EXCRETION OF DYSENTERY BACTERIOPHAGE BY MOUSE KIDNEYS IN EXPERIMENTAL DYSENTERY INFECTION

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EXCRETION OF DYSENTERY BACTERIOPHAGE BY MOUSE KIDNEYS IN EXPERIMENTAL DYSENTERY INFECTION

/Following is the translation of an article by K. S. Zobnina in the Russian-language journal Byulleten' Eksperimental'noy Biologii i Meditsiny (Bulletin of Experimental Biology and Medicine), Moscow, No 9, 1963, pages 84-88./

From the Institute of Epidemiology and Hygiene (Director: Docent N. A. Nemshilova, Consultant -- Corresponding Member of the Academy of Medical Sciences USSR A. D. Ado), Kazan'

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The excretion of toxic, antigenic substrates and viruses with urine has currently been demonstrated (1, 6). L. A. Zil'ber (5) first observed the excretion of viruses by immune and nonsusceptible animals and posed the question of the role of this process in the defense of the organism against infection. In the laboratory he directs (8, 9), the first attempts have been made to use physiological methods in studying the mechanism of their excretion (using a phage model). A. D. Ado and coworkers, also examining excretion of antigens as a physiological defense mechanism, have expressed the opinion that the mechanism of kidney excretion of antigens (2, 10, 12, 13), is similar to the excretion of proteins (14, 15, 16).

We studied excretion of bacteriophages by the kidneys and the regularities of the circulation of the bacteriophage in the organism during dysentery infection in mice nonimmune and immunized with homologous culture or phage.

Experimental Methods

The Flexner bacteriophage (0.6 ml) was administered intravenously. In 6 hours an intraparenteral injection of dysentery culture No 938 (500 million microbial bodies) was made. Urine was collected in urine containers [mochepriyemniki]. Six clearance periods were established in 2, 12, 24, 48, 72, and 96 hours after administration of phages over the course of 2-6 hours.

To intensify diuresis, 3 ml of distilled water was injected subcutaneously. The phage in the blood and urine was determined by titration on a dense nutrient medium without preliminary accumulation. The concentration index and the purification index (according to the van Slyke formula) were calculated. Preliminary immunization of the mouse with dysentery Flexner culture was carried out three times at doses of 250 million, 250 million, and 500 million microbial bodies subcutaneously at an interval of seven days. Immunization with phage was also carried out three times, by introducing subcutaneously each four days culture in doses of 0.1 ml, 0.2ml, and 0.3ml. The experiment with immunized animals was carried out ten days after the last injection of culture or phage. The factorial analysis method according to Pomorskiy was used to estimate the reliability of deviation of the mean indices.

The inoculability of the organs (liver, kidney, spleen, lymph nodes) by the phage and causative agent were determined for the dehematized mice (the animals were selected at random). The state of the mouse organism reactivity was estimated from phage reproduction in blood and organs upon injection (7, 11) and from the antibody content (agglutinins) in the blood of randomly dehematized immunized animals.

Experimental Results

Propagation of phage in the blood and an increase in its concentration (Figure 1) was observed in nonimmune animals (30) during the first hours following injection of culture. The purification index was reduced to one-hundreth compared to the original value and increased during the course of injection to 0.09 ml/min (after 48 hours of observation), which is related to change in the permeability of the renal filtrate. The concentration index varied with the same regularity (cf Table).

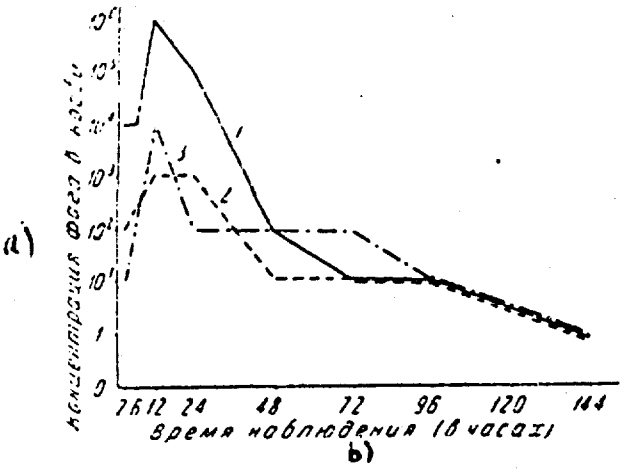

Figure 1. Change in concentration of dysentery bacteriophage in the blood of nonimmune (1), dysentery culture-immune (2), and bacteriophage-immune (3) mice. Scale is arbitrary.
LEGEND: a) phage concentration in the blood; b) period of observation (in hours).

In mice immunized with dysentery bacillus (33), the original phage concentration in the blood was lower than for the nonimmune animals (cf Figure 1). Phage propagation following injection was lacking in 60 % of the mice. The reliability of the difference (Θ) of the mean phage concentration by 24 hours of observation was 72.96 for these groups of mice. At $V_2 = 294 = 00$ and $V_1 = 1$ the reliability indices equalled 3.84 (degree I), 6.64 (degree II), and 10.84 (degree III).

Excretion of phage by the kidneys in mice immunized with dysentery bacillus occurred more intensively than in nonimmune mice, and the purification index during the first days was several dozens and thousands of times higher than during the last few days. The maximum purification (0.9 ml/min) was noted in 48 hours (cf Table), and the difference between the purification value in nonimmune mice was wholly reliable: $\Theta = 10.27$.

The agglutinin titer in the blood ranged from 1:40 to 1:2560. the intensity of phage excretion by the kidneys did not depend on antibody titer, and for the same titer value the purification indices varied widely.

In mice immunized with bacteriophage (21), the phage concentration in the blood also was lower than in the nonimmune. Propagation of the phage after injection together with its active excretion in urine was not observed for 30 % of the animals. Purification of the blood from phage in these mice was more intense than in the nonimmune. The maximum purification index after 24 hours was 0.85 ml/min, that is, by an earlier time than for mice immunized with culture. The difference between the averages for this period was not significant ($\Theta = 0.44$).

Mean Concentration Indices and Purification Indices in Mice

(a) Время наблюдения (в часах)	(b) Неиммунные		(c) Иммунизированные дизентерийной культурой		(d) Иммунизированные бактериофагом	
	(e) концентрационный показатель	(f) показатель очищения (в *мл/мин*)	(e) концентрационный показатель	(f) показатель очищения (в *мл/мин*)	(e) концентрационный показатель	(f) показатель очищения (в *мл/мин*)
2	0,065 ($1\cdot10^{-5}$ — $1\cdot10^{-2}$)	0,002 ($2\cdot10^{-7}$ — $3\cdot10^{-2}$)	1,12 ($1\cdot10^{-4}$ — 10)	0,043 ($4\cdot10^{-6}$ — 0,24)	0,6 ($1\cdot10^{-3}$ — 10)	0,02 ($2\cdot10^{-5}$ — 0,64)
12	0,0007 ($1\cdot10^{-7}$ — $1\cdot10^{-2}$)	0,00003 ($5\cdot10^{-8}$ — $2\cdot10^{-4}$)	2,33 ($1\cdot10^{-4}$ — 10)	0,083 ($5\cdot10^{-8}$ — 0,59)	6,23 ($1\cdot10^{-6}$ — 100)	0,23 ($2\cdot10^{-5}$ — 7,48)
24	0,082 ($1\cdot10^{-7}$ — 1,0)	0,007 ($5\cdot10^{-9}$ — $9\cdot10^{-2}$)	5,76 ($1\cdot10^{-3}$ — 100)	0,46 ($8\cdot10^{-5}$ — 9,11)	13,7 ($1\cdot10^{-4}$ — 100)	0,85 ($5\cdot10^{-8}$ — 8,7)
48	0,72 ($1\cdot10^{-4}$ — $1\cdot10^{-2}$)	0,09 ($4\cdot10^{-6}$ — 1,6)	10,7 ($1\cdot10^{-2}$ — 100)	0,9 ($7\cdot10^{-2}$ — 8,9)	1,8 ($1\cdot10^{-4}$ — 10)	0,09 ($7\cdot10^{-6}$ — 0,8)
72	1,16 ($1\cdot10^{-2}$ — 10)	0,08 ($5\cdot10^{-4}$ — 0,8)	2,63 ($1\cdot10^{-2}$ — 10)	0,2 ($9\cdot10^{-4}$ — 1,04)	1,0 ($1\cdot10^{-4}$ — 10)	0,08 ($7\cdot10^{-6}$ — 7,07)
96	(g) Фаг не обнаружен		1,36 ($1\cdot10^{-2}$ — 10)	0,12 ($1\cdot10^{-5}$ — 0,74)	(h) Фаг обнаружен у 2 мышей	

LEGEND: a) time of observation (in hours); b) nonimmune; c) immunized with dysentery culture; d) immunized with bacteriophage; e) concentration index; f) purification index (in ml/min); g) phage not detected; h) phage detected in two mice.

Antibodies to the bacillus, even at a low titer, were discovered in one of the 14 mice examined. In spite of the antigenic detachment of the phage from the bacterial cell, common regularities in variation of excretory function of the kidneys in mice immunized with cultural phage were observed. Evidently, the protective mechanisms of the organisms are not restricted only to immunological specific reactions, but are related with change in individual physiological functions and systems of the organism.

Diuresis in mice of all the experimental groups during the first hour after injection remained unchanged (from $13 \cdot 10^{-4}$ to $16 \cdot 10^{-4}$ ml/min), and by 24-48 hours of observation it had increased two-nine times. From comparison of data in Figures 1, 2, 3, it is clear that the purification index does not depend on phage concentration in the blood and increases during the course of infection as well as diuresis. However, no direct relationship between the extent of purification and diuresis was discovered.

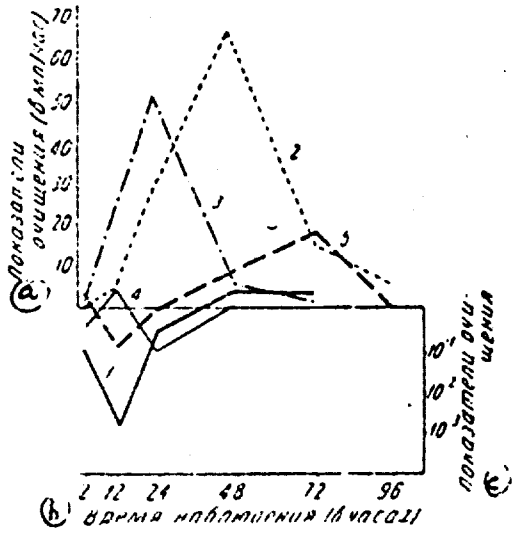

Figure 2. Change in mean indices of blood purification from bacteriophage in infected non-immune (1), dysentery culture-immunized (2), bacteriophage-immunized (3), typhus abdominalis culture-immunized (4), and staphylococcus vaccine-immunized (5) mice. The purification indices were calculated on the basis of one hour. Scale is arbitrary. LEGEND: a) purification indices (in ml/hour); b) period of observation (in hours); c) purification indices.

To resolve the question of the specificity of activation of the excretory function of kidneys in immunized animals we performed experiments on 29 mice previously immunized with typhus absominalis culture or staphylococcal vaccine. The dynamics of phage circulation in the organism of these animals was the same as for nonimmune mice. Excretion of phage, however, was more active. The mean purification indices ranged from 0.002 to 0.29 ml/min in mice immunized

with staphylococcal vaccine, and from 0.012 to 0.088 ml/min for those immunized with typhus abdominalis culture. The difference in the purification indices in nonimmune mice lay within the limits of random error.

The purification indices in mice immunized with dysentery culture essentially exceeded during individual periods (by the 48th hour of observation) those values for mice immunized with typhus abdominalis culture (Θ = 6.2) or staphylococcal vaccine (Θ = 4.89). This supported the relative specificity of activation of phage excretion in immune animals.

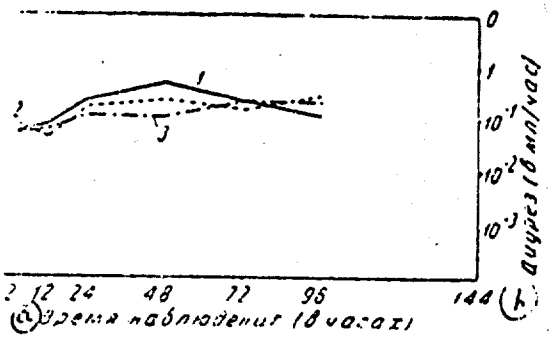

Figure 3. Change in mean indices of diuresis in nonimmune (1), dysentery culture-immunized (2), and bacteriophage-immunized (3) mice. Scale is arbitrary.
LEGEND: a) time of observation (in hours); b) diuresis (in ml/hour).

The highest inoculability of organs with phage (observations on 75 mice) was noted for the nonimmune animals; phage multiplied intensively. Much the same was phage propagation in organs of mice immunized with phage. For most of the mice immunized with dysentery culture, phage propagation proved less pronounced or was totally lacking, and it disappeared sooner, which can be accounted for by the more active breakdown of phage and bacillu in the immune organism.

The inoculability of organs with causative agent in all cases was slight: of 372 inoculations (without prior accumulation) causative agent was found in nine.

We attempted to establish a relationship between excretion of phage in urea and excretion of dysentery antigen, using the complement fixation reaction in the cold. We investigated 2-4 mice during each period of observation in all of the experimental groups. The appearance of antigen in the urine agreed with the maximal purification index (in nonimmune mice -- in 48-72 hours, in dysentery culture-immunized -- in 24-48 hours, and in phage-immunized -- in 12-24 hours). This allows us to suggest that purification of blood from phage by the kidneys occurs in parallel with purification of the organism from the bacterial antigen.

The rise in the index of purification from phage and the diuresis level during the course of infection cannot explain kidney excretion of the phage by means only of the filtration mechanism, since it is known that enhanced filtration is considerably dependent on disturbance of reabsorption in the tubules (3, 4). By means of this same mechanism, several authors explain the more active excretion of antigen in immunized animals (2, 10, 12). The lack of any direct relationship between purification and phage concentration in the blood and the diuresis level allows us to suggest that participation of renal tubules in phage excretion is not limited to resorptive processes; evidently, phage is actively excreted through the lumens of tubules as the organism is freed from infection.

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