

*Summary.* The intravenous injection of a mixture of amino-acids (enzymic hydrolysate of casein) was followed in a few hours by a significant increase in the serum albumin concentration of fasting dogs rendered hypoproteinemic by a severe hemorrhage. Since the serum globulin concentration and the relative red cell volume both decreased, it is inferred that the increase in serum albumin concentration was due to a regeneration.

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#### Further Studies of Antigenic Properties of Bacteriophage.

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Although bacteriophage is antigenic, it has not been found possible to induce active anaphylaxis in guinea pigs with bacteriophage,<sup>1</sup> even when employing for the test-injection active purified bacteriophage-protein containing  $2.6 \times 10^{18}$  lytic units, (1.79 mg of specific protein,<sup>2</sup> equivalent to 5 liters of crude phage). In seeking another method of demonstrating the interaction of bacteriophage with its antibody *in vivo*, we attempted to utilize the Schwartzman test.

Four rabbits were sensitized by repeated injections of crude *B. coli* bacteriophage, and their sera, 10 days after the last (ninth) injection, were found to be capable (in a dilution up to 1:320) of neutralizing an equal volume of undiluted crude phage having a titer of  $10^9$  lytic units per cubic centimeter. Two of these rabbits were then prepared by the intracutaneous injection of 0.25 cc of meningococcal filtrate, kindly sent us by Dr. Schwartzman, and 24 hours later one of them received intravenously 5 cc of crude phage and the other 9.5 cc of freshly prepared active purified phage containing a total of  $5.7 \times 10^{18}$  active lytic units (or 3.42 mg of specific protein equivalent to 11.4 liters of crude phage).

The animal that had received crude phage responded within 3 hours with a typical reaction at the site of the preparatory injection, while the one that had received the purified phage had no reaction. This difference indicated that the reaction in the animal receiving

<sup>1</sup> Bronfenbrenner, J., and Kalmanson, G. M., *J. Bact.*, in press.

<sup>2</sup> Kalmanson, G. M., and Bronfenbrenner, J., *J. Gen. Physiol.*, 1939, **23**, 203.

crude phage might have been due not to the bacteriophage itself but rather to other constituents of the crude phage.

In order to inquire further into the nature of this response, the other 2 sensitized rabbits were similarly prepared with meningococcal filtrate, and 24 hours later one of them received 5 cc of sterile broth intravenously, and the other 5 cc of a Berkefeld filtrate of an 18-hour broth culture of *B. coli*. The rabbit receiving broth had no reaction, while the one receiving the culture-filtrate had a mild but definite reaction. Furthermore, 2 out of 3 normal rabbits gave positive reactions at the prepared sites when injected intravenously with crude bacteriophage, thus indicating the presence of a directly reacting factor in the crude lysate.

Similarly we failed to obtain a Shwartzman reaction in a test of reverse passive sensitization to bacteriophage: a normal rabbit was prepared by injection of meningococcal filtrate and, after an interval of 24 hours, was given an intravenous injection of 11.2 cc of purified active phage containing  $7.84 \times 10^{18}$  units (or 4.7 mg of specific protein equivalent to 15.7 liters of crude phage), followed in one-half hour by 5 cc of pooled antiphage-serum intravenously. There was no reaction.

Thus, attempts to demonstrate the *in vivo* interaction of bacteriophage with its antibody by means of the Shwartzman test were not successful in rabbits sensitized to bacteriophage either actively or passively, despite the fact that amounts of purified phage-protein equivalent to many liters of crude bacteriophage were used for the test-injections.

We have been able to carry out only few experiments thus far, because lots of 15 to 20 liters of phage yield sufficient purified phage for only one test, and because it is impossible to accumulate a greater quantity since purified phage loses its potency on standing.