An Herbarium-inhabiting Bacteriophage

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On March 8th, 1951, a bacteriophage and its susceptible organism were isolated from a nutrient agar plate which had been sprinkled with dust from plant specimens in the herbarium of Central State College. The susceptible organism was found to be *Bacillus cereus* Frankland, and from all indications, the phage may be specific for this organism alone. The type strain of *Bacillus cereus* showed a positive reaction to the phage, but the variety *mycoides* of *B. cereus* was negative. All of the other species of *Bacillus* available at the time were subjected to the concentrated phage and none showed a positive reaction. The plaques of bacteriophage were small, averaging 100 to 500 microns in diameter. Three days were required, on the average, before the plaques became visible to the naked eye, and six days elapsed before complete clearing.

Isolation was accomplished in the following manner: Herbarium dust was blown out of a folder near a nutrient agar plate, and the plate incubated until colonies bearing clear plaques had developed. The susceptible bacteria were isolated from around one of the plaques, and transferred to nutrient agar broth. After this had incubated 24 hours, a stab was made through the plaque and shaken in the broth culture. The combination was then incubated for 48 hours and the bacteriophage separated from the bacilli by filtration through a Seitz bacterial funnel or by centrifuging 5 ml. portions for 3 minutes. The ultra-filtered broth was proven bacteria-free, and the supernatant fluid from the centrifuged broth contained very few bacilli. This bacteriophage concentrate was kept in the icebox, and

increased when needed by reinoculations into cultures of the susceptible organisms.

After a number of phage tests had been run on the herbarium strain of *B. cereus*, certain irregularities in the growth of the surviving bacteria became apparent. In affected colonies, the margin bordering a plaque was raised, and clearing began in the center of this raised portion. There did not appear to be any changes in morphology or in biochemical behavior of the bacilli.