other studies(8-12) for which at least 30 injections over a 6-week period are required for the earliest deposition of amyloid. Whether the number of injections or the time interval is most important for amyloid deposition is not answered by this experiment; but earlier workers(9,11,12) employing the caseinate method, observed that both the number of injections and duration of administration rather than the quantity of material introduced are the significant factors.

Summary. A method has been described for the induction of amyloidosis in mice by injection of the Freund-type adjuvant. The *M. butyricum*, or oil and emulsifying agent alone were not effective. *M. butyricum* with either the oil or emulsifying agent was capable of inducing amyloid. *N. asteroides* or Group A streptococci could be substituted for *M. butyricum* but the incidence of amyloidosis was less. The present method, as compared with previous ones, requires fewer injections, and it produces a higher incidence of amyloidosis in a shorter time.

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Heat Studies on a Thermophilic Bacteriophage.* (20809)

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Inactivation of bacteriophage by heat has been observed by several investigators, and seems to be a general phenomenon(1-3). Nanavutty(2), who exposed coli-dysentery phage to various temperatures and Smith and Krueger(3), employing vibrio-phage, found that their respective phages were not inactivated at a constant rate; heat inactivation curves (50° to 65°C) obtained by them were concave and became asymptotic with the time-axis. In contrast, the inactivation of a staphylococcus phage at various temperatures followed a monomolecular reaction and the temperature coefficient was characteristic of protein denaturation(4).

Koser(5,6) isolated a thermophilic phage from sewage-polluted river water and observed the optimum temperature for the lytic activity of the virus to be about 50°C, its ability to lyse the host at 57° to 58°C, its survival at 70°C for 30 minutes, and its destruction at 75°C. Heat resistance tests demonstrated that despite activity at higher temperatures, the phage was inactivated at about the same temperature (70° to 75°C) as the coli-dysentery phages. A thermophilic phage studied by Adant(7) was found to be active at 52°C, but was destroyed at 75°C when exposed for 30 minutes.

Recently we isolated from green house soil a bacteriophage capable of lysing a thermophilic bacterium at 65°C. Because the virus was active at high temperatures, heat inactivation studies were made since it was thought that it might be more resistant to heat than phages thus far reported. This investigation

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was made to determine the rate at which the virus was destroyed and the temperature at which no further lytic activity was demonstrable.

Materials and methods. The host organism is a thermophilic bacterium designated No. 10 in a group of bacteria isolated from the hot springs of Yellowstone National Park and characterized by Marsh and Larsen(8). The optimum temperature for growth of this organism is 65°C, the same temperature at which the virus exhibits maximum activity.

The phage lysate was prepared in Trypticase broth (20 g Trypticase [Baltimore Biological Laboratory] per liter, at pH 7.0). The lysate was allowed to ripen for a few days in the refrigerator, then centrifuged at 4000 r.p.m. for 20 minutes to remove bacterial debris and finally passed through an ultrafine sintered glass filter. (When a Berkefeld filter was used, the titer of the filtrate was reduced 50%.)

Because of the high temperatures employed, a constant temperature oil bath was used in these studies. Small test tubes were prewarmed at the desired temperature and 0.5 ml aliquots of the lysate were introduced into these tubes which were immediately returned to the bath. At one-half hour intervals, samples were removed and diluted at once with Trypticase broth to stop the action of the heat. The residual active phage was determined by plaque count using the agar layer technic. Because of the higher temperature of incubation, 2.0% Trypticase agar was used in the plates and 0.9% to 1.0% Trypticase agar in the layer. An unheated aliquot was always plated simultaneously with the heated aliquots to determine the activity of the phage at the start of each run. Three runs were made at each temperature on consecutive days.

Results. The phage lysate was exposed to temperatures which ranged from 70°C to 100°C to determine the rate of inactivation as well as the temperature at which there was no further evidence of phage activity. When the logarithm of residual active phage was plotted against time of heating (Fig. 1), it was observed that straight lines were not obtained, indicating that inactivation did not follow a unimolecular reaction.

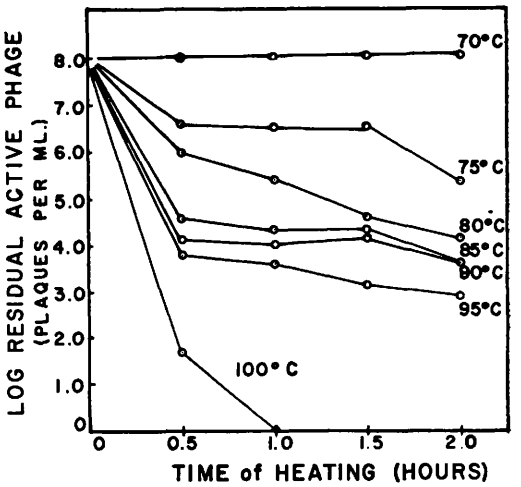

FIG. 1. Heat inactivation of a thermophilic bacteriophage in a broth lysate at pH 7.0.

At 70°C there was no appreciable decline in activity. After an exposure of two hours at 75°C, there remained about 340000 phage particles capable of producing plaques as compared to one hundred million per ml at 70°C. Two hours at 80°C reduced the number to 13000.

At 100°C there were but 50 plaques per ml detectable after the phage was exposed for thirty minutes with complete inactivation at the end of one hour. At temperatures of 85°C, 90°C and 95°C most rapid inactivation occurred during the first half hour, after which time the rate decreased quite markedly. Plaque-producing phage particles observed at 0.5 hour were 35000 at 85°C and 6000 at 95°C, while at the end of two hours there still remained 3500 and 1000 respectively per ml.

A precipitate was observed in the lysate within a few minutes after heating at 95°C. Its significance and character were not investigated. Even after the precipitate appeared, the phage was still active after a two hour exposure at 95°C. This would indicate that the substance precipitated exhibited little or no protective effect on the phage.

It was also noted that after exposure to the higher temperatures (85°C to 95°C) the plaques were considerably larger than at 65°C to 80°C, otherwise they appeared to be the same.

Discussion. It can be concluded from the

data presented in the graph that the rates of inactivation of this thermophilic virus are very similar to those of the phage systems reported by Nanavutty(2) and Smith and Krueger(3). However, the temperatures at which the phage becomes susceptible to inactivation are much higher than those of the other reported phages. The virus is quite stable at 70°C but above this temperature the curves become concave. The most rapid inactivation occurs in the first half hour at temperatures above 80°C with a decided decrease in rate for the remainder of the period. There is no evidence of plaque formation after exposure to 100°C for 60 minutes and the virus is assumed to be completely destroyed.

This virus begins to be inactivated at about 75°C, at which temperature the thermophilic phages reported by Koser(5,6) and Adant(7) were completely destroyed. These data bear out the hypothesis that the virus used in this investigation is more heat resistant than any previously reported.

Summary. A thermophilic bacteriophage has been isolated which is inactivated by heat at a rate which is not constant but decreases with time. The phage remains viable after two hours exposure at 95°C, but is destroyed at 100°C, indicating that it is much more heat resistant than phages previously reported.

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Blood Eosinophil Levels in the Young and Adult Pyridoxine-Deficient Rat. (20810)

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The 24-hour rhythm of the blood eosinophils has been shown to synchronize with the 24-hour cyclic variations of the urinary excretion of ketosteroids(1,2) as well as with that of the blood lymphocytes(1,3). These correlations with currently accepted indices of adrenal function make possible the use of the blood eosinophil levels taken at a specific phase in their rhythm as an additional index of adrenal activity. A characteristic eosinophil level may be established for any species and strain provided constant conditions are maintained(1,4).

In the present experiment, the blood eosinophil levels of the pyridoxine-deficient male rat were determined throughout the progress of the deficiency. In order to evaluate the role of inanition in these changes, pair weighed and normal controls were also studied.

Materials and methods. Young animals. To develop an early and severe pyridoxine deficiency(5), on the 15th day of age litters were divided into 3 groups. One-third of the males were placed with lactating mothers receiving a pyridoxine-deficient ration (Table I) and the remaining males were left with mothers receiving the same diet containing pyridoxine. On the 21st day of age the young animals were placed in individual metabolic cages. Three groups of 8 animals per group were fed as pyridoxine-deficient, pair weighed and *ad libitum.* The young animals received a high protein diet (Table I) which resulted in a more severe pyridoxine-deficiency than lower protein levels would have induced. A complete vitamin mixture (Table I) was fed 3

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