The Influence of Antibacterial Substances on the Interaction of Bacteria and Bacteriophages

1. The Influence of Penicillin

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SUMMARY: Penicillin in concentrations up to 100 units/ml. in broth or synthetic media has no demonstrable effect, after 20 hr. incubation at 37°, on the activities of *Staphylococcus* K phage, Coli-phage C 36, Coli-dysentery phage S13, a Streptococcal phage and a *Bacillus subtilis* phage.

The simultaneous action of penicillin and phage on young cultures of *Staphylococcus aureus* (Oxford) in broth or synthetic medium at 37° produces, under certain conditions, a more rapid lysis than occurs in the presence of penicillin or phage alone.

The phenomenon of accelerated lysis through the joint action of penicillin and phage occurs with other organisms besides *Staph. aureus*, e.g. *B. subtilis* and *Streptococcus pyogenes*, Group C, differing from that with *Staph. aureus* only in degree.

Penicillin does not affect the adsorption of phage by the organisms. When the amount of antibiotic is sufficient to interfere adversely with the growth of the cell then the multiplication of phage decreases. It is suggested that certain balanced intracellular reactions of metabolism are disturbed by the action of penicillin, and as a result, intermediates essential to growth both of cell and phage cease to be available.

A phage-inhibiting substance was demonstrable in certain instances when *Staph. aureus* (Oxford) cultures were lysed by penicillin.

Bacteriophages, or bacterial viruses, as they are now frequently called, are parasites requiring the environment prevailing within the actively multiplying host cell for their continued propagation. There are certain well-defined chemicals and also substances of biological origin, antibiotics, whose chemical structure is not in every case known, but all of which, in varying degree, interfere adversely with the normal metabolism of the bacterial cell, arresting growth and often causing death. Studies of the combined action of such antibacterial agents and phages on bacteria, therefore, may furnish evidence as to whether a particular substance interferes with the intracellular reactions prerequisite for the multiplication of the respective bacteriophages. The chemical nature of the interfering substances being known, some indication may be gleaned of the type of reaction involved, and of the underlying processes determining both bacterial and virus multiplication. It was with such possibilities in mind that the present studies were initiated.

Its effectiveness as an antibiotic against a wide range of organisms made penicillin an obvious choice for early investigation. It is now available in highly purified form, notably as the crystalline sodium or calcium salt (Report, 1945). The present paper describes a detailed study of the influence of penicillin upon the interaction of *Staphylococcus aureus* and an anti-staphylococcus phage. Its influence on some other organisms, and their respective phages has been studied, but in less detail.

MATERIALS AND METHODS

Organism. The Oxford 'H' strain of *Staph. aureus*, widely employed in penicillin studies in recent years, has been used, and the S3K strain originally obtained from Dr A. P. Krueger of the University of California. Both strains are readily susceptible to Staph. K phage, but the results recorded here refer to the Oxford strain unless otherwise stated. The culture media were papain broth, tryptic digest broth (Hartley, 1922) and the defined medium of Fildes & Richardson (1937). The growth curves have been determined both by the plate count of viable organisms and by the combined visual and photoelectric estimation of the turbidities of cultures.

Bacteriophage. The anti-staphylococcus bacteriophage Staph. K, also originally obtained from Dr Krueger, has been used throughout. Determination of phage concentrations have been made by the plaque-count method, employing 1% nutrient agar.

Penicillin. I am much indebted to my colleague, Mr P. Bruce White, for furnishing accurately standardized solutions of penicillin in phosphate buffer at pH 7. The concentrations of penicillin are expressed in terms of international units/ml. The early experiments were made with solutions of the calcium salt from commercial preparations of comparatively low grade, 150 u./mg. Later, solutions of the crystallized sodium salt of penicillin G—potency 1600 u./mg.—were used. The data presented, unless otherwise stated, are for the sodium salt. The solutions were sterilized by filtration through gradocol membranes of porosity 0.4–0.5 μ . Penicillin solutions may be filtered through such membranes without detectable loss of activity.

Abbreviations used. Staph. O = *Staphylococcus aureus*, Oxford strain; $[S]_0$ = initial concentration of staphylococci in organisms/ml.; P = penicillin; $[P]$ = concentration of penicillin u./ml.; $[\phi]_0$ = initial concentration of bacteriophage in particles/ml.; ϕ = bacteriophage; ϕI = phage-inhibitor.

THE ACTION OF PENICILLIN AND PHAGE ON
*STAPHYLOCOCCUS AUREUS**The action of penicillin on Staph. O*

Fleming (1929), in his original paper on the effect of penicillin on a culture of *Staph. aureus*, states 'the staphylococcus colonies became transparent and were obviously undergoing lysis'. This observation has since been supplemented by numerous studies of the action of penicillin under varied conditions, and many of the published facts have been confirmed in the course of the present work. All experiments were made with the staphylococcus culture in its logarithmic phase of growth, since not only is this most favourable for the bacteriophage but penicillin also has been found to exert its action most strikingly on the multiplying organism (Bigger, 1944; Hobby & Dawson, 1944*a, b*; Todd, 1945*a*; Fisher, 1946). On the addition of an appropriate amount, say 0.1 u./ml. of penicillin, to such a culture the organisms appear to multiply normally for a short period; then there is a decrease in the rate of

increase of viable organism, soon followed by a decrease in the viable count, which falls off rapidly. The turbidity increases less rapidly soon after the viable count has begun to fall, and finally decreases as lysis commences. This continues until the culture is relatively clear, only opalescent. The quantitative aspect of the action of penicillin in relation to its concentration is clearly shown in Fig. 3. The findings confirm those of Rantz & Kirby (1944), Nitti, Fossaert & Faguet (1944), Lee, Foley & Epstein (1944), and Todd (1945*a*), amongst others. Optical studies with the ultra-violet light microscope and also the electron microscope, supplementing the observations recorded in this paper (Smiles, Welch & Elford, 1948), conclusively demonstrate, in agreement with Gardner (1940), Smith & Hay (1942) and Fisher (1946), that the organism quickly swells in the presence of penicillin to almost twice its normal size before lysis.

The action of phage on Staph. O

Staph. aureus 'Oxford' was more readily adapted to grow in synthetic medium than the S3K strain. The course of phage action on this organism was very similar to that observed on S3K, the cultures in broth and synthetic media being lysed completely. The phage titres of such lysed cultures were invariably of the order 5×10^9 particles/ml.

The action of penicillin on Staph. K phage

Known concentrations of penicillin in (a) 0.9 ml. papain broth, and (b) 0.9 ml. defined medium, each received 0.1 ml. of a 1/100 dilution, in the corresponding medium, of Staph. K phage (0.7 μ . membrane filtrate of a lysed culture of Staph. O). The appropriate controls without penicillin were included, and all systems, in $3 \times \frac{5}{8}$ in. tubes with rubber caps, were incubated for 20 hr. at 37°. Then 1/10,000 dilutions were made in saline broth and plated in triplicate with Staph. O. The results of a number of such experiments showed that penicillin, in concentrations up to 100 u./ml. in broth or synthetic medium, and in one test up to 400 u./ml. in broth, was without significant effect on Staph. K phage. This agrees with the findings of Neter & Clark (1944), Himmelweit (1945), Jones (1945), Nicolle & Faguet (1947) and Rountree (1947).

The combined action of penicillin and phage on Staph. O

The first series of experiments made in 1944 demonstrated that young cultures of Staph. O, in broth or synthetic medium, were lysed more rapidly by the combined action of penicillin and phage than were control cultures containing either penicillin or phage alone. The observations in a typical experiment are given in Table 1. The accelerated lysis was so pronounced that a detailed study was undertaken. Meanwhile, similar observations were independently recorded by Himmelweit (1945), and confirmed and extended by Nicolle & Faguet (1947).

(i) *The effect of time of contact between Staph. O and penicillin before the addition of phage, on the times of lysis and yield of phage.* Five ml. amounts of

Staph. O culture in the logarithmic phase of growth in papain broth at 37° were inoculated with 0.1 ml. penicillin solution to give $[P]=0.1$. After chosen periods of incubation 0.1 ml. phage filtrate was added to each system as indicated in Table 2, which gives the phage concentrations after 1, 3 and 24 hr. Fig. 1 presents the turbidity data from photoelectric measurements in a

Fig. 1. Turbidity/time curves for *Staph. aureus* broth cultures inoculated with Staph. K phage after different periods of treatment with 0.1 u./ml. penicillin at 37° . $[Staph.]_0 = 4.4 \times 10^7/\text{ml}$. $[\phi]_0 = 1.5 \times 10^7/\text{ml}$. $P_{10, 20, 40, 80}$ = time (min.) culture had been incubated with penicillin before infection by phage.

similar experiment. Clearly, the phenomenon of accelerated lysis by $P + \phi$, although still present, becomes less pronounced as the initial contact with P is extended. The yield of phage decreases progressively with the time of exposure of Staph. O to penicillin. It is also of importance to note the maximum level of turbidity reached by the various systems before lysis sets in.

(ii) *The action of phage on a penicillin-resistant variant of Staph. O.* A penicillin-resistant (P.R.) variant of Staph. O was trained to grow in the presence of 40 u./ml. penicillin by Mr J. Bligh. This resistant variant grew more slowly and the colonies on agar were smaller and less opaque than those

Table 1. The combined action of 1.4×10^7 phage particles/ml. and varying quantities of penicillin on Staph. O in papain broth at 37°

Staph. O ([S] ₀ = 4.8×10^7) treated with	Turbidities at times (min.)											
	0	15	35	50	70	80	90	100	113	130	160	180
Nil	+	+	+	+	+	+	+	+	+	+	+	+
φ	+	+	+	+	+	+	+	+	+	+	+	+
[P] = 10	+	+	+	+	+	+	+	+	+	+	+	+
[P] = 10 + φ	+	+	+	+	+	+	+	+	+	+	+	+
[P] = 1	+	+	+	+	+	+	+	+	+	+	+	+
[P] = 1 + φ	+	+	+	+	+	+	+	+	+	+	+	+
[P] = 0.1	+	+	+	+	+	+	+	+	+	+	+	+
[P] = 0.1 + φ	+	+	+	+	+	+	+	+	+	+	+	+
[P] = 0.01	+	+	+	+	+	+	+	+	+	+	+	+
[P] = 0.01 + φ	+	+	+	+	+	+	+	+	+	+	+	+

±, +, +, +, + = slight to maximum turbidities of culture; - = no turbidity.

Table 2. Influence of time of contact between Staph. O ([S]₀ = 7.9×10^7) and penicillin ([P] = 0.1) before addition of phage ([φ]₀ = 2.5×10^7), on yields of phage

Time of contact of Staph. O with penicillin before adding phage (min.)	Phage particles/ml. after			
	1 hr.	3 hr.	24 hr.	
0	3.9×10^8	3.1×10^9	2.5×10^9	
5	2.3×10^8	6.7×10^7	4.5×10^7	
10	1.8×10^8	5.6×10^7	1.7×10^7	
20	1.08×10^8	1.6×10^7	3.1×10^6	
40	9.4×10^7	4.8×10^6	1.2×10^6	

of the normal sensitive organism. A broth culture of this P.R. variant was completely lysed by Staph. K phage at 37°. Demerec (1945) has reported a similar finding in his experiments with penicillin-resistant mutants of *Staph. aureus*. When our phage was titrated against P.R. Staph. on 1% agar the plaques were definitely larger (0.5–1 mm.) than those usually developed against Staph. O (0.1–0.25 mm.). The P.R. Staph. would appear to be abnormally sensitive to the phage, but the more probable explanation lies in the rate of propagation of the phage relative to the leisurely growth characteristic of this P.R. variant (cf. the interpretation of influence of penicillin on plaque size in next paragraph).

(iii) *Phage plated with staphylococci on agar containing penicillin.* Staph. K phage was plated with Staph. O on 1% nutrient agar containing known concentrations of penicillin. $[P] = 0.1$ completely inhibited the organism. The combined action of phage and penicillin resulted, under certain conditions— $[P] = 0.01$ —in the formation of larger plaques, 0.5–1.0 mm., than normally observed. It appears that, as observed in broth systems, penicillin retards the active growth of the staphylococci, and in effect, enhances the zone of influence of each phage particle so that cells become infected and lysed over a greater area before their age and concentration arrest the process.

(iv) *The influence of the initial concentration of staphylococci on the outcome of the combined action of penicillin and phage.* Staph. O in the logarithmic phase of growth in papain broth at 37° was diluted to give a serial range of concentrations 10^5 – 10^9 /ml. Four experimental systems were prepared for each $[S]_0$; $[\phi]_0 = 3 \times 10^7$ and $[P] = 1$ in all cases. The results are shown graphically in Fig. 2. This type of experiment provided much information. First, there is the quantitative aspect of the action of a given $[P]$ on different $[S]_0$. Secondly, the yield of phage from a standard $[\phi]_0$ in relation to various $[S]_0$ is shown, and thirdly, our chief interest, the yield of phage and the incidence of lysis when the same $[P]$ and $[\phi]$ act together on different $[S]_0$.

When $[S]_0$ is 10^9 the growth is poor; presumably competition for metabolites is so great that the organisms are unable to multiply many times. When $[P] = 1$ there is only a slight decrease in viable count, suggesting that either the antibiotic is deterred through the sluggish growth of the organisms, or, that the amount of penicillin is inadequate. The curves for the growth of Staph. O alone and for Staph. O plus penicillin demonstrate very clearly that penicillin acts with greatest effect on cultures in which the organisms are growing freely. Conditions most favourable for the multiplication of phage obtain when the number of actively growing organisms is greatest. The multiplication of phage in the presence of penicillin is diminished according to the extent to which the antibiotic has interfered with the activity of the organisms. The form of the curves showing the falling off of phage activity with time suggests that a phage-inhibiting substance may be present (see below).

In summary, therefore, the degree in which $[S]_0$ affects the outcome of the combined action of P and ϕ is a function of the vitality of the culture within the first few hours of incubation. Under the conditions of our experiment, i.e. for $[P] = 1$ and $[\phi]_0 = 3 \times 10^7$, lysis is accelerated most in the zone $[S]_0 = 10^7$ – 10^8 .

It may be noted that in these conditions each organism would be infected by a single particle (Delbrück & Luria, 1942). In more concentrated cultures the growth of *Staph. O* is less prolific and in consequence the lytic potency of *P* and ϕ is reduced, whereas for the lower values of $[S]_0$, when multiple infection by ϕ can occur, all sensitive organisms are rapidly eliminated even by ϕ alone.

Fig. 2. Influence of initial concentration of *Staph. aureus* on the outcome of the interaction with penicillin and phage. ○ = *Staph.* control, × = *Staph.* + penicillin, ⊙ = *Staph.* + phage, ▼ = *Staph.* + phage + penicillin. $[P] = 1$ u./ml. $[\phi]_0 = 3 \times 10^7$ /ml.

(v) *The influence of the concentration of penicillin.* The influence of $[P]$ has been investigated in both broth and defined medium with similar results. The curves in Fig. 3 show how, for a given $[S]_0$ and $[\phi]_0$, the development of the culture can be influenced by the presence of penicillin in concentrations ranging from 40 to 0.0004 u./ml. Little growth of the staphylococci occurs when $[P] = 0.4$ to 40 and visible lysis sets in sooner than in the control (*Staph. O* + *P*) or (*Staph. O* + ϕ) systems. The phage titre increases slightly during the first hour, but, thereafter, decreases as the action of penicillin combines to lyse the organisms. A notable increase in the development of phage occurs at $[P] = 0.04$, where the initial slope of the *Staph. O* growth curve approximates that of the control. However, it is not until the growth curve of the organism approaches even more closely that of the control that the yield of phage approaches that in the culture containing no penicillin. Even with $[P]$ as low as 0.0004 a broth culture of staphylococci is clearer in 4 hr. than in the absence of penicillin, although otherwise the curves are much alike.

Another interesting point concerns the shape of the curve showing the variation of ϕ concentration with time. There is a definite maximum after about 1 hr. for $[P]=0.4-40$, but at $[P]=0.04$ the maximum is much later and less pronounced, while for yet smaller $[P]$ the curve becomes very like the

Fig. 8. Influence of penicillin concentration on the interaction of penicillin and phage on *Staph. aureus*. $\circ$ = concentration of phage, $\bullet$ = turbidity of $S+P$, $\times$ = turbidity of $S+P+\phi$. $[Staph.]_0 = 6.5 \times 10^7/\text{ml}$. $[\phi]_0 = 4.9 \times 10^7/\text{ml}$.

control where $[P]=0$. This falling off in phage titre in the presence of penicillin (see also Tables 2 and 8) may be explained in one of two ways: (a) the lysis of the organisms may be accompanied by the liberation of a phage-inhibiting substance (ϕI); or (b) phage becomes irreversibly adsorbed to cellular debris and so is not available for infecting susceptible organisms. Early experiments

with a low-grade penicillin gave evidence favouring the former of these interpretations, since the presence of ϕI was readily demonstrated in 0.7μ . membrane filtrates of the staphylococcus culture lysed by penicillin. The filtration end-point for ϕI was close to that of the phage itself—namely $100 \text{ m}\mu$. It was found, furthermore, that the ϕI could be precipitated from the filtrate at 0° by adding 60 % ethanol, and this provided a means for its partial purification and concentration. A preparation concentrated in this way showed an interesting

Table 3. *The multiplication of phage in Staph. O cultures ($[S]_0 = 8 \times 10^8$) with and without 1 u./ml. penicillin, for various values of $[\phi]_0$*

$[\phi]_0$	Ratio of final to initial concentrations of phage after			
	8 hr.		24 hr.	
	Without penicillin	With penicillin	Without penicillin	With penicillin
8×10^8	28	7	33,000	13
8×10^5	50	10	730	5
8×10^7	32	6	18	4.7
8×10^9	3	1.8	1.9	0.4

dilution phenomenon. The undiluted fluid exhibited only a slight ϕ -inhibiting power, but when diluted 10- or 100-fold it proved strongly inhibiting for ϕ , suggesting that the medium might contain a substance which blocked the action of ϕI substance. This was found to be the case, since by treating the papain broth with 60 % alcohol a precipitate was formed, which, when taken up in water was found to possess the property of blocking the action of ϕI . In experiments, however, with purified penicillin acting on the staphylococci in papain broth or defined medium, though the culture was lysed, no clear evidence of the presence of ϕI in 0.7μ . membrane filtrates could be obtained, although the opalescent unfiltered liquid was strongly inhibitory. Two possible explanations of this change in behaviour were considered. The low-grade penicillin might have contained as an impurity an enzyme which, in combination with penicillin, facilitated the more complete disintegration of the cell structure than can be achieved by penicillin alone. Unfortunately, the batch of penicillin with which the early experiments were made was exhausted before the matter could be thoroughly investigated. An alternative explanation may be sought in mutation of the organism yielding a variant with altered susceptibility to penicillin. Evidence that mutation can occur has been met twice during the studies with Staph. O in broth and synthetic medium, colonies manifested by their subnormal size (s.c.) accompanying normal-sized colonies. On subculturing, the s.c. variant was found to be more sensitive to penicillin and also yielded larger plaques with Staph. K phage—1 mm. as against the usual 0.25 mm . This variant reverted after a period to the normal colony type, and the phage plaques likewise reverted to normal small size. The s.c. variant lysed by penicillin and then filtered through 0.7μ . membrane yielded a filtrate that reduced the ϕ -titre by 75 % during overnight incubation at 37° . A phage-inhibiting substance has been extracted from autolysed staphylococci by Levine & Frisch (1932) and Gough & Burnet (1934), and also from staphy-

lococci digested with trypsin by Freeman (1937). Burnet & Gough concluded that the inhibiting substance was a complex polysaccharide. It is interesting that filtration indicated the size of the inhibiting substance liberated by penicillin to be of the same order as that of the phage itself.

(vi) *The influence of the initial concentrations of phage.* The phenomenon of accelerated lysis of Staph. O cultures through the joint action of penicillin and bacteriophage was most clearly manifested within a range of $[\phi]_0 = 10^7$ to 10^9 when $[S]_0 = 10^7$ to 10^8 , i.e. for single particle infection of organism by phage. Where multiple infection could occur, $[\phi]_0 > 10^9$, the times of lysis with and without penicillin did not differ significantly. On the other side of the optimum zone the phage can infect initially only a fraction of all organisms, and hence lysis is much delayed.

The profound effect of penicillin on the multiplication of the phage is well illustrated by the ratio of the concentration of phage after 3 and 24 hr. to the initial phage concentration (Table 3). It is also interesting to note that in addition to the inhibition of phage following lysis of staphylococci when penicillin is present, so also, for high concentrations of phage alone, when lysis is complete, partial inactivation of phage can apparently occur.

THE EFFECT OF PENICILLIN ON THE ADSORPTION OF PHAGE ON STAPHYLOCOCCI

Adsorption experiments were made in Hartley's broth and defined medium, and in each instance there was no significant difference between the amount of phage adsorbed by normal staphylococci and organisms that had been exposed to the action of penicillin in concentrations up to 5 u./ml. for periods up to 2 hr. It is unlikely, therefore, that penicillin interferes with the initial step in process of infection of staphylococci by phage. Rountree (1947) found that staphylococci after 3 hr. contact with 5 u./ml. penicillin at 37° but not lysed by it, adsorb phage as readily as normal staphylococci.

Inactivation of penicillin by cysteine

It was confirmed that cysteine in appropriate concentration inactivates penicillin at pH 6.8–7.0. Penicillin so treated no longer affects the lysis of staphylococcal cultures in the presence of phage.

The filterability of Staph. K phage from culture lysed by the joint action of penicillin and phage

The filtration end-point for phage from Staph. O culture lysed by penicillin plus phage was the same as for phage propagated in absence of penicillin, namely 100 m μ .

The effect of penicillin on burst size

The experimental procedure described by Delbrück & Luria (1942) was used to ascertain whether penicillin has any effect on (a) the latent period elapsing between the moment of infection and the liberation of phage following lysis of the organism; and (b) the 'burst size' or, the average number of phage particles

liberated per infected cell. The results are summarized in Table 4, and some typical step-wise growth curves are given in Fig. 4. It will be seen that when $[P] = 0.1$ or less, in a concentration known to be definitely inhibitory for the continued growth of the staphylococci, the process of infection and phage

Table 4. *Burst sizes for Staph. O lysed by Staph. K ϕ with and without penicillin*

Medium	Latent period (min.)	Burst size	$[P]$	$[S]_0$	$[\phi]_0$	Remarks
Hartley's broth	39	47	—	2×10^8	1.2×10^7	2nd rise after 36 min.
Hartley's broth	38	52	0.01	2×10^8	1.2×10^7	2nd rise after 31 min.
Hartley's broth	38	54	—	2.75×10^8	1.1×10^7	2nd rise after 33 min.
Hartley's broth	35	49	0.1	2.75×10^8	1.1×10^7	No 2nd rise in $1\frac{1}{2}$ hr.
Hartley's broth	38	45	—	2.3×10^8	9.2×10^6	2nd rise after 33 min.
Hartley's broth*	34	47	0.1	2.3×10^8	9.2×10^6	Gradual rise after 40 min.
Hartley's broth	40	40	—	1.4×10^8	9.2×10^6	2nd rise after 24 min.
Hartley's broth*	37	21	2.0	1.4×10^8	9.2×10^6	No 2nd rise in 2 hr.
Synthetic	65	25	—	5×10^8	1.8×10^7	2nd rise not investigated
Synthetic	65	23	0.2	5×10^8	1.8×10^7	—
Synthetic	60	15	0.5	2.6×10^8	1.8×10^7	No 2nd rise in 4 hr.
Synthetic	60	24	—	5.8×10^8	1.8×10^7	2nd rise after 180 min.

* Penicillin present during initial adsorption.

Fig. 4. Step-wise growth curves for Staph. K phage on *Staph. aureus* in broth, with and without penicillin.

multiplication appears to proceed with little if any departure from the normal course. The first abnormality noted as $[P]$ is increased is the belated onset of the second step-wise increment in phage concentration and its eventual elimination. Ultimately, the average yield of phage per infected cell is reduced, but the latent period is not appreciably affected. Clearly, if all the cells are infected initially their lysis would be expected to occur simultaneously in (Staph. O + ϕ) and (Staph. O + $P + \phi$) systems. This has been observed to be so when conditions of multiple infection prevail. The general effect of penicillin on the course of phage action is the same in broth and synthetic medium. The mean generation time of Staph. O in defined medium was 40–45 min.

compared with 22–25 min. in papain broth. The corresponding latent periods of phage multiplication were 60–65 min. and 35–38 min., and the ‘burst sizes’ 25 and 48 respectively.

THE INFLUENCE OF PENICILLIN AND PHAGE ON ORGANISMS OTHER THAN *STAPHYLOCOCCUS AUREUS*

The action of penicillin on other bacteriophages. Tests similar to those described for Staph. K phage were made with coliphage C36, a coli-dysentery phage S13, a subtilis phage and a streptococcus phage. Concentrations of penicillin up to 100 u./ml. in broth medium were without significant action on these bacteriophages, after 20 hr. at 37°.

The combined action of penicillin and phage

Bacillus subtilis. The strain of *B. subtilis* and its phage were received through the kindness of Dr Wahl of the Pasteur Institute, Paris. The phenomenon of accelerated lysis was readily demonstrable in young broth cultures of *B. subtilis* at 37°, infected by phage in the presence of 10 u./ml. penicillin.

Bact. coli. The strain of *Bact. coli* used was partially inhibited when penicillin (100 u./ml.) was added to a young broth culture at 37°. The onset of lysis in the presence of phage C36 and penicillin 100 u./ml. was only very slightly earlier than that occurring with phage alone. The quantitative estimation of viable organisms and phage confirmed that the penicillin was exerting a definite antibacterial effect.

Shigella flexneri Y6R (National Collection of Type Cultures). Penicillin definitely inhibited *Shig. flexneri* Y6R growing logarithmically in broth at 37° when the concentration of antibiotic reached 100 u./ml. With $[P]=100$ and S13 phage there was no evidence of accelerated lysis. However, the yield of phage was low, undoubtedly a consequence of the antibiotic action exerted by the penicillin.

Streptococcus pyogenes, Group C, type 7. Streptococcus C, type 7, growing actively in yeast-Lemco-broth at 37°, was inhibited by 0.02 u./ml. penicillin. Accelerated lysis by penicillin plus phage was readily demonstrated. Further, the onset of lysis with $(P+\phi)$ was strikingly sharp. The multiplication of ϕ was decidedly less in the presence of penicillin than in the control.

Thus, though the phenomenon of accelerated lysis by penicillin and phage appeared to be general for organisms readily susceptible to the antibiotic, there were differences of degree according to the organisms concerned.

DISCUSSION

The most generally accepted view is that penicillin exerts its antibiotic action by interfering, directly or indirectly, with some essential reaction in the metabolism of the cell. Several writers have suggested that penicillin modifies the permeability of the cell wall, and, in consequence, the assimilation of essential growth factors (Smith & Hay, 1942). Recently Gale & Taylor (1946) have shown that penicillin does influence the assimilation of glutamic acid by

Staphylococcus aureus. The upset of oxidation-reduction balance within the cell has been considered by others to be the basis of the mode of action of penicillin (Mulè, 1946; Krampitz, Green & Werkman, 1947; Dufrenoy & Pratt, 1947). The course of action of penicillin proceeds thus—arresting of growth → loss of viability → final lysis. The lytic phase has been attributed by Todd (1945*b*) and Fisher (1946) to the action of bacterial autolysin, which, when growth ceases, becomes exceptionally active. Now that our knowledge of the chemical nature of penicillin is fairly complete, extended studies of the type of reaction into which it can enter (e.g. interference with normal function of —SH groups) should eventually throw light on its interaction with the cell. In contrast to penicillin, which is a relatively simple substance chemically, bacteriophage is much larger and more complex. The few phages so far submitted to analysis have been found to be nucleoproteins. Essentially a bacterial parasite, the phage finds conditions for its growth satisfied by the intracellular environment, but the precise requirements have not yet been elucidated. Some intermediate in the cell's synthetic processes (nucleic acid metabolism?) may be also a substrate for which the phage actively competes. As a result of infection by phage the cell generally succumbs and is finally lysed.

When a young culture of *Staph. aureus* is attacked simultaneously by penicillin and Staph. K bacteriophage, it is lysed more rapidly than when penicillin or phage acts alone. Certain conditions of infection are needed to ensure the ready demonstration of this striking effect. The concentration of the young culture should be 10^7 – 10^8 organisms/ml. and the initial concentration of phage such that most of the organisms become infected. When $[\phi]_0 \gg [S]_0$ then almost all the cells will be infected by at least one phage particle and no appreciable acceleration of lysis is observed. If, however, the clearing of the culture is dependent on at least two cycles of infection then the accelerated lysis by $P + \phi$ is readily demonstrated. This suggests that the staphylococci which have been exposed to the action of penicillin are more easily lysed by phage than normally. A phage-free ultra-filtrate of a lysed Staph. O culture does not accelerate lysis of Staph. O in the presence of penicillin; neither does heat-inactivated phage. The accelerated lysis is, therefore, the outcome of the combined action of intact phage and penicillin. Detailed analysis of this joint action has shown that:

(i) The presence of penicillin, in concentrations far in excess of that inhibitory to cell growth and acting for periods longer than the mean generation time, does not influence the adsorption of phage by the cell. This indicates that the phage receptor groups are not affected, and any modification in the course of infection should be looked for in an altered rate of penetration and possible changes in the intracellular processes.

(ii) When penicillin has seriously affected the growth of the cell then the yield of phage falls. This supports the hypothesis that phage is dependent upon some intracellular intermediate as substrate in its own multiplication.

(iii) Cells adversely affected by penicillin, while unable to support the normal rate of phage multiplication are, nevertheless, lysed, perhaps even more rapidly than normally, certainly more abruptly.

(iv) The determination of growth curves for Staph. K phage in cultures of Staph. O, with and without the presence of penicillin in different concentrations and acting for different periods, have shown that the liberation of phage from those organisms initially infected occurs in bursts in all cases and after a constant latent period for given cultural conditions.

Penicillin at concentrations known to inhibit the continued growth of staphylococci does not significantly affect the rate and degree of multiplication of phage within the cells initially infected. Strictly, inhibition and eventual loss of viability may mean that the cell is so affected that it cannot divide more than once or twice and thus is rendered incapable of forming a visible colony. The fact that penicillin does not modify the first step in the phage growth curve is then not surprising. Penicillin in higher concentrations, however, does influence the yield of phage by each infected cell. After it has acted on the cell for periods comparable with the latent period of phage multiplication and mean generation time of the organisms, then the step-wise liberation appears to be replaced by a gradual process and finally there is no evidence of phage increase. Compared with the control, the yield of phage is very much reduced. Here, maybe, the $[P]$ is such that the synthesis of phage-precursor or essential substrate is rapidly retarded. This interpretation accords with the observations of Parker & Marsh (1946) on the lethal action of penicillin on staphylococci. In the (Staph. O + $P + \phi$) systems, cells not initially infected by ϕ are continuously under the influence of penicillin, whereas the corresponding cells in the control can multiply logarithmically. Hence the actual amount of 'lytic work' to be done by ϕ is greater in the absence of penicillin. The observed more rapid clearing of a Staph. O culture in ($\phi + P$) systems than in systems where P or ϕ acts alone, is attributable, therefore, to the following factors:

(1) Penicillin inhibits the growth of the organisms initially not infected with phage so that at each stage lysis in the presence of penicillin produces a proportionally greater reduction in the turbidity.

(2) Cells whose growth has been affected through the action of penicillin, while they no longer support the normal multiplication of phage are, nevertheless, equally susceptible to lysis.

(3) The lytic potencies of penicillin and phage are supplementary.

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