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Effect of Penicillin on the Reaction Between Phage and Staphylococci.

A. P. KRUEGER, T. COHN, AND N. NOBLE.

From the Department of Bacteriology and Office of Naval Research Task V, University of California.

In Fleming's original paper on penicillin,[1] he described the lysis of staphylococci by this agent. Since then the lytic process and ancillary morphologic changes in the bacteria concerned have been studied by several investigators.[2-8] We wish to report here the effect of penicillin on the lytic action of bacteriophage.*

The phage employed in our experiments is the "K" race; it and the homologous strain of *Staphylococcus aureus* have been used in studies described in a series of papers appearing since 1929. Stock cultures were grown in Roux flasks for 18 hours at 36°C. In order to have a substrate of actively growing cells, 1 x 10^8 staphylococci/ml were suspended in 100 ml of broth contained in a 500 ml flask; aeration was provided by placing the flask in a shaker operating in a water bath set at 36°C. When growth had progressed to a level of 1 x 10^9 bacteria/ml, the cell suspension was removed from the shaker and used for the experiments described below. To determine [bacteria] when values were >5 x 10^8 staphylococci/ml, the direct microscopic count method was employed. For lytic curves, visual comparison was made with standards covering a range from 2 x 10^7 to 5 x 10^8 staphylococci/ml in formalinized broth. The fluid medium throughout was tryptose phosphate broth. Phage determinations were carried out by Gratia's method[9] of counting plaques and the values noted below are in plaques/ml.

A 10 ml mixture containing 5 x 10^8 staphylococci/ml, 5 x 10^8 phage units/ml and 10 units of sodium penicillin/ml was placed in a test tube and shaken at 36°C. Turbidity readings were made every 0.2 hour. This was also done with a suspension from which the phage was omitted and with one containing no penicillin. Using as an end-point the reduction of turbidity by 50%, *i.e.*, to 2.5 x 10^8 staphylococci/ml, it was observed that the suspensions lysed in the following order: Phage + penicillin, 0.8 hours; phage only, 2.0 hours; penicillin only, 2.7 hours.

When the initial concentration of phage was reduced to 5 x 10^7 phage units/ml, the respective times of lysis were: Phage + penicillin, 1.1 hours; phage only, 3.8 hours.

The accelerating effect could not be demonstrated in suspensions made up in Locke's solution; lysis was considerably delayed in the phage + penicillin suspension and the lytic curve paralleled that for penicillin alone.

Repetition of the experiment with concentrations of penicillin varying from one unit/ml to 1,000 units/ml showed that the acceleration of lysis took place uniformly in all concentrations and was independent of (penicillin) within this range, 0.01 unit of penicillin/ml and lesser concentrations had no measurable effect in speeding up phage-engendered lysis.

An experiment was performed to determine how long a period of penicillin action was required in a mixture of bacteria and phage

1 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 1.

* Since preparation of this article for publication, Dr. Winston Price of Rockefeller Institute has informed us in a personal communication of similar studies he has completed using another phage and bacterial substrate.

2 Gardner, A. D., *Nature*, 1940, **146**, 837.

3 Fleming, A., *Lancet*, 1941, **241**, 761.

4 Smith, L. D., and Hay, T., *J. Franklin Inst.*, 1942, **233**, 598.

5 Weiss, L. J., *Proc. Indiana Acad. Sci.*, 1943, **52**, 27.

6 Miller, C. P., and Foster, A. Z., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 205.

7 Todd, E. W., *Lancet*, 1945, **248**, 74.

8 Fisher, A. M., *J. Bact.*, 1946, **52**, 539.

9 Gratia, A., *Ann. Inst. Pasteur*, 1936, **57**, 652.

to produce the characteristic acceleration. Several 9 ml mixtures of phage and bacteria in test tubes were prepared containing 5 x 10^8 staphylococci/ml and 2.5 x 10^8 phage units/ml. The tubes were placed in the shaker at 36°C and at intervals of 10, 20, 40, and 60 minutes from the time of mixing, one ml of penicillin solution (10 units) was added to successive tubes.

The addition of penicillin at 10 minutes and 20 minutes produced 50% lysis 0.7 hours ahead of the tube containing phage only. When penicillin was added after 40 minutes had elapsed, the lytic end-point occurred 0.5 hours before that in the phage control. With these particular concentrations of bacteria and phage, a typical acceleration could be secured if the penicillin acted at least 0.9 hours on the cellular substrate. Exposure to penicillin for a period of from 0.4 to 0.7 hours reduced the time of lysis by 0.5 hours.

In many of the experiments performed when the initial concentrations of bacteria were 5 x 10^8 staphylococci/ml or greater, the initial phage concentration 5 x 10^8 phage units/ml and the concentration of penicillin between 10 and 1,000 units/ml, clearing of the suspensions occurred without any increase in turbidity. Despite the absence of obvious bacterial reproduction, there were increases in [phage], up to a maximum of 10-fold, as determined by the plaque count. The methods used for detecting any increase in [bacteria] were not critical, however, and this point should be reinvestigated with more sensitive procedures.

A detailed account of further experiments with penicillin and phage action will be published elsewhere.

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A Bacteriophage for *Mycobacterium smegmatis.**

GRACE M. GARDNER AND RUSSELL S. WEISER. (Introduced by E. J. Ordal.)

From the Department of Microbiology, University of Washington School of Medicine, Seattle, Wash.

During investigations on the isolation of microorganisms antagonistic to the mycobacteria a bacteriophage active for *Mycobacterium smegmatis* was encountered.[1] The finding of this bacteriophage is of interest because it is, to our knowledge, the first definite evidence of a bacteriophage among the mycobacteria. Steenken[2] described spontaneous lysis in old colonies of *Mycobacterium tuberculosis* H37Rv which may have been due to bacteriophage action. He determined that the lytic factor was filterable but did not identify it as a bacteriophage by all of the usual criteria. Lysis appeared when the cultures were 3 to 4 months old and the pH of the medium had dropped to values of about 4.2 to 4.6. It began in the center of the colonies and spread to the periphery. The filtrate produced lysis of living bacteria at pH 4.2 to 4.8 and of both living and heat-killed bacteria at pH 2.2 to 4.0. Secondary resistant colonies developed in old liquefied areas.

The fact that bacteriophages for the mycobacteria have not been reported previously suggests that they may be very limited in their distribution. The successful isolation of one of them in the present work may have been due to the particular enrichment treatment employed.

The enrichment was carried out on 200-g samples of moist leaf compost to which a small amount of calcium carbonate was added. The

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1 Weiser, R. S., and Gardner, G. M., unpublished results.

2 Steenken, W., *Am. Rev. Tub.*, 1938, **38**, 777.