11. ———, *J. Dairy Sci.*, 1950, v33, 842.
12. "Student", *Biometrika*, 1908, v6, 1.
13. Fisher, R. A., *Statistical Methods for Research Workers*, 1936, Oliver and Boyd, Edinburgh.
14. MacLeod, J., *Amer. J. Physiol.*, 1943, v138, 512.
15. ———, in *The Problem of Fertility*, edited by E. T. Engle, 1946, Princeton Univ. Press.
16. Tosic, J., and Walton, A., *J. Agric. Sci.*, 1946, v37, 69.
17. ———, *Biochem. J.*, 1950, v47, 199.
18. Prince, P. W., and Almquist, J. O., *J. Dairy Sci.*, 1948, v31, 839.
19. Gunn, R. M. C., *Austral. Council Sci. and Ind. Res. Bull.*, 1936, v94, 1.
20. Van Demark, N. L., Salisbury, G. W., and Bratton, R. W., *J. Dairy Sci.*, 1949, v32, 353.
21. Van Demark, N. L., Bratton, R. W., and Foote, R. H., *J. Dairy Sci.*, 1950, v33, 661.
22. Campbell, J. A., *J. Physiol.*, 1932, v76, 13 P.

Received September 8, 1952. P.S.E.B.M., 1952, v81.

Thermal Inactivation of a New Vibrio Phage.*† (19879)

LUCILE SCHULTZ SMITH AND ALBERT PAUL KRUEGER.
From Department of Bacteriology, University of California, Berkeley.

Susceptibility of bacteriophages to inactivation by heat has been observed by several workers to be a general phenomenon(1-4a). Nanavutty(5) studied rates of inactivation of a coli-dysentery phage exposed to various temperatures. He plotted the logarithms of residual active phage against time and obtained concave curves. He also observed a difference in heat susceptibility of the phage when different suspending fluids were used. Application of an accurate analytical method for phage assay to experiments on the thermolability of staphylococcal phage demonstrated that: 1) heat inactivation at various temperatures follows the course of a monomolecular reaction; and 2) the temperature coefficient is of a magnitude characteristic of protein denaturation(6). Other races of bacteriophage have since been shown to behave similarly during heat inactivation(7,8).

Recently we have been making a survey of the properties of a new Vibrio-phage system. The present papers deal with some interesting aspects of the heat inactivation of this particular phage.

Materials and methods. The virus-host system under study was recently isolated from San Francisco Bay mud. The bacterium is of the genus Vibrio, and will not grow in ordinary media without the addition of NaCl. A concentration of 3% sodium chloride supports maximum growth of the organism and optimal phage production. The phage lysate was prepared in yeast extract mineral 3% salt medium‡ and was passed through a Seitz filter (Type ST) to secure a bacteria-free suspension of phage at pH 7.9. When it was desired to compare the susceptibility of the phage suspended in various fluids, the lysate was ultracentrifuged, the supernatant fluid decanted, and the phage resuspended in the medium indicated, *i.e.*, M/15 phosphate buffer pH 7.9, M/15 phosphate buffer pH 7.9 plus 3% NaCl, 1% aqueous yeast extract plus NaCl in concentrations of 5, 4, 3, 2, 1, 0.5, 0.25, and 0.125%, respectively and no NaCl. The phage was exposed to a given temperature by dispensing 0.5 ml aliquots into prewarmed test-tubes and immediately placing these in the water bath at the desired temperature. At the end of each time interval a sample was removed from the water bath and immediately

* This investigation was supported in part by grants from ONR Task 21, the Board of Research, University of California, and State of California Cancer Research Fund.

† This work is partial fulfillment of the requirements for the degree of Ph.D. in Bacteriology at the University of California.

‡ The composition of Yeast Extract Mineral 3% salt medium is:

Yeast Extract (Difco)	%	Bacto dehydrated	1%
			%
$MgSO_4$	.05	NaCl	3.0
K_2HPO_4	.1	Tap water	10
NH_4NO_3	.1	Dist. water q.s.	90

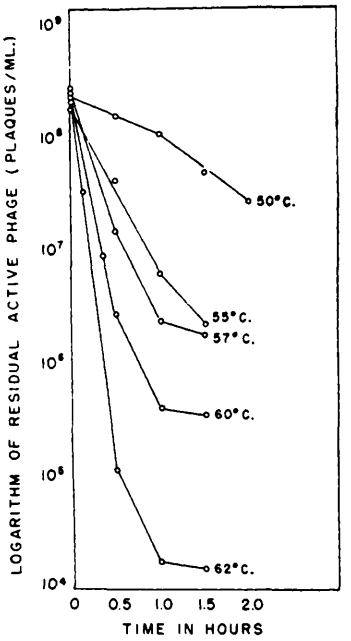

FIG. 1. Heat inactivation of Vibrio phage in yeast extract mineral 3% NaCl medium.

diluted in nutrient broth containing 3% NaCl. The residual active phage was determined by plaque count according to a modification of the original method of Gratia(9). The procedure employed, *i.e.*, using separate small aliquots of the phage suspension for each experiment, has several technical advantages; it is open to the objection that the results may not apply to a single large volume. We have checked this point by conducting parallel experiments with both methods and have obtained identical results.

Results. When phage lysates were exposed to various temperatures, and the logarithm of the numbers of remaining phage particles plotted against time, it was observed that the rate of inactivation did not remain constant. Inactivation at 50°C became faster after one hour; at 55°C the plot most nearly followed a straight line, but at higher temperatures, 57°C, 60°C, 62°C, there was a break in the curve at 0.5 hour and the rate decreased after this time (Fig. 1).

For comparison of the behavior of the phage in various environments it was resuspended in each of the following fluids, M/15 phosphate buffer pH 7.9, M/15 phosphate buffer pH 7.9 plus 3% NaCl, and 1% aqueous yeast extract. Susceptibility to heat inactivation was found to be different in each case. However, the same tendency to produce concave curves with rising temperatures was revealed (Fig. 2 and 3).

Discussion. From the data presented in Fig. 1, 2, and 3, it is possible to reach certain conclusions: 1) Susceptibility of the Vibrio phage to inactivation by heat is influenced by the nature of the suspending fluid. Of those used in this work M/15 phosphate buffer at pH 7.9 rendered the phage most vulnerable. The addition of 3% NaCl to the buffer increased the resistance. Likewise phage in 1% yeast extract was more resistant than in the buffer alone. However, phage activity was most resistant to high temperatures when the filtered lysate (containing both yeast extract and 3% NaCl) was exposed. This finding is in accord with the reported properties of other bacteriophages. The influence of suspending fluid on susceptibility to inactivation by heat has been demonstrated for a coli-dysentery phage by Nanavutty(5), for

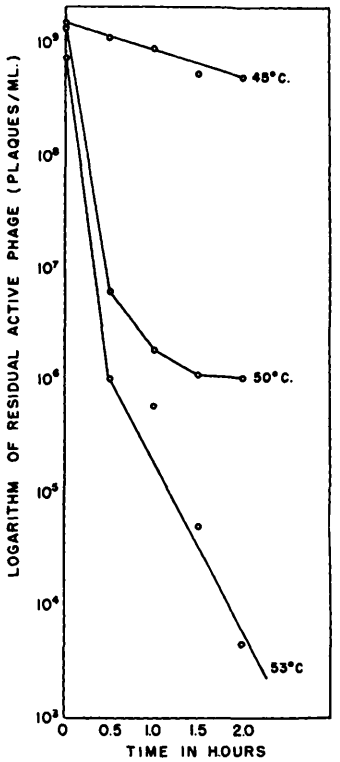

FIG. 2. Heat inactivation of Vibrio phage suspended in M/15 phosphate buffer pH 7.9.

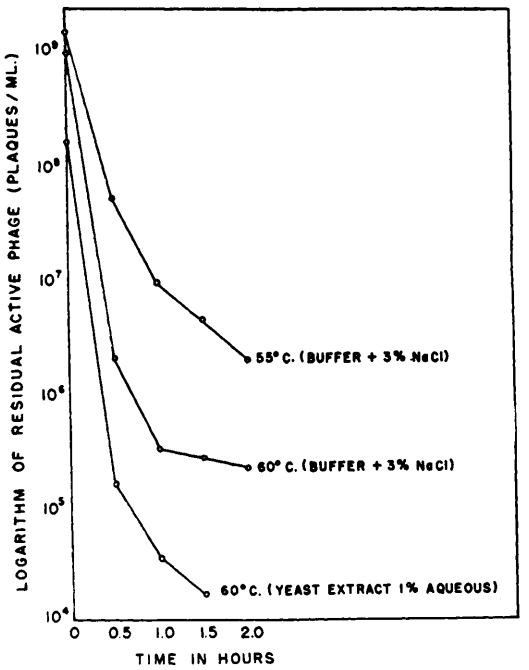

FIG. 3. Heat inactivation of Vibrio phage suspended in M/15 phosphate buffer pH 7.9 + 3% NaCl and in 1% aqueous yeast extract.

coli and staphylococcus phages by Burnet and McKie(10), for a megatherium phage by Gratia(11), and for the group of "T" coli phages by Adams(8). 2) Changes in the rate of phage inactivation with time occur in all of the suspending fluids. This indicates that the shape of the curves is not due to either the presence of a high concentration of electrolytes or the yeast extract alone. 3) Approaching the highest temperatures at which the inactivation can be studied, marked decreases in the rate occur after 90% of the phage activity has been lost. 4) In general, if essential experimental conditions are met, the curves for heat inactivation might be expected to be straight line plots of the sort reported by Krueger for staphylococcal phage(6). We have considered several possible explanations of the deviations noted above. It is conceivable for example that we are dealing with two races of phage which are characterized by different degrees of thermoresistance. If this were the case exposure to 60°C for 2 hours should completely destroy the more thermolabile race and should leave only the thermoresistant one. Such an experiment was performed and numerous plaques were picked from the remaining active phage. New phage preparations were developed separately on suspensions of the host cell and filtered lysates were made. The thermal inactivation characteristics of these lysates were determined and it was found that they behaved exactly as did the original phage from which they had been derived, *i.e.*, the experiment gave no indication that the observed changes in rate of thermal inactivation were due to separate races of phage. 5) Since reactivation of thermally inactivated phage has been reported by Krueger and Mundell(12) one might explain the present data in such terms if conditions were such that reversal of denaturation occurred only after 0.5 hour. However, experiments described in the accompanying paper on thermal shock(13) seem to preclude this possibility. 6) The presence of a hypothetical protective substance developing after the first 0.5 hour of heating could account for the reduced rate of inactivation. To investigate this possibility lysate was heated at 60°C for 0.5 hour, diluted with warmed (60°C) broth containing 3% NaCl and exposure to 60°C was continued for 1.5 hours longer. Theoretically, dilution of the postulated protective substance should diminish its effect on phage and inactivation should continue at the original rate. Actually no such result was obtained; the curve for inactivation corrected for dilution was the same as that of the undiluted control. 7) A skewed curve of distribution of thermoresistance among the phage particles composing the original lysate could account for the sets of curves in Fig. 1, 2, and 3, without involving any genetic factor. We have no evidence disproving the existence of such distribution.

Conclusions. 1. Susceptibility of the Vibrio phage to thermal inactivation is influenced by the nature of the suspending fluid. Decrease in rate of inactivation with time occurs in all of the fluids tested. 2. Several possible explanations for deviation of the inactivation curves from the plots predicted by the Mass Law have been considered.

1. Kabeshima, T., *Compt. rend. Soc. Biol.*, 1920, v83, 219.

2. Weinberg, M., et Aznar, P., *Compt. rend. Soc.*

Biol., 1922, v87, 136.

3. Tchang Kouo Ngen et Wagemans, J., *Compt. rend. Soc. Biol.*, 1922, v87, 1253.

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

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

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

7. Dreyer, G., and Campbell-Renton, M., *J. Path. and Bact.*, 1933, v36, 399.

8. Adams, M. H., *J. Gen. Physiol.*, 1949, v32, 579.

9. Gratia, A., *Annales Inst. Pasteur*, 1936, v57, 652.

10. Burnet, F. M., and McKie, M., *Australian J. Exp. Biol. and Med. Sc.*, 1930, v7, 183.

11. Gratia, A., *Compt. rend. Soc. Biol.*, 1940, v133, 443, 445.

12. Krueger, A. P., and Mundell, J. H., PROC. SOC. EXP. BIOL. AND MED., 1936, v34, 410.

13. Smith, L. S., and Krueger, A. P., PROC. SOC. EXP. BIOL. AND MED., accompanying paper.

Received September 12, 1952. P.S.E.B.M., 1952, v81.

Comparison of Creatinine and Inulin Clearance in the Dog During Hypoxia.* (19880)

EWALD E. SELKURT. (With technical assistance of Helen K. Grodeck.)

From the Department of Physiology, School of Medicine, Western Reserve University, Cleveland, O.

The creatinine/inulin clearance ratio has been reported by Shannon to be 0.99 in unanesthetized dogs(1,2). This agreement was found to be maintained during anesthesia (chloralose), but discrepancies began to appear in the isolated perfused dog kidney probably subjected to varying degrees of anoxia at low urine volumes where the ratio averaged 0.86(3). The desire to gain assurance that the creatinine clearance remained an index of glomerular filtration rate during hypoxic conditions in the dog prompted the present investigation in which this clearance was compared to the accepted standard of reference, the inulin clearance.

Methods. Pentobarbitalized animals were used in these experiments. Arterial blood pressure was recorded with a mercury manometer by direct cannulation of a femoral artery, and the trachea was cannulated for connection to a Douglas bag. Urine was collected by direct catheterization of the ureters. Substances to be analyzed for clearance were infused at a constant rate by a motor driven syringe. A moderate diuresis was instituted by infusion of Ringer's solution prior and during the experiment. *Hypoxia* was produced by inhalation of an 8% oxygen, 92% nitrogen mixture. In animals which tolerated the procedure well, alternate stages of low oxygen mixture and room air were observed, with a pair of urine collection periods of ten minutes duration at each stage, for a total of five stages (Table I). In some animals difficulties were encountered as the result of continued breathing of the low oxygen mixture (fall in blood pressure, respiratory paralysis) so that experiments were of briefer duration. Although inhalation of 8% oxygen produced the lowest level of blood oxygen content compatible with survival, in two experiments the kidney was subjected to an even greater degree of hypoxia by a technic of perfusion of the kidney *in situ* with venous blood while the animal breathed 8% oxygen. This was done with the aid of a pump previously described (4), which took blood from the right ventricle through a cannula introduced via the external jugular vein and delivered it into the renal artery at pressure kept at arterial pressure levels by proper adjustment of the stroke volume of the pump. An experiment of this type appears in Table II. *Blood oxygen* content was determined by the method of Van Slyke and Neill(5) in samples taken mid-way in each stage of the experiment. Since these were single determinations they cannot be taken as average values for oxygen content, but serve only as a relative index of the degree

* This investigation was supported by a grant from the U. S. Public Health Service.