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Relationship of Bacteriophage to Toxin and Antitoxin.

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Some investigators have implied that bacteriophage action was identical to the action of toxin and antitoxin. d'Herelle suggested that bacteriophage was in fact an antitoxin. The observations on the clinical application of bacteriophage have awaited an adequate theoretical basis before they could be interpreted and a foundation laid for intelligent formulation of a rationale of treatment and prophylaxis. Staphylococcus toxin has only recently been developed to a point where it may be safely assumed that such a toxin exists, and where means of measuring its activity and potency have been sufficiently standardized to permit of fruitful study. Having then a satisfactory staphylococcus toxin and a bacteriophage highly active against staphylococci, the necessary elements for a study of the relationship between toxin and bacteriophage have become available. The following report is a preliminary study of this relationship from which it is believed definite conclusions can be drawn.

The production of staphylococcus toxin followed the method given by Dollman[1] and based upon the earlier work of Burnet[2, 3] and his coworkers. Plates of beef extract broth to which 0.5% agar had been added were inoculated with staphylococcus and incubated for 48 hours in an atmosphere of 15% CO_2. The semi-fluid material after incubation was passed through filter paper, the reaction adjusted to pH 6.6 and 1:8000 merthiolate was added. Six toxins were prepared in this manner and their potency tested first by hemolysis of rabbit red blood cells. The results are shown in Table I.

Bacteriophage itself incubated under similar conditions with rabbit red blood cells produced no hemolysis and hence has none of the hemolytic properties of staphylococcus toxin.

Neutralization of staphylococcus toxin with bacteriophage was attempted by incubating 0.1 cc. of toxin with bacteriophage undiluted in the following amounts, 0.1 cc., 0.2 cc., and 0.5 cc. of phage. Toxin in dilutions of 1:50 and 1:100 was also incubated with similar amounts of bacteriophage. In no case was there any inhibition of hemolysis.

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[1] Dollman, E. C., *Canadian Public Health J.*, 1932, **23**, 125.

[2] Burnet, F. M., *J. Path. and Bact.*, 1929, **32**, 717.

[3] Burnet, F. M., *J. Path. and Bact.*, 1930, **33**, 1.

TABLE I.

Toxin dilution 1:50.
Rabbit red blood cells, 1% suspension. 0.2 cc. in each tube.
Total volume .4 cc., tubes 85 x 9 mm.

Toxin	.2 cc.	.1 cc.	.06 cc.	.04 cc.	.02 cc.	.01 cc.
1909†	++++	++++	++	+	—	—
1968	++++	++++	+	—	—	—
1908	++	—	—	—	—	—
1910	++++	—	—	—	—	—
1904	++++	++++	++	+	+	—
1912	++++	—	—	—	—	—
	1:100	1:200	1:333	1:500	1:1000	1:2000

++++ Complete hemolysis.
+ Supernatant fluid colored.
— Colorless supernatant.
Incubation, 1 hour 37° in water bath.
†These cultures were all susceptible to bacteriophagy and have been used in preparation of bacteriophage for therapeutic use.

Two rabbits were bled and the serums tested for antitoxic properties. Neither serum inhibited the hemolytic power of the toxin used (1904). One rabbit was then given 2 intravenous injections of toxin 1:50 dilution, 0.2 cc. followed by 0.5 cc. 24 hours later. The other rabbit was given 2 injections of bacteriophage, 2.0 cc. followed by 2.0 cc. after 24 hours. Both animals were bled after 5 days and the serum tested for antitoxin. The results are shown in Table II.

TABLE II.

Toxin dilutions 1:10, 1:25, 1:50, 1:100, 1:200, 1:400.
0.1 cc. of toxin dilution + 0.1 cc. of serum incubated 1 hour at room temperature. 0.2 cc. 1% rabbit cells added, incubation 1 hour at 37° in water bath.

Final toxin dilution	1:40	1:100	1:200	1:400	1:800	1:1600
Serum 733*	+++	++	+	—	—	—
" 737*	++	—	—	—	—	—
No serum	++++	++++	++++	+	—	—

*Serum 733 Produced by injections of toxin
" 737 " " " " bacteriophage
Toxin 1904

This test was repeated with various dilutions of serum and toxin but the results presented here are typical.

Staphylococcus toxin is not only hemolytic; it has strong dermonecrotic properties, and in order to check the above results, a series of skin tests were performed on rabbits. Staphylococcus toxin when injected intradermally into rabbits gives a skin reaction that consists at first of an area of erythema later deepening and having a necrotic center. The readings in this case were made after 70 hours and the size of the reaction zone was measured. The dermo-

necrotic titre is usually less than the hemolytic titre with a given serum. Toxin 1904 gave a measurable area in 1:20 dilution and visible reactions up to 1:80. Undiluted antitoxic serum from the rabbit immunized with bacteriophage neutralized the toxin in 1:10 dilution. When the antitoxic serum from the same rabbit was diluted 1:2 it still neutralized the toxin in 1:10 dilution. In 1:40 dilution this serum failed to neutralize. The serum itself gave a very faint skin reaction. The antitoxin produced by inoculating a rabbit with the staphylococcus toxin failed to neutralize the dermonecrotic toxin even when the serum was undiluted.

As bacteriophage antiserum was definitely antitoxic for staphylococcus toxin, the question arose as to whether the bacteriophage itself was responsible for the production of the antitoxin or whether there were in the bacteriophage filtrate other substances capable of producing the antitoxin. Bacteriophage was purified according to the method described by Krueger,[4] *i. e.*, electrophoresis. A purified phage was produced containing less than 0.1 mg. of nitrogen per cc. and this product injected into a rabbit, two 5 cc. intravenous injections being given, the second 48 hours after the first. The rabbit was bled after 10 days and the serum tested for antitoxin. The results are shown in Table III.

TABLE III.

Final toxin dilution	1:20	1:40	1:80	1:160	1:320
Toxin	++++	++++	+++	+	—
Toxin and serum 733	++++	++	+	—	—
Toxin and serum 736	++++	+	—	—	—

Serum 733 Produced by injection of toxin
" 736 " " " " purified bacteriophage

It appears from this experiment that the purified bacteriophage produced an antitoxin comparable to that produced by injection of unpurified phage and hence that the bacteriophage itself is the antigen concerned with the production of staphylococcus antitoxin.

It appears reasonably certain that bacteriophage when used as an antigen produces an antiserum capable of neutralizing staphylococcus toxin and that it is the bacteriophage itself which has this property. Bacteriophage is not a toxin or antitoxin, and is apparently in the nature of a toxoid.

[4] Krueger, A. P., and Tamada, H. T., *J. Gen. Physiol.*, 1929, **13**, 145.