will take just a few days.

Genetic modifications serve to change the phage specificity and increase the efficacy of phage therapy. To achieve this, the most aggressive phages are fitted with recognition structures binding them with the target bacteria. In addition, virus genomes are imbedded with genes coding the proteins toxic for bacteria, which disrupt metabolism – such phages are more deadly for bacteria.

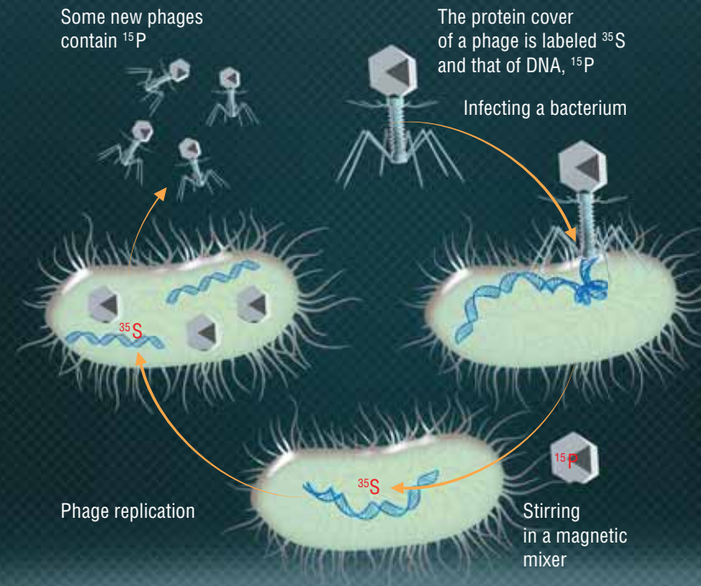
BACTERIOPHAGES AS FRUIT FLIES OF MOLECULAR BIOLOGY

In 1946, at the 11th Symposium held in the famous Cold Spring Harbor Laboratory, the one gene- one enzyme hypothesis was enunciated. The bacteriologist A. Hershey and a "former" physicist, molecular biologist M. Delbrück reported an exchange of genetic traits between different phages when *E.coli* cells were simultaneously infected by them. This discovery made at the time when the physical carrier of a gene was not known suggested that recombination, a shuffling of genetic traits, is inherent not only in higher organisms but in viruses as well. This discovery made possible the later detailed study of molecular replication mechanisms. In the years that followed, experiments with bacteriophages allowed establishing the principles of generic programs' structure and functioning.



Max L.H. Delbrück and his colleagues investigated mutant bacteriophages of the so-called T series targeting *E.coli*

In 1969, A. Hershey, M. Delbrück and their colleague S. Luria were awarded the Novel Prize "for their discoveries concerning the replication mechanism and the genetic structure of viruses



The experiments conducted by the Alfred Hershey and Martha Chase used bacteriophages labeled with sulfur and phosphorus isotopes and proved that DNA carries genetic information

In 1952, A. Hershey and M. Chase showed by experiments that the genetic information of bacteriophage T2 is encoded, contrary to a popular belief, not in proteins but in DNA molecules (Hershey & Chase, 1952). The researchers observed reproduction in two groups of bacteriophages, one with radioactively labelled proteins and the other with DNA molecules. Bacteria were infected with these phages, and it turned out that only the viral DNA is transferred to infected cells, which proved the role of DNA in storing and transferring genetic information.

In the same year, the American geneticists J. Lederberg and N. Zindler carried out experiments using two strains of Salmonella and bacteriophage P22. They showed that in the process of reproduction a bacteriophage could incorporate the DNA fragments of the host bacterium and transfer them to other bacteria infected by it (Zinder & Lederberg, 1952). This gene transfer from a host bacterium to a recipient was called transduction. The experimental results confirmed again the role of DNA in transferring information for inheritance.

In 1972, R. Beard and his colleagues, when investigating the replication (making a copy of cell information) of *E.coli* DNA, used bacteriophages as probes that can build into a bacterial cell genome and revealed that the process of replication goes in two directions along the chromosome (Stent, 1974)

ENZYMES "FROM BACTERIOPHAGE"

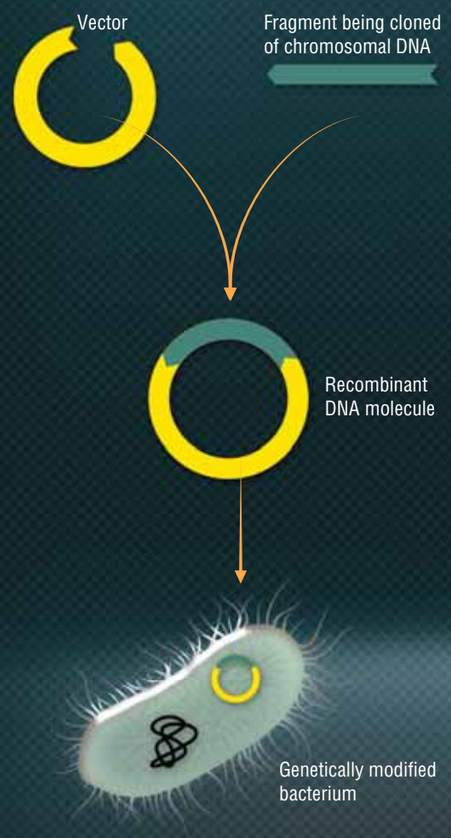
Research into bacteriophages resulted in the discovery of a great number of enzymes widely used today in molecular biology and genetic engineering. An example is restriction enzymes – a group of bacterial nucleases breaking down DNA. Back in the early 1950s, it was discovered that phages isolated from the cells of one bacterial strain tend to reproduce poorly in closely related strains. This meant that bacteria have a system for suppressing viral reproduction (Luria & Human, 1952). As a result, a restriction-modification system was found, a defense mechanism protecting bacteria against the invasion of alien DNA. The discovery of restriction enzymes (restriction endonucleases) supplied microbiologists with a priceless tool allowing manipulations with DNA: they learned to build some sequences into others and to cut the fragments needed, which ultimately led to the creation of recombinant DNA.

Another enzyme widely used in molecular biology is bacteriophage T4 DNA ligase, which joins the sticky and blunt ends of DNA and RNA double-stranded molecules. Recently, genetically modified variants of this enzyme have been developed that feature a higher activity.

Bacteriophages are the progenitors of most RNA ligases used in laboratory practices, which join single-stranded RNA and DNA molecules. In vivo, their main function is to repair broken RNA molecules. The most popular with researchers is bacteriophage T4 RNA ligase, which helps to attach single-stranded polynucleotides to RNA molecules in order to label them. The same technique applies to exploring the RNA structure, the binding sites of RNA and proteins, oligonucleotide synthesis, and so on. Newcomers to the class of routinely used enzymes are thermally stable RNA ligases extracted from bacteriophages rm378 and TS 2126 (Nordberg Karlsson, *et al.*, 2010; Hjorleifsdottir, 2014).

Some polymerases, vitally important enzymes, have also been derived from bacteriophages. An example is the highly accurate phage T7 DNA polymerase, which has found application in various areas of molecular biology such as site-directed mutagenesis though it is mostly used to determine the primary structure of DNA.

The chemically modified phage T7 DNA polymerase was suggested as an ideal tool for sequencing DNA back in 1987 (Tabor & Richardson, 1987). A modification of this polymerase increased its efficiency by several times: DNA polymerization rate is higher than 300 nucleotides per second, so it can be used to amplify large DNA fragments. This enzyme became the predecessor of sequenase – a genetically modified enzyme used for Sanger sequencing. Sequenase is highly efficient and is able to include into a DNA sequence the nucleotide analogs used to improve the sequencing results.



Conventional cloning technique (incorporation of an alien DNA) using plasmid – an extrachromosomal genetic element typical of many bacterial stains – as a vector begins with cutting the plasmid DNA and cutting out the necessary fragment of chromosomal DNA with the help of the restriction enzyme. Then the fragment of the DNA being cloned is built into the plasmid inserted into a bacterium; as a result, the latter acquires the ability to produce alien protein encoded in the built-in fragment

The first fully sequenced DNA-genome was the genome of the phage φ174, over 5,000 nucleotides long (Sanger *et al.*, 1977). The sequencing was performed by the group of the English biochemist F. Sanger, the author of the DNA sequencing method of the same name

Originating from bacteriophages are basic RNA polymerases (DNA-dependent RNA polymerases), enzymes catalyzing the process of transcription (reading RNA copies from a DNA matrix). Here belong SP6-, T7- and T3-RNA polymerases called after the respective bacteriophages SP6, T7 and T3. These enzymes are used to synthesize in vitro antisense RNA transcripts, labeled RNA probes, etc.

Polynucleotide kinases catalyze the transfer of the phosphate group from the molecule ATP to 5' end molecule of nucleic acid, exchange of 5' phosphate groups or phosphorylation of 3' ends of mononucleotides. The most commonly used in laboratory practices is the polynucleotide kinase of bacteriophage T4. Its applications include experimenting with DNA labeling using phosphorus radioactive isotope, seeking restriction sites, DNA and RNA dactylography, and substrate synthesis for DNA or RNA ligases.

Molecular-biological experiments actively engage such bacteriophage enzymes as phage T4 polynucleotide kinase, commonly used for DNA labeling with radioactive phosphorous isotope, DNA and RNA dactylography, etc., as well as the DNA cleaving enzymes used to obtain single-stranded DNA matrices for sequencing and analyzing nucleotide polymorphism

Bacteria have several mechanisms protecting them from antibiotics and bacteriophages; one such mechanism is the destruction of virus genomes by *restriction enzymes* targeting specific nucleotide sequences. The therapeutic potency of phages can be improved through "reformatting" their gene sequence at the expense of gene code degeneration in such a way as to minimize the number of nucleotide sequences "sensitive" to the enzymes, at the same time preserving their coding properties.

A universal method for protecting bacteria from any environment are the so-called *biofilms*, films containing DNA, polysaccharides and proteins and made jointly by some bacteria, which are proof against both antibiotics and therapeutic proteins. These films are pain in the neck for doctors as they facilitate the destruction of enamel, form on the surface of implants, catheters, and artificial joints as well as in airways, on the skin surface and so on. To deal with biofilms, special bacteriophages were designed containing a gene coding the specific lytic enzyme that destroys bacterial polymers.

The synthetic biology methods have helped to develop the bacteriophages equipped with most sophisticated weapons, which are used by bacteria against the phages themselves. These are *CRISPR-Cas* bacterial systems, which combine a DNA-cleaving nuclease and an RNA-sequence directing this enzyme towards a specific fragment of the virus genome. The "pointer" is a bit of the phage DNA that the bacterium keeps "for memory" in a special gene. When this protein-nucleotide complex discovers an analogous fragment inside a bacterium, it destroys it.

Having handled the principle of CRISPR-Cas operation, researchers tried to equip phages with a similar "weapon": for this purpose, a complex of genes was introduced into the phage genome that codes the nuclease and RNA addressing sequences complementary to the specific sites of the bacterial genome. "Targets" can be the genes responsible for multiple drug resistance. The experiments were a complete success: the phages efficiently defeated the bacteria to which they had been "tuned."

Phage antibiotics

Therapy does not necessarily require phages as such. Over millions of years of their evolution, bacteriophages have developed an arsenal of specific proteins – tools for recognizing the target microorganisms and manipulations with the victim's biopolymers, based on which antibacterial drugs can be designed. The most promising proteins of this kind are the endolysin enzymes used by phages to destroy the cell wall when leaving a bacterium. These enzymes as such are powerful antibacterial substances not toxic for humans. Their efficacy and ability to hit the target can be improved by altering their addressing structures – proteins specifically binding with certain bacteria.

With respect to the cell wall structure, most bacteria fall into gram-positive (their membrane is covered with a very thick layer of peptidoglycans) and gram-negative (the peptidoglycan layer is between the two membranes). The application of natural endolysins is especially efficacious in gram-positive bacteria (staphylococci, streptococci, etc.) as their peptide-glycan layer is external. The gram-negative bacteria (*Pseudomonas aeruginosa*, salmonellas, colibacillus, etc.) are a target more difficult to hit since the enzyme has to penetrate the outer bacterial membrane in order to approach the internal peptide-glycan layer.

To deal with this problem, the so-called *artilysins* were created – modified variants of natural endolysins containing polycationic or amphipathic peptides, which destabilize the outer membrane and deliver endolysin directly to the peptide-glycan layer. Artilysins feature a high germicidal activity and have proved efficacious in treating otitis in dogs (Briers *et al.*, 2014).

An example of modified endolysin selectively targeting certain bacteria is the drug P128 developed by the Canadian company *GangaGen*, Inc. It is a biologically active fragment of endolysin combined with *lysostaphin* – an addressing protein molecule binding with the surface of staphylococci cells. The fusion protein obtained is highly active against a variety of staphylococcus strains including these with multiple drug resistance.

Bacteria "counters"

In addition to being diverse therapeutic drugs and "disinfectants," bacteriophages are valued by microbiologists as convenient and precise analytical tools. For instance, owing to their high specificity they are natural analytical reagents capable of detecting the bacteria of a certain species and strain.

In the easiest version of this investigation, diverse diagnostic bacteriophages are added, drop by drop, in the Petri dish with a nutritional medium seeded with a bacterial culture. If a bacterium turns sensitive to a phage, the bacterial "lawn" develops a "patch" – a transparent area with killed and lysed bacterial cells.

Analyzing phage replication in the presence of target bacteria, we can evaluate the number of the latter. Since the number of phage particles in the solution increases in proportion to the number of the bacterial cells it contains, to evaluate the number of the bacteria we need only to determine the titer of the bacteriophage.

The specificity and sensitivity of this analytical reaction is quite high; the procedures are easy to carry out and do not require any sophisticated equipment. Importantly, the bacteriophage-based diagnostic systems signal the presence of a living pathogen itself whereas other methods, such as PCR and immuno-analytical, show only the presence of the biopolymers belonging to this bacterium. The diagnostic methods of this kind are especially convenient to use in ecological research as well as in food industry and agriculture.

Today, to detect and estimate the number of the various strains of microorganisms, special *reference species* of phages are used. Very fast, functioning virtually in the real-time mode, analytical systems can be created on the basis of genetically modified bacteriophages, which, on entering a bacterial cell, launch the synthesis of fluorescent (or capable of luminescence) reporter proteins, such as *luciferase*. When the necessary substrates are added to such a medium, it will generate a luminescent signal with the intensity proportional to the content of bacteria in the sample. These "light-labeled" phages have been developed to detect dangerous pathogens – agents of plague, tuberculosis, as well as of plant infections.

Probably, modified phages will help to deal with an old problem of global importance: the development of cheap and rapid techniques for detecting tuberculosis agents at an early stage of the disease. This is a big challenge as the mycobacteria causing tuberculosis grow very slowly when cultivated in laboratory conditions. Therefore, conventional diagnostics may take a few weeks.

Phage technology speeds up this task. The point of the technology is adding the bacteriophage D 29 targeting a wide range of mycobacteria to the blood samples being analyzed. After that, the bacteriophages are separated, and the sample is mixed with a fast-growing non-pathogenic mycobacterial culture, which is also sensitive to this bacteriophage. If the blood originally had mycobacteria infected with phages, the new culture will also produce the bacteriophage. In this manner, single mycobacterial cells can be detected, and the diagnostics takes just 2—5 days instead of 2—3 weeks (Swift & Rees, 2016).

Phage display

Today, bacteriophages are widely applied as simple systems to produce tailor-made proteins. We are talking about *phage display* – a most efficient molecular selection technique developed in the 1980s. The term was coined by an American, George Smith, who proved that *Escherichia coli* phages could be used to produce a viable modified virus with a foreign protein on its surface. To accomplish this, an appropriate gene is inserted into the phage genome, which merges with the gene coding a surface virus protein. These modified bacteriophages can be isolated from a mixture of wild-type phages owing to the "foreign" protein's ability to bind with specific antibodies (Smith, 1985).

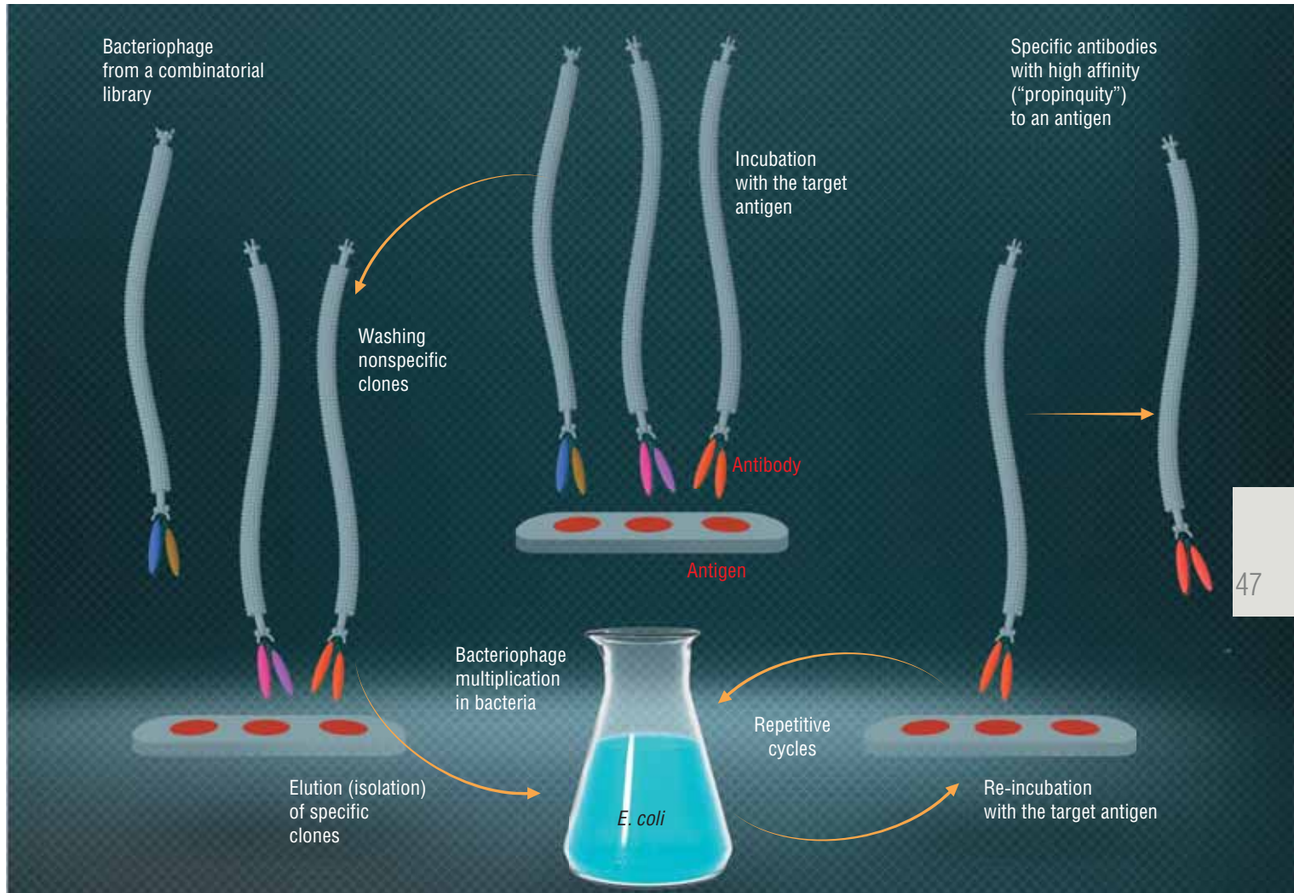
From Smith's experiments two important conclusions followed: first, using the recombinant DNA technology, you can generate a vast variety of populations numbering 10^6—10^{14} phage particles, each of them carrying on its surface different variants of proteins. These populations were called *combinatorial phage libraries*. Secondly, a specific phage (for example, a phage able to bind with a certain protein or an organic molecule) isolated from a population can be replicated in bacterial cells to produce an infinite number of descendants with given properties.

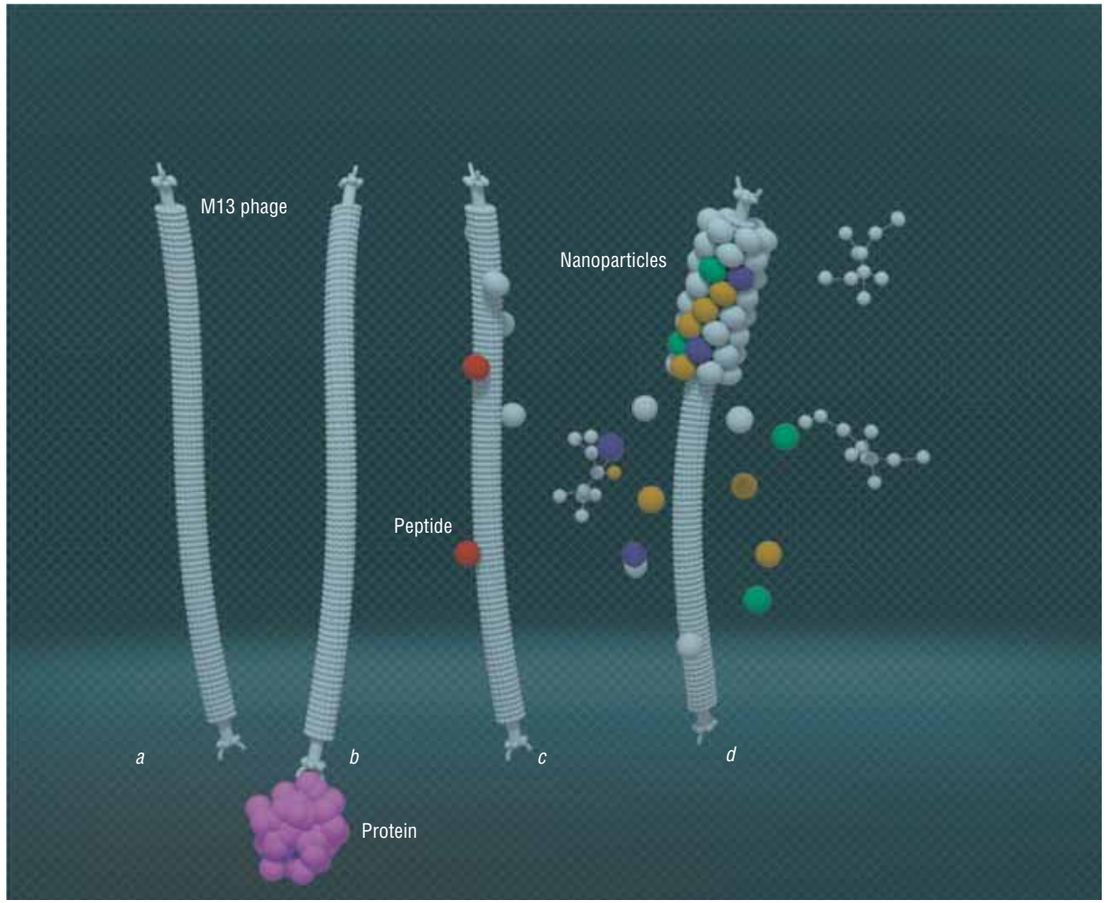
Today, phage display is used to make proteins that can bind selectively with therapeutic targets; for instance, the proteins exposed on the surface of the phage M13 are able to recognize and interact with tumor cells. The function of these proteins in a phage particle is to "pack" nucleic acids; therefore, they are well suited to produce gene therapy drugs, only in this case they form a particle with a therapeutic nucleic acid.

The two main current application areas of a phage display are the peptide-based technology and protein-based and domain-based technology. The former is used to study receptors and to map the antibody-binding sites, to create immunogens and nanovaccines as well as to map the substrate-binding sites in proteins-enzymes. The protein-based and protein domain-based technology helps to select antibodies with given properties, to study protein-ligand interactions, and to screen the expressed fragments of a complementary DNA and directed protein modifications.

Phage display can be used to introduce recognition groups into all species of surface virus proteins as well as into the main protein forming the bacteriophage body. The introduction of peptides with given properties into

The schematic diagram of biopenning – selection of recombinant antibodies highly specific to a concrete antigen target – from the combinatorial library of the phage display based on filamentous bacteriophages. From: (Tikunova, Morozova, 2009)





The filamentous bacteriophage M13 replicating in an ordinary Escherichia coli (*a*) can carry on its surface alien recombinant proteins such as antibodies (*b*) or peptides (*c*). Also, it can serve as a template for making nanodevices and nanomaterials such as nanocrystallic catalyst with a known surface area and the necessary pore distribution (*d*)

surface proteins gives you a whole range of valuable biotechnological products. For example, if this peptide imitates the protein of a dangerous virus or bacteria recognized by the immune system, the modified bacteriophage is a vaccine that can be produced easily, quickly and safely.

If you "address" the end surface protein of a bacteriophage to cancer cells, and add reporter groups (e.g., fluorescent or magnetic) to another surface protein, you will obtain a tumor detector. And if you add a cytotoxic drug to the particle (which can be done easily with the help of modern bioorganic chemistry), you will get an anti-tumor drug.

An important application of the protein phage display technique is the creation of the phage libraries of recombinant antibodies, where the antigen-binding fragments of immunoglobulins are placed on the surface of phage particles fd or M13. Of special interest are the libraries of human antibodies because these antibodies can be used in therapy without limitations. In the recent years, the pharmaceutical market of the USA alone has offered more than a dozen of therapeutic antibodies developed using this method.

Technologies allowing arranging filamentous bacteriophages one by one (tail-to-tail) have been developed. The resulting multiphage structures are ordered nano-matrices that can be applied to make transistor and diode devices

"Industrial" phages

Along with the uses described above, the phage display technique has found a completely unexpected application. Bacteriophages are primarily nano-sized particles of a certain structure with proteins located on their surface. With the help of phage display, the phages can acquire the property of binding specifically with the necessary molecules. These particles offer a widest range of opportunities to create materials with a given architecture and smart molecular nano-devices using eco-friendly production technologies.

Since the virus is a rigid structure governed by a certain dimensional equation, it can be used to make porous nano-structures with a given surface area and the necessary distribution of pores within its structure. As is known, the surface area of a catalyzer is a critical parameter defining its efficiency. Current technologies for forming a thinnest layer of metals and their oxides on the bacteriophage surface make possible catalyzers with an extraordinarily developed regular surface with the dimensions prescribed. (Lee *et al.*, 2012).

The researcher from the Massachusetts Institute of Technology A. Belcher used bacteriophage M13 as a template for growing rhodium and nickel nanoparticles and nanowires on the surface of ceric oxide. The catalyst nanoparticles obtained encourage the conversion of ethanol to hydrogen – in this manner, this catalyst may prove valuable for modernizing the existing hydrogen fuel cells and generating new ones. As compared with an analogous "conventional" catalyst, the catalyst grown from a virus template has a higher stability and is less prone to ageing and surface deterioration (Nam *et al.*, 2012).

The coating of filamentous phages with gold and indium dioxide has produced electrochromic materials – porous nanofilms changing color with a change of the electric field 1.5 times quicker than the analogues known. Such materials are promising for making energy-conserving ultrathin displays (Nam *et al.*, 2012).

In the Massachusetts Institute of Technology, bacteriophages have become the basis for the production of powerful and exceedingly compact batteries. For this purpose, living and genetically modified phages M12 were used, which are benign for people and are able to attach to their surface the ions of various metals. The self-assembly of these viruses resulted in the structures of a preset configuration, which, when coated with metal, formed sufficiently long nanowires that became the basis for the anode and cathode. The self-formation of the anode material employed the virus capable of attaching gold and cobalt oxide, and in the case of the cathode material, it was a virus capable of attaching iron phosphate and silver. The final phage also had the ability to "connect" the ends of the carbon nanotube, which is necessary for an efficient transfer of electrons.

In addition, bacteriophage M13, titan dioxide and single-wall carbon nanotubes have served to create materials for solar batteries (Dang *et al.*, 2011).

The recent years were marked with extensive studies of bacteriophages, which find themselves new applications not only in therapy but also in bio- and nanotechnologies. An evident practical result should be the emergence of a new large area of personalized medicine as well as the development of a wide range of technologies in food industry, veterinary medicine, agriculture and production of modern materials. We expect that another hundred years of bacteriophage research has at least as many discoveries in store as the first one.

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Viruses and Bacteria: The Great Confrontation

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The modern genome editing technology, which has been used successfully on various animals, plants, fungi, and bacteria, is based on research about CRISPR-Cas bacterial systems. Originally, they were thought to participate in DNA repair, but in 2007 scientists found out the real purpose of these systems: they combat bacterial viruses, i.e., bacteriophages. It took scientists as little as nine years to make a giant leap from understanding the mechanism of bacterial immunity to human genome editing, and now they are making the first experiments on editing the DNA of human embryos. Moreover, bacteria have other immune mechanisms too, and studying them might lead to new breakthroughs in biomedicine

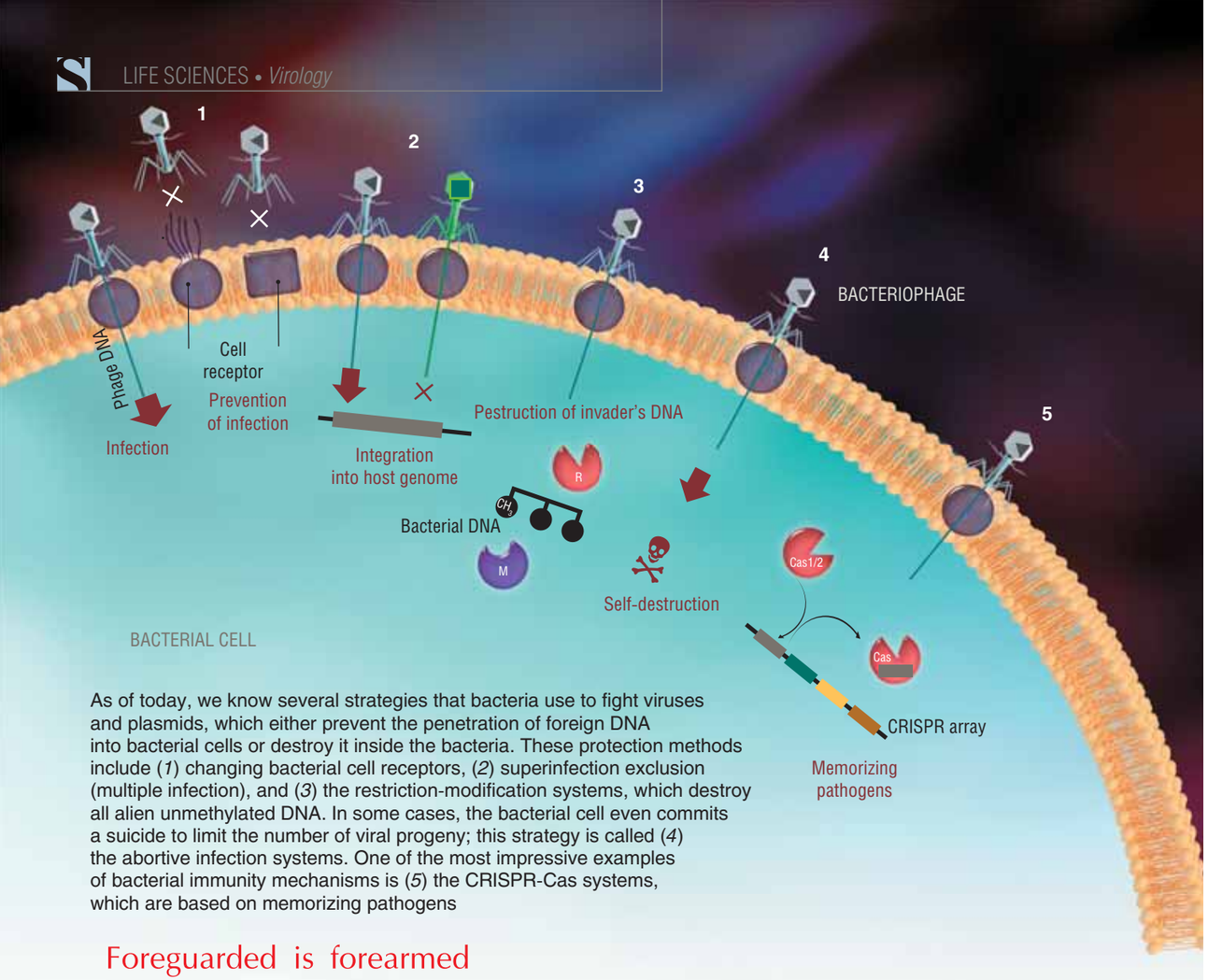
Key words: bacteriophages, bacterial defense mechanisms, CRISPR-Cas systems, genome editing

Bacteriophages are viruses that exclusively attack bacteria. During infection, they take under control all the life processes of the bacterial cell, virtually transforming it into a factory to produce viral progeny. Eventually, the cell dies, and the new viral particles come out to infect new bacteria.

Although natural phages are very abundant and highly diverse, we rarely encounter them face-to-face. However, there are situations when the activity of these viruses does not go unnoticed. For example, producers of cheeses, yoghurts, and other lactic acid products often have to deal with viral attacks on milk-fermenting bacteria. In most of these cases, the phage infection spreads fast and "good" bacteria die, leading to significant economic losses (Neve *et al.*, 1994).

To address this challenge of the dairy industry, scientists have obtained bacteriophage-resistant strains of lactic acid bacteria. It was these applied studies that revealed the specific mechanisms of how bacteria can avoid infection. Simultaneously, this research revealed the ways of how viruses overcome bacterial defenses (Moineau *et al.*, 1993).

In the course of evolution, there has been and still is selection for bacteria able to survive viral infection, which, in turn, motivates bacteriophages to improve their aggressive strategies. This "arms race," which has gone on for billions of years, i.e., for as long as bacteria and their enemies exist, has given rise to a range of sophisticated defense and attack mechanisms



As of today, we know several strategies that bacteria use to fight viruses and plasmids, which either prevent the penetration of foreign DNA into bacterial cells or destroy it inside the bacteria. These protection methods include (*1*) changing bacterial cell receptors, (*2*) superinfection exclusion (multiple infection), and (*3*) the restriction-modification systems, which destroy all alien unmethylated DNA. In some cases, the bacterial cell even commits a suicide to limit the number of viral progeny; this strategy is called (*4*) the abortive infection systems. One of the most impressive examples of bacterial immunity mechanisms is (*5*) the CRISPR-Cas systems, which are based on memorizing pathogens

Foreguarded is forearmed

As of today, we know five major—quite cunning—defense mechanisms that bacteria have developed in their incessant struggle against viruses: changing the cell membrane receptor; superinfection exclusion; the abortive infection systems; the restriction–modification systems; and, finally, the CRISPR-Cas systems.

A viral attack begins when a phage attaches through a specific receptor on the surface of a bacterial cell. However, if the receptor is lost or its structure is changed, there is no viral binding. Bacteria can change their receptors depending on environmental conditions such as density and diversity of microorganisms in the media and availability of nutrients (Bikard *et al.*, 2012). An interesting example is bacteria of the species *Vibrio anguillarum*, which can create a biofilm, i.e., a dense cell layer attached to a surface. These bacteria have quorum sensing feature: when the density of the cells increases, they lower the production of the receptor the virus can bind. Thus, the biofilm becomes almost totally resistant to infection (Tan *et al.*, 2015).

However, the bacterium may suffer from losing its receptors since they perform a variety of important functions, e.g., the transport of nutrients or the formation of intercellular contacts (Lopez-Pascua *et al.*, 2008). Thus, each bacterium–bacteriophage pair has found, in the course of the evolution, an optimal solution ensuring an acceptable level of protection while preserving the possibility of bacterial growth in various environmental conditions.

The next protective mechanism is superinfection exclusion. It is known that bacteriophages can infect bacteria in two main ways: the lytic way, which leads to the rapid death of the infected bacterium with a release of viral progeny, and the lysogenic way, when the viral genetic material integrates into the bacterium's genome and replicates with the host DNA, without causing any damage to the cell. When a cell becomes a lysogen, infection with

In 1978, the discovery of restriction enzymes was awarded with the Nobel Prize, which went to a Swiss geneticist Werner Arber and American microbiologists Daniel Nathans and Hamilton Smith. Research into the restriction–modification systems led to the development of molecular cloning technology, which is now widely used throughout the world. Restriction enzymes help cut out genes from the genome of one organism and insert them into that of another organism to obtain chimeric recombinant DNA that does not exist in nature. Scientists use various modifications of this approach to isolate and investigate individual genes. In addition, this enzyme is widely used in pharmaceutics, e. g., for the production of insulin or therapeutic antibodies: all such drugs have been developed by molecular cloning, i. e., are a product of gene modification

any other phage is unwanted for the intracellular virus (prophage).

Indeed, many viruses that have embedded their DNA into the cell genome will restrain a newly arrived bacteriophage (superinfection) by means of special repressor proteins that do not allow the invader's genes to work (Calendar, 2006). Moreover, some phages even prevent other viral particles from penetrating into the infected cell by affecting its receptors. As a result, a bacterium infected with the virus has an obvious advantage over its uninfected siblings.

During infection, all the resources of the bacterial cell are channeled into the production of new viral particles. If any vulnerable bacteria happen to be near such a cell, the infection will spread quickly and kill most of them. However, for such cases, the bacterium has the so-called abortive infection systems, which guide it to a programmed death. Of course, this altruistic mechanism will not save the infected cell itself, but it will stop the spread of the viral infection for the benefit of the entire population. The abortive infection systems in bacteria are very diverse, but the details of their functioning have not been adequately studied yet.

Another type of antiviral defenses in bacteria is the restriction–modification systems, which include genes coding two enzyme proteins—restriction enzyme and methylase. Restriction enzyme recognizes certain DNA sequences 4–6 nucleotides long and makes double-strand breaks in them. Methylase, on the contrary, covalently modifies these sequences by adding methyl groups to individual nucleotide bases to make them unrecognizable for the restriction enzyme.

All the DNA sites in a bacterium containing such a system are modified. Then, if the bacterium is infected with a virus whose DNA contains no such modification, restriction enzyme will protect it from infection by destroying the viral DNA. Many viruses combat the restriction–modification systems by not using genome sequences recognizable by restriction enzyme; obviously, virus variants with a different strategy have not left any offspring.

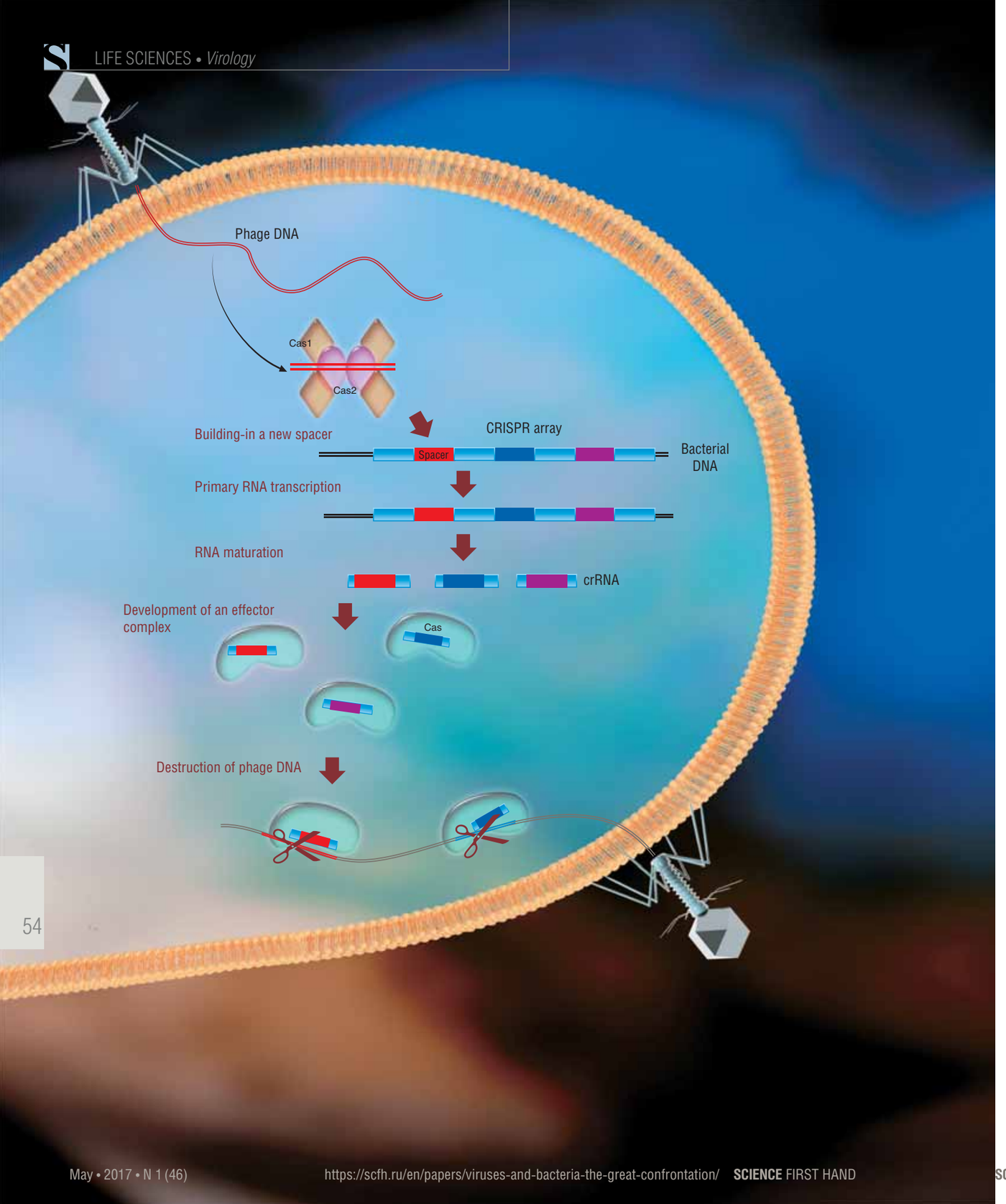
The last and currently most fascinating system associated with bacterial immunity is the CRISPR-Cas system, which allows bacteria to record information about the phages they have encountered in their life into their genome and pass it on to the daughter cells. These memories make it possible to recognize phages DNA and to resist it more effectively in the case of repeated infections. The CRISPR-Cas systems are currently in focus of research as they are the core of the revolutionary genome editing technology, which might help future generations to treat genetic diseases and create new breeds and varieties of agricultural animals and plants.

Know your enemy

The CRISPR-Cas systems are a unique example of adaptive immunity in bacteria. When a phage injects DNA into the cell, Cas proteins incorporate viral DNA fragments of 25—40 nucleotides in lenght into a specific region of the bacterial genome (Barrangou *et al.*, 2007). These fragments are called spacers; the chromosome region where spacers are incorporated is called a *CRISPR array* (Clustered Regularly Interspaced Short Palindromic Repeats); and the very process of acquisition of spacers is called adaptation.

For a cell to use spacers in its fight against phage infection, there is another process controlled by Cas proteins, the so-called interference. Its idea is as follows: the CRISPR array transcription creates a long RNA molecule, which is cut by Cas proteins into short sequences called protective CRISPR RNAs (crRNAs), each containing one spacer. The Cas proteins together with the crRNA molecule form an effector complex, which scans the cell's entire DNA for sequences identical to a given spacer (protospacers). When the effector complex finds protospacers, the Cas proteins cleave them (Westra *et al.*, 2012; Jinek *et al.*, 2012).

CRISPR-Cas systems have been found in most prokaryotes—bacteria and archaea. All the known CRISPR-Cas systems work on the same principle; however, the details of their individual mechanisms may differ considerably. The greatest differences are associated with the structure and functioning of the effector complex, which is why researchers distinguish between several types of CRISPR-Cas systems. As of today, the literature contains description of six types of unrelated CRISPR-Cas systems (Makarova *et al.*, 2015; Shmakov *et al.*, 2015).



The most studied system is the type I CRISPR-Cas system, which is found, e. g., in the bacterium *Escherichia coli*, a favorite object of molecular biology research. The effector complex in this system consists of several small Cas proteins, each of which is responsible for different functions: cutting the long non-coding crRNA, binding short crRNAs, and searching for and then cutting the target DNA.

The effector complex in the type II systems is formed by one big protein Cas9, which can do all the work on its own. It is the simplicity and relative compactness of these systems that guided the development of DNA editing technology. This is a method whereby the bacterial protein Cas9 and crRNA, which is called guide RNA (gRNA), are delivered into eukaryote cells (e.g., in humans). This gRNA contains, in place of a viral spacer, a target sequence consistent with a genome region that is of interest for research, e. g., where there is a disease-provoking mutation. Today, it is quite easy to obtain gRNA that would suit any taste.

The Cas9–gRNA effector complex makes a double-strand break in a DNA sequence matching the guide RNA. If we incorporate into the cell, together with Cas9 and gRNA, a DNA sequence without mutation, the broken region will be restored from the matrix of the correct copy! Thus, we can use different gRNAs to correct unwanted mutations and make directed changes in target genes. The Cas9–gRNA complex can be programmed to recognize targets with a very high accuracy, and this method is, on the whole, so simple that it triggered an exponential growth of research on genome editing of plants and animals (Jiang & Marraffini, 2015).

In the development of adaptive bacterial immunity, Cas1 and Cas2 bacterial proteins insert fragments of viral DNA as spacers into a CRISPR array, in which adjacent spacers are separated by DNA repeats (Nuñez *et al.*, 2014, 2015a, b). The CRISPR array is transcribed to form a long noncoding RNA. Cas proteins, as well as (in some cases) other bacterial proteins, cut this RNA into short CRISPR RNAs (crRNAs), each containing one spacer and a part of the repeat. In the course of interference, Cas proteins together with crRNAs form an effector complex that scans the cell's DNA in search of sequences corresponding to the crRNA spacer and cuts them (Westra *et al.*, 2012, Jinek *et al.*, 2012)

Being an environmental factor, bacteriophages induce targeted changes in bacterial genome, which are inheritable and give bacteria a distinct advantage by protecting them against repeated infection. Therefore, we can consider the CRISPR-Cas systems as an example of Lamarckian evolution, whereby acquired characteristics are inheritable (Koonin *et al.*, 2009)

Arms race

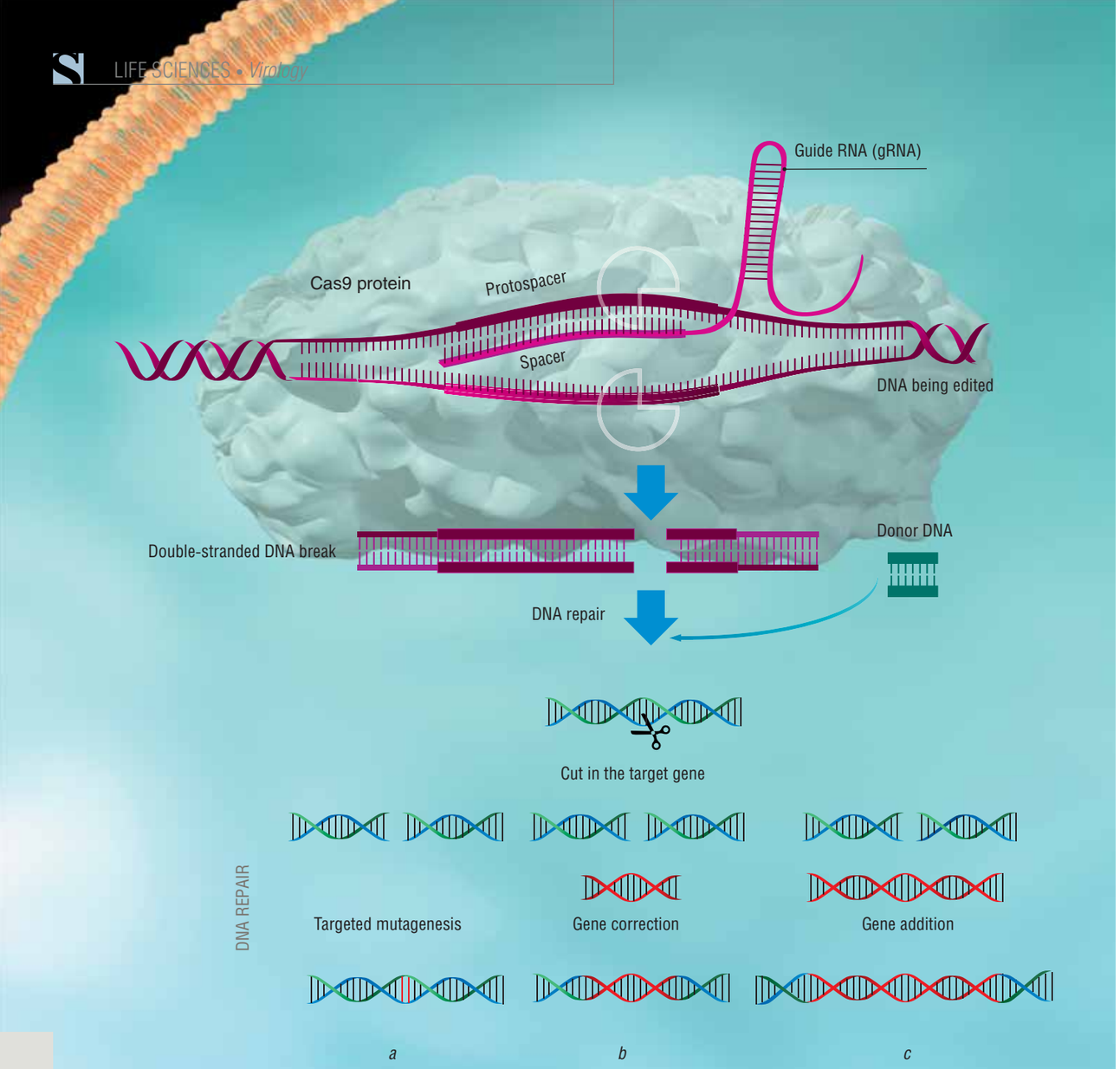
In the course of evolution, bacteria and bacteriophages developed a range of "arms" that either give them an advantage in fighting their enemy or an ability to evade the enemy's attack.

Speaking about CRISPR-Cas systems, if a phage develops a mutation in the protospacer, the effector complex will be less effective in recognizing the phage, giving it an opportunity to infect the cell. However, the bacterium will not ignore the attempt to circumvent CRISPR-Cas either: it will respond with a dramatic increase in the efficiency of acquisition of new spacers from the DNA of the already familiar, albeit mutated, phage. This phenomenon, called primed adaptation, multiplies the protective effect of CRISPR-Cas systems (Datsenko *et al.*, 2012).

Some bacteriophages respond to CRISPR-Cas systems in a bacterial cell by producing specific anti-CRISPR proteins that can bind with Cas proteins and block their functions (Bondy-Denomy *et al.*, 2015). Another contrivance is to replace the viral genome regions targeted by the CRISPR-Cas system by genome regions of related viruses with a different nucleotide sequence (Paez-Espino *et al.*, 2015).

The results obtained by our laboratory show that infected cells do die even when they have CRISPR-Cas protection, but they limit the quantity of viral progeny. Therefore, it would be more correct to consider CRISPR-Cas as abortive infection systems rather than real immune systems.

Given the ongoing advancement of bioinformatic search algorithms, coupled with the expansion of research scope to include more prokaryotic genomes into the analysis, it is reasonable to expect the discovery of new types of CRISPR-Cas systems in the near future. Another task is to find out the detailed mechanisms underlying the operation of many recently discovered systems. For example, an article published in *Science* in 2016 on the analysis of the type VI CRISPR-Cas system describes a C2c2 protein forming an effector complex with crRNA in order to degrade RNA rather than DNA (Abudayyeh *et al.*, 2016). In the future, such an unusual property may be used in medicine to regulate the activity of genes by changing the number of RNAs encoded by them.



The CRISPR-Cas system used in genome editing includes guide RNA (gRNA) and the Cas9 protein. The latter helps the gRNA attach itself to the protospacer, i. e., to a region of the viral DNA that matches the gRNA spacer (or, in the case of an artificial system, to a region of the target eukaryotic cell gene). Once the Cas9 protein recognizes a protospacer, it cuts the DNA strand in one strictly defined location. DNA repair at the cut site may take the form of (*a*) non-homologous end joining, which very often results in mutations. Another option is to deliver into the cell an artificially synthesized donor molecule matching the cut site; this way one can either (*b*) replace the gene site or (*c*) make directed insertion of a transgene. Thus, the CRISPR-Cas system can help correct genetic disorders or make the desired changes

Studies of the strategies used by bacteria in their struggle against bacteriophages might seem too theoretical and remote from the challenges of practical medicine. However, this research has brought invaluable benefits to mankind, as evidenced by the methods of molecular cloning and genome editing, i. e., directed introduction or removal of mutations and changes in the level of transcription of certain genes.

The rapid advancement of molecular biology methods allowed the development, only a few years after the discovery of the CRISPR-Cas action mechanism, of a working genome editing technology that is able to fight diseases that were previously deemed incurable. Widely available and simple, this technology might serve as a basis for human and veterinary medicine, agriculture, and biotechnology in the future, which will broadly apply directed and safe gene modifications.

There is no doubt that further research exploring the interactions between bacteria and their viruses will open new vistas that we now cannot even imagine.

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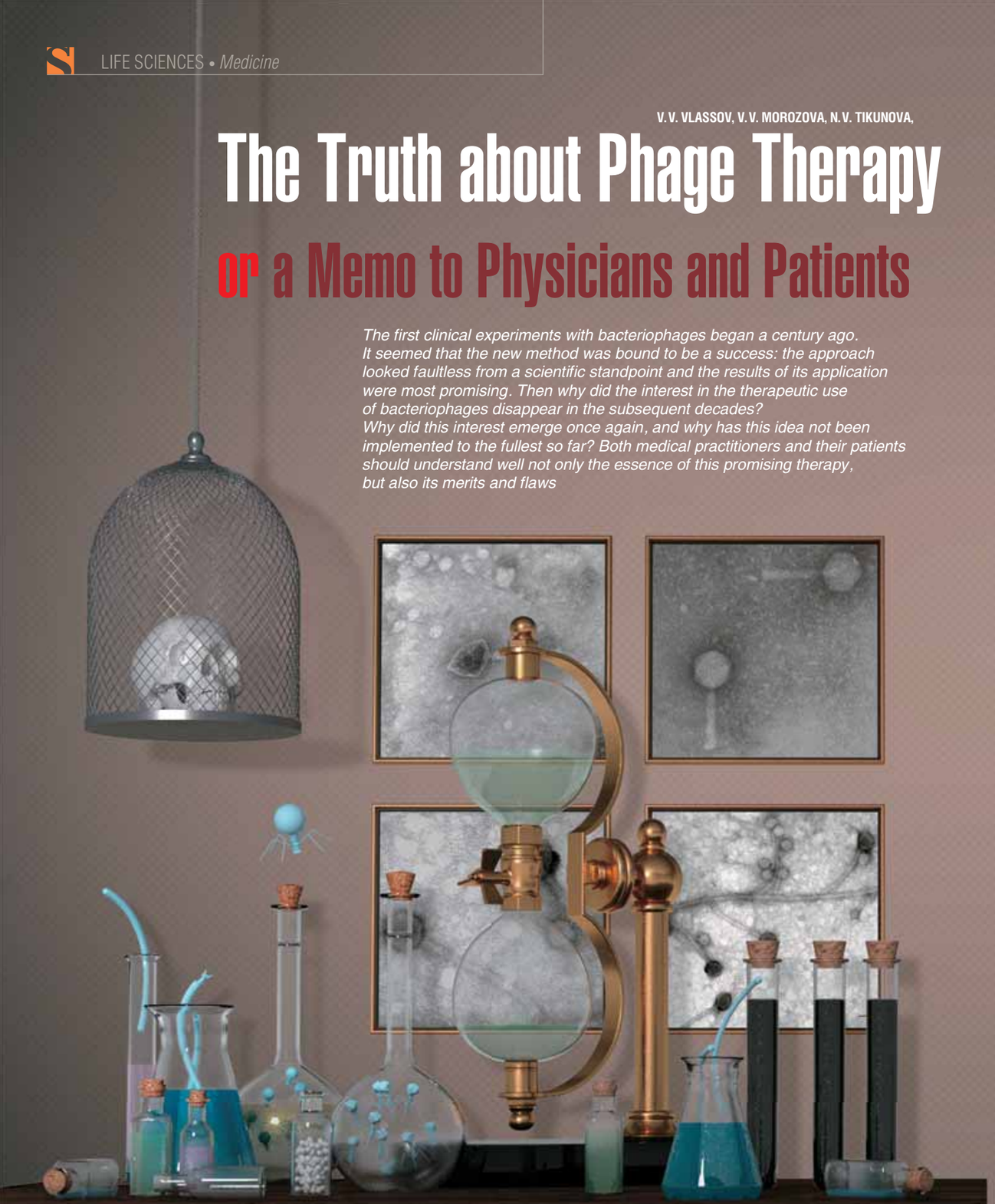
The Truth about Phage Therapy

or a Memo to Physicians and Patients

The first clinical experiments with bacteriophages began a century ago. It seemed that the new method was bound to be a success: the approach looked faultless from a scientific standpoint and the results of its application were most promising. Then why did the interest in the therapeutic use of bacteriophages disappear in the subsequent decades? Why did this interest emerge once again, and why has this idea not been implemented to the fullest so far? Both medical practitioners and their patients should understand well not only the essence of this promising therapy, but also its merits and flaws



Bacteriophages are not just common drugs. They are neither simple chemicals, like antibiotics and most other medicines, nor true living organisms since they, like all the other viruses, are able to reproduce only in the cell of their host. Actually, bacteriophages are nano-automatons with their own genetic program, which can penetrate into a bacterial cell and reproduce there causing its destruction.

Therefore, standard pharmacological approaches to bacteriophages are not always satisfactory. Although phage preparations are now produced and applied in medicine, our knowledge about the diversity of these viruses, about mechanisms of their interaction with bacteria, and about competition with their cognates is still insufficient to use their full therapeutic potential.

Safe and Efficient

Phage therapy emerged almost immediately after bacteriophages were discovered; however, expanded trials of these antibacterial tools were launched in the Soviet Union only in the late 1930s. The trials proved the efficiency of bacteriophages as preventive measures against dysentery and cholera epidemics, and using them for healing wounds and curing pyoinflammatory diseases demonstrated their potential as an alternative to antibiotics.

However, the results achieved at that time were often contradictory: in some cases the phages immediately inhibited the progression of infection but sometimes they were of no use. The specialists grasped the reason right away: the treatment was successful only when they used phages that were able to infect the particular bacterial strain that had caused the target infection. Thus, it was necessary to isolate the pathogen that caused the epidemic, assay the available phages for their ability to inhibit this agent, and produce the most efficient bacteriophage as a drug.

Unfortunately, the results of the studies performed in the Soviet Union were not properly documented and described in scientific literature; moreover, they were conducted not in compliance with the currently recognized protocols for clinical trials. Nonetheless, the major results of that work were undoubted: phages demonstrated their safety and high efficiency in real situations. Since then they have been

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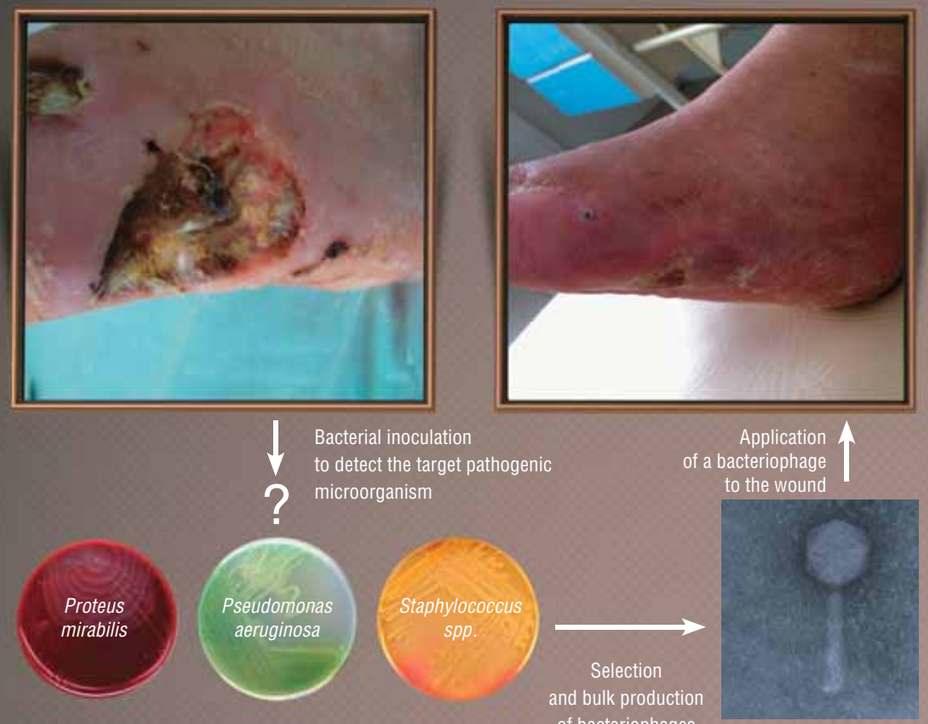
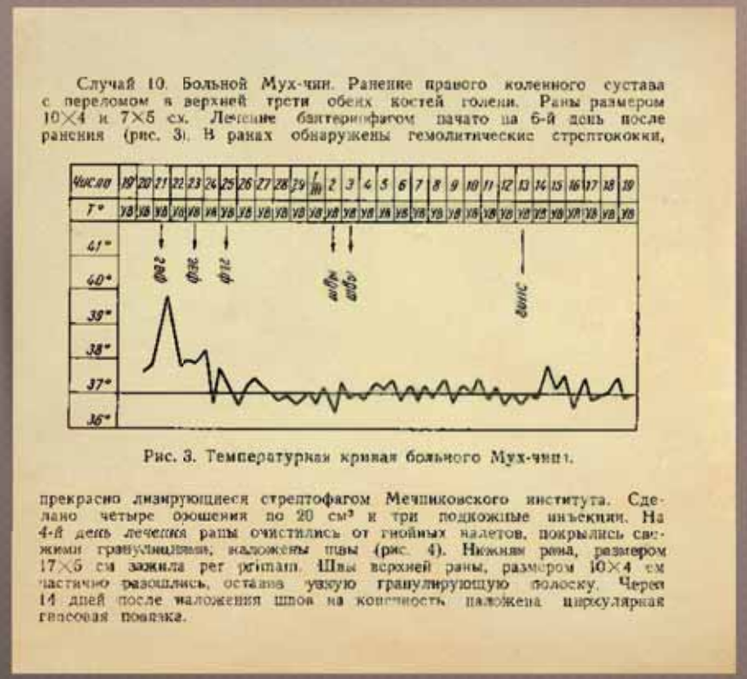
Key words bacteriophages, phage therapy, phage-based preparation



Diabetic foot, a severe complication of diabetes with potential development of gangrene, foot loss, and disablement, is experimentally treated in a Novosibirsk clinic. Bacterial infection is one of the factors underlying this pathology. Phage therapy comprises the following stages: swabbing the affected tissues to isolate the pathogenic bacterium; selecting the bacteriophage that can lyse the target bacterium from a phage collection; and applying the bacteriophage preparation (on a sterile pad) to the wound. The treatment takes about a week



Случай 10. Больной Мух-чин. Ранение правого коленного сустава с переломом в верхней трети обеих костей голени. Раны размером 10×4 и 7×5 см. Лечение бактериофагом начато на 6-й день после ранения (рис. 3). В ранах обнаружены гемолитические стрептококки,

Рис. 3. Температурная кривая больного Мух-чин.

прекрасно лизирующиеся стрептофагом Мечниковского института. Сделано четыре орошения по 20 см³ и три подкожные инъекции. На 4-й день лечения раны очистились от гнойных налетов, покрылись свежими грануляциями; наложены швы (рис. 4). Нижняя рана, размером 17×5 см зажила per primam. Швы верхней раны, размером 10×4 см частично разошлись, остался узкую грануляционную полоску. Через 14 дней после наложения швов на конечность наложена циркулярная гипсовая повязка.

ANTIBIOTICS

Advantages:

Broad spectrum; and
Simple patenting

Disadvantages:

Destroy the microflora of the body, creating a threat of secondary infections;

Are unable to accumulate at the infectious lesion;

Cause side effects, such as allergies and gastrointestinal disorders;

Cause emergence of drug-resistant bacterial strains; and

Require much time and money for developing new antibiotics

BACTERIOPHAGES

Advantages:

High specificity, which makes it possible to find an individual bacteriophage killer for any bacterium;

Search for a new target phage takes only several days or weeks;
Inexpensive and ecologically friendly production;

Never cause dysbacteriosis;

Nontoxic and have no side effects; and

Are eliminated from the body after destroying the target pathogen

Disadvantages:

Very high specificity: to guarantee successful treatment it is necessary to identify the target pathogen;

Difficulties in patenting

COME TO KNOW—FECAL MICROBIOTA TRANSPLANTATION

A common aftereffect of antibiotic therapy is rapid propagation of an aggressive bacterium, *Clostridium difficile*, which causes severe diarrhea and resistance to drugs. This is a very serious problem: not long ago it caused about a thousand lethal outcomes in the United States annually. A very simple means of curing diarrhea was found quite recently: the fecal microflora of a healthy donor is administered to the patient's intestine. The recovery is almost immediate, literally on the following day. Evidently, the "transplantation" of feces gives the patient a complete set of "proper" microorganisms that had been killed by antibiotics and bacteriophages that control the abundance of pathogenic strains.

Initially, the FDA restrained the spread of this approach, trying to apply the regularity rules approved for ordinary drugs. However, the protests of both therapists and patients came into play, and the method was approved with common precautions, i. e. selection of healthy donors and performance of the procedure by specialists and in healthcare facilities. The method has recently become widespread in the United States and shows good results. It is likely that only the prejudice of physicians still hinders the use of fecal microbiota transplantation in several European countries; in Russia, this treatment is available only at the Center for New Medical Technologies in Novosibirsk Akademgorodok

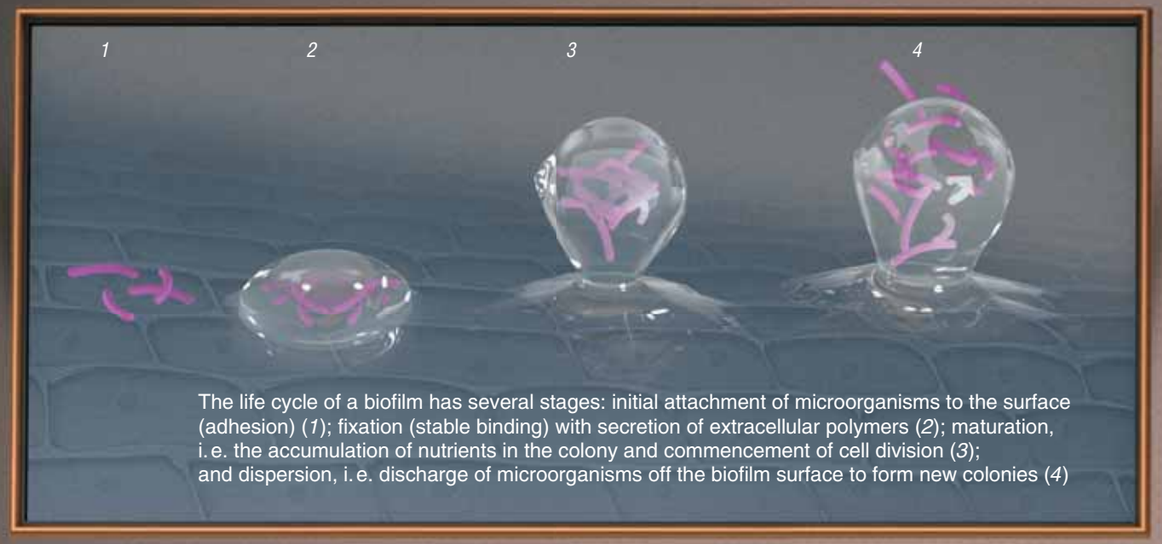
used in clinical practice in this country along with common therapeutic tools.

With the advent of antibiotics, western countries lost interest in phages; however, the emergence of antibiotic-resistant bacterial strains made several countries begin to elaborate phage preparations and conduct clinical trials, which in fact were the same as the ones that had been performed in the Soviet Union. The new results confirmed the safety of bacteriophage preparations, which was confirmed by the Food and Drug Administration (FDA).

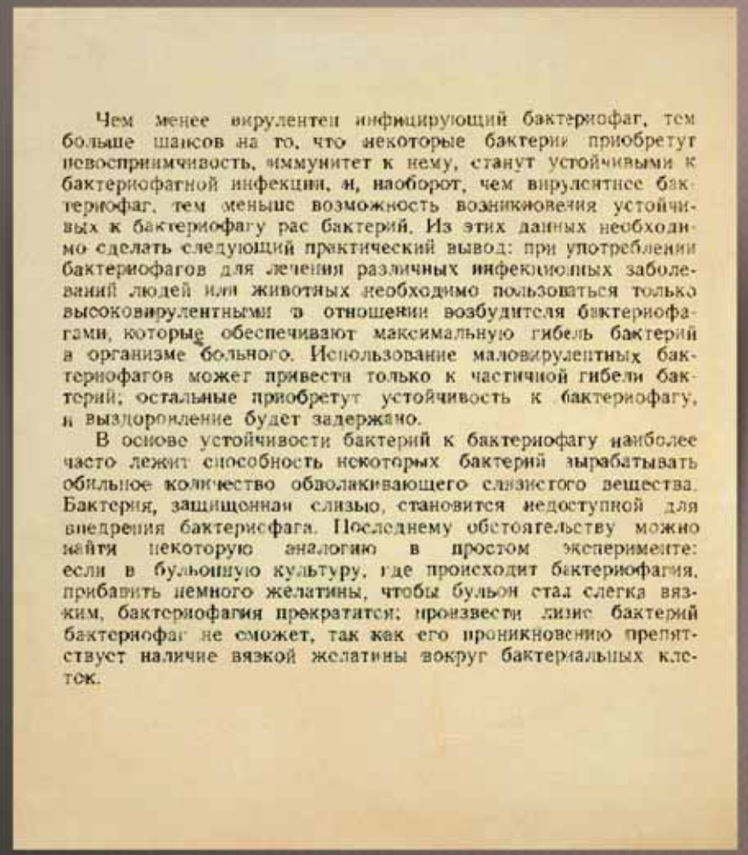
In the United Kingdom, experiments on treating chronic otitis caused by have proved to be successful. Under the Phagoburn project, seven medical centers in France, Belgium, and Switzerland are involved in the clinical trials of a phage cocktail for preventing infections in burn injuries. Several United States companies (Intralytix, Enbiotix, and AmpliPhi) report testing their original phage cocktails for a wide range of diseases, though none of these large-scale clinical trials has been completed yet.

What is a "medicinal bacteriophage?"

In Russia, bacteriophage preparations are available in pharmacies, but unlike other drugs with their precise chemical formula and concentration of the components, a bacteriophage preparation is a nonstandard solution containing live virus particles. Preparations that have the same name but were manufactured at different facilities or at different times may differ in their composition and/or ratio of phages.



The life cycle of a biofilm has several stages: initial attachment of microorganisms to the surface (adhesion) (*1*); fixation (stable binding) with secretion of extracellular polymers (*2*); maturation, i.e. the accumulation of nutrients in the colony and commencement of cell division (*3*); and dispersion, i.e. discharge of microorganisms off the biofilm surface to form new colonies (*4*)



Чем менее вирулентен инфицирующий бактериофаг, тем больше шансов на то, что некоторые бактерии приобретут невосприимчивость, иммунитет к нему, станут устойчивыми к бактериофагной инфекции, и, наоборот, чем вирулентнее бактериофаг, тем меньше возможность возникновения устойчивых к бактериофагу рас бактерий. Из этих данных необходимо сделать следующий практический вывод: при употреблении бактериофагов для лечения различных инфекционных заболеваний людей или животных необходимо пользоваться только высоковирулентными в отношении возбудителя бактериофагами, которые обеспечивают максимальную гибель бактерий в организме больного. Использование маловирулентных бактериофагов может привести только к частичной гибели бактерий; остальные приобретут устойчивость к бактериофагу, и выздоровление будет задержано.

В основе устойчивости бактерий к бактериофагу наиболее часто лежит способность некоторых бактерий вырабатывать обильное количество обволакивающего слизистого вещества. Бактерия, защищенная слизью, становится недоступной для внедрения бактериофага. Последнему обстоятельству можно найти некоторую аналогию в простом эксперименте: если в бульонную культуру, где происходит бактериофагия, прибавить немного желатины, чтобы бульон стал слегка вязким, бактериофагия прекратится: произвести лизис бактерий бактериофаг не сможет, так как его проникновению препятствует наличие вязкой желатины вокруг бактериальных клеток.

It is believed that the overwhelming majority of microorganisms in their natural environment (flow conditions) exist at the interface of two media as "biofilms," a sort of "colonies" with a specific spatial and metabolic structure. The bacterial cells in such "microbial cities" are submerged into an extracellular mucous matrix formed of polymeric substances secreted by cells. The biofilm can be attached to the surface of either inanimate objects (for example, calculi, catheters, or joint implants) or to a part of an animate object (intestinal walls, teeth, or skin). This mode of existence provides many advantages for microorganisms; in particular, the biofilm microflora, owing to the mucous matrix, is much less affected by various adverse factors, such as ultraviolet radiation, dehydration, or antibiotics. The matrix also protects the bacteria against the attacks of bacteriophages and host immune cells

All the differences are determined by the specificity of the phage selection procedure and their production. Bacteriophages are selected according to their ability to lyse an individual bacterial isolate; then a mixture of phages is grown on a specified bacterial culture, and goes into production, i.e. bacteriophages are grown in voluminous reactors (fermenters) with the help of bacterial strains.

As a result, a drug that can kill the necessary bacterial strain is created. For example, the *Pseudomonas aeruginosa* bacteriophage contains the phages that kill *P. aeruginosa*, but the physician does not know either the number of phages in the preparation or what phages it contains, what *P. aeruginosa* strains it can kill, and whether it is appropriate for a particular patient. The preparation will have an excellent effect if the patient is infected with the same bacterial strain as was used for phage production; otherwise, the only hope is that since the phage cocktail contains many components, one of the bacteriophages may be specific to the target pathogen.

Thus, it does not pay to buy a bacteriophage in a drugstore for self-treatment. It is up to the doctor to prescribe the treatment and drugs. The range of diseases susceptible to bacteriophage therapy is wide, including trophic ulcers, burn and wound infections, as well as various infections of respiratory, urogenital, gastrointestinal organs, and bones. In these cases, the causative agents are notorious bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, pathogenic *Escherichia coli* strains, salmonellas, *Proteus*, and streptococci, including their drug-resistant variants. In fact, it is possible to find naturally occurring bacteriophages against any bacteria, including those that cause plague and anthrax. Bacteriophages can also be used to prevent communicable bacterial diseases; for example, they were successfully used in kindergartens to prevent a dysentery epidemic.

Bacteriophage preparations are administered either locally, to the lesion, or orally. Advertisements allege that phages can spread within the human body and pass from the stomach to bloodstream; however, there is no clear and unambiguous scientific proof yet. Note that a bacteriophage preparation may contain most different bacterial viruses with most diverse fates in the human body.

In certain situations, it is difficult for bacteriophages to hit their "victims." For example, tuberculosis bacteria reside within the body cells where phages cannot get, while some bacteria form *biofilms* which are impenetrable for both phages and antibiotics. Then, to destroy the biofilms it is necessary to use enzymes synthesized by specially constructed phages.

In an infected organism, phages reproduce until the majority of sensitive bacterial cells are killed. The patient in whose body bacteriophages fight with bacteria will finally get well when his/her immune system starts to work in full force, and will further protect the body for a long time independently of whether the pathogen is present or not.

Besides, thanks to their specificity, bacteriophages do not kill "good" microorganisms, i.e. unlike antibiotics, they do not damage the human microbiome. It is now known that the intestinal flora disturbance may cause severe consequences, namely certain problems with the gastrointestinal tract, various allergies, and functional impairments of the central nervous system. Another advantage is that bacteriophages do not interfere with the effects of other therapeutics, and are not influenced by them either.

An individual cocktail for everyone

Why have bacteriophages failed to become the main tool for controlling infections, and there are frequent complaints of unsuccessful treatment in the social networks? This is explained in part by the administering of inadequate preparations. A decade ago, numerous "healthcare facilities" advertised stem cell preparations as drugs to cure any disease; now, extracts of "bacteria isolated from permafrost" and "preparations involving bacteriophages" (with no bacteriophages detectable) are intensively advertised. When buying a preparation, you should be sure that it was produced by a reliable manufacturer.

The main cause of treatment failures is an awkward selection of phages for individual patients. Each particular phage is efficient against one or a few bacterial strains, while an infection similar in its appearance, for example, angina, can be caused in individual patients by different streptococcal strains. To cure a patient, it is necessary to isolate the culture of the pathogen and test it for sensitivity to different phages, i.e. bacteriophage therapy should follow the pattern of personalized medicine. Alas, current medicine is not ready to do this yet.

The experience of the Soviet Union, Georgia, and Poland has shown that a successful use of bacteriophages requires not only a clinical facility, but also a laboratory production site with a collection of phages and the staff skilled in identifying bacteria as well as in selecting and isolating bacteriophages for individual patients.

The question here is whether large-scale production of bacteriophage preparations is reasonable. The answer is yes, because the problem of narrow specialization of bacteriophages is solved in part by making phage cocktails containing several (sometimes, several tens of) different phages that can hit different target pathogen strains. Evidently, it is easier and faster to select the

V. V. Morozova and Yu. N. Kozlova (Laboratory of Molecular Microbiology with the Institute of Chemical Biology and Fundamental Medicine, Russian Academy of Sciences, Novosibirsk) titrate a bacteriophage preparation

ON THE WAY TO PERSONALIZED PHAGE THERAPY

In the Novosibirsk Science Center, the consortium of the Institute of Chemical Biology and Fundamental Medicine (Russian Academy of Sciences) and Center for New Medical Technologies is involved in developing the technologies for personalized bacteriophage therapy; this is done in collaboration with a team of clinicians from the Tsiv'yan Novosibirsk Institute of Traumatology and the Railroad Clinical Hospital.

The large collection of bacteriophages at the Institute of Chemical Biology and Fundamental Medicine contains unique strains that can fight the newly emerged but already widespread agents of hospital infections, such as the gram-negative bacteria *Acinetobacter baumanii* and *Stenotrophomonas maltophilia*. The phages that can lyse a broader range of bacterial strains, including drug-resistant variants, more efficiently than the bacteriophages available as commercial preparations have already been isolated and characterized. This set includes the bacteriophages of the *Pseudomonas aureginosa*, *Proteus*, *Staphylococcus aureus*, *S. epidermidis*, *Klebsiella*, and pathogenic *Enterococcus*. Genome sequencing of the most promising bacteriophages has allowed the researchers to find strains that differ from the known ones.

Clinicians participated in accumulating the expertise to use bacteriophages for treating infections that accompany the diabetic foot syndrome, osteomyelitis, and surgical wound infections. A few cases of curing respiratory infections caused by drug-resistant pathogens and urogenital diseases have been reported. For example, a 6-month-old girl with a congenital laryngeal abnormality was completely cured of severe tracheobronchitis, which had developed after tracheotomy, by inhalations of the *Pseudomonas aureginosa* bacteriophage. The pathogen that had caused her disease was identified as the *Pseudomonas aureginosa* strain, resistant to almost all antibiotics approved for infants. The girl received the bacteriophage twice a day for a week; after the pathogen and infection disappeared, the tracheotomy tube was removed; now the girl is quite well.

As for treating chronic drug-resistant urinary infections, it has been found that the bacteriophage should be administered directly onto the bladder. This cured a patient with post-surgery chronic cystitis caused by a "bouquet" of antibiotic-resistant enterobacteria. A set of specific bacteriophages had been administered for 10 days on a daily basis; the urine tests after the treatment showed no pathogenic flora

necessary phage cocktail for an individual rather than to test many individual phages from a large collection.

For all that, bacteriophages are not likely to replace antibiotics completely: these preparations are complementary and applicable to different situations. When a patient is seriously ill, with a good reason to suspect a bacterial infection, there is no time for experiments in selecting the proper phage preparation. The only satisfactory solution then is a broad-spectrum antibiotic.

However, bacteriophage therapy is preferable when you deal with a chronic infection or with a disease caused by multidrug resistant bacteria. In the case of chronic illnesses, such as otitis, the physician has enough time to administer a phage cocktail or to select a specific phage. Another example is a post-surgery infection with an antibiotic-resistant bacterial strain, which causes rapid deterioration of the patient's state; here phage therapy can be the only option.

Wide experience in the clinical use of bacteriophages acquired over the last 100 years demonstrates the promising future of phage medical technologies. Further efforts of the experts working in this area, in combination with synthetic biology tools, will certainly create preparations with incomparably higher efficiency than that of the currently available phage cocktails.

However, several factors unrelated to science hinder the advances in designing and producing "medicinal" bacteriophages. The fact is that bacterial viruses are very easily reproduced, which offers exciting possibilities for their counterfeiting, thereby infringing on the rights of bona fide manufacturers. The requirements for phages as therapeutics have not been established yet either. It is only clear that they should be different from the requirements for synthetic drugs. Bacteriophage genomes are diverse; so, if a personalized approach is used, they should be selected individually.

Still, biotechnologists as well as researchers and physicians hope that these safe and efficient preparations will take their rightful place in the toolkit of therapies for infectious diseases.

The Russian Federation has currently the largest-scale production of bacteriophages. Mikrogen, a research and production facility, and the world leader in this area, manufactures a wide range of phage preparations. Therapeutic bacteriophages are also produced in Georgia, at the Eliava International Phagotherapy Center, which comprises both production facilities and a clinic with a vast collection of bacteriophages. European and the United States clinics, where phage therapy has not been officially approved yet, cooperate with this center under a medical tourism program. The Phage Therapy Unit with the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences produces bacteriophage preparations for experimental clinical application when treating patients who are non-sensitive to antibiotics

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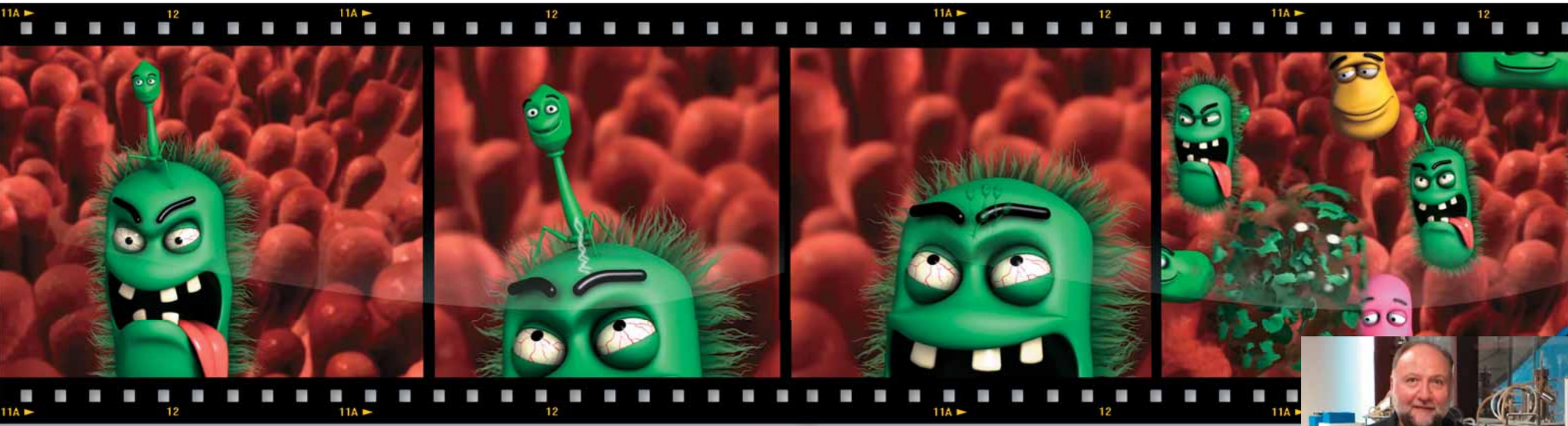
A. SULAKVELIDZE



PHAGEBIOTICS
FOR A HEALTHY LIFE

Our health and longevity are closely related to the "health" of our microbiome – the community of microorganisms inhabiting the human body. The condition of this important "microbial organ" is continuously impacted by many factors, ranging from antibiotics to diet to stress. The use of bacterial viruses (phages) as probiotics having a gentle and targeted impact on microflora can help fine-tune the microbiome and, as a result, may help prevent both infections and non-infectious diseases, ranging from diarrheal diseases to certain forms of cancer. With the modern advances in phage biotechnology, bacteriophages are becoming an attractive platform technology based on which various commercial products can be developed for numerous applications, ranging from preventing and/or treating people and animals to improving the safety of our foods

Key words bacteriophages, phage therapy, probiotics, phagebiotic, phage-based preparations, phage biocontrol

Although the temptation to use phages for any and all applications where bacteria present a problem may be strong, a careful and comprehensive analysis (cost, technical feasibility, competitive landscape, etc.) is warranted before embarking on a new phage-based product development program. Also, an important consideration would be the marketing strategy for bacteriophages. As explained later in this essay, although some phage preparations for human applications may be developed as "dietary supplements/probiotics," others may be best suited for a typical "drug" development cycle. For example, a phage preparation that targets major diarrheagenic bacterial pathogens may be developed and marketed as an over-the-counter dietary supplement to be used prophylactically by people traveling in or to developing countries where the incidence of diarrheal bacterial diseases is high. Indeed, the FSU's scientific literature contains many publications reporting the successful use of *Shigella* phages in humans (for a review, see (Goodridge 2013)).

A more complicated scenario is using lytic phages to prevent and treat wounds often colonized/infected by multiple bacterial species. This situation is addressed by using complex/multivalent phage preparations containing several bacteriophages active against several bacterial pathogens known to interfere with wound healing. Two examples of such preparations are "Pyo-bacteriophage" produced by the Eliava Institute of Bacteriophages, Microbiology and Virology in Tbilisi, Georgia, and "Complex pyobacteriophage" produced by NPO *Microgen*. In the same context, the first phage therapy trial for human wound infections in the USA utilized a multivalent phage preparation containing eight different phages lytic for three bacterial pathogens commonly found in infected wounds: *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Rhoads, Wolcott *et al.*, 2009).

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He is the author of 14 issued patents and several pending patents in the field of bacteriophages

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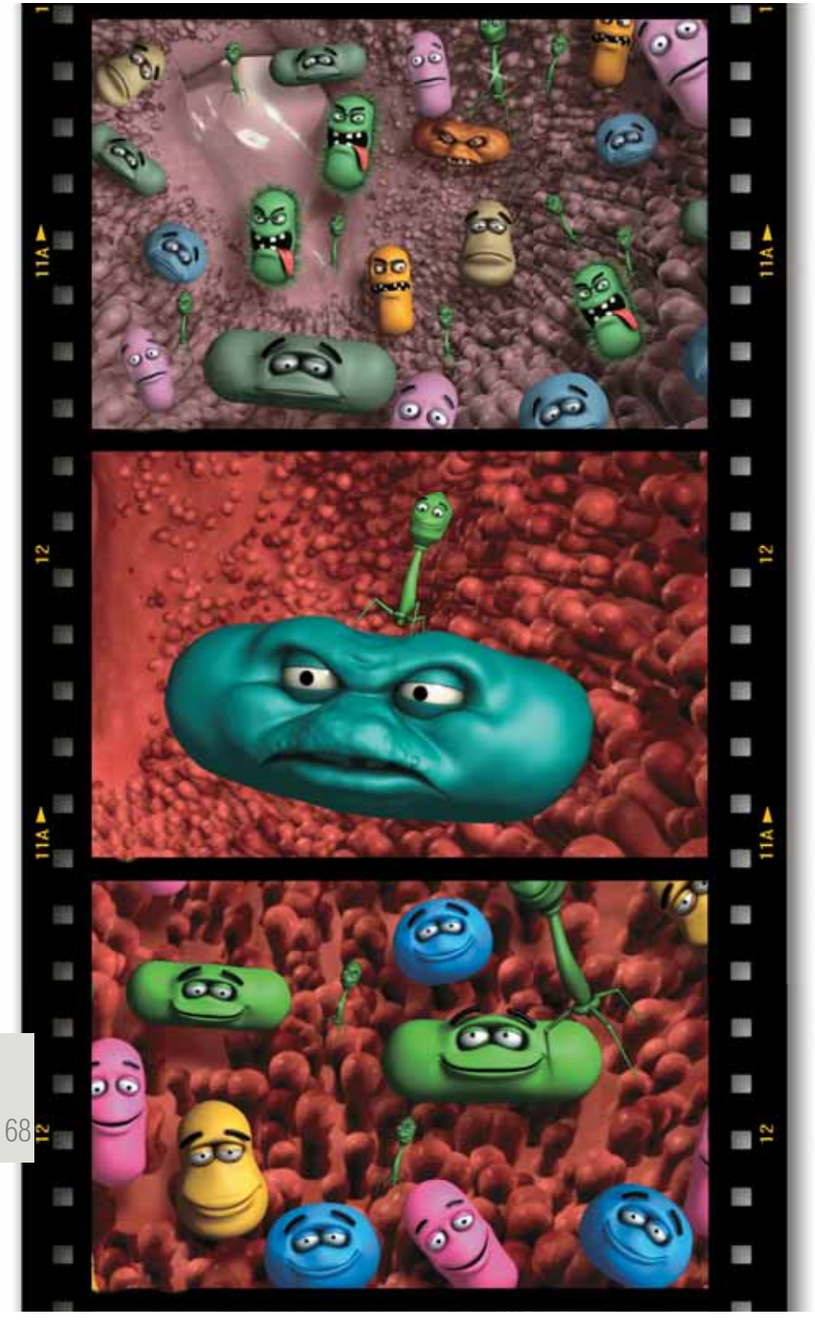


Lytic bacteriophages can be selected to specifically attack pathogenic bacteria in the gut, without impacting the normal and useful microflora

The production and control over the quality of these preparations requires much more effort; therefore, it may be preferable to develop and sell them as conventional drugs.

It goes without saying that the commercialization of different products will vary essentially in terms of development costs, statutory regulation strategies, and timeframe.

Wholesale and custom-designed

Phage-based therapeutic preparations offer unprecedented flexibility for keeping up with the emergence of new highly pathogenic clones of various bacterial species, and with the emergence of phage-resistant mutants in bacterial populations. Phages have been co-evolving with their host bacteria for >3 billion years (Lenski 1984); therefore, when they are needed, it is relatively easy to isolate new environmental phages that can kill newly emerged pathogenic clones and/or phage-resistant bacterial mutants. From a practical standpoint, that approach will require monitoring the targeted pathogen's phage-sensitivity, and updating the phage preparations as needed. The first part is not novel or particularly difficult because bacterial isolation and antibiotic-sensitivity testing are routine practices in all major hospitals, and similar testing could be implemented for bacteriophages – with some updates in medical infrastructure (e.g., high throughput phage sensitivity testing equipment) and by implementing phage-specific training protocols, but, in general, without too much difficulty. However, phage substitutions may be challenging from a regulatory standpoint. Updating phage preparations by replacing old phages with new, more effective phages has been commonly and successfully done in the FSU and EE. However, that practice may be novel for Western regulatory agencies accustomed

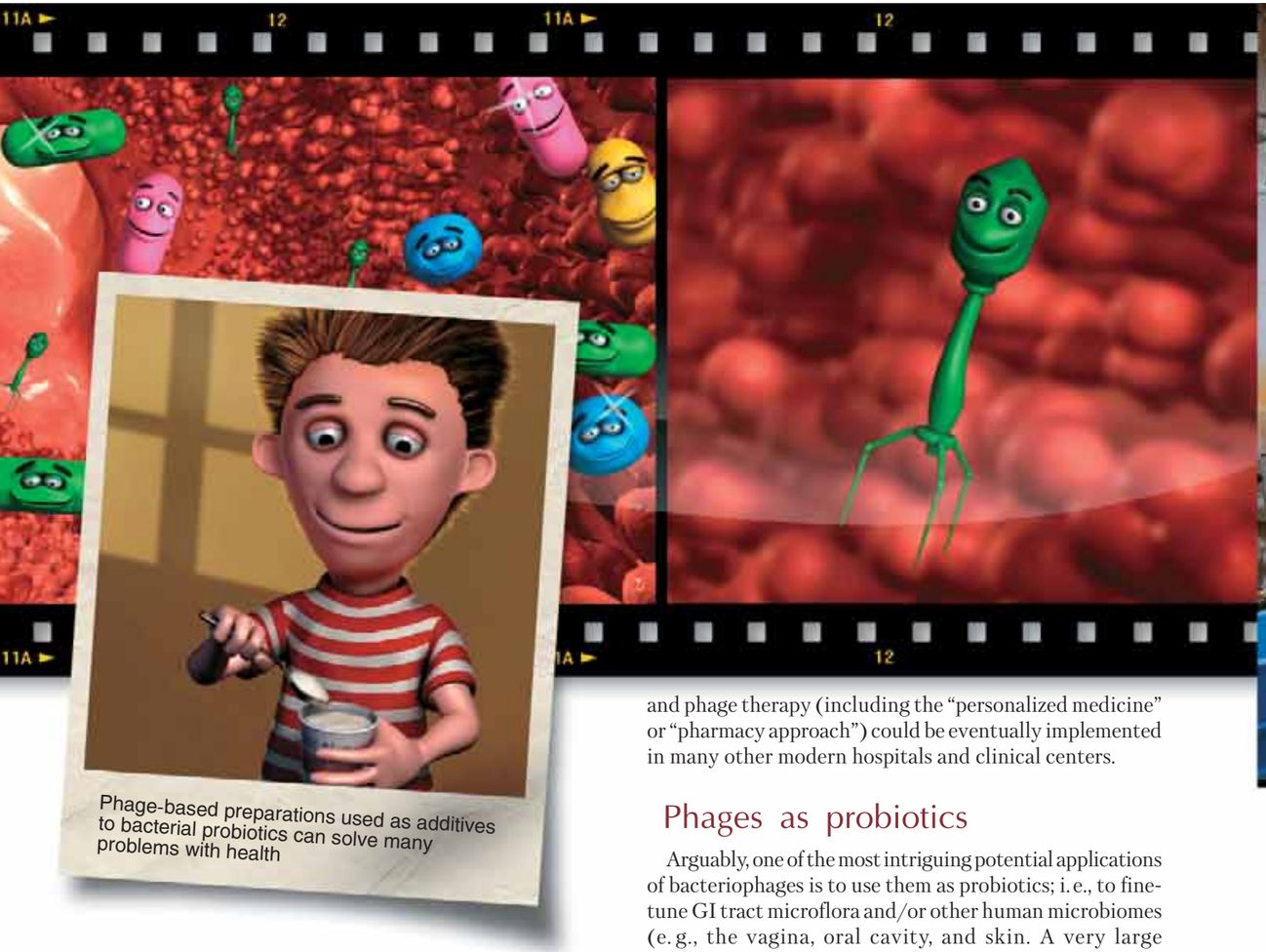
Phages are arguably the most ubiquitous organisms on Earth. For example:
– The total number of phages on Earth has been estimated to be 10^{30}-10^{31} plaque-forming units (PFU) (Bergh, Borsheim et al., 1989, Brussow and Hendrix 2002).
– One tablespoon (approx. 15 mL) of unpolluted water has been estimated to contain ca. 3 x 10^{9} phage particles (Bergh, Borsheim *et al.*, 1989).
– Phages are present – often in billions of PFU – in all of the fresh, unprocessed foods we eat. For example, bacteriophages have been commonly isolated from ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardines, raw skim milk, cheese, and many "healthy foods," including yogurt (reviewed in (Sulakvelidze and Kutter 2005))
– Phages are common in the human mouth, where they are harbored in dental plaque and saliva (reviewed in (Sulakvelidze and Kutter 2005)).
– Phages are common in animal feeds, feed ingredients, and poultry diets (Maciorowski, Pillai et al. 2001).
Phages are the most common or the second most common commensals (after bacteria) of the normal GI tract's microflora, where they coexist with various bacteria (Breitbart, Hewson *et al.*, 2003, Sulakvelidze and Kutter 2005)

to approving defined chemicals and requiring that each change in a preparation must be the subject of a new regulatory application. Having similar requirements for preparations containing naturally occurring phages that target a single or only a handful of bacterial pathogens will impede the development of new, effective phage-based therapeutics (Sulakvelidze 2012).

A positive development in that regard is the FDA's flexibility regarding the use of phages for food safety applications. For example, that agency has allowed future updates of several "food safety" phage preparations in response to the emergence of new, phage-resistant strains of foodborne bacterial pathogens (Woolston and Sulakvelidze 2015). It remains to be seen whether a similar approach can be implemented for human therapeutic applications, but it should be pursued because it would enable optimal utilization of phage therapy's potential public health benefits.

Some additional challenges may arise due to the specificity of bacteriophages. Since lytic phages are highly specific, mainstream commercial phage products may not be as effective against one or more strains of the targeted species that happen to predominate in a particular hospital or clinical center, or are isolated from a given patient (as has been reported by various investigators in the FSU; e.g., (Zhukov-Verezhnikov, Peremitina

et al., 1978)). One possible approach for addressing this potential challenge is to (i) examine all isolated bacterial strains for their *in vitro* sensitivity to various phages (the availability of high throughput phage sensitivity screening equipment could be invaluable for the process), and (ii) select and use the phages exhibiting strong lytic potency against the strains causing the patients' diseases. This type of "personalized medicine" approach is gaining attention in the West for many other medications. For example, it is similar, in principle, to how antibiotics are currently used in many clinics in the West, where bacterial strains are screened for their sensitivity to various antibiotics before prescribing the most effective antibiotic for a given patient. Thus, in order for phage therapy to reach its full potential, such "custom-designing" also must be implemented. From a technical standpoint, that approach should be doable: e.g., large libraries of well-characterized lytic phages could be assembled, and the technology for rapidly screening their activity against the isolated bacterial pathogens could be developed. However, it will require some creative, "out-of-the-box" thinking from the West's regulatory agencies. Finally, logistics must be developed for this approach to be commercially viable. Thus, for optimal clinical applications, it may be feasible to initially establish a small number of reference phage clinics or centers where the appropriate technologies could be implemented by highly trained, specialized medical personnel caring for local patients or patients willing to travel to those institutions. The number and/or size of those reference centers could be increased as the required technology and well-trained personnel become available,



Phage-based preparations used as additives to bacterial probiotics can solve many problems with health

The therapeutic use of phages declined after antibiotics became widely available in the 1940s and 50s, and eventually all but stopped in the West. On the other hand, phage therapy continued to be utilized in the former Soviet Union (FSU) and Eastern Europe (EE) – and, on a much smaller scale, in France, Switzerland and Egypt (Sulakvelidze and Kutter 2005). Several hundred publications reporting various therapeutic applications of bacteriophages are available; however, most of them are in non-English (mostly Russian and Georgian) biomedical journals not widely available to the Western medical establishment. However, that situation is now gradually improving, with the recent publication (e. g., (Alisky, Iczkowski *et al*., 1998, Sulakvelidze and Kutter 2005) of several English-written reviews of the relevant FSU and EE literature

and phage therapy (including the "personalized medicine" or "pharmacy approach") could be eventually implemented in many other modern hospitals and clinical centers.

Phages as probiotics

Arguably, one of the most intriguing potential applications of bacteriophages is to use them as probiotics; i. e., to fine-tune GI tract microflora and/or other human microbiomes (e. g., the vagina, oral cavity, and skin. A very large proportion of the bacterial cells is found in the GI tract, which is colonized by an abundant and diverse microbiota that plays a significant role in mucosal protection, regulation of GI immune tolerance, digestion of fecal matter, and vitamin K synthesis. However, numerous factors (e. g., antibiotic treatment, diet, and psychological and physical stress) may lead to physiological disturbances in the gut's microflora. Such alterations may contribute to many chronic and degenerative diseases, including rheumatoid arthritis and various inflammatory bowel diseases and "intestinal dysbioses."

One of the approaches used to alleviate those disorders has been the ingestion of probiotic microorganisms; i. e., nonpathogenic microorganisms that improve health when ingested in sufficient quantities, presumably by beneficially altering the microbial balance of the human GI tract. Traditionally, various bacterial species have been

The production facility where phages are produced by the American biotechnology company *Intralytix, Inc*. (Baltimore, Maryland, USA). *The photo is the courtesy of the author*

used as components of such "probiotic cocktails," with lactobacilli and bifidobacteria being the most commonly used and delivered in various commercial dietary supplements, healthy beverages, infant formulas, and other foods. A brief overview of probiotics is presented in several review articles are available; e. g., (Schrezenmeir and de Vrese 2001). On a somewhat oversimplified level, the rationale for using bacterial probiotics is that they colonize the gut, inhibit or prevent colonization by – and/or the proliferation of – potentially pathogenic microorganisms, and help to restore the healthy microbial balance of the GI tract. Bacterial probiotics have been markedly gaining in popularity in the USA and other countries around the world.

A novel probiotic approach is to use lytic phages as probiotics/dietary supplements for targeting "problem" bacteria. The key difference between bacterial probiotics and lytic phage-based probiotics ("phagebiotics") is that the former use nonpathogenic bacteria to interfere with the ability of pathogenic bacteria to colonize and cause disease; whereas, the latter use lytic phage to kill specific pathogenic bacteria while preserving the commensal community. Phagebiotics are expected to have a very gentle effect on the overall microflora because of their high specific activity against specific bacterial species, which may further enhance their protective effects. Also, they are expected to be compatible – and, in fact, synergistic – with bacteria-based probiotics. Thus, the phage-based probiotic approach may serve as a platform technology for developing a new class of "phagebiotics" or "super-probiotics" for improving human health.

Many health issues could be potentially addressed by the "phagebiotics" approach, because phage-based probiotics can be used as new preventive means against numerous bacterial infections, including diarrheal diseases (e. g., those caused by *Shigella* spp.), diseases of the oral cavity (e. g., dental caries caused by *Streptococcus mutans*), skin and ocular diseases (e. g., acne, chronic blepharitis, and endophthalmitis caused by *Propionibacterium acnes*),

Foods sometimes contain bacteria that cause intestinal infections/foodborne diseases. To reduce the risk of catching a disease, foods can be treated with phage preparations that specifically kill those disease-causing bacteria

and "women diseases" (e.g., bacterial vaginosis caused by *Fusobacterium nucleatum*).

Moreover, the phagebiotics approach may lead to new strategies for improving human health by advancing the prevention of *noninfectious* diseases elicited by one or more bacterial species in the human GI tract; e.g., obesity and some types of cancer that may be caused by certain bacteria inhabiting the GI tract (e.g., B2 *E. coli*, *Bacteroides fragilis*, and *Salmonella* Typhi sometimes called "oncobacteria"). For example, chronic *S.* Typhi infections of the gall bladder have been reported (Dutta, Garg *et al.*, 2000) to be associated with hepatobiliary cancers. Thus, phages that target *S.* Typhi may potentially help to reduce the risk of hepatobiliary cancers in susceptible populations.

Finally, phages may be valuable tools for advancing our understanding of the important physiological roles that specific bacteria have in various mammalian microbiomes. For example, laboratory animal studies characterizing the local and systemic physiological changes occurring after using phages to eliminate or significantly reduce the levels of specific bacterial species in the GI tract may provide clues to the roles of those bacteria in that microbiome (i.e., a probiotic version of the "gene-knock-out" approach). This is truly a unique feature of bacteriophages since no other currently available antibacterial agents offer such a targeted approach against a specific subgroup of bacterial strains or species.

Phages as biocontrol agents

The concept of using phages for food safety applications has been slowly but steadily gaining acceptance in the USA, and the trend is likely to continue as more food processors recognize the advantage of using bacteriophages for improving the safety of the foods they produce, and consumers learn more about bacteriophages, including how ubiquitous they are in the environment and the foods we eat.

Naturally occurring bacteriophages may provide one of the safest and most environmentally-friendly approaches for reducing contamination of foods with foodborne bacterial pathogens (e.g., *Listeria monocytogenes*, pathogenic *E. coli* strains, and *Salmonella* spp.) without deleteriously affecting the nutritional value of the foods and their normal, and often beneficial, microflora. The approach of using phage-containing food safety products for direct food applications is based on adding an appropriate concentration of lytic bacteriophages active against the pathogenic bacteria contaminating the foods. If the foods are contaminated with pathogenic bacteria targeted by the bacteriophage preparations, the phages will eliminate or significantly reduce the concentrations of those bacteria in the foods, thus making them safer to eat. If the foods are not contaminated with the targeted foodborne bacteria, the phages will simply dissipate over time.

During the last few years, the FDA has approved several phage-based preparations for food safety applications. (Sulakvelidze 2012, Woolston and Sulakvelidze 2015). The first phage-based preparation for food safety applications cleared by the FDA was ListShield™, which was developed and marketed by Intralytix, Inc. This was the first and, to date, the only phage-based product approved as a *food additive* by the FDA. The preparation is active against *L. monocytogenes* in various foods, including ready-to-eat foods.

Several more recent food safety approvals for phages have been under the GRAS (Generally Recognized As Safe) status, and it is likely that most, if not all, future phage products for food safety applications (and possibly for "probiotic" applications, see below) also will be marketed in the USA under the GRAS status.

Many of the phage preparations for food safety applications (including ListShield™) are free of preservatives and do not alter the general composition, taste, aroma or color of foods. Also, some of them are Kosher- and Halal-certified, and are listed by the Organic Materials Review Institute (or equivalent), which makes them suitable for use in the production of organic foods (Woolston and Sulakvelidze 2015).

Surely, to make phage therapy widely available throughout the world, some problems including technical ones need to be handled (Sulakvelidze and Kutter 2005, Sulakvelidze 2011). However, given the ever-increasing threat of antibiotic-resistant mutants, and potential of bacteriophages to provide a safe and effective approach for managing various bacterial diseases caused by multi-antibiotic-resistant bacteria, robust efforts to integrate this natural antibacterial approach into modern day medicine are long overdue.

The "probiotic" use of phages, in particular, is very intriguing, and several phage-based probiotic preparations are likely to be developed during the next several years. Initially, they may be designed to prevent and treat diarrheal diseases of well-defined bacterial etiology (e.g., shigellosis).

Ultimately, the phagebiotic approach can help to support the overall normal bacterial flora, which will prevent many diseases including non-contagious ones. Being a unique tool for studying, fine-tuning, and improving the most important "microbial organ" of the human body, phages can play an important role in our lives.

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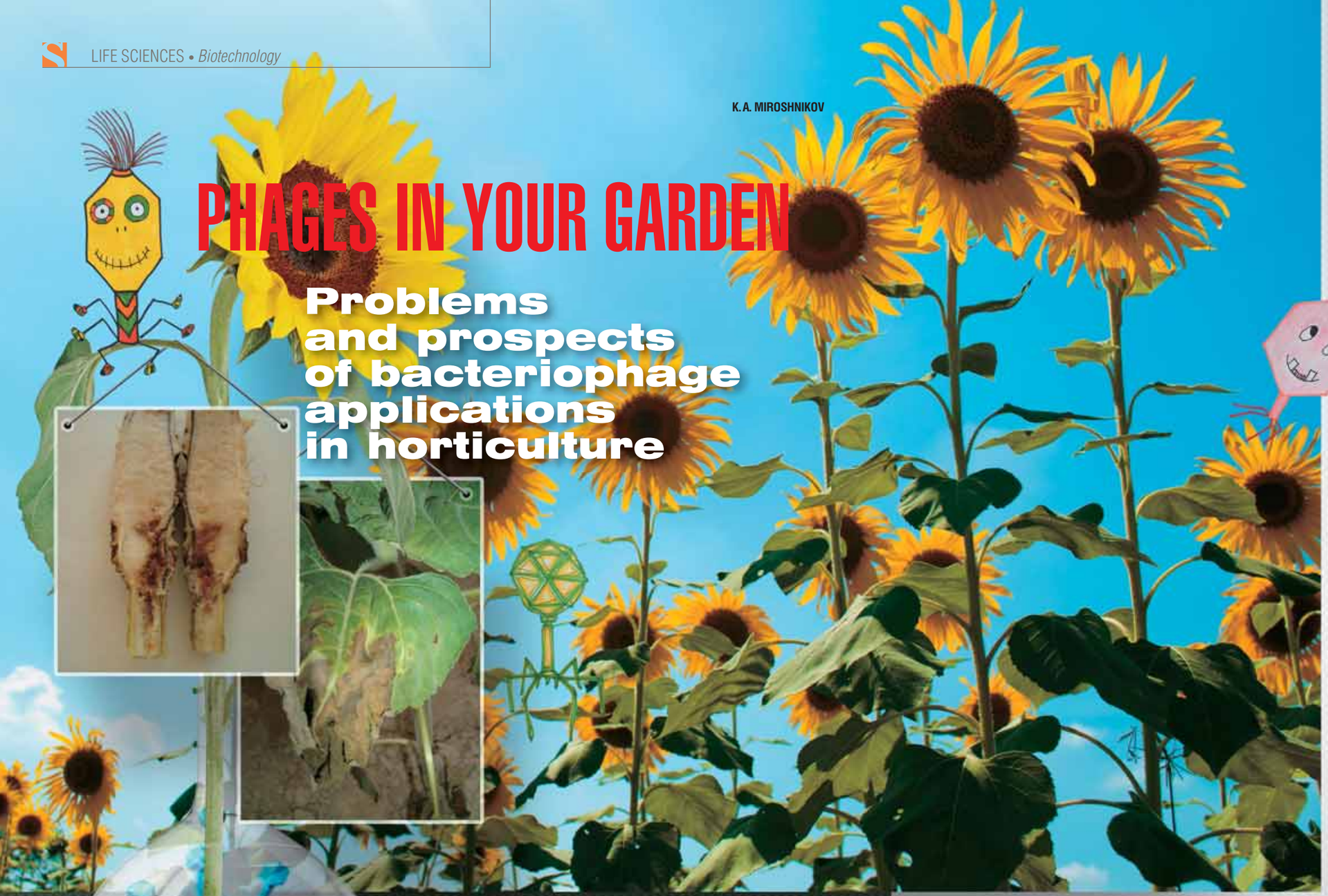
K. A. MIROSHNIKOV

PHAGES IN YOUR GARDEN

Problems and prospects of bacteriophage applications in horticulture



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Bacterial encounters are often a disaster for a plant, leading to tissue degradation and damage to the root and vascular systems. Even when bacteriosis does not immediately kill a plant, it slows down its development, reducing the size and vendibility of the crops. Phytopathogenic bacteria have been long and rather successfully controlled by using special agricultural products, which were, as a rule, crude and cheap versions of medical antibiotics of the streptomycin and tetracycline series. However, this path may have dangerous and unpredictable consequences both for the global environment and human health

Left: bacterial blight and stem rot of sunflower plants; the disease is caused by *Xanthomonas campestris* pv. *campestris*. This bacterium also affects the root system, which then necrotizes and rots as the high-molecular polysaccharide secreted by the bacterium leads to vessel blockages. This disease, which was first described in 1981 in the United States, has become increasingly widespread in recent years.
Photo of sunflowers: © Creative Commons

Phytopathogenic bacteria cause extensive damage to global agriculture. Fighting these infections is always a challenge. As a rule, agriculturally important plants are cultivated on large land plots, and individual monitoring and treatment of each plant is very time-consuming and expensive, if at all possible. Weeds, pest insects and fungi are controlled through the mass use of herbicides, pesticides and fungicides.

In the last decade, the international medical community has been alarmed by the rapid spread of varieties (*strains*) of pathogenic bacteria resistant to several classes of antibiotics used in clinical practice. The infections caused by these bacteria lead to most severe complications and impede the treatment of diseases, leading to thousands of deaths and huge economic losses.

Key words: diseases of agricultural plant, bacteriophage, phage therapy, protein engineering, enzymology, molecular diagnostics

Importantly, the greatest danger is associated with nosocomial infections, which are caused by strains circulating in hospitals. Constantly exposed to pressure resulting from the use of antimicrobial agents, these bacteria have adapted to these agents and acquired multiple resistance to antibiotics.

The likelihood of catching such an antibiotic resistant infection in everyday life is, as of today, relatively low because medical antibiotics affect environment only in the places of their permanent use. However, this "setback" is by far compensated by agricultural antibiotics: industrial farmers worldwide use them on a scale of tens of thousands of tons every year to combat bacterial diseases in animals and plants. It is the excessive use of agricultural antimicrobials that is the most likely culprit for the mass development of opportunistic pathogenic bacteria, which are common in nature and do not pose any particular risk for human health, but can affect people with a weakened immune system. However, it is virtually impossible to get rid of those "opportunists" because of their resistance to antibiotics.

Therefore, legislative measures to restrict the use of antibiotics in agriculture, especially for preventive rather than curative purposes, is a very smart move in terms of global health. In light of this situation, organic horticulture, which is focused on the maximum diversity and productivity of plants with the minimum use of xenobiotics, becomes a rational response to the current challenges rather than a fashionable trend.

On the road to organic horticulture

Organic horticulture is not at all about those next-to-idiotic concepts saying, "Yes, we grow small and stunted vegs and in medieval quantities, but we grow them on the dung and remove all the worms by hand." It is a harmonious blend of modern achievements of agricultural science—new resistant and productive varieties, modern mechanized farming technologies, calculated use of a combination of organic and inorganic fertilizers, monitoring crop sizes and plant diseases, modern storage and processing technologies, etc. This mix also includes science-based tailored applications of biological control agents.

We define *biological control* as the use of living organisms, i. e., specific predators or parasites, to suppress pest populations in agricultural areas. In particular, an appealing strategy to combat bacterial infections in plants is the use of *bacteriophages*, or bacterial viruses targeting specific pathogens. This concept has many advantages. Phages are natural inhabitants of the same ecological niches as bacteria; hence, their use does not affect the state of major ecological systems. Phage breeding technologies and applications are logical, relatively simple and inexpensive. Agricultural phage therapy can be combined with most other chemical and biological control methods and is safe both for the treated plants and for humans and animals.

However, it should be understood that phage phytocontrol is not a panacea against plant pathogens. Successful development of any phage therapy, including in plants, requires detailed understanding of the biology and genomics of bacteriophages and target organisms, research efforts to explore their interactions, and technologies of selective breeding of targeted phages. At first glance, this method is so hi-tech that it is intimidating. But are these fears realistic and what are the prospects for agricultural phage therapy?

How it all began

It all began at the beginning of the twentieth century, when Félix d'Hérelle proposed his theory of infectious lysis of bacteria by viruses. As the awareness and acceptance of this theory was growing among researchers, global enthusiasm about the potential of bacteriophages was growing. After all, it was a time before the discovery as well and mass use of antibiotics, which became later the gold standard in the treatment of bacterial infections. In the 1920s–1930s, there were virtually no effective methods to control diseases caused by bacteria. No wonder that one immediately began testing phages as therapeutic agents for the treatment of virtually all known diseases of microbiological origin.

The earliest documented experiments to control and treat bacterial diseases in plants were conducted at the University of Michigan (USA). In 1924, Mallmann and Hemstreet showed that the filtrate of cabbage tissue affected by *Xanthomonas campestris* can inhibit growth of the pathogen under laboratory conditions. The following year, Kotila and Coons showed that phages with specific activity against the causative agent of blackleg disease in potatoes can be isolated directly from soil. When the bacteriophage and the pathogen, i. e., the bacterium *Erwinia carotovora* (its modern name is *Pectobacterium carotovorum* subsp. *carotovorum*), were simultaneously applied to potato tubers and carrot roots, rotting was observed only in those samples where there was no phage.

In 1934, the English researcher Robert Massey gave an explanation of the intriguing fact that the incidence and severity of bacterial blight disease in field-grown cotton, which is caused by the bacterium *Xanthomonas malvacearum*, was lower on lands that were flooded by waters of the Nile River. He suggested that a key factor in limiting the severity of the disease was the phages

Xanthomonas campestris pv. *campestris*, the causative agent of vascular bacteriosis, affects all cruciferous crops including turnips (*left*), but is the most damaging to cauliflower and cabbage (*right*)

transported by river waters. The following year, he proved his hypothesis by showing that phages were found only in the soil of the areas that had experienced floods.

Despite these results, the early works provided little understanding of how bacteriophages work in natural conditions, and the number of failed phage therapy attempts far exceeded that of successful ones. At that time, there was an apparent lack of information on how to select, characterize, cultivate and use bacteriophages in medicine and in agriculture.

A trigger for a new round of interest in bacteriophages, which occurred in the 1960s–1970s, was the discovery that these microscopic organisms can be conveniently manipulated. Bacteriophages helped discover and describe many of the fundamental processes that are now the classics of molecular biology. However, there was much less attention to practical applications of phages, which is not surprising given the total domination of antibiotics in the treatment of bacterial infections in human and veterinary medicine and in horticulture.

The majority of these experiments were conducted in Japan, a country that did not participate in the first stage of phage research. Although the scientific press of that time contains a number of successful reports on phage therapy, including in agriculture, the attempts to summarize the data and develop a framework concept were unsuccessful. Moreover, some researchers even wondered how one could have obtained any repeatable positive results at all. In the context of the widespread introduction of antibiotics and other chemical agents (e. g., copper compounds) in agriculture, using phages to treat plant diseases looked as an exceedingly complicated and unpromising alternative.

Since then, despite the increase in information about the structure, genetics and action mechanisms of bacteriophages, the interest in phage applications for the treatment of bacterial diseases of plants has remained stably low. Every year, microbiological and agricultural journals publish a few papers about successful cases of phage therapy of a given plant–pathogen combination, propose a phage preparation, which is sometimes even patented and produced commercially (e. g., Multifag in Belarus and Pentafag-C in Ukraine). However, the response to these publications and the commercial success of the products is low because many of the logical questions posed by skeptics still remain unanswered.

The five cons

There is a number of fundamental limitations arising from the biology of the interaction between a virus and its host, which complicate the use of phage therapy and serve as a basis for criticism of varying degrees of validity.

The major limitation of bacteriophages is their high infectious specialization with respect to host bacteria. Each type (clone, or race) of bacteriophages infects a fairly narrow group of strains within a taxonomic species of bacteria. The choice depends on the bacterium's receptor molecule to which the virus attaches itself, on the adaptation to the metabolic cycle of the host bacteria,

The universally popular potatoes are prone to a wide range of bacterial diseases.
Above: potatoes affected by blackleg, which is caused by the bacterium *Dickeya solani*; *bottom:* tubers affected by soft rot, which is caused by the bacterium *Pectobacterium carotovorum* subsp. *carotovorum*

and on other factors. Phages that are able to parasitize bacteria of different species or genera are extremely rare, and the bacteria infectable by these phages are, as a rule, closely related.

The narrow spectrum of the infectious action of phages presents a fundamental contrast to the broad spectrum of antibiotics. There is a well-founded opinion that if the causative agent is not clearly identified, the phage is likely to fail. Therefore, an extremely important step in phage therapy is a highly accurate diagnosis of the pathogen (up to a strain group). Given that plants can demonstrate similar symptoms when infected with different microbiological agents and that agricultural diagnostics is much less developed in comparison with medical diagnostics, this requirement is often considered as a critical limitation.

The next limitation is the rapid adaptability of bacterial pathogens. Opponents of phage therapy always point out that bacteria can mutate and become resistant to individual phages. Indeed, the classical experiments conducted by Luria and Delbrück showed that the probability of a phage resistant mutation is about 10^{-9}. That is, any large bacterial population will soon produce a mutant uninfectable with the phage. Therefore, the use of bacteriophages in a large ecological system will at best slow down the progression of a disease rather than stop it completely.

Another factor that cannot be ignored is the complexity of microbial communities. Apart from the above mentioned ability of different bacteria to cause diseases with similar symptoms, the infection may likely involve several strain groups of the same or similar pathogens. Many phytopathogens have not yet been studied by modern methods, and there are cases where bacteria with the same set of morphological and biochemical properties are found to be quite different genetically.

An example is the bacterium *Xanthomonas campestris* pv *Vesicatoria*, which causes bacterial leaf spot (BLS) on tomatoes and peppers. This bacterium, which was previously considered a separate species, is in fact a mix of at least four different species, each of which contributes to the development and manifestation of BLS symptoms. The high specialization of bacteriophages will likely render useless any phage applications against such uncharacterized microorganisms.

It is generally accepted that a key requirement for successful phage therapy is a high concentration of the phage. Indeed, for phage control to be effective, phages should be present in an amount exceeding a certain threshold relative to the target bacteria (*multiplicity of infection*). Most publications give numbers of 10^6–10^8 particles per ml, depending on the density of bacterial population. At concentrations below this threshold, the therapeutic effect will be insignificant. However, the production of high-concentration bacteriophage preparations to spray fields will most likely be economically unfeasible.

Moreover, regardless of the concentration ratio between the phage and the bacterium, phage therapy will not succeed unless the target microorganism is physically available to the phage. Environmental microbiologists use the term *spatial refuge* for the primary means by which a microorganism avoids an attack. Bacterial populations on the plant surface (*phyllosphere*) and in the soil layer adjacent to the roots (*rhizosphere*) are very heterogeneous in terms of density and are physically mobile. Viruses, however, cannot move on their own; therefore, their penetration into the plant tissues is a challenge even for preparations with a high bacteriophage concentration.

The vulnerability of phage particles deserves special attention. Field and laboratory studies have shown that viruses become inactive at high temperatures, at high and low acidity, when exposed to solar radiation, and in a medium with insufficient or excessive humidity. Thus, the conditions that are natural for the phyllosphere are detrimental to phages, leading to a strong and rapid decrease in the bacteriophage population on the treated plant. Introducing bacteriophages into the soil extends their circulation period, which may reach a few days, and the small size of the phages allows them to penetrate, together with the flow of fluid, directly into the vascular system of plants.

However, if the phage does not find the target bacterium to multiply, its lifetime in the colloidal solution will be limited. Although bacteriophage particles are completely biodegradable and do not accumulate in the environment, like inactive antibiotics and chemical protection products, the short period of existence (*persistence*) of phages on the plant surface and in the soil under natural conditions is a prime factor that virtually eliminates the possibility of their preventive use.

These are, of course, objective challenges. But are they really so insurmountable as is believed by phage opponents? As a rule, each of these problems has a solution.

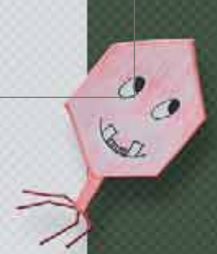
Problems solved

The narrowness of phages' infectious spectrum can be addressed by pre-screening, i.e., selective breeding of phages with a wider action spectrum or finding a combination of several phages to cover the most likely infectious range of the pathogen or the set of pathogens. This approach is used in the design of medical phage drugs, both those that have been produced for years in Russia and Georgia and those recently launched in a number of countries around the world. Some of these preparations include 30–40 different bacteriophages to target as many bacteria as possible.

However, there is a downside to this solution: combining a large number of different phages in high concentrations in one preparation may lead to interactions (aggregation, nonspecific adsorption, and unspecific recombination) between the phages. According to many researchers involved in the design of phage compositions, the optimal number of different bacteriophages in one preparation is 5 to 10 strains.

Designing phage products that combine several viruses infecting the same pathogen is an important step to address bacterial resistance to bacteriophages. There is now a good understanding of how bacteria develop this resistance; it is even possible to perform artificial selection of phages against bacteria that emerged as a result of the most likely mutations.

For example, let us assume that the fastest and most effective defensive solution for a bacterium is to discard or alter the receptor to which the bacteriophage attaches itself. These receptors are different molecules on the surface of bacteria: the proteins of *pili* (hair-like appendages for bacterial conjugation) and transport channels, surface



In the press, you may come across publications about horticultural applications of phage lysins, i. e., phage enzyme proteins that destroy bacterial cells. But these results look more like a ridiculous story than serious science: the undisputed limiting factor is the high cost of recombinant proteins and the need for point-like application of the products. Even if a particular enzyme product has proved effective for a few indoor plants, it is difficult to imagine a person walking with a brush across an endless potato or corn field in search of spots infected by bacterial pathogens

polysaccharides and peptidoglycans, etc. Excluding these molecules from the metabolism of the bacterial cell often has a strong impact on its viability: there is extensive evidence that phage-resistant mutants of pathogenic bacteria are less aggressive and the host microorganism can better cope with them. If a preparation contains several phages that attach to different receptors, then this combination will, first, reduce the probability of a mutant resistant to all phages and, second, the resulting mutant will be even less pathogenic.

Given the tremendous diversity of microorganisms in nature, the number of bacteria and their strains with significant pathogenic effect on plants is large yet limited. Modern molecular diagnostics (PCR and immunochemical methods) can already—or will soon be able to—detect most of these pathogens. Although more expensive, this diagnostics is faster and more accurate, compared with conventional microbiological methods. Therefore, it could be a viable solution for large-scale agricultural businesses to conduct regular microbiological monitoring and set up their own diagnostic laboratories.

The task of choosing a panel of bacteriophages capable of infecting phytopathogens that are most common in certain cultures in a particular locality is huge yet not infinite. In recent years, a new term has come into use: *personalized medicine*, which includes, *inter alia*, personalized selection of drugs based on the causative agent of the disease and the patient's condition.

In horticulture, it is virtually impossible to diagnose and treat each plant. However, when it comes to the treatment of large plots in one locality with one plant culture coming from a single seed source, then the organization of diagnostic monitoring followed by the selection and production of a phage preparation adapted to a specific pathogen is a realistic action plan fitting into the concept of high-tech agriculture.

Recently, smart solutions have emerged for how to maintain the necessary concentrations of phage-therapeutic agents and increase the time span of phage preparations. A well-known method of biological control and soil bioremediation in horticulture is the use of non-pathogenic bacteria belonging, as a rule, to the *Bacillus*, *Agrobacterium*, *Rhizobacterium* and *Pseudomonas* genera. When introduced into the soil, they act as antagonists of pathogenic microorganisms, preventing their reproduction and the transfer of infection on plants. This approach also has some fundamental limitations, but it has been quite successful in agriculture.

With the increase in our knowledge about the biology of bacteriophages, it was found that some of these bacteria may act as intermediate hosts for phages that are active against their close pathogenic relatives. The lytic activity of these phages is considerably lower, but when they are introduced into the rhizosphere together with the bacterial antagonists, their concentration will longer remain at a stable level than when phages are put there alone.

For example, certain strains of the bacterium *Pantoea agglomerans*, which serve as antagonists of *Erwinia amylovora*, a fruit-tree pathogen, may act as an intermediate reservoir for phages that are highly infectious towards this bacterium. The effect of this combination product was comparable with that of streptomycin (Balogh *et al.*, 2010). Another study described a successful use of a nonaggressive strain of *Ralstonia* as an intermediate host for a phage active against the solanaceous pathogen *R. solanacearum* (Fujiwara *et al.*, 2011). A further development of this approach may be the selection of phages that can multiply at a low rate in bacteria belonging to the normal soil flora.

To simplify and reduce the cost of applications, both bacteriophages and bacterial antagonists are typically used in the form of culture solutions. However, this approach makes it difficult to ensure the necessary concentration and viability of active agents during the storage of the phage preparation.

Recently, new technologically advanced and cost-effective methods have appeared whereby populations of bacteria and bacteriophages are confined in polymer capsules. This method stabilizes the biological product and ensures its gradual release into the rhizosphere. The treatment of seeds with these encapsulated products provides protection against pathogens in the early stages of plant development.

To implement phage control of plant diseases in modern agriculture, it is very important to have a clear understanding in what agronomic situations bacteriophages are the most effective. Phage therapy is most successful in closed biological systems with controllable physical conditions. It is almost impossible to achieve these ideal conditions on large agricultural fields; however, this does not mean that phage products cannot be effective there as well.

Today, an increasingly popular technology in commercial agriculture, especially, in the production of seeds, is greenhouse horticulture with fixed-composition substrates, drip irrigation and hydroponics. In these cases, the use of phage preparations against timely and accurately diagnosed pathogenic bacteria that cause plant diseases is fully justified and has a high chance of success.

Summarizing all the above, it can be argued that there are no fundamental obstacles to introducing bacteriophages for control and treatment of bacterial diseases in industrial horticulture. However, it is necessary to take some technological and methodological measures to make this promising approach an integral part of organic agricultural technologies.

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A NANOVERSION of Dr. Doolittle

BACTERIOPHAGES AS AN ALTERNATIVE TO ANTIBIOTICS IN VETERINARY

Key words: salmonella, chicken, bacteriophages, antibiotics, efflux, drug resistence, infectious safety

Infectious diseases of farm animals head the list of illnesses causing a significant economic loss due to the mortality and decreased productivity of the animals and because of considerable expenditures on the prevention and control measures. Of special importance are infections that also affect humans; these are anthrax, rabies, brucellosis, leptospirosis, and salmonellosis

One of the key and intensively developing branches of agriculture in many countries is poultry farming, giving about 300 million tons of meat annually, which is obtained from over 500 billion broilers. However, approximately 5 % of the birds, i. e. every second over 800 chickens, die of various diseases. Pathogenic bacteria, including those infecting humans, are among the major causes of avian mortality. The most widespread pathogens in this country are *Listeria*, *Yersinia*, *Salmonella*, *Campylobacter*, and some *Escherichia coli* strains.

Note that sooner or later every animal farm encounters the problem of infectious diseases, regardless of whether it breeds cattle, sheep, goats, pigs, or fur animals. Health support and safety of the products require combatting pathogenic microorganisms. Since the discovery of penicillin in 1928,

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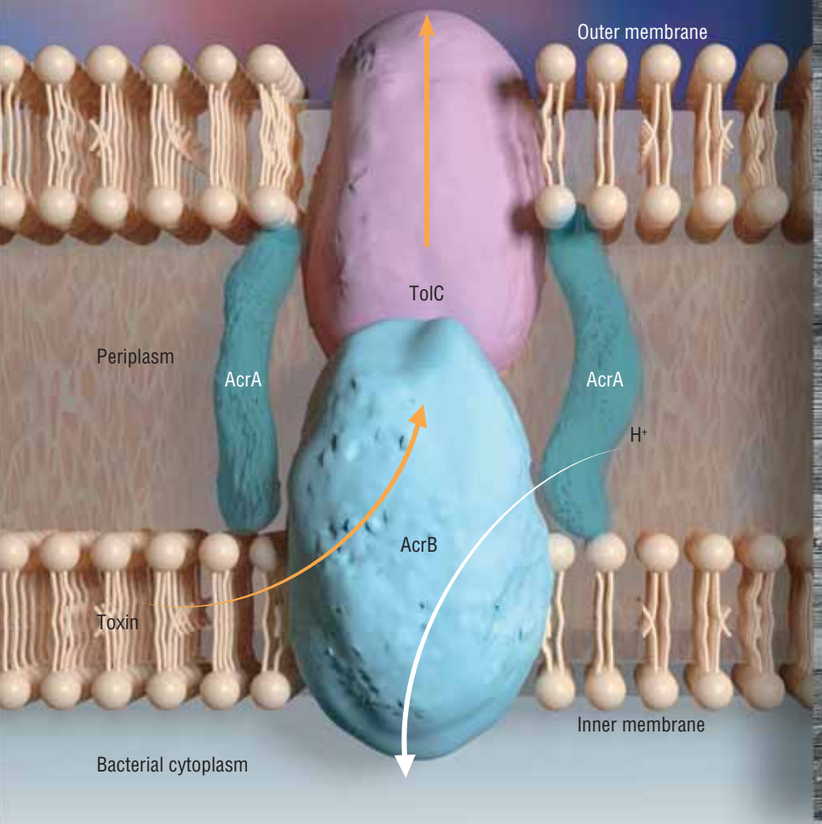


In the last decade, the *Salmonella* and *E. coli* strains resistant to a wide range of antibiotics have been detected in increasing frequency. The resistance of these microorganisms is often determined by the efflux, an active pumping-out of toxins from the bacterial cell implemented by transport (pump) systems of protein nature (Hernando-Amado *et al.*, 2016). The efflux of substances is triggered with the help of an intricate regulatory system that responds to various impacts, such as oxidative stress or exposure to some cation disinfectants used by poultry breeding facilities instead of antibiotics. Owing to the efflux, the bacteria become resistant to gentamicin, chloramphenicol, florfenicol, and other antibiotics, thus preventing their binding to the targets inside the cell

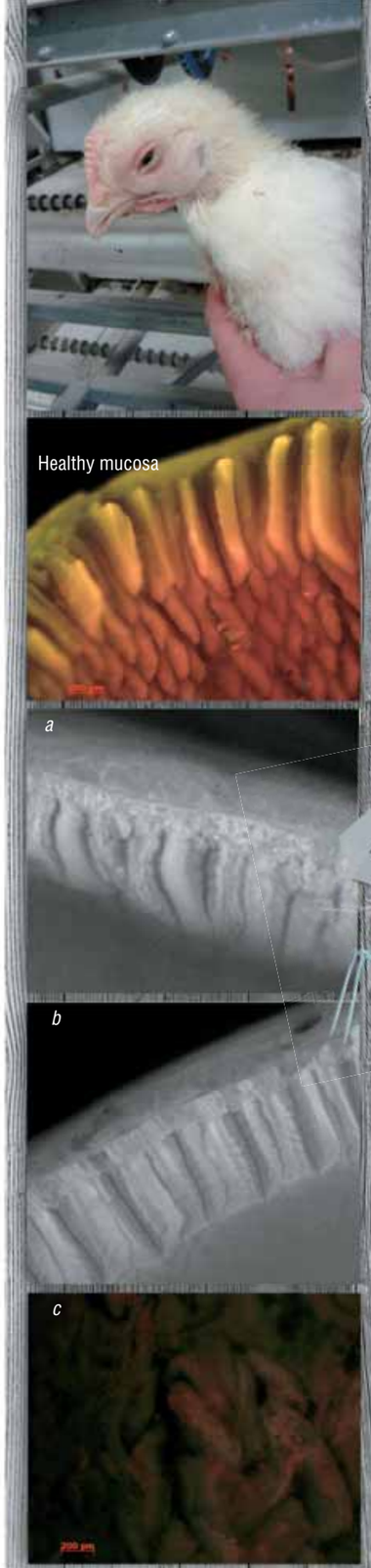
Bacterial efflux pump is a channel connecting the cell cytoplasm with the environment; it is used to eliminate the positively charged toxins. The channel is formed of the proteins AcrA and TolC, while the energy-dependent elimination of toxic compounds from the cytoplasm and periplasm is carried out by the protein AcrB (Klaas, 2009)

antibiotics have been a major tool of controlling bacterial infections. However, despite constant development of new drugs, the emergence of multiresistant bacterial strains displaying resistance to almost all available antibiotics has become a serious problem not only in public health care, but also in veterinary medicine.

The use of antibiotics in animal and poultry breeding is often criticized because, on the one hand, the drugs can be retained in the foodstuff and, on the other hand, they can induce the emergence of new drug-resistant pathogens. In addition, the use of antibiotics in agriculture is not always justified and is sometimes harmful. In particular, antibiotic therapy in the case of swine diseases is frequently a two-edged sword. For example, the bacteria causing clostridiosis, while defending themselves from the antibiotic, sometimes produce spores and synthesize toxins, thereby causing a *toxic infection* and injuring the intestinal mucosa. Another example is the *reproductive and respiratory syndrome:* antibiotics frequently aggravate the course of this viral infection inducing lung inflammation.

However, limited administration of antibiotics to animals could cause an increase in human infection by the corresponding pathogenic bacteria, resulting in turn in the increased use of antibiotics in the human population. In addition, today we know that there are mechanisms that can induce antibiotic resistance in bacteria even if they are not in contact with these drugs.

This challenge stirs up interest in searching for new therapeutic tools that will be able to replace or supplement antibiotics in controlling infectious diseases. The search for alternative means to treat bacterial infections immediately highlighted *phage therapy* and *phage prophylaxis* (Akimkin *et al.*, 2010).



This chicken, infected with a metapneumovirus that causes a severe respiratory disease, can easily die if infected by some bacteria, since in this case antibiotics turn out to be ineffective.
Photo by the courtesy of V. Afonyushkin

From a laboratory to a poultry plant

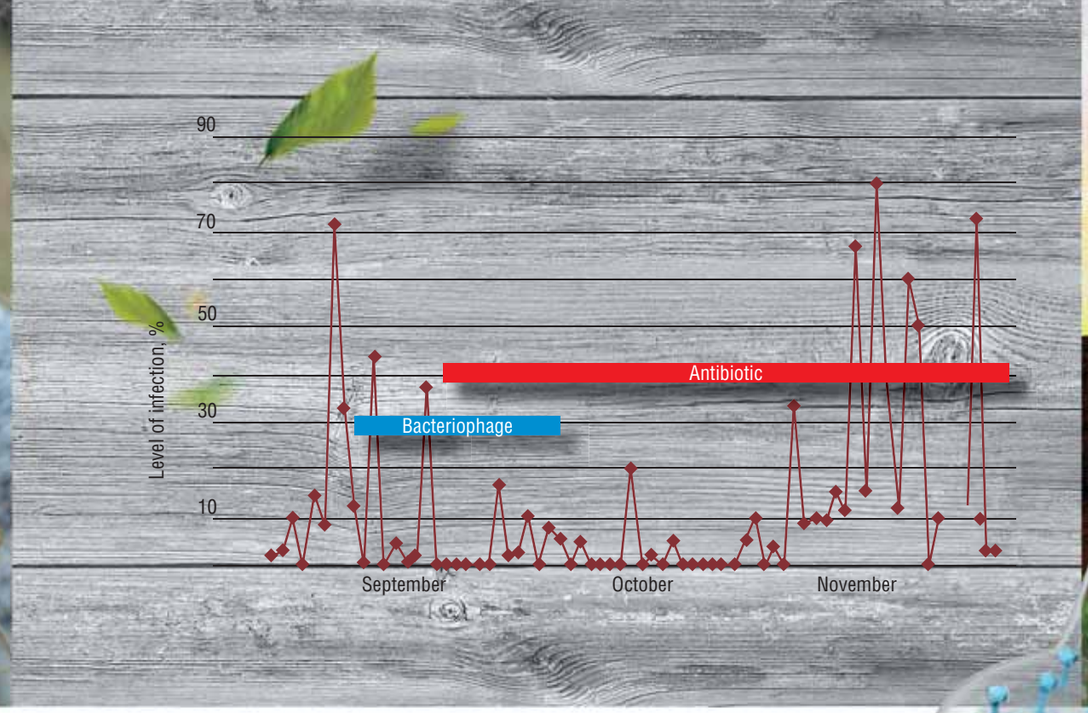
W. Smith and his colleagues from the Institute for Animal Disease Research (Great Britain) may be regarded as pioneers in using phages to treat animals (Smith *et al.*, 1987). They studied the laboratory mice experimentally infected with *E. coli* and found that even a single administration of the bacteriophage preparation significantly reduced the number of viable bacterial cells in the gastrointestinal tract. Later they did the same experiments on the calves, lambs, and guinea pigs infected with a virulent *E. coli* strain that caused diarrhea. Phage therapy reduced the bacterial counts in the gastrointestinal tract as well as alleviated the symptoms associated with this infection, such as dehydration. As a result, almost all infected animals survived.

The administration of bacteriophage preparations in large agricultural facilities has its own specific features favoring this kind of therapy. Biological safety systems in large agricultural facilities efficiently limit the diversity of infections; correspondingly, the number of pathogenic microbial species is considerably smaller than in the human population. That is why the infections present in such plants are highly reproducible, so the diagnosis made at one poultry house can be extrapolated to other houses. However, it should be kept in mind that bacteria can defend themselves against phages. For example, the experiments on bacterial monocultures have shown that administering therapeutic phages leads to the emergence of phage –resistant bacterial cells; this takes only a few hours. In addition, while antibiotics have a relatively wide range of action, there is no superbacteriophage that can attack a large number of different microbial species and strains. Thus, it is more practical to use complex bacteriophage cocktails.

Today, it is already technically and economically feasible to search for the necessary bacteriophages for an individual farm and to produce the corresponding biological preparations. This does not exterminate the overall range of pathogenic bacteria but makes it possible to select and use efficient bacteriophages against the bacteria most dangerous from a sanitary or epidemic standpoint.

Bacteriophage preparations against salmonellas and *E. coli* have been successfully used in large poultry plants. For example, an abnormally high salmonella infection rate (50—70 %) of broiler chickens at a poultry plant was decreased to an unrecordable level over several months. Besides, the

The chickens had dystrophic and inflammatory changes in the small intestinal mucosa; in this case, administration of antibiotics is not only inefficient but even increases the mortality rate: (*a*) viral enteritis of the duodenum complicated by bacterial infection; (*b*) edema of the duodenal mucosa against the background of an unknown, presumably viral infection; and (*c*) necrosis of the small intestinal mucosa against the background of *Clostridium* and *Eimeria* infections.
The courtesy of V. Afonyushkin



Experimental administration of bacteriophages at a Russian poultry plant,* conducted by a team from the Siberian Federal Research Center of Agrobiotechnologies (Russian Academy of Sciences), showed that phage therapy helped to decrease the level of salmonella infection. The infection level remained relatively low some time after the administering of the bacteriophage was discontinued, which was due to its spontaneous circulation in the facility. Then the level of salmonella carriage returned to its initial value though the antibiotic ciprofloxacin was used.
**Each point on the plot corresponds to an individual poultry house with 40 000 broiler chickens 41 days old*

FOR CREATURES GREAT AND SMALL

Our pets, such as cats and dogs, are also affected by bacterial infections. They come into close contact with people, thus becoming a more dangerous source of infections, such as leptospirosis, which affects the kidneys and liver, or intestinal yersiniosis, accompanied in humans by chronic abdominal pain and diarrhea, which can sometimes even lead to death. Highly pathogenic variants of *E. coli*, salmonella, *Campylobacter*, and *Clostridium* can also enter the human body from cats and dogs, causing intestinal infections, bacterial sepsis, and hemolytic-uremic syndrome. In this case bacteriophage therapy for animals would also be a good prevention measure for their owners who would not run the risk of being infected with antibiotic-resistant bacteria from the pets.

Unfortunately, the veterinary toolkit still lacks bacteriophage preparations against such severe diseases as leptospirosis and yersiniosis, although research work aimed at designing polyvalent anti-yersiniosis bacteriophages is in progress. The web search, including the forums of pet owners, demonstrates that the list of drugs for animal treatment contains some “human” phage preparations, such as staphylococcus and streptococcus bacteriophages, used for the prevention of purulent skin and mucosal infections as well as of other infections caused by these bacteria, and complex pyobacteriophage (pyopolyphage), which displays a wide range of antibacterial activities and is used for treating several purulent and inflammatory diseases. The specific therapy of puppies and kittens infected with pathogenic *E. coli* strains, which frequently cause a lethal outcome, requires the use of the bacteriophage against paratyphoid fever and colibacteriosis

administration of bacteriophages, vaccine virus strains, and useful probiotic bacteria in large poultry and pig plants is organized after an epidemic pattern, which considerably reduces the cost of infection control and increases its efficiency.

In the above example with salmonellas, the bacterial serotype changed in the course of phage therapy, bringing about a new strain, *Infantis*, resistant to the bacteriophage that was used. This suggests that bacteriophages may act as a factor of interspecific competition for bacteria. The fact is that the concentration of *Salmonella* bacteria in the intestines is relatively low, which could suggest that there is no competition between different species and subspecies of this genus (Antunes *et al.*, 2016). However, the succession of different salmonella serotypes in chicken populations indicates the nonrandom character of this phenomenon.

Thus, at present the salmonella serotypes *Gallinarum* and *Pullorum* almost do not occur in the intestines as distinct from the serotype *Enteritidis*, which, in turn, occurs much less frequently than the new serotype *Infantis*. Interestingly, it was during the increase in the rate of chicken infection with the *Infantis* salmonellas that the research team from the Institute of Chemical Biology and Fundamental Medicine (Siberian Branch, Russian Academy of Sciences) and from the State Research Center of Virology and Biotechnology *Vector* (Novosibirsk, Russia) isolated a large amount of bacteriophages active against the *Enteritidis* serotype. However, the bacteriophages against the *Infantis* serotype were rather few. In fact, there may be several explanations for this; the idea that bacteriophages are involved in displacing closely related bacterial groups looks very probable.

The relevant research literature widely covers the phenomenon of *polyhostality* (the ability to infect a wide range of bacterial species) of bacteriophages and the phenomenon of different phage resistance of individual bacterial strains within the same species towards the same bacteriophage. Evidently, the bacterial strain that can maintain the existence of a bacteriophage without killing the host cell will be able to acquire an evolutionary advantage because it will cause death to the competing bacteria sensitive to the bacteriophage. This example shows how many different biological ideas can appear when interactions between bacteria and bacteriophages are observed. In addition, by studying the spontaneous application of bacteriophages in poultry and pig plants it is possible to quite safely simulate epidemics.

Bacteriophages are unlikely to replace antibiotics in the nearest future. Nonetheless, bacteriophages are undoubtedly useful when antibiotics do not work and when it is necessary to prevent the emergence of antibiotic-resistant bacterial strains.

The use of bacteriophages is much more promising in veterinary medicine than in human medicine. It takes a shorter time for new veterinary drugs to enter the market as compared with human drugs. In addition, the diversity of infections at a large animal breeding farm is considerably ower as compared with the infections diagnosed in humans, which simplifies the identification of bacteria and selection of the corresponding bacteriophages.

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This is how a filamentous bacteriophage looks like after counterstaining with phosphotungstic acid in the field of vision of a Jem 1400 (JEOL, Japan) electron microscope by E. Ryabchikova

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