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Thermal Inhibition of Phage Production in Mixture of Phage and Growing Susceptible Bacteria.*

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One of the most interesting aspects of the entire phage problem has been the question of how phage is formed. In earlier studies it appeared that phage was produced only in the presence of an actively growing bacterial substrate. This relationship found experimental verification in quantitative studies on the phage-bacterium reaction and led to the adoption of an equation in which phage production was expressed in terms of bacterial reproduction.[1] Further work, however, proved this relationship to be more apparent than real for it was found possible to impose conditions such that cell division was inhibited while phage continued to be developed at a very considerable rate.[2, 3, 4] The fact that phage can be produced at all in the absence of cellular reproduction indicated that the latter is not an essential conditioning factor for phage formation, even though the two phenomena ordinarily proceed concurrently and require careful adjustment of pH, temperature, electrolyte concentration,[5, 6] etc., to be dissociated.

Attention was then devoted to the possibility that phage production might involve the autocatalytic transformation of an inactive precursor into phage.[7] Formal proof of the existence of the precursor in cell-free solution has not yet been obtained, although traces can be detected irregularly.[8] However, there is satisfactory evidence that the substance from which phage is developed exists within susceptible cells.[9] To demonstrate it, staphylococci are subjected to active growth in an oxygenated medium, after which they are washed and

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2 Krueger, A. P., and Fong, J., *J. Gen. Physiol.*, 1937, **21**, 2.

3 Northrop, J. H., *J. Gen. Physiol.*, 1939-40, **23**, 59.

4 Ellis, E. L., and Spizizen, J., *Science*, 1940, **92**, 91.

5 Scribner, E. J., and Krueger, A. P., *J. Gen. Physiol.*, 1937, **21**, 1.

6 Krueger, A. P., and Strietmann, W. L., *J. Gen. Physiol.*, 1938, **22**, 131.

7 Krueger, A. P., *Science*, 1937, **86**, 379.

8 Krueger, A. P., and Baldwin, D. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 393.

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

stored in Locke's solution at 5°C. These "activated" cells when mixed with phage in appropriate proportions, bring about a tenfold increase in phage titre within 2 minutes.[10] The reaction does not implicate any obvious cellular activity beyond supplying preformed precursor, for the bacteria are in a resting state and hence are metabolically inactive. As a further precaution, the amounts of phage and activated bacteria can be adjusted to the critical lytic ratio, resulting in destruction of the cells and their complete removal from the reacting mixture.[11]

Experimental work has shown that the intracellular precursor is much more labile than the reproductive mechanism of the bacteria. For example, amounts of iodoacetic acid[12] and methylene blue[13] which will totally abolish the phage-augmenting effect of activated cells, are insufficient to interfere with their reproductive capacity. This is also true of heat inactivation;[14] the precursor is rapidly destroyed at temperatures ranging from 45°C to 50°C, although at these temperatures resting, precursor-containing cells fail to show any reduction in plate count. Further, the thermal inactivation reaction has a high critical thermal increment, μ being 90,000. This would suggest that the precursor, like phage itself, is a protein.

If the precursor theory is correct and if bacterial growth and phage formation are not related as cause and effect, it should be possible to select an environment for the phage-bacterium reaction in which cellular reproduction progresses normally while phage formation does not occur either because of destruction of precursor or inhibition of precursor synthesis. In unreported experiments by Krueger, Scribner and Mecracken, we attempted to accomplish this by exposing mixtures of growing bacteria and phage to the action of known precursor inactivators, *e.g.,* iodoacetic acid, KCN, and methylene blue + light. But in every case tested, the concentration of chemical required for inactivation of precursor also interfered with bacterial growth. More recently we have obtained positive results by the simpler procedure of carefully selecting the temperature, thereby avoiding certain technical difficulties inherent in the use of chemicals.

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13 Krueger, A. P., Scribner, E. J., and Mecracken, T., *J. Gen. Physiol.*, 1940, **23**, 705.

14 Krueger, A. P., Mecracken, T., and Scribner, E. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 573.

A mixture of phage and staphylococci in nutrient broth of pH 7.4 containing 5×10^7 bacteria/ml and 1×10^7 phage units/ml was placed in the water bath shaker at 42.3°C. Samples were removed every 0.25 hours for determining:

a. [Total bacteria] (nephelometer).
b. [Viable bacteria] (plate count).
c. [Phage].

The turbidity measurements were conducted with the Klett-Summerson photoelectric nephelometer and the numbers of viable cells were determined by the standard plate count method. The phage content of serial dilutions of each sample was determined by the activity titration procedure.[15] As controls there were included:

a. Suspensions of staphylococci activated by the customary technic and subsequently maintained along with the experimental mixtures at 42.3°C to observe the effect of this temperature on pre-formed precursor.

b. Standard phage solution in broth kept at 42.3°C to detect possible thermal inactivation.

The results of 4 experiments are averaged in Fig. 1. Within 1.5 hours [bacteria] increased 450%, the viable count being approximately 65% of the total count. During this period there was no

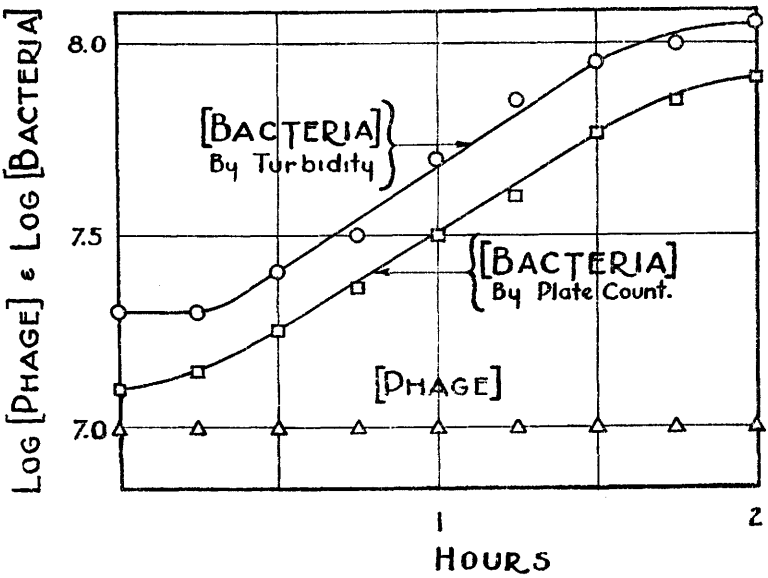

FIG. 1.
[Bacteria] and [Phage] in a broth mixture kept at 42.3°C.

[15] Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 557.

detectable rise in [phage] and consequently the suspension never lysed. This maintenance of a constant titre cannot be ascribed to continuous thermal inactivation of phage just sufficient to balance the production of new phage, for in keeping with earlier work the control solutions held at 42.3°C showed no drop at the end of 2 hours. There remain two alternative explanations for the phenomenon: it may depend upon the thermal inactivation of precursor as it is produced in the growing organisms or it may involve inhibition of the synthetic mechanism. The first possibility appears remote because it has been shown in experiments on activated resting cells that the rate of precursor inactivation is relatively slow. The velocity constant at 42.3°C is 0.031 and it takes almost 1 hour to abolish the store of precursor in fully activated bacteria under the conditions of the present experiment.

Experimentally the fact is that when a mixture of phage and growing susceptible staphylococci is kept at 42.3°C the bacteria grow without inducing the formation of phage. The conclusion seems valid that this result depends upon inhibition of the precursor-producing mechanism of the cell rather than upon thermal destruction of phage or phage-precursor.

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Nature of the Blood Iodine. II. Nature of the Plasma Iodine.

SOLOMON SILVER. (Introduced by H. Sobotka.)

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In a previous publication[1] it was indicated that all the iodine normally occurring in the blood is in a form which does not pass through a cellophane membrane when the blood is dialyzed against water.

This study is an attempt to cast further light upon the state in which the iodine exists in the plasma.

It has been known since the early work of Baumann and Oswald that much of the iodine present in the thyroid gland exists in combination with protein, particularly globulin. The presence of a specific iodized globulin—thyroglobulin—has been established. Because of the presence of thyroglobulin as the chief iodine-con-

[1] Silver, S., *J. Mt. Sinai Hosp.*, 1940, **7**, 97.