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Protective Values of Various Types of Vi-Phage on Experimental Typhoid Infection in Mice.

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The importance of the kind of phage employed in determining the efficacy of phagotherapy in experimental typhoid infection has been indicated by the work of Asheshov, Wilson and Topley.[1] These workers have found that 2 strains of Vi-phages specific for V-form *B. typhosus* were effective in preventing deaths of infected mice, while a non-specific phage, capable of lysing both V- and W-forms of *B. typhosus in vitro,* was ineffective. More recently Craigie and Yen[2] found that the Vi-phages can be grouped into 4 serological types which are all specific for V-form *B. typhosus* but differ in their thermal death point, particle-size and relative lytic activity for various strains of V-form *B. typhosus.* In view of the latter findings, it is of practical interest to ascertain whether or not these serological types of Vi-phages would differ in their protective action on experimental typhoid infection. In this communication our observations are limited to the determination of their relative efficacy on mice experimentally infected with a strain of V-form *B. typhosus.*

The Ty2 strain was used throughout in this experiment. For infecting the animals, 3- to 5-hour broth cultures were prepared from an 18-hour agar growth. When sufficient growth was reached, the broth culture was chilled in ice-water and diluted with ice-cold broth to an opacity of 10% No. 4 standard of a Pulfrich photometer. At this opacity the broth culture contained 100 million organisms per milliliter. The phages employed were the original strains of Type I, II, III, and IV Vi-phages isolated by Craigie and Yen.[2] All these phages were propagated on the Ty2 strain. The titers of the phages were expressed in plaque-units, being the number of discrete plaques as determined on an agar-surface growth except for the type I Vi-phage in which instance the plaque-counting was made by the pour-plate technic,[3] using 0.7% agar so as to render the plaque more easily visible.

Normal mice weighing from 15-18 g were kept in separate groups

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

2 Craigie, J., and Yen, C. H., *Canad. Pub. Health J.,* 1938, **29**, 448.

3 Yen, C. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1006.

TABLE I.
Survival-rate of Infected Mice with and without Phagotherapy.

		Phage treated				
		Types of Vi-phage				
Tests	Phage units given	I	II	III	IV	Controls
1	5 x 10^8	2*	4	2	—	1
2	7.5 x 10^8	2	7	7	7	0
3	1.5 x 10^9	1	8	8	8	0

*The figure represents number of animals surviving over a week from each group of 10 animals tested.

of 10 each. All the animals were given intraäbdominal infections of 100 million organisms of the Ty2 culture. In the phage-treated groups, Vi-phages of various potencies were given in 1 cc amounts subcutaneously immediately following the infection. The animals were kept under observation for a week and the number of survivals are summarized in Table I.

Results. Death of the animals usually occurred within 48 hours after infection and in the majority of instances within 24 hours. The Type I Vi-phage was found to show no significant protective action under the experimental conditions. On the other hand the Type II, III, and IV Vi-phages were found to be equally effective in preventing about 70 to 80% of the infected mice from dying, when the phage-units given approximated 8 to 15 times the number of organisms contained in the infecting dose. Ordinary broth and Vi-phages inactivated by immersing in boiling water for 10 minutes were separately given to a few groups of infected mice but all failed to show any beneficial effects.

Comments. It may be pointed out here that in contrast to the other 3 Vi-phages the Type I Vi-phage produced very minute plaques on agar-surface growths, and highly potent preparations were obtained with difficulty. Furthermore, using similar concentration of phage-units as the other types, the clearing of a given broth culture was not only slower but also less complete. It is of interest, therefore, to correlate the lack of demonstrable protective action *in vivo* with its slower rate of lysis of the organisms *in vitro*. As the phagotherapy is given subcutaneously following infection, the evaluation of the protective action of these phages are rather more strict than if they were given intravenously as employed by others.[1] Therefore the non-protective action of Type I Vi-phage is relevant only under the specified conditions. Also the phage-susceptibility of various strains of V form *B. typhosus* vary greatly not only with the type of Vi-phage used for testing but also with the strain of organisms

employed for the propagation of the phages.[2, 4] Hence our findings here cannot be justifiably generalized for the *in vivo* action of these phages towards all strains of V form *B. typhosus.* These observations, however, are sufficient to demonstrate a significant difference in the relative efficacy of these phages on experimental typhoid infection in mice.

Conclusion. Of the 4 serological types of Vi-phage propagated on Ty2 strain of V form *B. typhosus,* only the Type I Vi-phage failed to show noticeable protective action under the specified experimental conditions. The Type II, III and IV were found to be equally effective in their protective action.

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Influence of Specific Soluble Substance on Course of Experimental Pneumococcus Pneumonia.

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The specific soluble substance (SSS) of pneumococci has been shown by many workers to inhibit the action of natural or acquired antibodies, either partially or completely. The purpose of this investigation was to determine the effect of this material* on the course of experimental canine Type I pneumococcus pneumonia when administered in sufficient quantity to completely neutralize the humoral defense mechanism.

Healthy 10K dogs were used in this study. They were infected by the intrabronchial route using a uniform dose of 0.001 cc of actively growing Type I pneumococci suspended in 1 cc of starch-broth mixture.[1] This quantity was employed since in the experience of this laboratory such dose seldom produces bacteremia and characteristically results in a localized lobar lesion. Five control dogs in this series confirmed this finding. Hourly samples of blood were taken for culture and serum pneumococcidal-promoting power.

4 Craigie, J., and Yen, C. H., *Trans. Roy. Soc. Canada,* 1937, **37**, Sec. V, 79.

* The SSS used in this study was supplied by the Lederle Laboratories, Inc., through the courtesy of Dr. W. G. Malcolm.

1 Terrell, E. E., Robertson, O. H., and Coggeshall, L. T., *J. Clin. Invest.,* 1933, **12**, 393.