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Effect of pH on Heat Inactivation of Bacteriophage.*

A. P. KRUEGER AND E. J. SCRIBNER.

From the Department of Bacteriology, University of California, Berkeley.

It has been shown by Krueger[1] and confirmed by Dreyer and Campbell-Renton[2] that the heat inactivation of bacteriophage follows the course of a monomolecular reaction. In Krueger's data the increase in rate of inactivation with rise in temperature was very rapid (μ in the van't Hoff-Arrhenius equation = 101,000). Critical thermal increments of this order of magnitude are uniquely characteristic of protein denaturation and the experimental results with phage were consequently interpreted as indicating that inactivation involved denaturation as a significant factor. The present paper deals with the effect of pH on the heat inactivation of the same anti-Staphylococcus phage used in previous work.

2.5 ml. of standard anti-Staphylococcus phage containing $1x10^{10}$ activity units per ml.[3] was added to 2.5 ml. of beef infusion broth containing sufficient normal HCl to bring the final pH to the desired point. The samples were placed in a water bath adjusted to 57°C. for one-half hour and the residual (Phage) determined by the activity titration method.[3]

Results are summarized in Table I and indicate that the phage suspension is most thermostable in the region of neutrality. At pH 7.0, 6.5, and 6.0 between 34% and 32% of the total original phage remains active; at pH 5.75 there is a sharp drop to 3% survival and at pH 5.5 to pH 4.0 less than 1% of activity is retained. On the alkaline side of neutrality the percentages of phage surviving the half-hour period of exposure to 57°C. are respectively: pH 7.5 = 50%, pH 8.0 = 24%, pH 8.5 = 10%, pH 9.0 and pH 9.5 = less than 1%. With the exception of the data obtained at pH 5.5 and pH 9.5 the experimental results are regularly reproducible within narrow limits. It is likely that the discrepancies noted at pH 5.5 and pH 9.5 are due to minor variations in pH since these points apparently represent zones of maximal thermolability and very slight differences in $[H^+]$ or $[OH^-]$ exert a considerable influence on the percentage of phage surviving.

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1 Krueger, A. P., *J. Gen. Physiol.*, 1932, **15**, 363.

2 Dreyer, G., and Campbell-Renton, M. L., *J. Path. and Bact.*, 1933, **36**, 399.

3 Krueger, A. P., *J. Gen. Physiol.*, 1929, **13**, 557.

TABLE I.
Effect of pH on Heat Stability of Phage.
Initial Phage = 5 × 10^9 Activity Units/ml.

pH	Log Phage Activity Units/ml. Remaining after Exposure to 57° C. 0.5 hr.		% Total Original Phage Surviving
9.5	<4.0		<1.0
9.0	7.6		<1.0
8.5	8.7	Aver. of 7 exp.	10.0
8.0	9.1		24.0
7.5	9.4		50.0
7.0	9.23		34.0
6.5	9.2		32.0
6.0	9.2		32.0
5.75	8.23		3.0
5.5	<7.0	Aver. of 10 exp.	<1.0
5.25	<3.0		<1.0
5.0	<2.0		<1.0
4.5	<2.0		<1.0
4.0	<2.0		<1.0

Controls consisting of Phage adjusted to all pH values from pH 9.5-pH 4.0 were kept at 20° C. 0.5 hour and then titrated. There was no loss of activity during this time.

It is known that hydrogen ion concentration markedly influences the rate of heat inactivation of many enzymes and it is commonly stated that hydrogen or hydroxyl ions catalyze the inactivation reaction. For example the observations of Morgulis and Beber[4] are explicable on this basis; they found that while the heat inactivation of catalase was slow at the iso-electric point, pH 6.0, it became very rapid at pH 4.0 to pH 5.0 and at pH 8.0 to pH 9.0. There seems to be no doubt, however, that additional factors are concerned in the heat inactivation of other enzymes. One special case of this sort is presented by trypsin, which is remarkably thermostable in dilute acid.[5] Crystalline trypsin has been proven to be a protein the denaturation of which is paralleled by loss of enzymatic activity[6] and resistance to high temperature is not a common property of either proteins or enzymes in solution. The explanation of trypsin's thermoresistance is to be found in the fact that as the solution is heated the protein is denatured and activity is lost, but upon cooling within the pH range obtaining in the experiment denaturation is reversed and enzymatic activity regained.[6]

The heat inactivation of phage has the high critical thermal increment characteristic of protein denaturation and with increases in $[H^+]$ or $[OH^-]$ the inactivation reaction is favored as in the case of

4 Morgulis, S., and Beber, M., *J. Biol. Chem.*, 1928, **77**, 115.
5 Mellonly, J., and Wooley, V. J., *J. Physiol.*, 1913, **47**, 339.
6 Northrop, J. H., *J. Gen. Physiol.*, 1932, **16**, 323.

many enzymes. The present data can be explained in two ways, first by postulating a simple catalysis of heat inactivation by hydrogen and hydroxyl ions and second by assuming that while Phage denaturation with corresponding loss of activity occurs at all pH values tested the reversal of denaturation and restoration of activity proceeds best within the pH zone close to neutrality. It is not possible to determine from the present experimental data which mechanism is actually concerned.

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V. Lymphatic Absorption in Simple Obstruction: Significance of Distention upon Its Occurrence.*

LOUIS SPERLING AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

It is generally conceded that the content of the obstructed bowel is toxic as is also that of the unobstructed gut. The matter of abnormal absorption from the obstructed bowel is still the subject of considerable debate and speculation. The possible avenues through which toxic material may be absorbed from the bowel are: (1) the mesenteric veins, (2) the lymphatics, and (3) transperitoneally by diffusion through the bowel wall. It has been well established by many investigators that under conditions of obstruction, venous absorption, at any rate for substances absorbed from the normal bowel, is decreased. Transperitoneal absorption apparently does not occur unless there is gross damage to the bowel wall by distention with impairment of its viability.[1]

In this study an attempt has been made to evaluate the occurrence of lymphatic absorption under conditions of simple intestinal obstruction and increased intra-enteric pressure. The absorption of dyes and of bacteria from the obstructed bowel was examined in the following manner:

Method and Results. Simple ileal obstruction was produced in 8 cats, and 20 cc. of 1% gentian violet or trypan blue was injected into

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[1] Scott, H. G., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 287; Sperling, Louis, and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**,