OBSERVATIONS ON THE EFFECT OF PENICILLIN ON THE REACTION BETWEEN PHAGE AND STAPHYLOCOCCI

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In an earlier paper, Krueger, Cohn, and Noble (1) reported certain changes in the reaction between phage and *Staphylococcus aureus* brought about by the presence of penicillin. Particular attention was paid to the following points: (1) When phage acts on a broth suspension of staphylococci, the time required for initiation of massive lysis is considerably reduced by penicillin. (2) With values of $[phage]_0$ between 1×10^9 plaques/ml. and 1×10^7 plaques/ml. the accelerating action is independent of [penicillin] over a fairly wide range. (3) Exposure of the cellular substrate to penicillin action for at least 0.9 hour is essential to secure the full acceleration effect. (4) In some mixtures of phage, bacteria, and penicillin, phage formation apparently occurs in the absence of bacterial reproduction.

Because these experiments were performed with insensitive procedures for detecting increases in [bacteria] they were repeated later in modified form, and it was found that phage can be produced under conditions precluding multiplication of staphylococci.

We wish to present here an account of additional experimental work on the same general topic not reported in our first paper.

Experimental Methods

Throughout this paper the following abbreviations are used: P = the K race of staphylococcal phage; [P] = concentration of phage in plaques/ml.; B = the K strain of *Staphylococcus aureus;* [B] = bacteria/ml. Subscripts are employed as follows to indicate the procedure used for determining cell concentrations: $[B]_D$ = direct count; $[B]_V$ = viable count; $[B]_K$ = total cell concentration secured with Klett-Summerson photoelectric colorimeter; $[B]_C$ = direct visual comparison with formalinized cell suspensions of known density; PN = sodium penicillin G (crystalline); [PN] = concentration of sodium penicillin G in Oxford units.

1. Preparation of Cell Suspensions.—Stock cultures of staphylococci were grown in Roux flasks on tryptose agar for 18 hours at 36°C. To produce "activated" cell suspensions, tryptose-phosphate broth was inoculated with staphylococci recently harvested from Roux flasks and rapid growth was promoted by shaking the flasks in a water bath set at 36°C. When growth had progressed to a level of 1×10^9 bacteria/ml. the suspensions were removed from the water bath and were used for the experiments described below.

2. Determination of Bacteria.—For many purposes, such as establishing the time of half-lysis, it was found that direct visual comparison with formalinized broth suspensions of staphylococci was adequate ($[B]_C$). Actually, very good agreement was obtained between values secured by direct visual comparison and by the Klett-Summerson colorimeter.

Direct counts in the Petroff-Hausser chamber were routinely made when [B] was $>5 \times 10^7$ staphylococci/ml. In the presence of phage, the cell suspensions were diluted in formalinized broth to inhibit continuing lysis.

In using the Klett-Summerson photoelectric colorimeter for determining [B], readings were converted into cells/ml. by reference to a standard curve plotted from points established by direct counts.

Viable counts were made by plating aliquots of test suspensions on the surface of tryptose agar plates taking care to spread the fluid evenly. On occasion, pour plates were used but these required longer incubation for reading and appeared to possess little advantage.

Determinations of phage were carried out by Gratia's method (2), using a separate pipette for each serial dilution.

EXPERIMENTAL RESULTS

1. Quantitative Aspects of the Penicillin Effect.—In order to ascertain the effects of varying [PN] and [P] on the time of lysis of mixtures containing bacteria, phage, and penicillin, several series of experiments were carried out using the standard "activated" cell suspension in tryptose-phosphate broth as a substrate. Ten ml. test mixtures were prepared, consisting of 5 ml. of cell suspension (1×10^9 bacteria/ml.), 1 ml. of phage solution, 1 ml. of penicillin solution, and 3 ml. of broth. These were placed in a 36° water bath and were shaken mechanically to maintain even suspensions. Turbidity readings were taken every 0.2 hour by direct visual comparison and the elapsed times required to reduce [B] to one-half the original value were recorded. The results are summarized in Table I.

The minimal [PN] producing an effect on time of lysis appears to be 1×10^{-2} units/ml. This amount in the absence of phage does not produce measurable lysis within the arbitrary period of 5 hours established as the limit of observation. Combined with [P]'s of 1×10^5 plaques/ml. or 1×10^6 plaques/ml., the times of half-lysis are respectively 5.1 and 4 hours; controls without PN and with [P] = 1×10^5 plaques/ml. do not lyse. With [P] = 1×10^6 plaques/ml. half-lysis occurs between 4.0 and 5.0 hours.

When higher concentrations of phage are employed; namely, 1×10^7 or 1×10^8 plaques/ml. the controls without PN lyse at 2.3–3.8 hours and 1.6 hours respectively while the suspensions containing both P and PN show moderate acceleration.

With the highest [P] tested, namely 1×10^9 plaques/ml., lysis proceeds at the same rate in control and test suspensions.

As [PN] is raised above 0.1 unit/ml. the lower concentrations of P (1×10^4, 1×10^5, and 1×10^6 plaques/ml.) appear to have little effect in accelerating lysis beyond the rates for PN alone. However, with [P] = 1×10^7 there is a pronounced speeding of lysis in the test mixtures as compared with PN alone or P alone.

Nearly all the suspensions made with a [P] of 1×10^8 plaques/ml. attain half-lysis by 0.8 hour. This is significantly less than the t (half-lysis) for 1×10^8 phage alone (1.6 hours) and as much as 1.9 hours less than the t (half-lysis) observed for 1×10^2 units PN alone (2.7 hours). When [P] = 1×10^9 plaques/ml.

TABLE I

Time of Half-Lysis in Mixtures of Phage, Penicillin, and Staphylococci in Tryptose Phosphate Broth at 36°C.

[Bacteria] determined by direct comparison with standards containing known concentrations of staphylococci.

[Penicillin] units/ml.	[Phage], *plaques/ml.*						
	0	1×10^4	1×10^5	1×10^6	1×10^7	1×10^8	1×10^9
	hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
0		5.0	5.0	From 4.0 to 5.0 hrs.	From 2.3 to 3.8 hrs.	1.6	0.8
1×10^{-2}	Usually no lysis to 5.0 hrs.	Usually no lysis to 5.0 hrs.	5.1	4.0	2.1	1.2	0.8
1×10^{-1}	1.9	1.7	1.7	1.5	1.2	0.8	0.8
1.0	2.1	2.0	1.7	1.6	1.3	0.8	0.7
10.0	2.1	2.1	2.0	1.9	1.3	0.8	0.7
1×10^2	2.5	2.5	2.3	2.0	1.3	0.8	0.7
1×10^3	2.7	2.7	2.4	2.2	1.3	0.8	0.7
1×10^4	2.7	2.5	2.5	2.3	1.8	0.9	0.7

t (half-lysis) is only 0.7 hour in the five mixtures having the highest [PN]'s. Evidently, the presence of PN exerts little influence on the rate of lysis in suspensions containing so much P, for the P control reaches half-lysis in 0.8 hour.

A somewhat broader view of the accelerative effect of penicillin can be obtained by comparing Fig. 1 with Fig. 2. Fig. 1 shows the lytic action on the standard cell suspension of [P]'s varying from 1×10^9 to 1×10^6 plaques/ml.; the curves for lysis are based on photoelectric colorimeter measurements secured in four separate experiments. Fig. 2 presents the data from a single experiment in which [PN] = 1×10^2 units/ml. and [P] varies from 1×10^9 to 1×10^4 plaques/ml. It is evident that the lytic process proceeds more rapidly when PN is present than when P acts alone.

PN alone lyses the test organism. In mixtures containing [PN]'s ranging

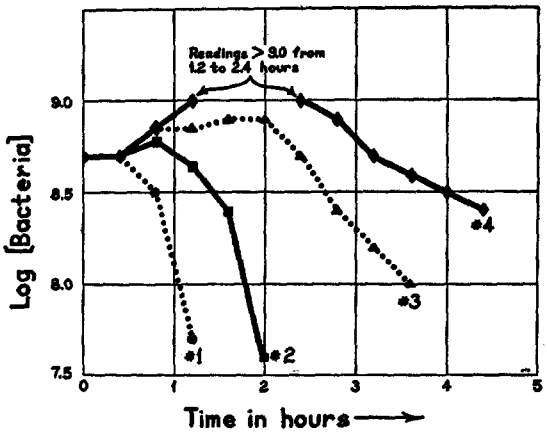

FIG. 1. Lytic curves for staphylococci and phage in tryptose-phosphate broth at 36°C. without penicillin. Points are averages of four experiments. Initial [bacteria]: 5×10^8/ml. Initial concentrations of phage: No. 1 = 1×10^9; No. 2 = 1×10^8; No. 3 = 1×10^7; No. 4 = 1×10^6. [Bacteria] determined by Klett-Summerson photoelectric colorimeter. Final [phage] after lysis (plaques/ml.): No. 1 = 5×10^9; No. 2 = 5×10^9; No. 3 = 4×10^9; No. 4 = 2×10^8.

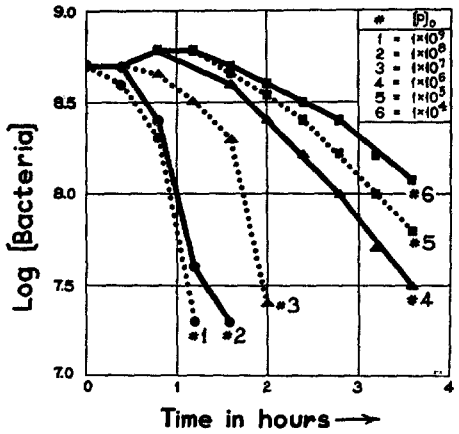

FIG. 2. Lytic curves for staphylococci + phage + penicillin in tryptose-phosphate broth at 36°C. Data of a single experiment. Initial [bacteria]: 5×10^8/ml. Initial [penicillin]: 1×10^2 units/ml. Initial concentrations of phage varied from 1×10^9 plaques/ml. to 1×10^4 plaques/ml. [Bacteria] determined by Klett-Summerson photoelectric colorimeter.

from 0.1 unit/ml. to 1×10^4 units/ml., the t (half-lysis) varies inversely with $[PN]_0$. This parallels Eagle's observations that bacteria were killed more rapidly with lower [PN]'s than with relatively concentrated PN (3).

2. The Production of Phage in Broth Mixtures of Phage, Staphylococci, and Penicillin.—In order to follow the course of P formation, plaque counts were made at brief intervals on samples taken from mixtures of P, PN, and B in tryptose-phosphate broth at 36°C. Four experiments were done employing

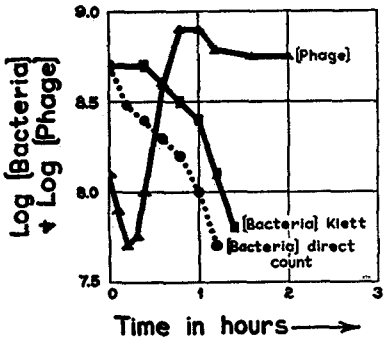

FIG. 3

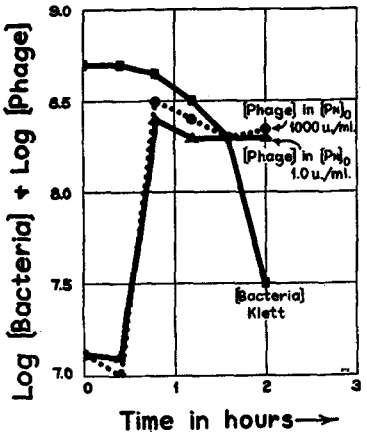

FIG. 4

FIG. 3. Curves for cellular lysis and phage formation in mixtures of staphylococci, phage, and penicillin in tryptose-phosphate broth at 36°C. (average of four experiments). "Activated" bacteria kept 1 hour at 21°C. and 0.5 hour at 5°C. Centrifuged and sediment suspended in broth containing 1×10^4 units PN/ml. and about 1×10^8 plaques/ml. Mixture shaken at 36°C. and samples taken at intervals for: (1) determination of [bacteria] by photoelectric colorimeter and by direct count, and (2) phage assay. Latter aliquots immediately diluted at 5°C.

FIG. 4. Curves for cellular lysis and phage formation in mixture of staphylococci, phage, and penicillin in tryptose-phosphate broth at 36°C. "Activated" bacteria kept 1 hour at 21°C. and 0.5 hour at 5°C. Centrifuged and sediment suspended in broth containing (*a*) 1.3×10^7 plaques/ml. and 1 unit PN/ml. and (*b*) 1.3×10^7 plaques/ml. and 1,000 units PN/ml. Samples taken at intervals for (1) determination of [bacteria] by photoelectric colorimeter, and (2) phage assay. Lytic curves for $[PN]_0$ 1.0 and 1×10^3 identical. Phage curves almost identical.

the concentrations shown in Fig. 3. The curve for [P] drops sharply to 0.2 hour and then rises rapidly to a peak at 0.8 hour. The maximal [P] is approximately six times the [P] initially present and sixteen times the lowest value reached during the reaction (at 0.2 hour). Subsequently, there is a 30 per cent decrease in [P] until a steady state is reached at 1.6 hours.

Judging from the $[B]_D$ curve, bacterial lysis begins as soon as the reactants are mixed and not only continues during the phase of P formation, but extends into the period when [P] falls off 30 per cent. The curve for $[B]_K$ (obtained by

means of the photoelectric colorimeter) shows a slower drop than that based on $[B]_D$; a possible reason for this discrepancy will be discussed later.

Other, essentially identical, experiments were performed with smaller concentrations of P and PN; the curves for B lysis so obtained agreed well with those presented in Fig. 2. Fig. 4 illustrates such an experiment, in which two different mixtures were followed. In each, $[B]_0$ was 5×10^8/ml. and $[P]_0$ 1.3×10^7 plaques/ml.; $[PN]_0$ in one was 1 unit/ml. and in the other 1×10^3 units/ml. The rate of lysis is seen to be the same for both suspensions and there is no significant difference in the curves for P formation. However, when the latter are compared with P curves secured with higher initial values of [P] (Fig. 3), certain differences are noticeable: (1) The maximal [P] formed is twenty-five times the initial [P] instead of six times this concentration. (2) There is no drop in [P] during the early phases of the reaction. (3) The period of P production is completed before massive cellular lysis is well under way.

In the experiments already described, P formation has occurred without demonstrable cellular reproduction, confirming earlier experiments performed with the K strain of *Staphylococcus aureus* in this laboratory and with *Staphylococcus muscae* in Dr. Winston Price's laboratory (4). However, the suspending medium for the reaction between PN and B was veal infusion broth in Dr. Price's experiments and tryptose-phosphate broth in our experiments, each medium favoring the growth of the respective organism in the absence of PN. While it is known that in some cases at least (*Escherichia coli* and T2 phage) B stop multiplying as soon as they are infected with P (5) one must consider the possibility that a certain amount of cell division, perhaps restricted to P-infected cells, could occur without detection by the procedures employed for determining [B]. For example, in the experiment of Fig. 4 only 1 cell in 40 is infected at 0.4 hour when [P] begins to rise, so that a generalized single fission among P-containing staphylococci would result in a mere 2.5 per cent increase in [B].

One way to test the possibility that selective cell division may be involved is to raise the P/B ratio in order to infect a considerable percentage of the total bacteria present; then, if infected cells are prone to divide, the increase should be experimentally detectable. This was done in the experiments summarized in Figs. 3, 5, and 6, unfortunately without settling the issue. Apparently, as the P/B ratio is raised the early drop in [P] is exaggerated (Fig. 5) and may continue to practical extinction of the P added (Fig. 6). In some cases there is measurable production of P but the final titre never exceeds the initial [P] (Fig. 5).

While the chance of any significant cell division seemed remote in the above experiments, we felt it would be advisable to limit this factor still further by attempting to produce P in mixtures of B, PN, and P suspended in Locke's solution. These experiments are described below.

3. The Formation of Phage in Locke's Solution Mixtures of Phage, Staphylococci, and Penicillin Containing 5 to 10 Per Cent Broth.—The course of P production in Locke's solution mixtures of P, B, and PN was obser ved as follows:

"Activated" B were centrifuged down from the tryptose-phosphate medium and were resuspended at a concentration of 4×10^8 cells/ml. in Locke's solution con-

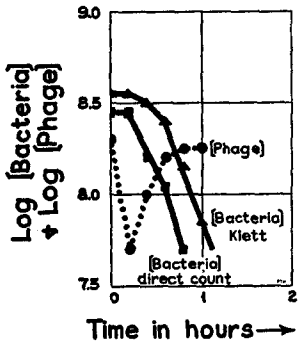

FIG. 5

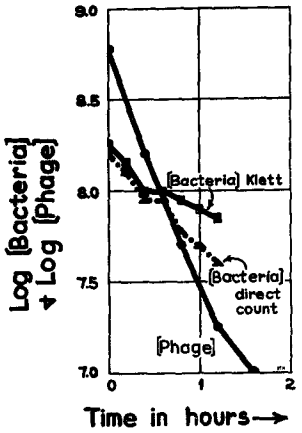

FIG. 6

FIG. 5. Curves for cellular lysis and phage formation in mixture of staphylococci, phage, and penicillin in tryptose-phosphate broth at 36°C. "Activated" bacteria kept 1 hour at 21°C. and 0.5 hour at 5°C. Centrifuged and sediment suspended in broth containing 2×10^8 plaques/ml., 1×10^4 units PN/ml., and 7×10^8 B/ml. Mixture shaken at 36°C. and samples taken at intervals for: (1) determination of [bacteria] by photoelectric colorimeter and by direct count, and (2) phage assay.

FIG. 6. Curves for cellular lysis and phage formation in mixture of staphylococci, phage, and penicillin in tryptose-phosphate broth at 36°C. "Activated" bacteria kept 1 hour at 21°C. and 0.5 hour at 5°C. Centrifuged and sediment suspended in borth containing 7×10^8 plaques/ml., 1×10^4 units PN/ml., and 2×10^8 B/ml. Mixture shaken at 36°C. and samples taken at intervals for: (1) determination of [bacteria] by photoelectric colorimeter and by direct count, and (2) phage assay.

taining 1.0 unit PN/ml. The suspension was held at 5°C. for 1 hour. At this time P was added (usually <1 ml. to each 9 ml. of the Locke's solution mixture) and the preparation was kept an additional hour at 5°C. It was then divided into 10 ml. aliquots in test tubes and the latter were shaken at 36°C. Samples were removed at intervals for plaque counts and for determination of $[B]_K$. The phage samples were diluted immediately in broth or Locke's solution at 5°C., the samples for $[B]_K$ were diluted in Locke's solution at 5°C. and were read at once.

Fig. 7 is a summarized graphic presentation of the data from four experiments. Cellular lysis without an increase in the total cell count begins shortly

after the mixtures containing PN are brought to 36°C. In the absence of PN, however, considerable B growth takes place and lysis is much slower.

The [P] curve shows a marked drop during the 1 hour period at 5°C. and rises sharply when the temperature is adjusted to 36°C. Some 60 per cent of the total P produced is formed during the phase of massive bacterial lysis (taking place between 2.6 hours and 3.4 hours). The yield of P is not remarkable, being only 6 × $[P]_0$.

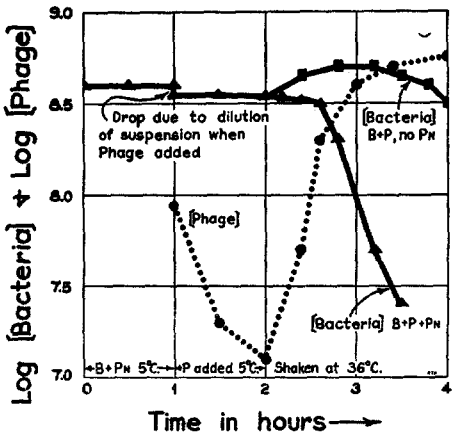

FIG. 7. Curves for phage formation and cellular lysis in Locke's solution. "Activated" bacteria 4 × 10^8/ml. held at 5°C. 1 hour in Locke's solution containing 1.0 unit PN/ml. Phage diluted in Locke's solution added and mixture kept additional hour at 5°C. Suspension transferred to tubes and shaken at 36°C. Samples taken at intervals for plaque counts and determination of [bacteria] by photoelectric colorimeter. Aliquots for phage assay immediately diluted at 5°C. The curves for [bacteria], B + P + PN, and [phage] are averages of four experiments; that for [bacteria], B + P, is an average of two experiments.

To confirm the lack of cellular reproduction noted above, an additional set of experiments was performed using parallel determinations of]B] by the photoelectric colorimeter and by direct count. The data are presented in Fig. 8. Again, as noted earlier in connection with the broth mixtures, there is a noticeable inclination of $[B]_D$ to drop in advance of $[B]_K$; both curves exhibit no evidence of an increase in [B]. Plaque counts were done only at the start and at the end of the experiments; they showed an average increase of four times $[P]_0$.

While it is reasonable to expect that cell multiplication would be discouraged in the experiments employing Locke's solution as a suspending medium, the inclusion of some nutrients cannot be overlooked. Concentrations of tryptose-phosphate broth between 5 and 10 per cent were added with the P and it is clear from the control curve (no PN) for [B] in Fig. 7 that even this dilute

medium suffices to support staphylococcal growth, providing no PN is present. However, when the test mixture contains both P and PN, the curve for [B] determined by direct counts shows a prompt and continuing downward trend.

Price has performed similar experiments with *S. muscae* (6), using suspensions of B, PN, and P in broth diluted 1/17 with Locke's solution. Since P formation was his primary concern no B growth curves are given and comparison with our data cannot be made.

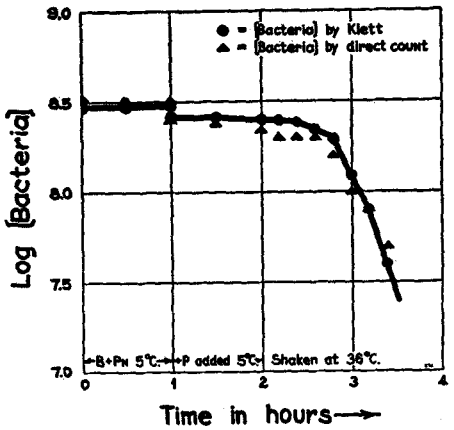

FIG. 8. Lysis without cellular growth in mixture of phage, penicillin, and staphylococci in Locke's solution. "Activated" bacteria 3.2×10^8/ml. held at 5°C. 1 hour in Locke's solution containing 1.0 unit PN/ml. Phage diluted in Locke's solution added to produce $[P]_0$ of 1×10^8 plaques/ml. and mixture kept additional hour at 5°C. Suspension transferred to tubes and shaken at 36°C. Samples taken at intervals for determination of [bacteria] by Klett-Summerson photoelectric colorimeter and by direct count (formalinized Locke's solution used as diluent for latter). Curve drawn through averaged values for three sets of Klett determinations. Direct count points are averages of these same three experiments and an additional one for which Klett values were not determined.

4. Mutual Sensitization of Staphylococci by Phage and Penicillin.—

(a) Preliminary Treatment of Cells with Penicillin Followed by Exposure to Phage: Several experiments were performed to determine whether a period of exposure to PN made staphylococci susceptible to accelerated lysis when removed and suspended in P. Table II summarizes one of these tests, in which the initial mixture consisted of 5×10^8 B/ml. and 10 units PN/ml. The suspension was kept at 5°C.; at intervals two 10 ml. aliquots were removed and centrifuged for 10 minutes at 5°C. One sediment was resuspended in broth; the other in P containing 1×10^7 plaques/ml. and both were shaken at 36°C. $[B]_D$ was determined frequently to establish the course of lysis, and the time of half-lysis was recorded for each mixture.

It appears that the cells exposed to 10 units PN/ml. at 5°C. become sensitive

to the action of P and that this sensitization process requires approximately 1 hour to produce maximal acceleration of lysis.

This type of experiment is open to the theoretical objection that small amounts of PN may be carried over into the final mixtures and from the evidence presented in Table I it is known that concentrations between 1×10^{-1} and 1×10^{-2} unit PN/ml. could speed up the lytic process. Probably such a mechanism does not apply to the present case for no lysis of the cells resuspended in broth occurs within the 6 hour period of observation.

TABLE II

Sensitization of Staphylococci to Phage Action by Previous Exposure to Penicillin

Preliminary Mixtures: $[B]_0$ 5×10^8; $[PN]_0$ 10 units/ml. Kept at 5°C. At various intervals two samples removed, centrifuged in angle centrifuge at 4,000 R.P.M. (at 5°C.). Supernatants decanted and sediments resuspended: (*a*) in broth, (*b*) in P of 1×10^7 plaques/ml. Course of lysis followed by comparison with B suspensions of known density.

Resuspended Mixtures: (In shaker at 36°C.)

Period of exposure to PN (including time of centrifugation)	Time of half-lysis (from time resuspended)	
	Broth suspension	Phage suspension
min.		
10	No lysis to 6.0 hrs.	3.4 hrs.
34	" " " 6.0 "	2.5 "
58	" " " 6.0 "	1.9 "
82	" " " 6.0 "	2.0 "

Controls: (Shaken at 36°C.)

$[B]_0$	$[PN]_0$	$[P]_0$	Time of half-lysis
			hrs.
5×10^8	0	1×10^7	3.7
5×10^8	10 units/ml.	0	2.2
5×10^8	10 " "	1×10^7	1.7

(*b*) *Preliminary Treatment of Cells with Phage Followed by Exposure to Penicillin:* It is not possible to determine experimentally whether P treatment conditions stapyylococci to subsequent action by PN. The difficulty encountered is that during preliminary exposure of the cells they rapidly adsorb P and carry it over into the second phase of the experiment when the B are resuspended in PN solution. Consequently, in effect, one is simply making mixtures of bacteria, phage, and penicillin.

5. Experiment to Detect Cellular Swelling during Reaction between Phage, Staphylococci, and Penicillin.—During the reaction between P, B, and PN we regularly observed a considerable lag in the curve for cellular lysis plotted from $[B]_K$ data when compared with that based on $[B]_D$ data (Figs. 3, 5, and 6). It seemed conceivable that this discrepancy might depend upon swelling of the cellular substrate prior to lysis, with the result that the turbidity readings

would remain high while the direct count values would drop. To test this idea the following experiment was performed:

"Activated" staphylococci were prepared in the usual fashion and were held at 5°C. for 1 hour. A mixture in tryptose-phosphate broth was made containing: 5×10^8 B/ml., 1×10^8 P plaques/ml., and 1×10^3 units PN/ml. It was shaken at 36°C. and at intervals samples were removed for: (1) determination of $[B]_K$; (2) determination of $[B]_D$; (3) microscopic measurement of cell size (wet preparations) with the ocular micrometer.

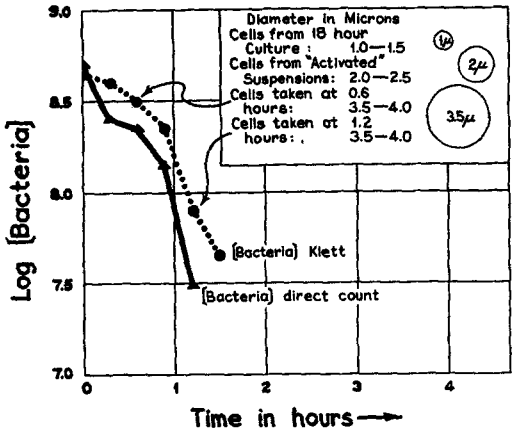

FIG. 9. Swelling of staphylococci during reaction with phage and penicillin. $[B]_0 = 5 \times 10^8$/ml. $[P]_0 = 1 \times 10^8$ plaques/ml. $[PN]_0 = 1 \times 10^3$ units/ml. Tryptose-phosphate broth at 36°C. Samples removed at intervals for determination of $[B]_D$ and $[B]_K$ and for microscopic measurement of cells with ocular micrometer.

The curves for $[B]_D$ and $[B]_K$ are plotted in Fig. 9 and the diameters of the staphylococci are recorded. Measureable swelling of the organisms occurs during the activation procedure (from an average diameter of 1.25 microns to one of 2.25 microns). A further increase to an average diameter of 3.75 develops by 0.6 hour from the time of mixing B, P, and PN. This appears to be the maximal amount of swelling, for samples taken at 1.2 hours give identical measurements.

SUMMARY AND CONCLUSIONS

The essential facts relating to the reaction between phage, sodium penicillin G, and the K race of *Staphylococcus aureus* are:

1. Except when [P] is very high, massive lysis of the cellular substrate occurs considerably sooner in the P-PN-B mixture than in preparations containing P alone or PN alone.

2. The accelerative effect is present in concentrations of PN varying from 0.1 to 1×10^4 units/ml.

3. Acceleration of lysis can be secured by exposing staphylococci to PN prior to treatment with P.

4. In certain concentrations of P and B in tryptose-phosphate broth, P formation apparently takes place without bacterial reproduction. The extent to which P is produced is influenced very little by $[PN]_0$ but is markedly dependent upon $[P]_0$. With low P/B ratios the [P] curve shows a lag followed by a rapid rise to a peak of 25 to 30 times $[P]_0$. When the P/B ratio approaches unity there is a considerable primary drop in [P] and later an increase which, however, fails to bring the total P produced above $[P]_0$. When P/B is still higher, the [P] curve drops profoundly as the bacteria lyse and never enters into a productive phase.

5. In Locke's solution mixtures of P-PN-B, containing 5 to 10 per cent broth, P formation occurs in the absence of detectable cellular reproduction to the extent of a four- to sixfold increase over $[P]_0$.

6. Direct microscopic examination of wet preparations removed during the P-PN-B reaction has disclosed swelling of the staphylococci. The swollen cells are three times the diameter of normal *S. aureus* secured from an 18 hour culture. Cellular swelling apparently accounts for the experimental observation that the curve for lysis plotted from $[B]_K$ lags considerably behind the $[B]_D$ curve. Increase in the size of individual cells would tend to keep the photoelectric colorimeter measurements high even while the direct count was diminishing.

7. When the P-PN-B reaction is carried out in broth, attainment of the peak in P production is followed by a moderate loss of P. This does not occur when P, PN, and B react in Locke's solution.

The reaction dealt with here between P, PN, and *S. aureus* is similar in a good many respects to that investigated by Price for P, PN, and *S. muscae*. For example, in both cases P is produced without bacterial reproduction. There are, however, certain noteworthy differences: (*a*) PN increases the time of half-lysis for *S. muscae* and lessens it for *S. aureus*. (*b*) The yields of P in ranges of [P] and [PN] permitting P formation without bacterial reproduction are higher for *S. muscae* than for *S. aureus*. (*c*) An increase in [PN] from 33 units/ml. to 833 units/ml. greatly reduces the final plaque count secured with *S. muscae* as a substrate but has no discernible influence on the reaction when *S. aureus* is used.

Currently studies are in progress on the P-PN-B reaction in a synthetic medium in order to obtain information on the mechanism involved.

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