LYSIS FROM WITHOUT OF S. AUREUS K_1 BY THE COMBINED ACTION OF PHAGE AND VIROLYSIN*

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ABSTRACT

Lysis from without (LFW) occurs in two steps: (1) sensitization of cells by phage, which renders the cells susceptible to (2) destruction of an essential cell structure by an extracellular lytic enzyme. Virolysin, from phage-infected cells, was used in these studies. Normal cell autolysin is also effective.

Evidence is presented that:

1. Neither phage nor lysin alone causes LFW.
2. Sensitization requires phage adsorption.
3. It can be caused by non-infectious particles. This establishes a new biological activity of the particle.
4. Heat, U.V., detergents, penicillin, and other damaging agents also sensitize cells.
5. Sensitization involves a non-lethal, reversible reaction.
6. Sensitization by phage prevents virus synthesis. Following adsorption, a cell can undergo sensitization or infection but not simultaneously. When only a few particles are adsorbed, infection can occur; when sufficient particles are adsorbed, sensitization takes place.
7. Quantitative aspects of LFW are described. Lysis proceeds logarithmically. The lysis end-point depends upon the phage concentration but is independent of the enzyme concentration.

INTRODUCTION

This paper is concerned with the lysis that begins immediately after the additions of phage and an extracellular lysin to living staphylococcal cells. The adsorbed phage is lost and the cells which lyse do not form new particles. Thus the phenomenon can be regarded as a case of lysis from without (Krueger and Northrop, 1930; Northrop, 1937, 1939; Delbrück, 1940). Virolysin, obtained from lysates of phage-infected cells, or autolysin, from autolysates of uninfected cells, may function as the extracellular lysin. The properties and differentiation of these two agents have been reported. Both lysins behave like enzymes (Ralston *et al.*, 1955, 1957).

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In the staphylococcal system, lysis results from the combination of two steps, sensitization of the cell by phage and lysis by a lysin. These are described; quantitative aspects of phage and lysin are given; and this system is compared with others.

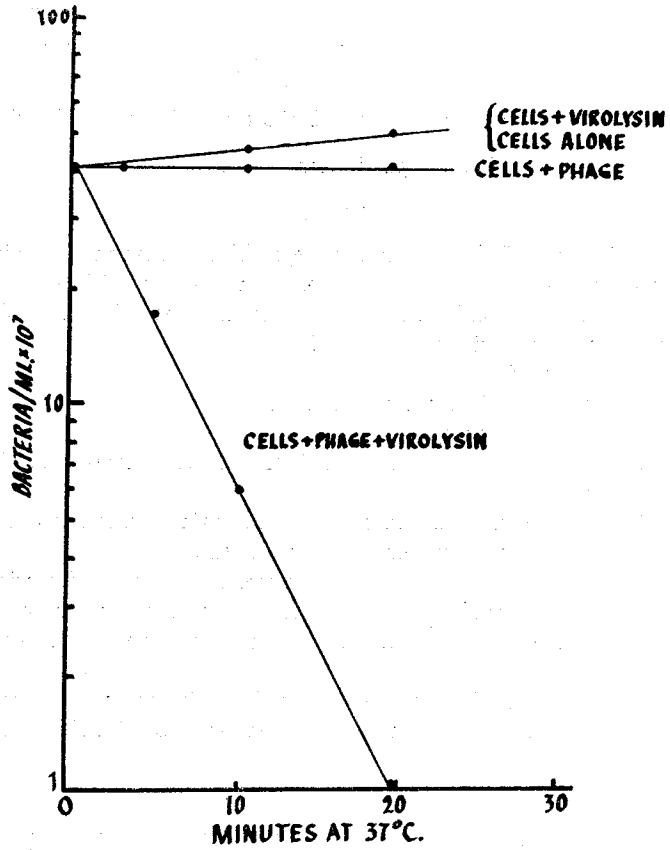

FIG. 1. Effect of virolysin, phage, and the two combined on 3 hour agar-grown cells. Test components: cell concentration, 4.1×10^8/ml.; 14 (K_1) purified phage concentration, 4.5×10^9/ml.; phage/cell ratio = 10/1; virolysin dilution, one-half in tryptose phosphate broth (ultracentrifuged supernate of 14 (K_1) phage lysate, residual phage = 1×10^7/ml.).

Experimental Results

I

Requirement for Both Phage and an Extracellular Lysin in Lysis from without of S. aureus K_1

Virolysin alone is incapable of lysing freshly harvested, rapidly dividing cells. The cells grow at the same rate as untreated controls (Fig. 1).

Phage alone is also incapable of producing lysis from without, even when large numbers of particles (close to saturation) are adsorbed to cells (Fig. 1). This suggests that phage does not activate the internal autolytic enzymes of the cells, even though the cells can be made to autolyze in the absence of phage and yield free autolysin, by adding toluene to them at 37° C. or by storage at 4° C.

In 1955 (Ralston *et al.*), we reported that phage preparations obtained by one cycle of ultracentrifugation produced slight but significant lysis of young cultures. Subsequent investigation has shown that this lysis resulted from the action of phage combined with traces of residual virolysin in the resuspended phage pellet. The virolysin can be removed in various ways: digestion with trypsin, adsorption on super-cel, heating at 37° C., and several cycles of differential centrifugation. The purified preparations no longer cause lysis from without. When virolysin is again added, dilutions as high as 1/250 restore the ability to cause lysis from without (Fig. 1).

Both phage and virolysin are required to produce lysis from without because the cell must undergo two reactions before lysis occurs: (1) a sensitization, which is accomplished by phage; followed by (2) the action of a lysin on a cell substrate.

II

The Lytic Step

Previous studies have characterized the action of virolysin on the substrate of killed cells — irreversibly sensitized by heating a 56–120° C. for 1 hour. The lysin was shown to have the properties of an enzyme (Ralston *et al.*, 1955, 1957). Evidence that virolysin is also responsible for the lytic step of lysis from without is based upon a comparison of the activities of virolysin-containing preparations for heat-killed and phage-sensitized cells. Activity is (1) destroyed at comparable rates at 37° C., (2) prevented by an identical set of inhibitors and remains unaffected by a second group (Table I), (3) requires the same divalent cations for its function (Table II), and (4) shows similar host specificity, in that it lyses all *S. aureus* strains, provided that the living cells can adsorb phage P_1 and P_{14}.

Virolysin preparations obtained from P_1 infection of K_1 and 145 cells and from P_{14} infections of *S. aureus* 145 substitute for the 14(K_1) virolysin in producing lysis from without of phage-sensitized cells (Table III).

III

The Step of Sensitization

Sensitization involves a damage to the mechanism for protection of the living cell against virolysin. Since the fundamental reaction is not known, its study must depend upon an indirect test—the lysis of sensitized cells by virolysin. Sensitization by phage occurs under complex conditions.

1. Sensitization Requires Phage Adsorption.—When it occurs, it follows immediately after adsorption. When versene is included in a mixture of phage, virolysin, and viable cells, both phage adsorption and lysis from without are inhibited. The addition of M/1000 Ca^{++}, Mn^{++}, Co^{++}, or Zn^{++} and M/50 Mg^{++}

TABLE I

Effect of Enzyme Inhibitors on Lysis of Heat-Killed and Phage-Sensitized Cells by Virolysin at 37°C.

Agent tested	Final concentrat on	Lysis of heated cells Per cent inhibition of virolysin activity	Lysis of phage-sensitized cells Per cent inhibition of virolysin activity
Inhibitor			
Merthiolate	0.1 per cent	76*	75*
HCHO	0.1 per cent	100	75
$CuSO_4 \cdot 5H_2O$	M/1000	60*	82*
$HgCl_2$	M/1000	92*	100*
$PbCl_2$	M/1000	82	48
$AgNo_3$	M/1000	71*	98*
Duponol WA	0.1 per cent	68	100
Non-inhibitor			
Proflavin	0.0005 per cent	0	0
NaN_3	M/1000	0	0
NaF	M/1000	10	0
NaCN	M/1000	10	0
Na-arsenite	M/1000	21	0
Semicarbazide	M/1000	0	0

* Inhibition was reversed by 0.1 per cent cysteine-HCl.

A 14 (K_1) lysate containing virolysin was mixed with inhibitor. Each sample was then mixed with a constant number of cells. For living cells, the initial cell phage ratio was 10/1. The samples were incubated at 37°C. for a specified time: for living cells, 20 minutes; for heated cells, 100 minutes. For each series the per cent inhibition of virolysin activity was calculated from the equation

$$\text{Per cent inhibition} = \frac{100\ (\text{lysis control} - \text{lysis with inhibitor})}{\text{lysis control}}$$

With heated cells, control samples lysed 63 per cent; with phage-sensitized cells, control cells lysed 28 per cent.

to the inhibited system permits adsorption and lysis from without (Table II). Lower concentrations of Mg^{++} permit adsorption but not lysis from without.

2. Sensitization Is a Separate Biological Activity of the Phage Particle.—The particle need not be infective in order to sensitize. This may be shown by the following treatments: damage to the particle by ultraviolet irradiation (Ralston *et al.*, 1955); damage to the particle by repeated freezing and thawing (Table IV); and host alteration of its infectivity by passage through controller

TABLE II

Effect of Metal Ions on Versene Inhibition of Virolysin and Phage Adsorption

Components of test sample	Phage adsorption to living cells	Effect on lysis	
		Lysis from without of phage-treated cells Per cent lysis	Heat-killed cells (no phage needed) Per cent lysis
1. Virolysin	+	46	31
2. Virolysin + versene	−	0	7
3. Virolysin + versene + Mg^{++} M/500	+	0	39
" + " + " M/200	+	10	34
" + " + " M/50	+	28	25
Virolysin + versene + Mn^{++} M/1000	+	42	35
Ca^{++} M/1000	+	44	40
Co^{++} M/1000	+	41	34
Zn^{++} M/1000	+	16	21

Virolysin was mixed with versene at final concentration = 0.02 per cent. Heat-killed cells tested at concentration of 7×10^8 cells/ml.; readings made after 60 minutes at 37°C. Lysis from without tested in presence of phage added at initial phage/cell = 5, cells at 7×10^8 cells/ml.; readings made after 40 minutes at 37°C. Free phage was assayed by plaque count at the end of the incubation period.

The following salts did not reverse the inhibition of lysis from without and did not allow phage adsorption to the cells: Pb^{++}, Cu^{++}, Sr^{++}, Fe^{++}, Ba^{++}, Sn^{++}, K^+, Na^+.

TABLE III

Interchangeability of Phage Pellets and Virolysins in the Lysis from Without of S. aureus K_1 at 37°C.

(A) Lysate source	Component Tested													
	Pellet only (from A)		Virolysin 1(145) + pellet from A		Virolysin 1 (K_1) + pellet from A		Virolysin 14(145) + pellet from A		Virolysin 14 (K_1) + pellet from A		Virolysin only from A			
											Heated cells	Viable cells		
	P/B	Per cent lysis 40 min.	P/B	Per cent lysis 40 min.	P/B	Per cent lysis 40 min.	P/B	Per cent lysis 40 min.	P/B	Per cent lysis 40 min.	Per cent lysis 40 min.	P/B	Per cent lysis 40 min.
1 (145)	1.4	2	1.5	23	1.4	16	1.4	21	1.7	22	19	0.08	+9*
1 (K_1)	1.9	+10*	2.0	15	2.0	20	2.1	13	2.2	13	15	0.30	+4*
14 (145)	0.9	+6*	1.0	21	0.9	10	0.8	20	1.1	15	19	0.02	+10*
14 (K_1)	3.7	4	3.7	23	3.5	24	3.7	21	3.7	25	17	0.18	+12*

Procedure—Phage lysates were ultracentrifuged at 30,000 × g. Pellets were resuspended in 0.85 per cent saline. Supernates were used as source of virolysin. For each test: 0.1 ml. pellet and 5 ml. virolysin diluted one-fourth in tryptose phosphate broth were mixed with cells at an initial input of 3.6 to 4.1×10^8 B/ml. Final phage in supernates, after dilution, varied from 1.4×10^7 to 1.0×10^8 ml.

(A), lysate source.

* Per cent growth in 40 minutes.

cells (Ralston and Krueger, 1952, 1954). The damaging treatments may have produced phage "ghosts," as described for the *coli* T phage system (Anderson, 1945; Herriott, 1951; Williams and Frazer, 1956).

In contrast to the T phage ghost—which shows an increased capacity per particle for producing lysis from without (Herriott, 1951)—destruction of infectivity of the staphylococcal P phages neither increases nor reduces their sensitizing properties.

3. Sensitization Can Be Accomplished by Phage P_1 as Well As P_{14} (Table III).—

4. Sensitization Can Be Accomplished by Non-Phage Substitutes.—Treatments such as heat at 56° C., ultraviolet irradiation, acetone extraction, detergent extraction, and exposure to penicillin have been found to sensitize cells. Most of these methods kill as well as sensitize the cells.

TABLE IV

Effects of Freezing and Thawing of Phage on Sensitizing Activity and Infectivity

Phage pellet frozen and thawed times	Lysis from without per cent lysis		Residual phage plaques/ml.	P/B (Active phage/cell, during lysis test)
	By phage alone	By phage + virolysin		
0	0	40	1.2×10^9	1.5
3	0	40	5.6×10^8	0.8
7	0	38	2.0×10^8	0.3

Phage 14 (K_1) in a mineral salts, buffered broth, at pH 7.0, frozen in a thin layer in tube immersed in acetone and dry ice. Thawed in cold water. Tested for residual sensitizing activity by mixing with viable agar-grown cells (8.3×10^8 cells/ml.) in presence of constant dilution of 14 (K_1) virolysin and incubating at 37°C. Turbidimetric readings made after 30 minutes. Samples plated for plaque count after freeze-thaw procedures.

5. Under Certain Conditions Sensitization May Be Reversed.—As shown in Table V, sensitization by phage can occur in the cold. When the temperature is raised to 37° C., a proportion of sensitized cells becomes resistant to virolysin. When phage is added to cells at 37° C. without the preliminary adsorption period at 4° C., no reversal of sensitization may be detected; *i.e.*, the number of sensitized cells remains constant throughout the latent period.

In the absence of phage, a reversible sensitization may be produced by heating viable cells in saline at 46° C. The sensitized cells may be restored to their resistant state by incubation in tryptose-phosphate broth at 37° C. This method of sensitization does not impair the viability of the cells, as measured by colony formation on agar (Table VI).

By appropriate treatment it may be possible to dissociate the lethal and sensitizing properties of the phage particle.

6. Sensitization Prevents Phage Formation.—An individual cell may be shown to be capable of undergoing two responses to phage—sensitization and

TABLE V

Sensitization by Phage at 4°C.; Reversal of Sensitization at 37°C.

Conditions of incubation	Total cells × 10^7 /ml.	Sensitized cells × 10^7/ml. A	Infected cells × 10^7/ml. B	A + B
Sensitization				
4°C., for 1 hr.	33	21	13	34
Reversal of sensitization				
37°C., for 15 min.	32	13	18	31
30 "	33	9	20	29
45 "	35	4	30	34

Method—Cells of *S. aureus* K_1, grown 5 hours at 37°C. on tryptose phosphate agar, were harvested in chilled tryptose phosphate broth. Phage 14 (K_1) (virolysin-free preparation) was added to make a cell:phage ratio of 5:1. Mixture was adsorbed for 1 hour at 4°C. Then anti-P_1 serum was added to inactivate all free phage. After 10 minutes, the phage-adsorbed cells were dispensed in 5 ml. portions. The temperature was raised to 37°C. At intervals, virolysin was added to determine the number of sensitized cells. Lysis was complete within 10 minutes. At this time samples were plated for the numbers of infected centers in the presence and absence of virolysin. Virolysin did not reduce the number of infected centers. The latent period was between 60 and 65 minutes.

TABLE VI

Sensitization of Living S. aureus K_1 Cells by Mild Heat and the Reversal of Sensitization (Reappearance of Lysin Resistance) upon Incubation in Tryptose Phosphate Broth at 37°C.

Sample treatment	Effect of Treatment on				
	Virolysin susceptibility			Cell viability	
	Cells/ml. exposed × 10^8	Cells/ml. lysed × 10^8	Per cent lysed	Cells/ml. × 10^8	S.D.
A. Untreated control; stored in saline at 20°C.	7.0	0.5	7.1	6.63	±0.33
B. Sensitized; Heated 8 min., 46°C., stored at 20°C.	6.6	3.3	50.0	6.50	±0.91
C. Reversed; Incubated sensitized cells 5 min. 37°C. in tryptose phosphate broth	7.0	0.36	5.0	—	—

Procedure—Three hour growth on tryptose phosphate agar, harvested in saline, and treated as outlined in table. For *lysis test*, samples of A and B were mixed in triplicate with active virolysin from 3 × ultracentrifuged supernate of 14 (145) phage lysate, diluted one-half in tryptose phosphate broth. Estimates of cells lysed made from turbidimetric readings using the Klett photoelectric colorimeter. For tests of *cell viability*, samples were taken after 5 minutes' incubation of triplicate cell control tubes at 37°C. Each experimental sample was assayed in duplicate dilution series, and plate counts were made from the proper dilution, in triplicate, on tryptose phosphate agar. Standard deviations were calculated for the mean of each sample. The colony counts were multiplied by 2 to obtain the total cells per milliliter, since greater than 95 per cent were in suspension as diplococci. Microscopic observation showed that the sensitizing and reversal of sensitization procedures did not alter the Gram-staining properties or cell groupings in the suspensions.

infection—but not simultaneously. Normally the living cell is resistant to virolysin; when only a few phage particles are adsorbed, the cell becomes infected, but its resistance to virolysin is not reduced; *i.e.*, it is not sensitized. When more particles are adsorbed, the cell becomes sensitized; such cells do not form phage (Fig. 2).

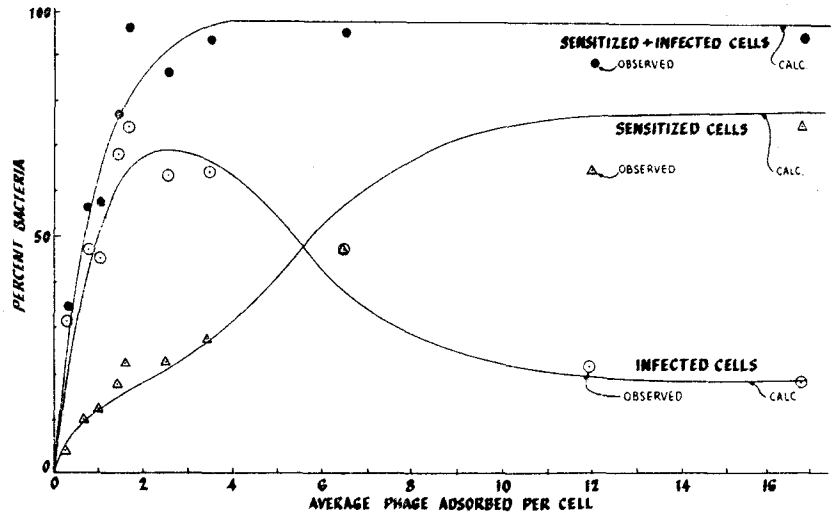

FIG. 2. Effect of phage concentration on the numbers of sensitized and infected cells in a population of *S. aureus* K_1.*

Method: S. aureus K_1 cells were harvested after 4.5 hours at 37°C. on tryptose phosphate agar. Duplicate aliquots containing 4.5×10^8 cells/ml. were exposed to varying concentrations of virolysin-free P_1 (K_1) phage for 15 minutes at 20°C. The phage adsorbed per cell varied from 0.3 to 17.

The numbers of sensitized cells were determined by exposure to strong concentrations of virolysin, to cause lysis from without; and the numbers of infective centers were determined by plaque count of serum-treated samples as follows: One sample of each phage-cell mixture was diluted two-thirds in active virolysin; the other was diluted in boiled virolysin. All samples were incubated at 37°C. and the extent of lysis from without was determined turbidimetrically. Only the samples exposed to active virolysin underwent lysis and this came to an end-point within 5 minutes. After 10 minutes at 37°C., the samples were placed at 20°C. and were exposed to phage P_1 antiserum for 5 minutes before assaying for the numbers of infective centers. The numbers of infective centers were the same in the presence and absence of virolysin.

Explanation of Curves

1. Infected Cells.—Experimentally observed values for the percentage of infective centers over the range phage/cell = 0.3 to 17, are shown together with the theoretical curve, calculated from the Poisson equation (Yule and Kindall, 1937) according to

* The analysis of the data for this figure was suggested by Dr. John H. Northrop.

IV

Quantitative Aspects of Lysis from Without

The curves representing the logarithms of the numbers of unlysed bacteria plotted against time at any given temperature depend upon the effects of phage and virolysin on the sensitizing and lytic reactions of the cell.

1. Effect of Phage.—(*a*) *On the lysis end-point.* In a given cell population, when the phage concentration is increased and the virolysin concentration is held constant, the lysis end-point is increased. This is due to the effect of phage on the sensitization of cells.

Fig. 2 shows that if the phage concentration is increased above that required for the adsorption of at least 1 particle per cell, the numbers of sensi-

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the assumption that at any given average phage adsorbed per cell, infective centers result from the adsorption of 1, 2, 3, 4, or 5 particles on 0.8 of the cells and of more than 5 particles on 0.2 of the cells. That is:

$$P \text{ infected} = 0.8\ (P\ 1, 2, 3, 4, \text{ or } 5) + 0.2\ (P > 5), \text{ in which}$$

$$P = \text{probability of a successful infection and}$$

$$P\ 1, 2, 3, 4, \text{ or } 5 = e^{-m}m + \frac{e^{-m}m^2}{2!} + \frac{e^{-m}m^3}{3!} + \frac{e^{-m}m^4}{4!} + \frac{e^{-m}m^5}{5!}$$

$$P > 5 = 1 - (e^{-m} + P\ \ 1, 2, 3, 4, \text{ or } 5), \text{ in which } m = \text{the mean phage/cell ratio.}$$

2. Sensitized Cells.—Experimentally determined values are plotted together with the theoretical curve calculated according to the assumption that sensitized cells result from the adsorption of >5 particles per cell on 0.8 of the population and 1, 2, 3, 4, or 5 particles per cell on 0.2 of the population. Therefore:

$$P \text{ sensitized} = 0.2\ (P\ 1, 2, 3, 4, \text{ or } 5) + 0.8\ (P > 5)$$

3. Infected Cells + Sensitized Cells.—Experimentally determined values for the sum of the percentages of infected cells and sensitized cells over the range phage/cell = 0.3 to 17, plotted with the theoretical curve of the fractions of cells expected to receive 1 or more particles. This is obtained by the equation:

$$P \text{ sensitized or infected} = 1 - e^{-m}$$

in which e^{-m} is the probability of receiving no particles at any given average phage/cell ratio, m.

At any phage/cell ratio, the entire population of bacteria B_t can be accounted for by the sum of the sensitized cells B_s, infected cells B_I and cells receiving no phage B_0, that is:

$$B_t = B_s + B_I + B_0$$

The approximate error in the determination of the sensitized cells is ±10 per cent; the approximate error in the plaque count is ±8 per cent.

tized cells are increased. Correspondingly, the numbers of infective centers are decreased. At each average phage/cell ratio, the entire population can be accounted for by the sum of the numbers of sensitized cells + infective cen-

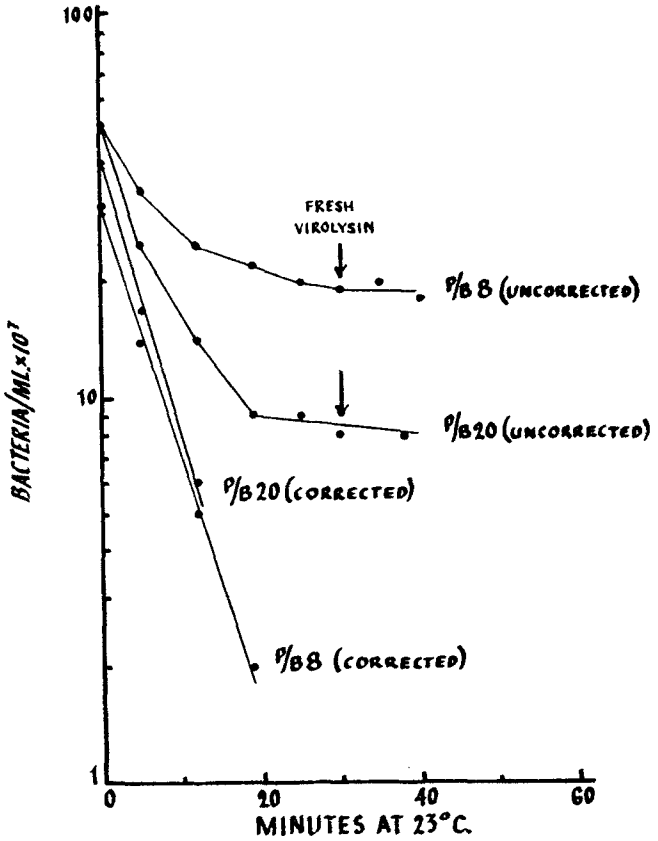

FIG. 3. The course of lysis from without of *S. aureus* K_1 before and after correction for the numbers of unsensitized cells. Phage P_1 (K_1) was added to 5×10^8 cells/ml. at cell:phage ratios of 8 and 20. After 10 minutes at 23°C., P_1 antiserum was added to stop further adsorption. After an additional 5 minutes, constant amounts of virolysin were added (one-half dilution) and the mixtures were incubated at 23°C. until the reaction had come to an end-point. Fresh virolysin was then added; this did not affect the end-point. The lysis was determined by turbidimetric readings.

ters + cells which have not adsorbed any phage. This indicates that sensitization is brought about when the cell has adsorbed a sufficient number of particles. Fig. 2 indicates that, according to calculations based upon the equation for the Poisson distribution, infective centers may result from the adsorption of 1 to 5 particles; sensitized cells may result from the adsorption of

more than 5. The entire population, however, is not homogeneous; a proportion of cells appears to be more resistant to sensitization. In the experiment of Fig. 2, this corresponds to about 20 per cent. It is possible to show that these cells may be sensitized by phage at phage/cell ratios greater than 17.

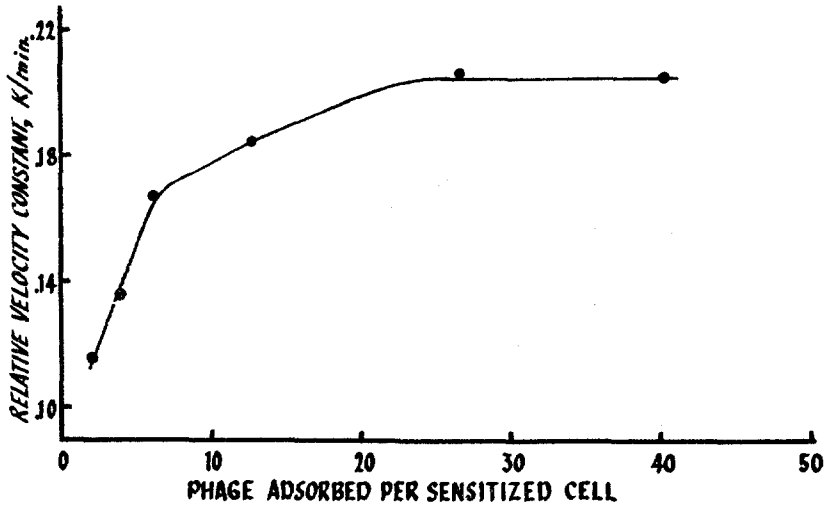

FIG. 4. Effect of phage concentration on the lysis from without rate constant. K_1 cells were grown in tryptose phosphate broth for 3 hours at 37°C., washed, and resuspended in tryptose phosphate broth (double strength). A constant amount of cells was mixed with increasing amounts of phage (prepared by ultracentrifuging a P_{14} (K_1) lysate). The cell concentration was 3×10^8 bacteria per ml.; the phage to cell input varied from 2 to 55. The phage-cell mixtures were allowed to adsorb at 23°C. for 10 minutes. Active virolysin (an ultracentrifuged supernate of a 14 (K_1) lysate) was added at a dilution of $\frac{1}{50}$ to one set of tubes, boiled virolysin to another. All tubes were placed at 37°C. and the lysis followed by taking Klett readings at intervals. After 10 minutes at 37°C., samples were removed, filtered through supercel, and plated for plaque count to determine the free phage remaining. Phage antiserum was then added to stop further adsorption. After 20 minutes at 37°C., samples were removed in order to determine the number of infective centers. The velocity constant K/minute was calculated after correction for the numbers of non-sensitized cells.

Another proportion of cells appears to be sensitized by less than 5 particles per cell; this also corresponds to 20 per cent. Different cell preparations have been observed to vary with respect to the proportion of sensitization-resistant cells and also with respect to the number of particles required to sensitize 100 per cent of the population.

In Fig. 3, the curves of lysis from without are corrected for the numbers of non-sensitized cells. The lysis appears to proceed logarithmically.

(*b*) *On the velocity constant, K.*—Fig. 4 shows that the value *K*, calculated for the lysis of sensitized cells by a given amount of virolysin, is increased as

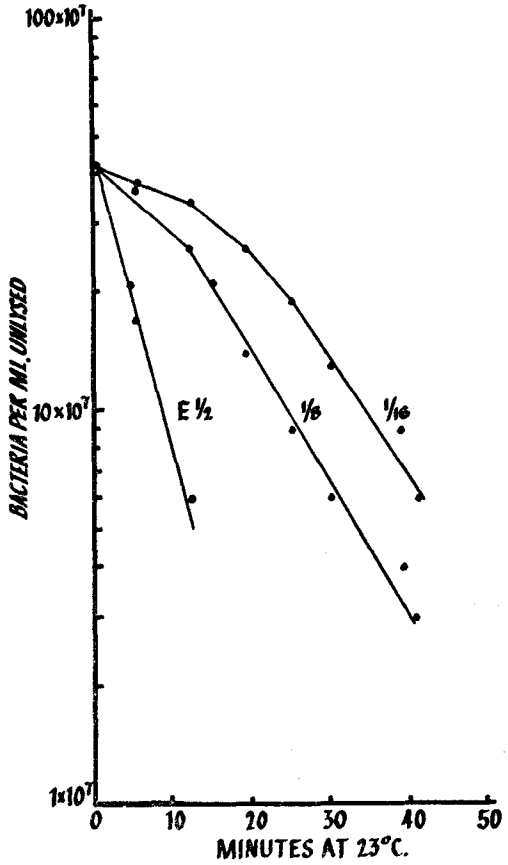

FIG. 5. The effect of virolysin concentration on lysis from without of phage-sensitized cells at 23°C. Phage P_1 (K_1) was mixed with 4 hour agar-grown cells at 23°C. at a phage/cell ratio of 20/1, for 10 minutes. Phage antiserum was then added for 5 minutes to stop further phage adsorption. A constant volume of 14 (145) virolysin was added so that the final dilutions of virolysin were one-half, one-eighth, and one-sixteenth. The lysis was determined turbidimetrically during incubation at 23°C. The data were corrected for the numbers of non-sensitized cells.

the average phage adsorbed per cell is increased. The value approaches a maximum as the cell becomes saturated with phage.

2. *Effect of Virolysin.*—For any given phage/cell ratio, an increase in virolysin concentration causes an increase in the velocity constant *K* (Fig. 5). The total lysis is independent of the virolysin concentration. With dilute

virolysin, an initial shoulder appears in the curves of lysis from without, represented by the plot of the logarithm of the surviving sensitized cells against

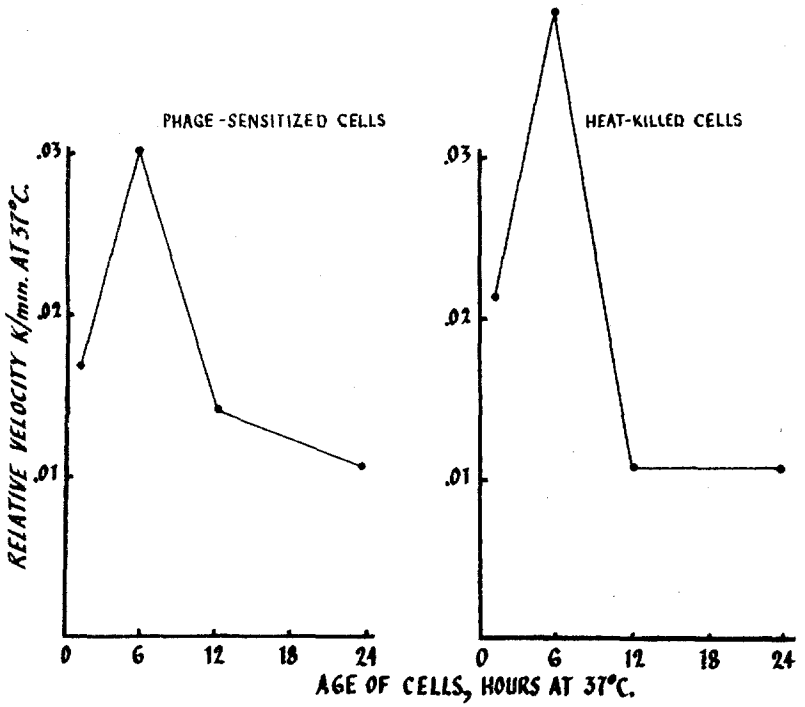

FIG. 6. Influence of age on relative velocity constant, K, for lysis from without of phage-sensitized and heat-killed *S. aureus* K_1 by a constant amount of 14 (K_1) virolysin. Cells were grown on tryptose phosphate agar for 1 to 24 hours. They were harvested in saline and divided into two portions: one portion was heated at 56°C. for 1 hour; the other was used for the studies of lysis from without. Heat-killed cells were mixed with virolysin in a dilution of one-half, and were incubated at 37°C. The cells were corrected for a constant percentage of resistant cells and the velocity constants were calculated. Viable cells were mixed with phage and one-half virolysin. The phage:cell ratio was 20, an amount of phage which sensitized greater than 95 per cent of the cells of each age. The samples were incubated at 37°C. and the velocity constants, K, were calculated for the lysis of the sensitized cells.

time. The non-sensitized cells are Gram-positive and are resistant to fresh lysin.

The action of virolysin has been studied on heat-killed cells (Ralston *et al.*, 1957). Lysis also proceeds logarithmically, the velocity constant, K, is proportional to the virolysin concentration. The end-point is independent of the lysin concentration. About 50 per cent lysis occurs; the remaining cells are Gram-negative and are resistant to fresh lysin.

3. Effect of the Test Cell.—The sensitizing and lytic reactions are affected by certain components of the test cell. These vary with age and medium of growth. For example, sensitization requires fewer particles in 3 hour cells than in 24 hour cells. The lytic step is affected by a heat-stable material; heat-killed and phage-sensitized cells show a similar age variation in their response to virolysin (Fig. 6).

Certain of these cell components may also affect the tendency to undergo spontaneous autolysis, but they do not influence the growth of the uninfected cells; nor do they influence the length of the latent period, the phage yield, or virolysin yield of infected cells.

DISCUSSION

The Relationship of Sensitization to the Infection Cycle.—The results suggest that sensitization, as defined, is not an early step. It is possible, however, that when infection occurs, the sensitizing reaction proceeds on a lesser scale, enabling a localized destruction of substrate by internal lysin, perhaps playing a role in "penetration." If this is so, there must be a separate reaction for activating the internal lysin, for phage sensitization does not involve enzyme activation—at least when it occurs on a scale great enough to render the cell susceptible to external lysin.

It is conceivable that a sensitization similar to that in lysis from without is involved in a terminal stage of infection; that it might be produced intracellularly by phage (or phage components); and that it might enable intracellularly formed virolysin to lyse the cells from within, thus releasing phage (Ralston *et al.*, 1955).

Lysis from Without: A General Mechanism.—Data available for several phage-host systems suggest that there is one fundamental mechanism for lysis from without—the action of an enzyme on a cell substrate. The substrate is essential to the structural integrity of the cell. The living cell may be protected against the enzyme by maintaining either the substrate or internal enzymes in a "masked" form. Systems may vary with respect to the location and state of activation of the enzyme and the "availability" of the substrate. As a result, specific components of the particle may function as (*a*) active enzyme, (*b*) activator for internal cellular enzymes, or (*c*) substrate-unmasking agent.

Whatever the function of the particle, the final conclusion depends upon experimental demonstration that it is associated with the particle and not with some contaminating material.

In systems in which the phage causes lysis of boiled cells, the particles most probably contain the enzyme. This is suggested by the work of Weidel on the T2r$^+$–*E. coli* B system (1951). In addition, Barrington and Kosloff (1956) and Brown and Kozloff (1957) have claimed that—with several T phages—an

enzyme is an integral part of the particle, located within the tail, and that it acts on a heat-stable substrate in trypsin-treated cell walls.

In systems in which the phage causes lysis only of living cells, it is difficult to distinguish between several possibilities: (*a*) phage acts as an enzyme activator for heat-labile autolytic enzymes of the cell; (*b*) phage contains an enzyme which acts on a heat-labile cell substrate; or (*c*) phage renders substrate available to already active autolytic enzymes of the cell (Northrop (1937), *S. muscae* phage; Herriott (1951), T_2–*E. coli* B and B/4; Puck (1953), T_2–*E. coli* B). Puck has suggested that since T_2 phage preparations are more stable at 60° C. than the bacterial cells, the phage might act as an enzyme activator.

In the *S. aureus* K_1–P_1 phage system, lysis from without is clearly enzymatic, but the enzyme is both extracellular and extraphage. Since the particle by itself functions neither as an enzyme nor as an enzyme activator but is necessary for the reaction, a new function is revealed—that of rendering substrate available to the extracellularly added enzyme. We have called this "sensitization."

Methods

The *S. aureus* K_1–phage P_1 or P_{14} system was used for the preparation of virolysin and phage, the K_1 strain was also used for the test cell. The phage-host system, its method of assay, the procedure for obtaining virolysin from phage lysates, the turbidimetric procedure for measuring lysis, all have been described previously (Ralston *et al.*, 1955, 1957).

Except where indicated, the studies were carried out by adding known quantities of phage and lysin to living cells and recording the lysis after incubation at a given temperature. Estimates of the lysis from without reaction rate constant were calculated according to the equation:

$$K = \frac{2.3}{t} \log (B_0/B_t),$$

in which B_0 = initial bacteria/ml., B_t = unlysed bacteria/ml. at time t, and t = minutes. As described under Experimental Results, the numbers of bacteria are corrected for the non-sensitized cells. This measure of lytic activity is intended only for purposes of comparison within a given experiment; the value K is not a true constant.

Cell Preparations—Agar-grown *S. aureus* K_1 cells were prepared by spreading tryptose-phosphate agar plates with 1 ml. aliquots of an 18 hour agar-grown inoculum, adjusted to contain 1×10^9 cells/ml. After incubation at 37°C. for a specified time, the cells were harvested in saline, washed once, and resuspended in saline or in double strength tryptose phosphate broth.

Broth-grown cells were prepared by adding a similar inoculum to double strength tryptose phosphate broth at an initial concentration of 2×10^8 cells/ml., incubating with shaking at 37°C. for a specified time, harvesting by centrifugation, washing, and resuspending in broth or saline.

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