EX-SOVIET DEVELOPMENTS IN THE ELIAVA INSTITUTE OF BACTERIOPHAGE, MICROBIOLOGY AND VIROLOGY
A WORLD PREMIER INSTITUTE IN BACTERIOPHAGE RESEARCH

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Introduction

At the beginning of the 20th century d'Herelle suggested that bacterial viruses (bacteriophages) might be applied to the control of bacterial diseases. In the West this idea was explored in a desultory fashion only and was eventually discarded largely due to the advent of extensive antibiotic usage. However, interest was maintained in the countries of the FSU where bacteriophage therapy has been applied extensively since that time. Central to this work was the Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi, Georgia, which was founded thanks to joint efforts of two professors George Eliava and Felix D'Herelle (Fig. 1). This institute remained continuously active in this field for more than 75 years.

Figure 1. Dr. Elena Makashvili (left), Professor Felix D'Herelle (in the centre), Professor George Eliava (right). Photo is taken in 1934 in Tbilisi, Georgia.

Vast collections of prophylactic and therapeutic phages have been gathered during the long history of the institute.

In the Eliava IBMV, phages were sought for bacterial pathogens implicated in disease outbreaks in different parts of the SU and were dispatched for use in hospitals throughout the country. Although infections caused by a wide variety of bacterial pathogens have been treated, much of this has been published in Russian and is not readily available in the West. By contrast, interest in the West has been limited to a small number of enthusiasts and academics and until very recently there has been little interest. The main
reason that the medical and scientific communities are now beginning to take notice, is the continuing world-wide rise in the incidence of multiple-antibiotic-resistant bacterial pathogens and the absence of effective means for their control.

The present report summarizes previous experience of use of bacteriophages in medicine as developed in the FSU countries.

The principle activities of bacteriophage relevant to their application in therapy

Bacteriophages are viruses for which bacteria are the natural hosts. There are two main groups of bacteriophages – temperate and intemperate. The initial stage of infection for both groups is an adsorption of an individual phage particle to a susceptible bacterium. This occurs usually through the interaction between the phage tail and a specific attachment molecule on the related bacterial surface. The phage injects its nucleic acid into the cell where it replicates itself (Fig. 2).

Figure 2. Attachment of the phage Sb-1 to the surface of S. aureus

The temperate phages form stable relationship with the host cell, which maintains for many generations. Replication of intemperate phages results in formation of the mature particles around the newly synthesized phage nucleic acid followed by lysis of the cell and release of the new daughter phages (Fig. 3).

Figure 3. Propagation of a virulent bacteriophage Sb-1 inside S. aureus bacterial cell.
The whole idea suggested by D'Herelle in early 1920s involves the exploitation and application of a biological system that is already in operation naturally and for which there is good evidence as to its ability to determine the outcome of natural and experimental infections. Thus, it is likely that the conditions exist under which the system operates optimally and all attempts must be made to reproduce these conditions to achieve optimal activity.

Clinical efficacy of bacteriophages in therapy and prophylaxis: experience from the Eliava Institute for Bacteriophage, Microbiology and Virology (IBMV)

Reviewing the old Soviet literature it becomes obvious that the mass application of bacteriophages was first facilitated in relation to the Finnish campaign (1938-1939) and then during the Second World War (1941-1945). The period between 1930s and 1940s coincides with the peak of a number of scientific publications on phage therapy. Hence, it is sometimes difficult to find access to the old data. This, in part, explains why most of the results of the clinical experiments are not properly designed and registered and the fact that the data for the control groups often are missing. Migration of the patients from a frontline hospital to the base one did not allow the doctors to accomplish a proper monitoring of the clinical effect of phage therapy. However, the period of war and lack of therapeutic preparations inspired the Soviet doctors to perform the new trials with phages and invent the novel methods for their administration. Ironically, this period turned into a genuine heyday for a creative search in the field of phage therapy.

The first mass experiment - prophylaxis of gas gangrene during the Second World War

Kokin (1947 - cited in Krestovnikova, 1947) describes application of the mixtures of anaerobic, Staphylococcus and Streptococcus phages (produced by the IBMV, Tbilisi, Georgia) for treatment of gas gangrene in soldiers. The mixture was applied to 767 cases and resulted in a decrease of the death rate to 18.8% instead of 42.2% in the control group treated with the traditional methods. The other group of authors have observed the low death rates of 19.2% in the group of soldiers treated with the same mixture of phages against 54.2% treated with other medications (Lvov, Pasternak, 1947 - cited in Krestovnikova, 1947).

In addition to therapy, this phage mixture has been used by the mobile sanitary brigades as an emergency aid for treatment of wounds (prophylactics for gas gangrene) (Krestovnikova, 1947). This paper summarizes the observations of the three mobile brigades. Observation was carried out within 2-6 weeks in course of the evacuation of the patients from the front-line hospital to the basic one. In the first group counting 2500 soldiers and treated with phages, only 35 (1.4%) showed the symptoms of gas gangrene. In the control group of 7918 wounded soldiers, 342 (4.3%) were infected. The second brigade applied phage therapy to 941 soldiers, only 14 (1.4%) of which were infected with gas gangrene, in comparison with 6.8% from the control group treated with other methods. The third brigade dealing with 2584
solders observed the development of the disease in 18 soldiers (0.7%) while in the control group, the disease has emerged just in 2.3% of cases. Comparing the data described by the three independent brigades it becomes apparent that the 3-fold decrease of incidence of the cases with gas gangrene is a direct consequence of the application of phage mixtures for prophylactic treatment of wounds (Krestovnikova, 1947).

Treatment of the deep forms of dermatitis caused by Staphylococcal infection

Bacteriophage therapy of the deep forms of dermatitis resulting from Staphylococcus was shown to be effective and is described in a number of articles (Izashvili, 1940; Gvazava, 1957; Khuskivadze, 1954; Vartapetov, 1947, 1957). More recent studies (Shvelidze, 1970) were performed on a group of 161 patients with chronic and frequently relapsing infections with the following diagnosis: 62 patients with furuncles (boils) and furunculosis, 57 with carbuncles and 45 with hydroadenitis. In all cases antibiotic therapy with penicillin, biomicon, streptomycin had been performed without a positive outcome.

Duration of the chronic diseases varied from 2-3 months to 15-20 years. All the patients claimed to have high temperatures (37-37.8°C), headaches, weakness, insomnia and movement difficulties. Most of the patients with furuncles had 1-12 boils in different parts of the body. Microbial analysis of specimens obtained from the 161 patients identified 139 strains of coagulase-positive Staphylococcus. The 83% of these strains appeared to be penicillin-resistant. Phages were administered topically as applications and injected subcutaneously around the infected locus. In total 95 % (152 cases) were completely cured, 4.3% (7 patients) showed significant improvement and in 1.3% (2 patients) the treatment had no effect. Long-term surveillance was performed over the next 4 years. Relapse was observed after 3-6 months in 8.5 % of cases only. These patients underwent an additional course of the phage therapy after which they were completely cured. The results are summarized in Table 1.
Table 1: Effect of phage therapy for treatment of certain forms of dermal infections caused by *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diagnosis</th>
<th>Number of patients</th>
<th>Type of therapy</th>
<th>Duration of treatment (min-max)</th>
<th>Complete cure (%)</th>
<th>Improvement (%)</th>
<th>Temporary effect (%)</th>
<th>No effect (%)</th>
<th>Re-infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. Gr 1</td>
<td>Furunculosis</td>
<td>62</td>
<td>Phage</td>
<td>3-10 days</td>
<td>97.7</td>
<td>3.3</td>
<td>-</td>
<td>0</td>
<td>4.8</td>
</tr>
<tr>
<td>Control 1</td>
<td>Furunculosis</td>
<td>62</td>
<td>Antibiotics</td>
<td>3 months 16 years</td>
<td>0</td>
<td>53.3</td>
<td>20.9</td>
<td>25.8</td>
<td>100</td>
</tr>
<tr>
<td>Exp. Gr 2</td>
<td>Carbunculosis</td>
<td>54</td>
<td>Phage</td>
<td>5-10 days</td>
<td>66.7</td>
<td>20.0</td>
<td>-</td>
<td>13.3</td>
<td>11.1</td>
</tr>
<tr>
<td>Control 2</td>
<td>Carbunculosis</td>
<td>54</td>
<td>Antibiotics</td>
<td>2 months 15 years</td>
<td>0</td>
<td>7.4</td>
<td>92.6</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

The control groups are presented by the same groups of patients previously treated with antibiotics (Shvelidze, 1970).

**Combined phage and antibiotic therapy**

The end of the 1950s and beginning of the 1960s was marked by the emergence of a new direction in Soviet medicine. The researchers started to elaborate regimens by combining phages and antibiotic therapies (Jakobson, 1956; Sheviakova et al, 1956: 1958: 1961; 1964). From these types of experiment, the work accomplished by Vepkhvadze (1974) is especially remarkable. She carried out *in vivo* studies on a mouse model. The 20 mice in each group were infected with antibiotic resistant *Staphylococcus aureus*. The groups were then treated with the specific *Staphylococcus* phage (a product of the IBMV, Tbilisi, Georgia) alone, with the antibiotics alone and the phages in combination with different antibiotics (dichlorotetracycline, erythromycin, pasomycin, oxacillin). In the first series of experiments the antibiotics and the phages were administered immediately after infection. In the second series, the phages were administered immediately after infection, while the antibiotics were added with different intervals of time (4, 8, 24 hours after infection). In the third series, the antibiotics were administered immediately after infection, while the phages were given 4-8-24 h. after this. It was shown that results of treatment performed separately with either sub-therapeutic doses of phages (app. titre $1 \times 10^7$ pfu/ml) or antibiotics (3200 µg/ml), are quite similar (Table 2). However, for the combined use of antibiotics and phages, the best results were achieved when the antibiotics were administered 24 hours prior to phage treatment. It was recommended to use the bacteriophages in combination with the antibiotics but with the shift of 24 hours. The data presented in Table 2, shows the greater efficacy when phage were administered 24 hours after administration of antibiotic and microbial challenge with 75% of mice surviving to day 15th.

From 40% to 65% of mice survived, correspondingly, in the cases of the 4 and
8 hours delayed administration of phages. The control groups, where only erythromycin was administered simultaneously with the challenge indicated 45% of survival and, in the group where only phage was administered after 24 hours the survival rate was 35% (Vepkhvadze, 1974).

Table 2: Combined application of the phages and antibiotics

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Administration</th>
<th>Number of mice</th>
<th>Number of mice survived through the 15th day</th>
<th>P</th>
<th>Average life expectancy (days after manipulation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Abs. Number</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phage + Ery</td>
<td>Simultaneously with infection after 4 hours</td>
<td>20</td>
<td>8</td>
<td>40</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Phage + Ery</td>
<td>Simultaneously with infection after 8 hours</td>
<td>20</td>
<td>7</td>
<td>35</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Ery alone</td>
<td>After 8 hours</td>
<td>20</td>
<td>9</td>
<td>45</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Phage + Ery</td>
<td>Simultaneously with infection after 24 hours</td>
<td>20</td>
<td>8</td>
<td>40</td>
<td>5.8</td>
</tr>
<tr>
<td>Ery alone</td>
<td>After 24 hours</td>
<td>20</td>
<td>8</td>
<td>40</td>
<td>6.6</td>
</tr>
<tr>
<td>Ery + Phage</td>
<td>Simultaneously with infection after 4 hours</td>
<td>20</td>
<td>8</td>
<td>40</td>
<td>6.4</td>
</tr>
<tr>
<td>Ery alone</td>
<td>Simultaneously with infection</td>
<td>20</td>
<td>9</td>
<td>45</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Phage alone</td>
<td>4 hours after infection</td>
<td>20</td>
<td>8</td>
<td>40</td>
<td>3.2</td>
</tr>
<tr>
<td>Ery + Phage</td>
<td>Simultaneously with infection after 8 hours</td>
<td>20</td>
<td>13</td>
<td>65</td>
<td>8.1</td>
</tr>
<tr>
<td>Ery alone</td>
<td>Simultaneously with infection after 24 hours</td>
<td>20</td>
<td>9</td>
<td>45</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Phage alone</td>
<td>8 hours after infection</td>
<td>20</td>
<td>7</td>
<td>35</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ery + Phage</td>
<td>Simultaneously with infection after 24 hours</td>
<td>20</td>
<td>15</td>
<td>75</td>
<td>9</td>
</tr>
<tr>
<td>Ery alone</td>
<td>Simultaneously with infection after 24 hours</td>
<td>20</td>
<td>9</td>
<td>45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Phage alone</td>
<td>24 hours after infection</td>
<td>20</td>
<td>7</td>
<td>35</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The table is borrowed from the Ph.D. dissertation of Dr. L. Vepkhvadze.

**S. aureus phage for intravenous use**

Although the trails of intravenously administration of phages started at the very early stages of clinical experiments in 1930s and 1940s, this type of therapy was rejected due to the unfavorable side effects including rising temperature, up to 38-39 °C, shivering, headaches, etc. However, a lethal outcome has never been reported (Tsulukidze, 1938; Ukelis, 1940). The highest and the most recent achievement among the activities of the Eliava Institute of Bacteriophage is elaboration of the apyrogenic *Staphylococcus* phage for intravenous use (Chirakadze, Chanishvili, 1964; Chanishvili T., et
al., 1974; Nadiradze M.M, 1984), the action of which was proved in clinical experiments on adults and children. Altogether 900 patients were involved in this study, among them 247 children aged between 1 day and 15 years and 653 adults in the aged 15-72. The 494 persons were treated with phages alone or in combination with antibiotics and/or immune-stimulating means. The 406 persons from the control group were treated with antibiotics only. Intravenous use of phages did not have any significant side effect.

**Figure 4. The phage Sb-1 related to S. aureus used as a component of the intravenous bacteriophage preparation.**

After this experiment the Staphylococcus phage was produced by the industrial part of the Eliava Bacteriophage Institute and successfully applied in many clinics throughout the whole territory of the former Soviet Union (Fig. 4). The phage was applied for intra-venous use in infusions, transfusions and injections. It was mostly used for treatment of the chronic septicaemia, for treatment and prophylactics of eye, ear, throat, lung diseases, for healing burned wounds, bacterial consequence of surgical operations on bones and skull, women infertility related with bacterial inflammation problems and so on (Chkhetia, 1984; Samsygina, 1985; Bochorishvili, 1988).

**Table 3: Effect of phage therapy in the cases of septicaemia among adults and children**

<table>
<thead>
<tr>
<th></th>
<th>Adults</th>
<th></th>
<th>Children</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cases</td>
<td>Fatal cases</td>
<td>Per cent of mortality</td>
<td>Number of cases</td>
</tr>
<tr>
<td><strong>Experimental Group</strong></td>
<td>215</td>
<td>0</td>
<td>0</td>
<td>149</td>
</tr>
<tr>
<td><strong>Control Group</strong></td>
<td>308</td>
<td>11</td>
<td>3.6</td>
<td>98</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>653</td>
<td>11</td>
<td>1.68</td>
<td>247</td>
</tr>
</tbody>
</table>
Prophylaxis

In the former Soviet Union the phages have been extensively used for prophylaxis as well, especially for communities where the spread of infections might be rapid such as kindergartens, schools, military accommodation etc. According to the review written by Krestovnikova, (1947) an experiment on prophylactic use of phages was successfully accomplished in 1935 on thousands of people in the regions with high incidence of dysentery. Later on different modifications of the dysenterial phages, namely dry tablet forms have been included into the clinical studies. One of the latest studies describes the results of preventive treatment performed with the phage tablet forms having an acid-resistant coverage (Anpilov, Proskudin, 1984). Experimental and control groups were formed according to accidental selection, one soldier was taken as an observation unit. The persons in the experimental and control groups, that were located in different geographical zones of the USSR, however were placed in the similar epidemic conditions. The coded bacteriophage preparations and placebo were given seasonally during the rise of morbidity (threat of epidemics), in particular in June-July and in September-October. The coded preparations were given to the persons from experimental and control groups 1.5 - 2 hours prior to meals, 2 tablets each time. One group of people was receiving these tablets at intervals of 3 days, while another group was receiving them at 5 day intervals. Calcium gluconate was used in placebo experiments. The phage and placebo was given to every second person, thus providing quantity and quality equity of the experimental and control groups. Efficiency of prevention of the phage prophylactics taken once per 3 days was 75%, and 67% when taken once per 5 days. Thus, a conclusion was drawn according to which it was recommended to use the phage tablets once per 3 days.

Phages versus antibiotics

One of the areas that have made the idea of phage therapy attractive once again is the increasing prevalence of antibiotic resistant bacteria. The widespread use of antibiotics in modern medical practice is related to their rapid antibacterial action and broad spectrum of activity. This last characteristic is crucial in the treatment of diseases where the pathogens have not been identified but therapy is required immediately. However, antibiotic usage has negative effects some of which are becoming increasingly important including, generalized, non-specific antimicrobial activity that damages the normal microflora enabling colonization by opportunistic pathogens.

- Side effects, such as allergy and toxicity, including effects on the immune system.
- Selection of antibiotic-resistant bacteria and enhanced rates of transfer in the absence of the normal flora
- Enhanced spread of fungal and yeast infections.
Phage therapy/prophylaxis has a number of attractive advantages:

- Bacteriophages are highly specific for the target bacteria without affecting the normal microflora and are effective against multiple drug-resistant bacteria.
- Following numerous studies in animals and from clinical experience no adverse side effects have been observed. Purified phage preparations do not cause significant side effects such as allergy, intoxication, etc.
- Because of their ability to multiply in the target hosts a single dose treatment can be envisaged for many diseases.
- The environment provides an almost inexhaustible source of new active phages when required. Ecological safety of phages is implied by their high specificity towards the target bacteria. Bacteriophages will therefore not accumulate in the environment.
- Production of bacteriophages is economical. It does not require sophisticated and expensive equipment or complicated and multi-stage technologies.

**SUMMARY**

In summary, despite the early failures in the West, countries in the former Soviet Union have continued to use bacteriophages for disease therapy and prophylaxis and sterilization purposes with apparent success. Given the increasing concern over multiple antibiotic resistance in a number of bacterial pathogens, this is an area of disease control whose potential is proven and should be reviewed.

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