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Protective Action of Typhoid Phage on Experimental Typhoid Infection in Mice.

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Asheshov, Wilson and Topley[1] have reported on the protective effect in mice of typhoid bacteriophage given separately and by routes different from those used in infecting the animals. The preliminary studies here reported confirm the findings of the above investigators and enlarge upon certain aspects of the problem.

A recently isolated strain of *Eberthella typhi* obtained by blood culture was employed. It forms smooth colonies, is motile, produces typical sugar fermentation reactions and agglutinates specifically with anti-typhoid serum. Organisms from 18- to 24-hour-old cultures grown on veal infusion agar were suspended in mucin* according to the methods of Rake[2] and Miller,[3] and mice were infected by the peritoneal route, all inoculations being made with a quantity of 1 cc of the mucin-organism mixtures.

The phage was originally isolated from sewage and is a crude phage which produces several different types of plaques. Filtrates which contained approximately 3.3×10^8 phage particles per cc were used for mouse injection.

Typhoid infection in mice was acute. Blood stream invasion with resulting bacteremia usually occurred within 1 hour following intraperitoneal inoculation and all of the fatally infected control mice died within 24 hours. The minimal fatal dose of the culture varied in 7 virulence tests from 1000 to 10 bacilli, thus the quantity of organisms used in the phage experiments represented from 1000 to 100,000 fatal doses.

The protective effect of phage was tested by various ways in 7 experiments, the results of which may be summarized as follows:

(1) Phage injected immediately before the intraperitoneal inoculation of 1×10^6 typhoid bacilli protected all of 32 mice when injected intravenously and all of 19 mice when administered by the subcutaneous method. Intravenously injected phage also protected

1 Asheshov, I. N., Wilson, J., Topley, W. W. C., *Lancet*, 1937, **232**, 5919, 319.

* Granular mucin manufactured by Wilson Laboratories, Chicago, Ill.

2 Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1523.

3 Miller, C. P., *J. Inf. Dis.*, 1936, **58**, 263.

against $1x10^7$ and $1x10^8$ bacilli, but failed to protect against $5x10^8$ organisms which may have been due in part to the primary toxicity of such a large number of organisms.

(2) The amount of phage required to protect mice against $1x10^6$ bacilli was found to be quite small since $1x10^{-4}$ cc afforded complete protection to 10 mice. Smaller amounts of phage showed a diminishing effect.

(3) In other experiments it was found that mice remain resistant to infection for as long as 24 hours following intravenous phage injection. The persistence of resistance following other methods of phage injection or for longer periods after intravenous injection has not yet been determined.

(4) The therapeutic effect of phage was tested by injecting phage intravenously at various periods of infection up to 4 hours after typhoid inoculation. Blood cultures taken in every instance before phage injection showed bacteremia to be present in 23 of the 29 mice of this experiment. The fact that all of these mice survived even when phage injection was delayed for 4 hours shows that phage exerts a protective effect even after generalized infection has taken place.

(5) Control mice were included in each experiment. Among the untreated controls 44 of 48 mice died and in another group 19 out of 20 mice treated with phage heated to 70°C for 50 minutes developed a fatal infection. Thus among the 68 control mice there was a mortality of 92.6%.

It is apparent from the results obtained in this study that bacteriophage exerts a protective action in experimental *Eberthella typhi* infection in mice. Asheshov, Wilson and Topley[1] who worked with the "Rejuvenated Rawlings" strain of *E. typhi* and both pure and mixed phages also demonstrated the protective effect of phage. They record, however, that 23 of 30 mice died when phage injection was delayed for 4 hours. The use of mucin in the present study made possible the inoculation of smaller numbers of bacilli which perhaps explains the more striking curative results obtained.