Fate of Bacteriophage Particles Introduced into Mice by Various Routes.* (24112)

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Bacterial viruses inoculated into an animal should, because of their size and biological composition, act in many ways as a physical simulant for animal viruses. Because of their inability to specifically adsorb and multiply in sensitive animal tissues and the inherent simplicity of quantitation, bacteriophage studies were undertaken to ascertain the effect of some of the anatomical and physiological barriers of the animal body to large doses of bacteriophage particles, to explain the occurrences that follow introduction of large numbers of virus particles into host animals. Nungester and Watrous(1) have shown that 2 hours after intravenous inoculation of staphylococcus phage into rats the spleens and livers contained greater amounts of viable phage particles than did the blood. Appelmans(2) showed that bacteriophage particles inoculated into guinea pigs were rapidly eliminated through urine and feces but bacteriophage preparations administered orally did not pass into the blood stream. This report deals with the persistence and localization as well as the translocation of bacteriophage particles introduced into mice.

Method. The phage strain was derived from *Bacillus megatherium* 899a (lysogenic), and is the clear plaque mutant C strain as described by Gratia(3). The sensitive strain *Bacillus megatherium* KM was utilized throughout this study for production of phage stock and assaying procedures. The medium for stock phage production and all assay procedures consisted of 2% Bacto-peptone in both broth and agar medium. Phage determinations were carried out by the pour plate method as described by Adams(4). Two-tenths ml of phage suspension containing 2 x 10^{10} particles/ml, was injected intraperitoneally into white Swiss mice. At indicated intervals, mice were etherized and blood samples taken by the cul-de-sac method. The animals were then immediately sacrificed and brains, livers, spleens, kidneys and intestinal tracts (large intestines and caecum) were isolated in sterile petri plates. The individual organs were homogenized in sterile containers utilizing a Virtis tissue homogenizer. After homogenizing, the tissue suspension was placed into sterile test tubes and the homogenizer cup washed with a volume of sterile distilled water to bring total volume to 2 ml. The combined suspension was centrifuged and the supernatant assayed for phage content. Blood samples taken by the cul-de-sac method were assayed directly. In experiments concerned with oral administration of phage suspensions, introduction was achieved by feeding one drop from an 18 gauge needle from which the bevel had been ground and also by the administration of 0.1 ml by gastric lavage. Care was taken to prevent any laceration to nasopharynx and gastric mucosa by utilizing polyethylene tubing which had been flamed to remove any rough edges. Mice were lightly anesthetized with sodium pentathol before insertion of tube. Titer of phage suspensions in the feeding experiments was 1 x 10^{10}/ml. Blood samples were taken by cardiac punctures with heparinized syringes. In experiments when urine and feces were assayed for phage particles the following technics were used. Two drops of urine were suspended in 1 ml of sterile distilled water and assayed, and a fecal pellet was expressed from each animal, placed into 2 ml of sterile distilled water, emulsified, and the centrifugate assayed.

Results. Fig. 1 illustrates number of active phage particles recoverable from lung, kidney, brain and intestines at different time intervals after intraperitoneal inoculation of a phage suspension. Fig. 2 gives the same in-

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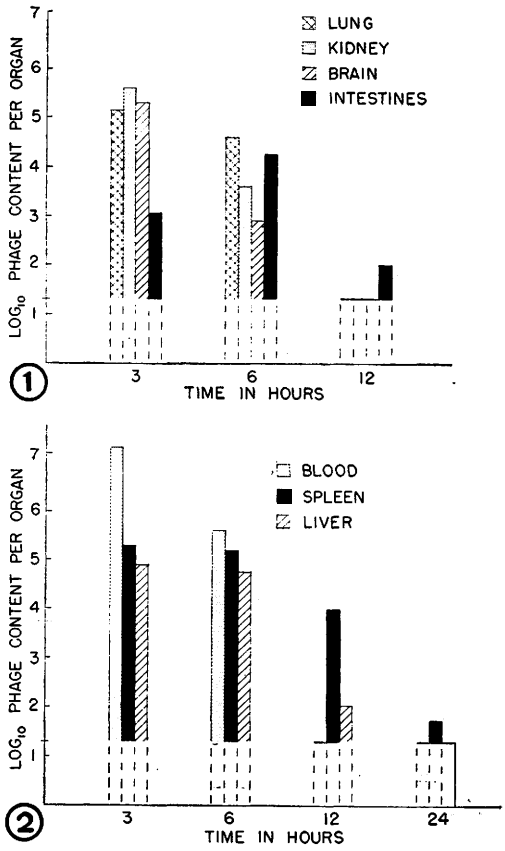

FIG. 1. No. of viable *B. megatherium* bacteriophage recoverable from lung, kidney, brain and intestine of mice calculated for content per total organ.

FIG. 2. No. of viable *B. megatherium* bacteriophage recoverable from blood, spleen and liver of mice calculated for content per total organ.

formation for blood, spleen and liver. For purposes of convenience and comparison all calculations have been made for active phage content/total organ. Since total phage inoculated was 4 x 10^9 particles, it can be seen that by the first time interval tested, 3 hours, there was a decrease of over 90% in number of viable particles titratable from blood. This may be in part explainable by the effect of phage neutralizing ability of properdin. Van Vunakis *et al.*(5) have shown this effect on T 2 phage of *Escherichia coli* using sera from mice. In a limited study we verified this effect with this phage strain. It is furthermore noted that 12 hours after inoculation, when no phage particles can be recovered from blood, lung, kidney and brain, virus particles can still be obtained from intestines, liver and in higher amounts from spleen. At 24 hours, viable particles in small amounts could still be recovered from the spleen. That the intestines consistently yielded bacteriophage particles when blood did not, may be explainable by the fact that we have been able to recover phage particles from fecal material collected within 3 to 6 hours after intraperitoneal inoculations of bacteriophage. From the Figures, it is obvious that organs of the reticuloendothelial system such as spleen and liver contain and release phage particles after homogenizing at time intervals when none could be detected in other organs tested.

The results of administration of high titered phage suspensions by gastric lavage are tabulated in Table I. The quantity of viable particles recovered from blood was random and irregular. Because of the wide range of difference in numbers of phage particles recovered at each time interval, the data have been cited for maximum number of phage particles in total blood volume. The proportion of mice which did not yield active particles can possibly be accounted for by the natural immune defenses, *i.e.*, properdin of individual mice, as we have noted wide variations in amount of phage recovered from individual mice after intraperitoneal inoculations of phage preparations. Bacteriophage could be recovered as early as 5 minutes after gastric

TABLE I. Numbers of *B. megatherium* Bacteriophage Recoverable from Blood of Mice after Gastric Lavage.

Time, min.	Max No. of phage particles in total blood vol ($\times 10^8$)	% of mice yielding active virus particles
5	44.0	75
10	3.2	85
15	1.0	60
20	22.0	79
25	16.0	67
30	85.0	77
35	2.0	56
40	19.0	86
45	61.0	67
50	13.0	54
55	8.9	78
60	20.0	77
	Avg %	71.8

Cumulative information of 14 experiments utilizing 135 mice.

lavage. Essentially similar results were noted when the experimental method was varied to administer the phage suspension by oral inoculations. As particles of this size range could pass the gastrointestinal barrier of normal mice, alternative experiments were carried out to obtain information on passage of bacteriophage into the gastrointestinal tract, as measured by assaying fecal material, and through the renal barrier as assayed by quantitating urinary samples. Experiments yielded again an irregular but repeatable amount of positive migration of phage particles. Particles were recovered in urine samples as early as 30 minutes. Fecal samples yielded phage particles within 3 to 6 hours after intraperitoneal inoculation.

Discussion. The results of the translocation studies indicate that *B. megatherium* bacteriophage, whose size as determined by Murphy(6) is 49 mμ for width of head and 330 mμ x 15 mμ for the tail, is able to pass apparently normal anatomical barriers such as gastro-intestinal tract and renal filters. Studies by Gordon *et al.*(7) have shown with feeding experiments of mice utilizing the bacterium *Serratia marcescens,* that organisms of this size in small amounts could pass the intestinal mucosa to the mesenteric lymph nodes and that such bacteria were localized at this site. These same studies cited that bacteria could not be isolated from the blood. It therefore appears that particles the size of bacteria can pass through the lymphatic system, but not through the small capillaries and blood vessels of the gastric mucosa. Whether the bacteriophage particles accounted for in the blood stream in our experiments passed through the lumen of the gut *via* the lymphatic system or blood capillaries was not determined, but on the basis of the short time necessary for the passage it would appear that a direct blood route is more likely. It thus appears that particles of this size range may not respect histological barriers such as the capillary endothelium. In this regard the speculation arises as to whether animal viruses such as the poliomyelitis virus which has been shown to have as one primary area of entrance, the intestines (8), could also enter directly into the blood circulation without prior infection and multiplication within cells of the alimentary mucosa and lymph nodes as is stated in a recent review(9). The results of the experiments dealing with the fate of bacteriophage intraperitoneally inoculated into mice indicate that biological particles of this size range are concentrated in organs of the reticulo-endothelial system such as the liver and spleen, and may in part explain why protection against challenge infections with *Shigella paradysenteriae* could be achieved up to 7 days after phage administration(10). This introduces the problem whether such filter-like organs also have ability to inactivate such localized particles by phagocytosis. A recent report(11) indicates that influenza virus, which has been shown to adsorb and to reduce the anaerobic glycolysis of guinea pig leucocytes(12), can be phagocytized by human and rabbit leucocytes. If phagocytosis of bacterial viruses can also occur and whether such phagocytized phage particles can be released from leucocytes subsequent to artificial breakage of such cells is being considered.

The preliminary information that bacteriophage particles pass through the renal filter has been extended and quantitated in studies with dogs in a separate report(13).

Summary. The fate of *Bacillus megatherium* bacteriophage intraperitoneally inoculated into mice has been investigated. Active bacteriophage particles were recovered from spleen and liver at a time when none were recovered from blood, lung, kidney, and brain. The relationship of this occurrence to possible phagocytosis of bacteriophage particles is discussed. Introduction of bacteriophage suspensions of high titer into the gastro-intestinal tract by feeding and gastric lavages led to an irregular but consistent recovery of active phage particles from the blood circulation. Conversely, phage particles inoculated intraperitoneally were recovered in samples of urine and feces.

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Provocation and Prevention of Potassium Deficiency by Various Ions.* (24113)

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Earlier work on the Electrolyte-Steroid-Cardiopathy that is characterized by massive, myocardial Necroses (ESCN) showed that, at least in the corticoid-conditioned rat, toxicity of sodium is decisively influenced by: (1) the anion to which Na is attached, (2) concurrent administration of various other cations. Thus, after sensitization with certain corticoids, Na_2HPO_4, NaH_2PO_4, $NaClO_4$ or Na_2SO_4 produced extensive myocardial necroses in rats, while equimolecular amounts of NaCl were ineffective in this respect. Furthermore, the ESCN elicited by corticoids plus sensitizing Na-salts was completely prevented by simultaneous administration of magnesium and potassium ions, particularly when these were given as chlorides. The nephro-calcinosis that normally accompanies the ESCN when the latter is produced with the aid of Na-phosphates was likewise prevented by KCl or $MgCl_2$(1). It has long been known, furthermore, that rats kept either on K-deficient(2, 3,4) or on Mg-deficient(3,5,6) diets also tend to develop focal myocardial necroses and nephrocalcinosis. In the case of Mg-deficiency, these changes are frequently accompanied by convulsions. Several observations suggested that an excess of Na can aggravate K-deficiency(7,8), but in none of these earlier studies were the effects of various Na-salts compared, because at that time there was no reason to suspect a dependence of Na-actions upon anions. The possibility of a replacement of K by Mg was also disregarded. Thus, for example, one group of workers claimed that administration of excess NaCl aggravates the myocardial necroses characteristic of severe K-deficiency(9,10). They arrived at this conclusion by substituting $MgCl_2$ for NaCl in their control rats. Schrader *et al.* (3) postulated that simultaneous Mg-deficiency does not significantly alter the course of K-deficiency in the rat; indeed, some workers believe that there exists an actual antagonism between Mg and K(11,12).

In view of our findings concerning participation of electrolytes in production of myocardial necroses by steroids, we wished to determine: (1) whether the cardiac necroses and other manifestations of dietary K-deficiency would be most markedly aggravated by those Na-salts that sensitize for production of the ESCN, and (2) whether the morbid changes usually ascribed to a lack of K are accentuated by a concurrent deficiency in Mg.

Materials and technics. In the first experiment, 60 female Sprague-Dawley rats, with a mean initial body-weight of 49 g (range 43-55 g), were placed on the "Low Potassium Diet" of the Nutritional Biochemicals Corp. (Cleveland, Ohio) for 7 days. This diet consists of: corn starch 64.2%; casein 30%;

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