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Heat Inactivation of Intracellular Phage Precursor.*

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Krueger and Mundell[1] recently reported a method for demonstrating intracellular phage precursor. The essential step in the method is the preparation of "activated" suspensions of staphylococci; this is accomplished by growing the organisms in a heavily oxygenated medium. The activated cells are separated from the broth, resuspended in Locke's solution and are then maintained at 5°C for 2 hours before they are used. To demonstrate intracellular precursor 4 ml of activated cell suspension containing 5×10^8 bacteria/ml is added to 1 ml of phage diluted with Locke's solution to contain 1×10^9 activity units/ml. The mixture is kept for 5 minutes at 5°C and is promptly titrated for total phage content. The end titer is 2×10^9 activity units/ml, an increase of 500% in phage concentration.

Krueger and Scribner[2] supplied additional evidence for the existence of the intracellular precursor. They made use of the fact that staphylococci activated in the presence of Mn^{++} have a relatively low lytic threshold and require only small amounts of phage to induce lysis. Starting with a small amount of phage it was possible to transform the phage precursor in successive lots of staphylococci into phage and to obtain the newly formed phage free in solution by lysing the precursor-containing cells. The original phage added at the start of the experiment has been diluted at least 1 to one million without any reduction in plaque count or activity titer.

It seemed likely from data already available that the phage precursor is more thermolabile than the bacterial cell which produces it. In the experiments of Krueger and Fong[3] the relationships shown in Table I between bacterial reproduction and phage formation at various temperatures were observed.

It is evident that phage production has a temperature optimum of approximately 35°C. At 40°C the rate of phage production is only

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1 Krueger, A. P., and Mundell, J., *Science*, 1938, **88**, 550.

2 Krueger, A. P., and Scribner, E. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 51.

3 Krueger, A. P., and Fong, J., *J. Gen. Physiol.*, 1937, **21**, 137.

TABLE I.
Rates of Bacterial Growth and Phage Production at Various Temperatures.
From Krueger and Fong.[3]

Temperature	% increase in [Bacteria]/hr.	% increase in [Phage]/hr.
30°C	50	2000
35°C	125	8000
40°C	175	1250
45°C	150	Drops

one-sixth of the 35°C rate. At 45°C there is no longer an increase in [phage] but rather a definite drop. This last mentioned result conceivably could be due to inhibition of the intracellular precursor-producing mechanism at the higher temperature or to heat inactivation of the precursor after it is produced.

We wish to report here experiments performed to determine whether the precursor content of staphylococci could be inactivated at temperatures that would not kill the organisms. Suspensions of activated staphylococci were prepared as described elsewhere. For each experiment 40 ml of a Locke's solution suspension of activated organisms containing 5×10^8 cells per ml was placed in a thin-walled glass container. A thermometer was immersed in the suspension and

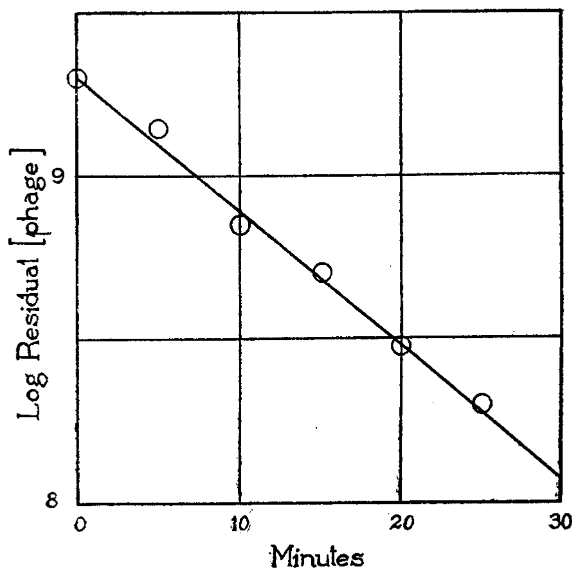

FIG. 1.
The rate of inactivation of intracellular phage precursor at 45°C. The ordinate represents the total phage formed when 4 ml of the heated sample was mixed with 1 ml of phage containing 1×10^9 activity units/ml. Each mixture was kept at 5°C for five minutes before titration. The points represent the average of six separate experiments.

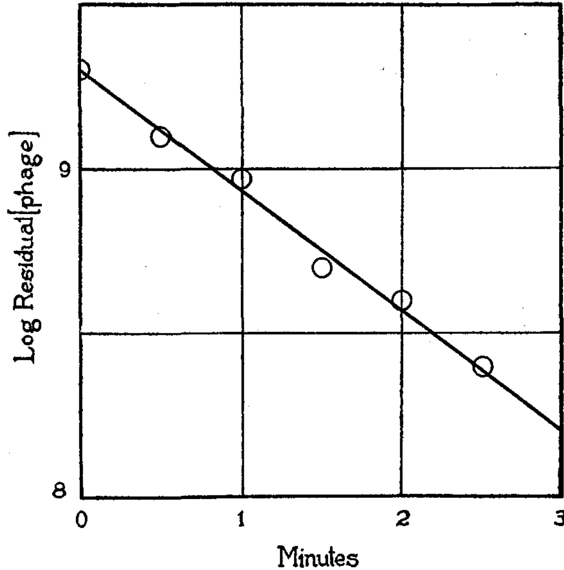

FIG. 2.
The rate of inactivation of intracellular phage precursor at 50°C. The points represent the average of five separate experiments.

the container was then placed in water at 70°C in order to attain the temperature of the experiment rapidly. As soon as the proper temperature was reached the container was transferred to a waterbath held at the desired temperature and samples were removed at short intervals to determine the number of viable organisms and the presence or absence of intracellular precursor in the bacteria. For the former purpose multiple samples were counted on agar pour plates after 36 hours of incubation at 37°C. For detection of precursor in the organisms each 4 ml sample was mixed with 1 ml of phage diluted in Locke's solution to contain 1 x 10^9 activity units per ml. The mixture was kept at 5°C for 5 minutes and was then titrated for total phage content by the activity method.[4]

Figures 1 and 2 summarize graphically the results of 6 experiments performed at 45°C and 5 performed at 50°C. After 25 minutes' exposure to 45°C the cells ceased to give a positive test for phage precursor. To obtain the same result at 50°C requires about 2½ minutes. During these periods of exposure the total viable cell count showed no significant reduction in any of the experiments; the essential data of the bacterial counts are summarized in Table II.

[4] Krueger, A. P., *J. Gen. Physiol.*, 1929-30, **13**, 557.

TABLE II.

Bacterial Counts During Heating of Activated Cell Suspensions at 45°C and 50°C. The values are all x 10^8 and represent averages of 3 dilutions prepared as agar pour plates and counted after 36 hours at 37°C.

45°C	Time of Sampling (Minutes)				
Exp. No.	0	15	30	45	60
1	4.75	4.85	4.47	4.19	3.19
2	5.28	6.25	6.40	4.47	2.54
3	6.05	6.46	5.43	2.30	1.53
4	7.15	6.1	5.81	2.42	
5	5.5	5.13	5.6	5.34	4.55
6	5.18	5.39	4.8	4.25	2.95

50°C	Time of Sampling (Minutes)					
Exp. No.	0	0.5	1.0	1.5	2.0	2.5
1	6.9	7.5	6.6	6.88	6.9	6.75
2	5.25	5.1	5.4	4.9	5.3	5.15
3	4.8	4.9	5.15	4.7	4.78	4.5
4	5.05	6.0	4.9	5.16	5.24	4.96
5	5.12	5.01	5.24	6.0	5.12	5.02

The velocity constants for the inactivation reaction were calculated from the equation:

$$d\ P/dt = k(P_o - P_i)$$

where P = [Precursor] at any time t, expressed as the total [Phage] formed upon the addition of 4 ml of activated cell suspension to 1 ml of Phage solution containing 1 x 10^9 activity units/ml.

P_o = Initial total Precursor/ml expressed in the same terms and P_i = Precursor/ml inactivated in time t.

For heat inactivation of precursor at 45°C k = 0.094; at 50°C k = 0.844. These values were substituted in the v'ant Hoff Arrhenius equation and μ, the critical thermal increment, was calculated to be 90,000.

μ for the destruction of phage precursor is of the order of magnitude characteristic of protein denaturation reactions in general and closely approximates the value reported for the heat inactivation of the homologous phage (101,000). The simplest assumption then would be that phage precursor is an intracellular protein similar to phage itself. However, it is possible that our experiments have not measured the actual rate of precursor inactivation but have followed the rate of denaturation of some entirely separate cell protein which when denatured alters permeability relationships and prevents access of phage to the cell's store of precursor. Whichever of these explanations proves to be correct it seems clear that the heat treatment of activated cells under controlled conditions results in loss of the phage-augmenting capacity characteristic of activated cells without entailing any significant number of cell deaths.