aging as a means of evaluating the severity of a given case of pneumonia on admission of the patient to the hospital and during the course of the disease. Further study of a large series of cases will be necessary to establish this point.

10304

Serial Production of Phage from Intracellular Phage Precursor.*

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

From the Department of Bacteriology, University of California.

Recently experimental evidence was advanced indicating that phage production occurs according to the reaction: inactive phage precursor + phage → phage.[1] The particular point of interest in this concept is the dissociation of phage production from what was previously held to be an essential conditioning factor, namely, bacterial growth. Furthermore, there exist authentic models for such a reaction in the autocatalytic transformation of inactive enzyme precursors into the active enzymes when the precursors are brought in contact with small amounts of active enzyme.

To summarize the experimental data it was found by Krueger and Baldwin[1] that under certain conditions cell-free ultrafiltrates of staphylococci added to solutions of bacteriophage resulted in 100% increases in phage activity. In studying the effect of salts on the phage-bacterium reaction it was found that the presence of sodium chloride[2] or sodium sulphate[3] during the phage-bacterium reaction produced a prelytic plateau in the bacterial growth curve for as much as 0.8 hour during which time phage production continued at a normal rate despite the absence of cellular reproduction. Later[4] experiments were reported in which phage production was dissociated from bacterial growth by means of changes in temperature and pH of the medium. Unfortunately these data do not offer conclusive proof that the phage precursor-phage reaction is the normal mode of phage production largely because the yields of pre-

*This work was supported by grants-in-aid from the Research Corporation, the American Medical Association, and several interested friends.

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

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

3 Krueger, A. P., and Strietmann, W. L., *J. Gen. Physiol.*, 1938.

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

cursor in ultrafiltrates are small and irregular and also because the methods used to detect Δ [bacteria] in the pH-temperature experiments were not sufficiently sensitive. Continued attempts to improve the methods for extracting precursor were unsuccessful and for the past year we have been working with a method which demonstrates the phage precursor within the bacterium. The present paper deals with the serial production of phage in separate aliquots of precursor-containing bacteria.

In the general procedure for demonstrating phage precursor[5] it is necessary to prepare suspensions of activated staphylococci by growing the organisms in a highly oxygenated medium. The details of this process are described in a paper now in press. After activation the bacteria are separated from the medium, resuspended in Locke's solution and maintained at 5°C for 2 hours in order to rule out the possibility of bacterial growth during subsequent reactions with phage. To demonstrate the precursor 4 ml of activated suspension containing 5×10^8 bacteria per ml is added to 1 ml of phage diluted with Locke's solution to contain 1×10^9 activity units per ml. The mixture is kept for 5 minutes at 5°C and is then titrated for total phage content by the activity method. The end titer is found to be 2×10^9 phage units per ml while that of the control prepared with nonactivated bacteria from a 16-hour agar culture is 2×10^8 units per ml.

The objection may be advanced that the increase in activity is a spurious one and involves some untoward effect of the activated cells on the titration system. This is apparently upheld by the fact that plaque counts show no increase in phage content of the activated bacteria-phage mixture. However, control experiments (Table I) have been run, adding the number of activated bacteria which would occur in samples of activated bacteria-phage mixtures when diluted for titration to standard titration mixtures. As shown in Table I these small amounts of activated bacteria do not influence the titration values. The failure of the plaque count to increase is explicable on the basis that no matter how much intracellular phage is formed per organism, the count will remain the same because each phage-containing bacterium becomes the locus of one phage plaque regardless of the amount contained in the cell.

The ideal test for phage precursor would be the demonstration that a small amount of phage added to successive aliquots of precursor would result in the continuous transformation of precursor in each aliquot into more phage. Since it is exceptional to obtain

[5] Krueger, A. P., and Mundell, J., *Science*, 1938, **88**, 550.

TABLE I.
Outline of Experiment to Test Effect of Activated Bacteria Added to Phage Titration System.

1. 9 ml broth blanks prepared containing three different concentrations of activated bacteria:
   5×10^5 activated bacteria/ml
   5×10^4 " " /"
   5×10^3 " " /"
2. Sets of each concentration used to dilute standard phage for titration (from 1×10^{10} activity units/ml to 1×10^7, 1×10^6, and 1×10^5 activity units/ml).
3. To 4 ml of each dilution of phage 1 ml of normal bacteria containing 12.5×10^7 cells/ml was added.
4. Time of lysis was determined.
5. Presence of 5×10^3, 5×10^4, or 5×10^5 activated bacteria/ml in titration system does not affect results.

sufficient concentrations of cell-free precursor to permit this test being done it is necessary to use intracellular precursor for the test. Immediately the difficulty arises that when phage is taken up by activated bacteria and converts the intracellular precursor into more phage practically all of the phage remains in or on the bacterium and consequently is not free to participate in transforming additional precursor into more phage. However, if appropriate conditions are chosen the cells may be lysed by the phage and the newly formed phage liberated free in solution.

Ordinarily there are required for lysis about 100 phage units per bacterium[6] and standard phage contains 1×10^{10} activity units per ml. These two facts limit the number of bacteria which can be lysed (without growth) unless a concentrated phage is used. In an average mixture when the concentrations requisite for lysis obtain there are 5×10^9 phage units per ml and 5×10^7 bacteria per ml. Under these conditions the phage concentration is so high and the number of bacteria so small that the increment of newly formed phage derived from the precursor-containing cells is both too small to detect and too small to permit a serial dilution experiment. This difficulty may be circumvented by carrying out the activation in the presence of $MnCl_2$ for it has been shown[7] that manganese-treated cells have a much lower lytic threshold than untreated bacteria, *i. e.*, they require less phage for the initiation of lysis.

Details regarding the strain of staphylococcus used, the media employed, etc., are given in earlier publications. For the present work 16-hour agar cultures of the staphylococcus were washed twice in Locke's solution and were then activated by growth in a medium through which oxygen was bubbled. The exact procedure

[6] Krueger, A. P., and Northrop, J. H., *J. Gen. Physiol.*, 1930, **14**, 223.
[7] Krueger, A. P., and West, N. S., *J. Gen. Physiol.*, 1935, **19**, 75.

for activation as described by Krueger and Scribner[8] was followed except that in this case the medium contained 0.0016M $MnCl_2$. Upon the completion of activation the bacterial suspension was placed in ice mixture and was kept for 2 hours before using.

Two controls were routinely employed in testing for activation:

1. A mixture containing 5×10^8 activated bacteria per ml and 2×10^8 phage units per ml was made in Locke's solution. This suspension was kept at 5°C for 5 minutes and was immediately diluted for titration, using for this purpose 0.0016M Na_2SiO_3 broth in order to prevent any effect of the manganese on the titration system.

2. A duplicate of number 1 except that nonactivated bacteria from the same culture were used and before mixing with phage these organisms were washed in 0.0016M $MnCl_2$ broth.

For transforming intracellular precursor into phage under conditions such that the cells would lyse and set free the newly formed phage, mixtures of the following composition were prepared: 6.3 ml of phage containing 1×10^9 activity units per ml, 2 ml of broth containing 0.008M $MnCl_2$, 1.7 ml of broth and 1 ml of bacterial suspension activated in the presence of $MnCl_2$ and containing 1×10^9 cells per ml. The mixtures were kept on ice for 0.75 hour and were then transferred to the waterbath shaker at 36°C. Lysis began between 0.5 and 0.6 hour and was complete between 0.8 and 0.9 hour after which the lysates were promptly titrated for phage content. As soon as the titer was known the lysate was again diluted to contain 1×10^9 phage units per ml and was used in preparing the next mixture in the series. In this way successive dilutions of the original amount of phage were prepared, adding each time new aliquots of activated bacteria.

It was necessary, of course, to prove that no cellular growth occurred during the interval before lysis took place. For this purpose plate counts were taken at short intervals from control mixtures made without phage and containing an aliquot of the organisms activated in the presence of $MnCl_2$. As an additional rough check all the mixtures were compared at 0.2 hour intervals with turbidity standards but reliance was placed only on the plate counts. In none of the control set did growth occur before lysis had taken place in the corresponding phage-containing mixture.

Results. As shown in Table II it has been possible by starting with a small amount of phage to transform the phage precursor in successive lots of staphylococci into phage under the conditions of

[8] Krueger, A. P., and Scribner, E. J., in press.

TABLE II.
Serial Production of Phage from Successive Aliquots of Precursor-Containing Bacteria.

	Mixture No. 1	Mixture No. 2	Mixture No. 3	Mixture No. 4	Mixture No. 5	Mixture No. 6
Initial [Phage]	6.3×10^8	6.3×10^8	6.3×10^8	6.3×10^8	6.3×10^8	6.3×10^8
	Diluted to	Diluted to	Diluted to	Diluted to	Diluted to	
Initial [Bacteria]	1×10^8	1×10^8	1×10^8	1×10^8	1×10^8	1×10^8
Final [Phage]	7.9×10^9	9.5×10^9	6.3×10^9	7.6×10^9	5.7×10^9	6.3×10^9
Total [Phage] *i. e.*, Initial [Phage] × Total Dilution	6.3×10^9	7.87×10^{10}	11.8×10^{11}	11.8×10^{12}	14.2×10^{13}	12.8×10^{14}

the experiment. In ordinary suspensions of activated organisms the serial dilution experiment is not possible because of the high lytic threshold, *i. e.*, the ratio phage/bacteria is so great that the ultimate yield of phage derived from precursor-containing cells is not measurable. The presence of $MnCl_2$ lowers the lytic threshold very considerably and makes it feasible to add relatively large numbers of organisms with the result that significant amounts of phage are released from the lysing bacteria. The phage produced in this way can then be diluted and employed to activate the precursor of a new lot of cells. The original phage used has been diluted to at least 1/1,000,000 without any reduction in activity or plaque count. This we interpret as indicating that the activated bacteria contain some sort of a phage precursor which is transformed into active phage by phage itself. An alternate explanation would postulate the phage to be a living autonomous unit, finding appropriate conditions for multiplication in the successive aliquots of activated organisms. However, there is ample evidence that phage is not a living organism but rather is a protein of high molecular weight possessing the general properties of an enzyme.[9, 10] It seems probable that phage production is not dependent upon bacterial reproduction *per se* but upon environmental conditions adequate for the normal synthesis of phage precursor.

10305

Experimental Pancreatico-Gastrostomy. Method for Conserving External Secretions of Pancreas.

E. COOPER PERSON, JR., AND FRANK GLENN. (Introduced by Bruce Webster.)

From the Department of Surgery, the New York Hospital and Cornell Medical College.

In any work on the pancreas, the well known danger of acute pancreatitis and peritonitis has long invested the gland with the reputation of a surgical *"noli me tangere."* The surgical attack has also been delayed because of the organ's relative inaccessibility, complicated functions and intimate relationship with major abdominal structures. Nevertheless, the need for a surgical procedure to pre-

[9] Northrop, J. H., *J. Gen. Physiol.*, 1938, **21**, 335.

[10] Krueger, A. P., *Physiol. Rev.*, 1936, **16**, 1.