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.

1. d'Herelle, F., *The Bacteriophage and Its Behavior,* The Williams and Wilkins Co., 1926, Baltimore, 292-296.

2. Nanavutty, S., *J. Path. and Bact.,* 1930, v33, 203.

3. Smith, L. S., and Krueger, A. P., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 371.

4. Krueger, A. P., *J. Gen. Physiol.,* 1932, v15, 363.

5. Koser, S. A., PROC. SOC. EXP. BIOL. AND MED., 1926, v40, 109.

6. Koser, S. A., *ibid.,* 1927, v41, 365.

7. Adant, M., *Compt. rend. Soc. Biol.,* 1928, v99, 1244.

8. Marsh, C. L., and Larsen, D. H., *J. Bact.,* 1953, v65, 193.

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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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TABLE I. Purified Diets Fed to Lactating Mothers, Young, and Adult Rats.

Constituents	Lactating mothers	Young rats‡	Adult rats‡
	%		
Casein	36	50	24
Hydrogenated vegetable oil	4	5	10
Salt mix(5)	4	4	4
Sucrose	54	41	62
Vit. B mixture*	1	0	0
Vit. A & D oil†	1	0	0

* Each kg of diet fed to mothers supplied the following vitamins in mg amounts: thiamine hydrochloride, riboflavin, pyridoxine, and folic acid, 2.0 each, calcium pantothenate and para-aminobenzoic acid, 10 each, niacin amide, 6.7, inositol, 250, biotin 0.27, and choline, 500.

† Each kg of diet contained the fat soluble vitamins in the following amounts: vit. A, 10000 I.U., vit. D, 1000 I.U., and mixed tocopherols, 100 mg, dissolved in 9 g of cottonseed oil.

‡ Water soluble vitamins dissolved in 20% aqueous ethanol and supplied each rat daily the following amounts in mg: thiamine hydrochloride, riboflavin, pyridoxine and folic acid, .02 each, calcium pantothenate and para-aminobenzoic acid, .10 each, niacin amide, .07, inositol, 2.5, biotin, .002, and choline, 5.0. Vit. A and D and mixed tocopherols were dissolved in vegetable oil and were supplied separately in amounts of 1000 I.U., 100 I.U. and 3 mg/rat/wk.

times weekly in individual cups; the deficient group received no pyridoxine in this mixture.

Adult animals. Adult male stock rats at an average age of 103 days were divided into the 3 groups mentioned above containing 8 animals each. They were given a diet containing 24% casein (Table I). The deficiency condition was induced by the addition of 30 mg of desoxypyridoxine per kg diet mixed directly into the ration of the deficient group and by the omission of pyridoxine from their vitamin mixture. *Eosinophil counting technic.* In young rats eosinophil counts were determined at weekly intervals for 9 weeks. In adult rats the circulating eosinophil levels were observed at irregular intervals from the 25th to 46th day. The eosinophil counts of 8 animals per group were used in the young and adult series. Blood was drawn from the tail vein into a white blood pipette to the 0.5 mark and diluted with phloxine eosinophil diluent (6). If a variation of greater than 10% occurred between counts of duplicate pipettes, the values were discarded. For young rats, both chambers of a Levy hemocytometer, and for adult rats, one chamber of a Fuchs-Rosenthal hemocytometer were counted for each of the 2 pipettes per animal. In order to eliminate diurnal variations all counts were completed during the hours of 9:00 a.m. and 12:00 noon in the same room in which the animals were housed in order to maintain the environmental conditions as constant as possible(4).

Results. Young animals. The average body weight gain at the termination of 9 weeks on the regimen was 68% for the deficient and pair weighed groups, whereas, the *ad libitum* group gained 450% above their respective weights on the 21st day of age. In the deficient animals acrodynia appeared in the 3rd and 4th weeks. Xanthurenic acid was detectable in the urine from the 14th to 16th day on the regimen. The rapidly growing *ad libitum* animals (Fig. 1) showed a continuous steep ascent of the circulating eosinophils from the 1st week with 27 $\pm$ 5 per mm^3 to 268 $\pm$ 38 per mm^3 in the 8th week when no further rise in the level occurred. In contrast, the deficient and pair weighed groups had a comparable but gradual rise in the eosinophil levels throughout the course of the experiment. The last two mentioned groups showed lower eosinophil levels ($p = \text{or} < .01$) than did the *ad libitum* group. First evidence of these depressed levels was observed in the

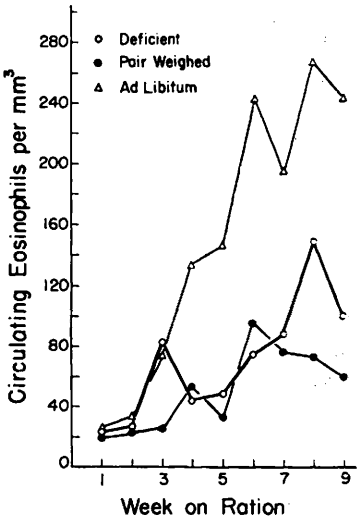

FIG. 1.
Circulating eosinophil levels in growing rats.

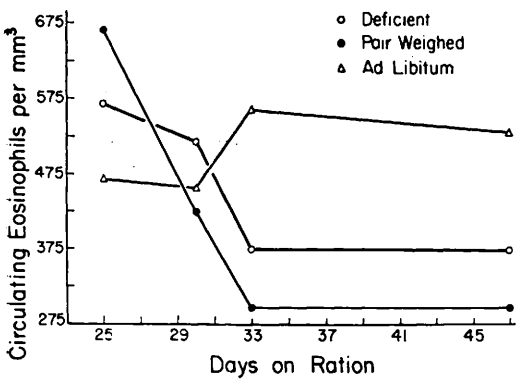

FIG. 2. Circulating eosinophil levels in adult rats.

deficient group at the start of the 4th week and in the pair weighed group at the beginning of the 3rd week. These significantly lower circulating eosinophil levels continued for the duration of the experiment.

Adult animals. The adult deficient and pair weighed groups lost 29% while the *ad libitum* group gained 3.5% of their initial body weight during the 8 weeks on the dietary regimen. Signs of acrodynia appeared within 2 weeks and progressively became more severe. On the 25th day of the experiment (Fig. 2) the eosinophil levels of the 3 groups were equal ($p = .3$ to $.7$). The *ad libitum* group showed no significant rise differences in the blood eosinophil levels throughout the period of observation. On the other hand, the eosinophil levels in the deficient and pair weighed groups were characterized by an equal and gradual fall until significantly lower levels ($p =$ or $< .01$) occurred simultaneously on the 33rd day and continued to remain low through the 46th day.

Discussion. These findings show that during rapid growth the eosinophil level in young normal animals ascends steeply until the 63rd day of age. In contrast, normal adult animals whose growth was insignificant maintained a constant eosinophil level for the duration of the experimental period. Clearly then, growth alone in the normal young rat caused an increase in the number of blood eosinophils.

An equal degree of endogenous eosinopenia gradually occurred in the young and adult pyridoxine-deficient animals as compared with their respective pair weighed controls. Inanition in the young calorie-restricted animals, whose growth was markedly retarded, produced a greater fall in the circulating eosinophils than it did in the adult animals similarly restricted. From these results it is inferred that inanition, not pyridoxine deficiency *per se*, was the specific cause of the eosinophilic leucopenia. Studies of chronic inanition have usually demonstrated a normal or hyperfunctional adrenal(7-9) although adrenal hypofunction has been reported by one group of workers(10). During calorie restriction in rodents, a greater amplitude without a shift of the 24-hour eosinophil rhythm(7), a greater deposition of liver glycogen under the influence of hypoxia(8), and a gradual development of a lymphopenia(9) are supporting evidences of a functional as well as a hyperfunctional adrenal. We also have demonstrated previously(11) that pair weighed and pyridoxine-deficient rats were capable of eosinopenic responses to stimuli equal to those of their *ad libitum* controls.

The depression of the circulating eosinophils in the pair weighed and deficient young and adult animals in the experiment here reported probably indicated an equal and augmented secretion of the adrenal 11-oxysteroid hormones comparable to that seen in cases of an endogenous eosinopenia(12). Since experiments were not performed on adrenalectomized animals, it is impossible to state categorically that the depression of the eosinophils was mediated solely by the adrenals.

Summary. The blood eosinophil levels of pyridoxine-deficient and pair weighed young and adult male rats were of the same order of magnitude and were significantly lower throughout the experiment than those of full-fed normal controls. These findings indicated that inanition alone was the cause of the chronic endogenous eosinopenia, therefore, excluding lack of pyridoxine *per se* as the agent.

1. Halberg, F., *Lancet*, 1953, v73, 20.

2. Pincus, G., Romanoff, L. P., and Carlo, J., *J. Clin. Endocrinology*, 1943, v3, 195.

3. Elmadjian, F., and Pincus, G., *J. Clin. Endocrinol.*, 1946, v6, 287.

4. Halberg, F., Bittner, J. J., and Visscher, M. B., *Blood*, 1946, v6, 832.

5. Hubbell, R. B., Mendel, L. B., and Wakeman,

A. J., *J. Nutrition*, 1937, v14, 273.

6. Speirs, R. S., and Meyer, R. K., *Endocrinology*, 1949, v45, 403.

7. Halberg, F., and Visscher, M. B., *ibid.*, 1952, v51, 320.

8. Hurley, L. S., and Morgan, A. F., *J. Biol. Chem*, 1952, v195, 583.

9. Shukers, C. F., and Day, P. L., *J. Nutrition*, 1943, v25, 511.

10. Mulinos, M. G., and Pomerantz, L., *Endocrinology*, 1942, v31, 276.

11. Butler, L. C., and Morgan, A. F., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 655.

12. Thorn, G. W., Forsham, P. H., Recant, L., and Hills, G. A., *J. Clin. Endocrinol.*, 1948, v8, 589.

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Action of Fibrinolysin (Plasmin) on Proteins.* (20811)

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Previous workers(1,2) have found that the products of the action of fibrinolysin (plasmin) on fibrinogen and fibrin are of high molecular weight. In the method of Ungar and coworkers(3) for the assay of fibrinolysin, unchanged fibrinogen is precipitated as fibrin and the material remaining in the filtrate is measured. If the fibrinogen is precipitated with trichloroacetic acid (TCA), the reaction products are precipitated along with the unchanged fibrinogen and cannot be found in the filtrate. Similarly, the soluble material formed by the action of fibrinolysin on fibrin is largely precipitable by TCA. The action of rennin upon casein is similar in some respects to that of thrombin upon fibrinogen. After exposure to the action of fibrinolysin if unchanged casein is precipitated with rennin, there is more soluble material present than when the casein is precipitated with TCA. These observations confirm the conclusions of previous workers(1,2) that the product of the action of fibrinolysin consists of proteins or peptides of high molecular weight.

Methods. The buffer in all cases was that of Ungar and coworkers(3) consisting of 0.15 M phosphate (sodium salts) of pH 7.4 in 0.1 M NaCl. Solutions of 0.1% bovine fibrinolysin† and 0.2% bovine fibrinogen (Armour) in buffer were made up daily as needed. Casein (Pfanstiehl) was dissolved in sufficient buffer to give a 2% solution by heating 10 minutes at the temperature of boiling water, cooled, made up to the original volume with distilled water, and filtered. It was stored in the refrigerator as long as one week. Thrombin† was used as a solution in isotonic saline containing 300 units per ml. It was stored in the frozen state for periods up to 2 weeks. Rennin (Nutritional Biochemicals) was dissolved in distilled water as needed to give a 0.1% solution. Fibrinolysin was assayed for its activity for fibrinogen and fibrin by the method of Ungar *et al.*(3). Activity for casein was determined similarly using rennin as the coagulant. The substrate (1.0 ml of 2% solution) was acted on by various concentrations of fibrinolysin in a total volume of 4.5 ml of buffer. After one hour at 37°C sufficient 0.1 M citric acid (1.1 ml) to bring the pH to about 5.3 and 0.1 ml of rennin solution were added. After 10 minutes at 37°C, the pH was adjusted to about 4.8 (0.3 ml of citric acid). The pH's were chosen to approximate the optima found by Lundsteen(4) for enzymatic action of rennin on casein and for coagulation of the product. After 30 minutes at 37°C the precipitates were filtered, and the optical density of the filtrates was read at 280 mμ against blanks without casein. Readings were corrected for the reading on casein with fibrinolysin absent. Although the isoelectric point of casein was approached at pH 4.8 and its solutions became milky in

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