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.**

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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.

samples were collected from various locations in the city. They were incubated at 37°C for 8 months and treated semi-weekly with 5 to 10 ml of a heavy well-washed suspension of a young culture of *M. smegmatis*. The suspension was prepared from organisms grown on glycerine broth for one week at 37°C. The culture mass was ground in a mortar and washed twice in physiological salt solution.

After 3 months of enrichment, tests for organisms antagonistic for *M. smegmatis* were begun by making fixation plates of dilutions of the compost ranging from 1:100 to 1:-100,000. The medium used for plating was nutrient agar containing 1% glycerine and heavily inoculated with *M. smegmatis*.

Soon after beginning the tests, bacteriophage plaques were noted on the plates made from two of the compost samples. They had smooth edges and displayed a halo of partial lysis about a central clear zone of complete lysis. Isolated plaques attained a diameter of about 3 mm. Smears made from the clear areas contained only an occasional acid-fast organism. The material from the clear areas, when sub-cultured on bacteriological media, likewise, yielded only acid-fast organisms.

A Berkefeld filtrate was prepared from plaque material and the bacteriophage subcultured several times on plates. Filtrates of plaque material from the sub-cultures contained the bacteriophage in a concentration of 300 billion particles per ml.

No particular attempt was made to determine that the bacteriophage was a pure strain. However, preliminary tests indicate that the bacteriophage is specific for our stock strain of *M. smegmatis*. It proved to be inactive for *Mycobacterium phlei* and a second strain of *M. smegmatis*.

The bacteriophage was inactivated by a temperature of 75°C for 10 minutes but remained active when held at 72°C for 10 minutes. It preserved well in 50% glycerine and by lyophilization.

As a test of the effectiveness of the method used for the isolation of bacteriophage for *M. smegmatis* the enrichment procedure was repeated using a sample of soil collected on the University campus. Precautions were taken in this trial to prevent possible contamination of the test plates with the specific bacteriophage we had previously isolated. Weekly tests for the presence of bacteriophage were negative until the third week of enrichment when plaques first appeared. Tests on the control unenriched sample of soil were negative for bacteriophage. The plaque characteristics of this newly isolated bacteriophage did not appear to be different from those of the previous isolate.

Discussion. In our work on the isolation of microorganisms antagonistic to the mycobacteria we have used both enriched and unenriched soil and compost. The bacteriophage was isolated from 2 of 6 samples of enriched compost and from one sample of enriched soil. It was not encountered in 8 samples of unenriched compost and 4 samples of soil. Apparently the isolation of the bacteriophage was facilitated by the specific enrichment employed, and possibly because the enrichment was carried out on the soil and compost samples preliminary to filtration rather than after filtration.

Bottcher and Hofer[3] have employed specific preliminary enrichment of soil for the isolation of bacteriophage for the legume bacteria by adding a special medium heavily inoculated with the organism. The present method differs from that of Bottcher and Hofer inasmuch as the organisms added were free of medium. It is possible that the lack of medium may operate to advantage by reducing the possibility of suppression of the specific organism or its bacteriophage by overgrowth of some other organism.

Summary. A bacteriophage specific for *M. smegmatis* was isolated from samples of compost and soil by specific enrichment with a heavy washed suspension of *M. smegmatis*. The method of enrichment employed may be useful in the isolation of other phages.

[3] Bottcher, J., and Hofer, A. W., *J. Bact.*, 1943, **45**, 407.