

phage, since greater specific inhibition of the anti-paratyphosus B phage was obtained depending upon the group of the test organism employed.³

A discussion of the significance of the observations made and their bearing on the nature of bacteriophage specificity is reserved for future publication.

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Observations on Phage Inhibition by Bacillary Extracts.

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The specific inhibition of bacteriophage by bacillary extracts recently described by us^{1, 2} and confirmed by Kligler and Olitski,³ Burnet,⁴ and Gough and Burnet⁵ may be employed to study the chemical properties of these complex cellular products with the aid of various manipulations such as fractionation and hydrolysis.

In a previous communication,² the observation was made that crude saline extracts of *B. aertrycke* boiled in N/2 alkali, still retained their phage inhibiting capacity and, consequently, it seemed desirable to study in detail the sensitivity of these substances to alkaline and acid treatment before attempting purification. For this purpose, a crude saline extract of *B. aertrycke* was employed and tested for phage inhibition before and after boiling in N/2 sodium hydroxide for 5 minutes* and subsequent neutralization. Such alkali treated extracts showed marked increase in phage inhibiting capacity although they gave a precipitin titre that was slightly lower than that of the untreated material. This observation, therefore, indicated that the degree of inhibition of phage did not run parallel to the precipitin titre of the crude extract. Striking confirmation of

³ Levine, Ph., and Frisch, A. W., *J. Exp. Med.*, 1934, **59**, 213.

¹ Levine, Ph., and Frisch, A. W., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 993; **31**, 46.

² Levine, Ph., and Frisch, A. W., *J. Exp. Med.*, 1934, **59**, 213.

⁴ Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1934, **59**, 213.

⁵ Burnet, F. M., *J. Path. and Bact.*, 1934, **38**, 285.

⁵ Gough, G. A. C., and Burnet, F. M., *J. Path. and Bact.*, 1934, **38**, 301.

* Under such conditions an inactive precipitate appeared which was removed after neutralization.

this view was found when these extracts were subjected to mild hydrolysis in N/15 and N/30 hydrochloric acid at 80°C. This procedure was adopted because boiling for 5 minutes or longer in N/2 acid completely destroyed both the phage inhibiting reaction and also the capacity to react with precipitins.

A crude extract of *B. aertrycke* was dissolved in saline, passed through a Seitz filter and then treated with N/5 hydrochloric acid in sufficient quantity to produce a final concentration of N/15 acid and held at 80°C. Samples were removed at various intervals, neutralized, brought to a dilution of 1:500, centrifuged to remove the precipitates, and sterilized by heating for one hour at 80°C. These solutions were then tested for their capacity to inhibit the action of the anti-paratyphosus B phage by means of the following method, a modification of the original adopted in order to permit the use of small quantities of extract. One drop of a 1:500 sterile solution of the extract was mixed with one drop of varying dilutions of the anti-paratyphosus B phage in serological tubes (100 mm. long and 20 mm. in diam.) and allowed to incubate over night at 37°C. (wet). The next day 2 cc. of beef extract broth was added to the sterile mixtures followed by one drop of a young broth suspension of *B. paratyphosus B*. Frequent turbidity readings were made to study the course of the lysis.

The results showed that specimens removed after treatment with mild acid for 30 to 90 minutes and somewhat longer intervals gave striking inhibition effects, considerably stronger than that of the untreated material. These solutions, however, showed a progressive diminution in their reactivity with precipitins (Table I). Somewhat

TABLE I.

Aertrycke extract	Precipitin titer with anti-aertrycke rabbit immune serum			Intensity of phage inhibition
	Dilutions of extract			
	5,000	50,000	200,000	
Untreated	++++	+++	++	+±
30 min. in N/15 acid	+	+	f. tr.	+++±
90 min. in N/15 acid	+	f. tr.	0	++++
4 hr. in N/15 acid	±	0	0	+±

analogous results were obtained with material previously boiled in N/2 alkali and subsequently subjected to similar acid treatment.

The data suggest the hypothesis that mild treatment with acid results in the appearance of partial hydrolytic products in which the specific reacting groups are unmasked as disclosed by the more intense phage reaction. This explanation seems likely, especially in

TABLE II.

Aertrycke extract	Precipitin titer.				Tested with antibody derived from:
	5,000	Dilutions of extract 50,000	500,000	5,000,000	
Untreated	+++ ±*	++± +	+± +	± tr	Rabbit serum Horse "
90 min. in N/15 acid	± +±	tr +±	0 ±	0 0	Rabbit " Horse "

*Zone effect.

Precipitin reactions recorded were made after the tests stood 2 hours at room temperature and over night in the ice box.

view of the characteristic behavior of the acid-treated solutions when tested with antibodies derived from rabbit and horse, namely better precipitin reactions with the anti-*B. paratyphosus B* horse serum (Table II). The assumption that the observations made are due to the appearance of hydrolytic products is further suggested by a similar finding of Heidelberger and Kendall,⁶ made with isolated hydrolytic products of the specific soluble substance of Pneumococcus type III, which gave precipitin reactions with specific antibodies obtained only from the horse and not from the rabbit.

The lack of parallelism between the precipitin reaction and phage inhibition may be understood if one assumes that for a precipitin reaction, as a rule, substances of higher molecular weight are required; whereas, specific inhibition reactions in general may be brought about by simpler chemical substances. (Landsteiner.)

Conclusive tests to determine whether the phage inactivating solutions obtained by mild acid treatment inhibit the precipitin reaction with rabbit antibodies, must await studies on the isolation and purification of the active phage inhibiting substances.

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Inactivation and Regeneration of the Glycolytic Enzyme System of Muscle Extract.

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An aqueous extract of muscle tissue, prepared according to Meyerhof,¹ contains the glycolytic enzyme which produces lactic acid

⁶ Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1933, **57**, 373.

¹ Meyerhof, O., *Biochem. Z.*, 1926, **178**, 395.