SOME BIOLOGICAL PROPERTIES OF THE PLAGUE BACTERIOPHAGE STRAINS

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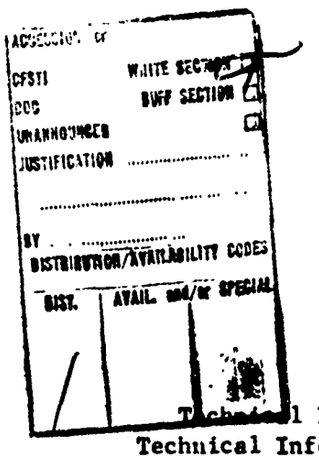

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[Following is the translation of an article by M. A. Shashayev, Alma-Ata, published in the Russian-language periodical Materialy Nauchnoy Konferentsii po Prirodnoy Ochagovosti i Profilaktike Chumy (Materials from the Scientific Conference on the Natural Focalness and Prophylaxis of Plague), Alma-Ata, Feb., 1963, pages 254--256. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Yershov and Bykova (1962), in studying the biology of plague phages, described the morphology of negative colonies and established the value of the neutralization constant of antisera for the D'Erell, Pokrovskaya, Berlin, Osolinker, and Romashovaya phages as equal to 2--25 minutes. We studied 13 strains of plague bacteriophage, isolated from various objects and in various places: D'Erell (1920), Pokrovskaya (1929), Berlin (1932), Osolinker (1936), No 322 Marine (1938), Mikhaleva (1944), Ivanov (1945), No 67 (1950), No 1497 Filimonovaya (1955), No 57 Kovalevaya (1956) and Nos 1048, 210, and 2938 Filimonovaya (1956).

All the phages were passaged five times on the No 319 virulent strain of the plague microbe, which subsequently was used as the strain indicator. The seedings were carried out on Hottinger broth with a pH of 7.3--7.4 and a residual amine nitrogen of 54-58 mg/%. Rabbits were immunized for obtaining the antiphage serum. Three animals were used for each phage. Each of them received subcutaneously 5 ml of the appropriate phage twice a week for a period of five weeks. After 14 days following the conclusion of the injections the antiphage sera were obtained. The serological properties of the phages were studied in the cross neutralization reactions with homologous and heterologous antiphage sera, according to Adams, with the calculation of the constant of the rate of phage neutralization according to the formula $K = \frac{2.}{T} \cdot D \cdot \text{Loq} \frac{Po}{P}$, [Formula illegible in the original.]

K -- constant of the rate of the reaction, D -- end dilution of the serum, Po -- amount of phage at the initial moment of time, P -- amount of phage by the time T. This formula is employed when 90-99% of the phage particles are neutralized by the antiphage serum.

The values of the constants from the 13 strains of plague bacteriophage were determined by us within the limits of 1--34.6 minutes.

Together with the serological properties we studied the morphology of negative colonies, by utilizing the method of agar layers. All the strains of plague bacteriophage studied formed transparent, large negative colonies, with a normal circular form, with a diameter of 3.6--4 mm. After 12--16 hours a second zone appeared around these colonies -- a zone of incomplete lysis.

Thus, all 13 tested strains of plague bacteriophage form large negative colonies and make up one serological group.

In the available literature we did not encounter any works devoted to the study of a single cycle of multiplication of the plague phage, therefore, the study of this problem was of specific interest.

In the test we used three strains of the plague phage: No 1497, Pokrovskaya and Ivanov.

Preliminarily we determined the amount of phage which is adsorbed in a specific segment of time. Adsorption of phage was studied according to Adams by the method of determining the amount of unadsorbed phage and supernatant liquid following the contact of the phage with the microbe and with a subsequent calculation of the percentage of adsorbed phage. Thus, in the course of five minutes 71.7% of phage No 1497, 73% of Pokrovskaya phage, and 78.2% of the Ivanov phage were adsorbed; in the course of 10 minutes -- 87.5% of phage No 1497, 87.3% of Pokrovskaya phage, and 90.6% of Ivanov phage.

The study of a single cycle of multiplication of the test phages was performed according to the method of Ellis and Delbruk (1939), with the help of which we determined the latent period, that is, the time of development of the phage following inplantation in the microbial cell, and the yield or "harvest" of phage, that is, the number of phage particles liberated by one infected bacterial cell.

When determining the duration of the latent period and the "harvest" of phage the corresponding bacteriophage was mixed with the indicator strain of the plague microbe in a ratio of 1:10, that is, one phage particle for 10 microbial cells. After five minutes 0.1 of this mixture was transferred to a second test tube with 0.9 ml of antiphage serum for neutralization of the unadsorbed phage. Then after five minutes the test sample from the second test tube was successively diluted by 10, 40, and finally 100 times. From the last two dilutions (4 and 5 test tubes), after a specific interval of time we took 0.1 ml samples and determined the titers of the spot forming particles. It was established that the duration of the latent period equaled 22 minutes for the Ivanov and No 1497 phages, and 23 minutes for the Pokrovskaya phage. The average "harvest" with the Ivanov phage equaled 98, Pokrovskaya -- 106, and No 1497 -- 111 phage particles.

Further we conducted tests on the study of the immunity to X-rays on the part of the Pokrovskaya plague phage and the Kotlyarovaya pseudotuberculosis phage. Irradiation of the phages was carried out on a RUM-7 X-ray device with an 0.09 mm aluminum filter at 50 kv, 10 mA, focus

distance 75 mm, and power of dose 11,000 r/min. The phages were irradiated in Hottinger broth pH 7.4, with a layer thickness of 1 mm. The concentration of Pokrovskaya plague phage comprised $9.9 \cdot 10^7$ phage particles in one milliliter of broth, Kotlyarovaya pseudotuberculosis phage -- $7.9 \cdot 10^7$. The dose of ionizing radiation, which equaled 110,000 r, inactivated 38.4% of spot forming particles of Pokrovskaya phage and 44% of Kotlyarovaya phage; with an increase of the dose of radiant energy up to 220,000 r, the indices of inactivation increased correspondingly up to 73.2 and 76%.