A STUDY OF PLAGUE PHAGES AND THE BACTERIAL MUTANTS RESISTANT TO THEM

REPORT I. BIOLOGICAL PROPERTIES OF THE PLAGUE PHAGES

A. I. Volosivets

From the All-Union Scientific-Research Institute of "Microbes", Dir., Prof. N. I. Nikolaev, Saratov
(Presented by Active Member of the Akad. Med. Nauk SSSR, N. N. Zhukov-Verezhnikov)
Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 9,
pp. 81-84, September, 1963
Original article submitted November 12, 1962

It has been established by numerous investigations that bacterial cultures are not uniform in their ability to adsorb phage, and also in their sensitivity to it. According to the contemporary data, phage resistance is acquired with lysogenization of bacteria, or as a result of their mutations. The features determining the appearance of resistance with lysogenization are sufficiently elucidated, since they are related to penetration into the cell of the DNA from the intermediate phage. As regards phage resistance arising as a result of mutation, two hypotheses are known. One of them holds that the phage resistant mutants are a result of spontaneous mutations of the bacteria, independent of their contact with phage, the role of which leads only to selection of the resistant variants. According to the other hypothesis, resistant mutants appear as a result of hereditary changes in the cells secondary to their contact with phage (adaptation). It has been proven that the phage resistant variants of the intestinal bacilli have a mutation origin [12, 13, 14]. In relation to other species of bacteria however, especially pathogenic ones, this hypothesis raises doubts. Thus, a study of the origin of phage resistance in various species of bacteria, combined with investigations on the properties of phages and the phage resistant variants, appears to be one of the means for demonstrating the general patterns that determine the nature of bacteriophagy.

In this report, we present the results of studying the biological properties of plague phages (EB, 1-17, d'Erell), which were used to obtain phage resistant variants of plague bacteria and to clarify their origin. The properties studied include the range of activity of the phages, their specificity, adsorption capacity, curation of intracellular development, and average harvest per single bacterial cell.

EXPERIMENTAL METHOD

Sensitivity to the phages EB, 1-17, and d'Erell, was tested on 100 strains of the plague microbe with varying virulence, in the R-, OR- and OS-form [4], by applying a drop of undiluted phage to the surface of an 18-hour culture, and then distributing it in the form of a trail, and also by the method of agar layers, using diluted phages (also 100 strains). The other properties (adsorption capacity, duration of intracellular development, and average harvest per single cell) were studied according to the methods described by Adams [8, 10]. In the experiments for demonstrating the adsorption capacity, the latent period, and the productivity, we used casein bouillon.

EXPERIMENTAL RESULTS

In studying the strains of the plague pathogen, isolated in mountainous and flat areas (Central Asia), we could not find any phage resistant variants of the bacteria among them (Table 1).

As can be seen from Table 1, all strains, regardless of the site of their isolation or their level of virulence, were sensitive to the available phages.

The literature describes the capacity of plague phages to lyse the pathogens of pseudotuberculosis, hemorrhagic septicemia, and representatives of the Salmonella family [1, 2, 5, 7]. In order to demonstrate the specificity of the test phages EB, 1-17, and d'Erell, raised on an avirulent indicator culture (EB), we set up an experiment using 211 strains of the plague pathogen, 40 of the pseudotuberculosis pathogen, and 202 strains of the microbes causing

TABLE 1. Range of Action of the Plague Phages (Number of Cultures That Were Lysed by the Phages)

Origin	Virulence of the culture	Name of the phages and degree of their dilution					
		EB		d'Erell		1-17	
		10^6	10^7	10^6	10^7	10^6	10^7
Lowlands	Avirulent		15		15	2	13
	Virulent*		50		50	7	43
Mountains	Avirulent		14		14	1	13
	Virulent		21		21	2	19
	EB-virulent		1(70)†		1(110)†		1(20)†

* The virulent strains of the plague pathogen were considered those that caused the death of guinea pigs in the course of 4-20 days.

† The figure in parentheses shows the number of negative colonies for the EB strain.

TABLE 2. Adsorption Capacity of the Plague Phages

Phage	Adsorption of phages (in %) after contact for a period of					Constant for the rate of phage adsorption (in million/min)
	1 min	3 mins	4 mins	5 mins	6 mins	
EB	25.9	45.9	54.2	66.7	66.7	$4.4 \cdot 10^9$
1-17	47.1	50.6	64.2	73	73	$5.2 \cdot 10^9$
d'Erell	31.7	38.7	54.6	59.2	72.8	$3.2 \cdot 10^9$

various infections (paracholera vibrios, Salmonellae, dysentery microbes, Brucellae, Pasteurellae, Listerellae, sporulating, encapsulated, pigment forming, cocci, etc.). The results of the experiments showed that, in addition to the plague pathogens, sensitivity to the phages was also shown by certain strains of the pseudotuberculosis microbes. In this case, the percent of the pseudotuberculosis pathogens that were lyzed was equal to 10 for the EB and d'Erell phages, and 12 for the 1-17 phages. Among the strains of the pseudotuberculosis pathogen that were lysed, we encountered the R- and OR-forms; among the resistant strains—R-, OR-, and S-forms. There was absolutely no lysis of representatives of the other species of microorganisms. This specificity is apparently the result of raising the phages on one strain (EB).

In the experiments studying the adsorption capacity of the phages, it was shown that the basic mass of phage corpuscles is adsorbed in a period of 5 minutes in the case of the EB and 1-17 phages, and 8 minutes for d'Erell phage. However, in certain experiments, the basic mass of phage particles was adsorbed in the 8th minute for the EB and 1-17 phages, and in the 10th minute for d'Erell phage. In the following period, the number of phage particles remained constant, or fluctuated between slight increases and decreases.

Table 2 shows that the dynamics and the constant for the rate of adsorption were different for the different test phages. The highest figure for the adsorption rate constant was noted in phage 1-17 (titer $11 \cdot 10^8$), a lower figure was observed for phage EB (titer $7 \cdot 10^8$), and the lowest number was seen with phage d'Erell (titer $2 \cdot 10^8$).

Thus, the adsorption capacity of the plague phages, as in the case of many others, is correlated with their lytic activity.

The next stage of the work was a demonstration of the interrelation between the lytic activity of the phages and the duration of their intracellular development, and also their productivity (Table 3).

In view of the fact that the size of the negative colonies of plague phage considerably exceeded that of the other phages, and they rapidly blended into one another, it was difficult to count them in the bounds of the first 2-3 hundred. For this reason, the end of the latent period was taken as that time when, after 6 hours of incubation at 28°, it was still possible to count the individual negative colonies, and when in the following interval we observed their fusion. Using this determination, the end of the latent period for the EB and 1-17 bacteriophages occurred on the 23rd minute, and for d'Erell, on the 25th minute. As in the case of the adsorption capacity, the period of intracellular development of the phages was correlated with their lytic activity.

TABLE 3. Latent Period and Harvest of Plague Phages

Phage	Latent period of development (in minutes	Average number of phage particles per single cell
EB	23	100
1-17	23	95
d'Erell	25	56

In addition to the experiment elucidating the average harvest of one cycle of development, we performed a supplementary study of this characteristic in individually isolated bacteria [10]. It was shown that the average harvest for the EB bacteriophage was equal to 110 corpuscles per cell, for phage

TABLE 4. Serological Properties of the Plague Phages

Phage	Duration of contact (in minutes)	Neutralization of the phages (in %) by the antiphage sera in a dilution of 1:10					
		antiserum of EB	K	antiserum of 1-17	K	antiserum of d'Erell	K
EB	5	95.4	6.1				
	10	98.5	4.1	97.5	3.6	87.5	2.0
1-17	5			86	3.9	86	
	10	95.2	3.0	98	3.9	92	1.9
d'Erell	5						4.8
	10	98.4	4.1	96.4	3.3	93	2.6

1-17—72 phage particles, and for d'Erell—54. Looking at all the obtained results, it may be noted that the average harvest of EB and d'Erell phages, ascertained by the two methods, remained more constant (EB-100-106-110; d'Erell-51-54-56), while the harvest of bacteriophage 1-17 ranged from 72-95 phage particles per bacterial cell.

It is known that one of the most reliable taxonomical characteristics for classifying bacteriophages is their antigenic properties [9], which are determined, in experiments, from their neutralization by antiphage sera. The neutralization reaction is characterized by the magnitude of K—the constant for the rate of neutralization. The capacity of the antiphage sera to neutralize phage was studied in dilutions of the sera of 1:10, 1:20, 1:40, 1:80 and 1:160. The results of the experiments indicated that the obtained antiphage sera possessed low activity, and neutralized 90-99% of the phage corpuscles in a dilution of 1:10 in the course of 5 and 10 minutes. Data on the neutralization of the phages by homologous and heterologous sera are presented in Table 4.

The results of the experiments show that the antiphage sera of one phage neutralize all the others with almost the same intensity, which is evidence of a close relationship among the plague phages studied, and coincides with the data of other authors [3, 6]. This makes it possible to unite them into one serological group. The neutralization rate of the plague phages by homologous sera was greater than by heterologous. The magnitude of K for the test phages ranged from 1.9-6.1. In the experiments, we did not establish any connection between the lytic activity and the antigenic properties of the phages, which confirms the existent point of view on the separation of the receptor and antigenic structures of phage particles.

Thus, the investigated bacteriophages EB, 1-17, and d'Erell, possess a wide range of action in relation to plague bacteria of different origin, but also lyse the pathogens of pseudotuberculosis (10-12%). Adsorption of the plague phages occurs in 5-8 minutes (EB and 1-17) or 8-10 minutes (d'Erell). The latent period of development of phages EB and 1-17 is equal to 23 minutes, and of d'Erell bacteriophage—25 minutes. The last two characteristics are related to the lytic activity of the phages. These phages compose one serological group.

SUMMARY

A study of the biological properties of plague bacteriophages EB, 1-17, and d'Erell, demonstrated that these phages possess not only a wide range of action in respect to the plague bacteria of various origin, but also lyse 10-12 percent of the representatives of the pseudotuberculosis pathogen. Adsorption of the plague phage particles occurs in 5-8 minutes (EB and 1-17) or 8-10 minutes (d'Erell). The period of intracellular development is 23-25 minutes. The average output of phage particles per bacterial cell differs for the various phages, ranging from 56 to 110 corpuscles. According to their serological properties, these bacteriophages belong to one group.

LITERATURE CITED

1. N. F. Adaeva, Works of the Institute of "Microbes" [in Russian], Saratov, No. 1 (1951), p. 260.
2. I. P. Druzhinina, M. S. Naumshina, and É. S. Knyazevskaya, Works of the Institute of "Microbes" [in Russian], Saratov, No. 4 (1960), p. 235
3. F. I. Ershov and Z. A. Bykova, Zh. mikrobiol., No. 4 (1963, p. 131.
4. E. I. Korobkova, Vestn. mikrobiol., Vol. 16, No. 1-2 (1938), p. 3-4.

5. G. A. Lalazarov, Works of the Scientific Conference Dedicated to the 25th Year Jubilee of the Institute of "Microbes" [in Russian], Saratov (1948), p. 153.
6. Yu. N. Mar'ina, Works of the Rostov-on-the-Don Scientific-Research Anti-Plague Institute [in Russian], Vol. 7 (1948), p. 17.
7. A. P. Yashuk, L. S. Prokhorova, and M. I. Sovetova, Works of the Institute of "Microbes" [in Russian], Saratov, No. 4 (1960), p. 240.
8. M. H. Adams, Meth. med. Res., Vol. 2 (1950), p. 1.
9. M. H. Adams, Ann. N. Y. Acad. Sci., Vol. 56, Art. 3 (1953), p. 442.
10. M. Adams, Bacteriophages [in Russian], Moscow (1961).
11. F. de Erell, Bacteriophage and Its Significance for Immunity [in Russian], Moscow-Leningrad (1926).
12. J. Lederberg and E. M. Lederberg, J. Bact., Vol. 63 (1952), p. 399.
13. S. E. Luria and M. Delbrück, Genetics, Vol. 28 (1943), p. 491.
14. H. B. Newcombe, Nature, Vol. 164 (1949), p. 150.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
