THE EFFECT OF IODOACETATE AND OTHER INHIBITORS ON PHAGE PRODUCTION AND LYSIS IN THREE PHAGE SYSTEMS*

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INTRODUCTION

This paper is concerned with the effects of minimal bacteriostatic concentrations of iodoacetate, azide, and proflavine on phage production and lysis in three host-virus systems, the *B. mycoides* N, *E. coli* B, and *M. aureus* 145. The results obtained by adding inhibitors at intervals during the latent period are compared with those of Foster (1948) using proflavine and of Bozeman *et al.* (1954) using chloramphenicol in the T phage systems.

Materials and Methods

1. Phage Assay Technique.—The Gratia layering method of plaque counting (1936) was used in the *B. mycoides* system and the Jones-Krueger (1951) slide technique in the *E. coli* and *M. aureus* systems.

2. Source of Inhibitors.—Proflavine sulfate (2,8-diaminoacridinium monohydrogen sulfate) was obtained from the Allied Chemical and Dye Corporation, iodoacetic acid from the Eastman-Kodak Co., and sodium azide from the Paragon Laboratories.

3. Experimental Cultures.—Lag phase cultures of *B. mycoides* or *E. coli* were prepared by growing the organism in Difco nutrient broth on a shaker at 30°C. for 18 hours and diluting with fresh broth to the desired concentration. Log cells of *M. aureus* were prepared by shaking lag cells at 37°C. in medium III (Ralston and Krueger, 1952) for 2 hours.

4. Inhibitor Studies.—Experiments on phage formation during the latent period were done with singly infected cells by the one-step growth method; those on the time of lysis were run on multiply infected, turbid suspensions. In both cases, adsorption was carried out in an ice bath for 1 to 2 hours. The adsorption mixture (diluted suitably in single infections) was distributed to a number of tubes which were shaken at 30 or 37°C. to allow phage multiplication to proceed. The inhibitor was then added to tubes at intervals during the latent period. Determinations of the degree of lysis and phage content of the treated tubes were made on samples removed at the time

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that normal lysis of an untreated control occurred. In addition, samples were withdrawn from one tube without drug at intervals during the latent period and plated immediately for plaque count.

RESULTS

1. Effect of the Minimal Bacteriostatic Concentration on Free Phage, on Adsorption, on Lysis, and Phage Yield When Added at the Onset of Infection

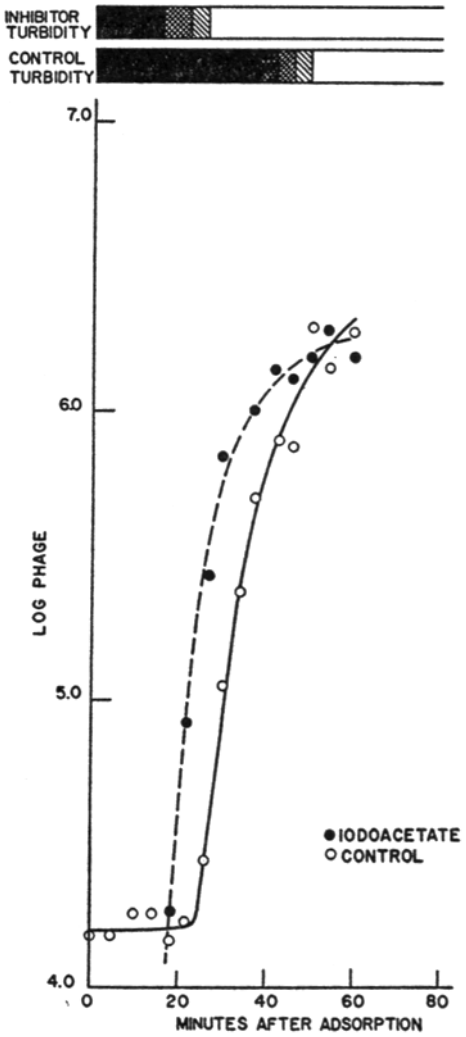
Iodoacetate was tested in all three phage systems; proflavine and azide in the coliphage system only. In every case, the minimal bacteriostatic concentration proved to be that concentration which completely inhibited phage production when added at the start of phage infection. (The minimal bacteriostatic concentration of iodoacetate for *B. mycoides* and *M. aureus* was M/1000; for *E. coli*, M/200; of proflavine, 0.0065 mg./ml. for *E. coli*; and of azide, M/5 for *E. coli*.) In the *mycoides* and coliphage systems, these concentrations also inhibited lysis completely when introduced at the onset of infection. In all systems, these concentrations had no effect on free phage or adsorption.

2. Effect of the Minimal Bacteriostatic Concentration of Iodoacetate, Azide, or Proflavine on Final Phage Yield When Added at Intervals during the Latent Period

When the minimal bacteriostatic concentration was added during the first part of the latent period of virus in *B. mycoides* or *E. coli*, no plaque-forming particles were released. If this concentration was added during the last part, there was a linear increase in phage that progressively approached the yield obtained in the absence of inhibitor (Figs. 1 to 4). The same findings hold for the *M. aureus* system (Fig. 5), except that phage was released from cells to which inhibitor had been added much earlier than in the other phage systems.

3. Effect of the Minimal Bacteriostatic Concentration on Lysis When Added at Intervals during the Latent Period

When the minimal bacteriostatic concentration was added at intervals during the latent period of phage formation in *B. mycoides* or *E. coli*, the tubes containing inhibitor could be divided into three sets at the time of normal lysis: (1) no lysis in tubes to which inhibitor was added early, (2) partial lysis in tubes to which inhibitor was added in the middle of the latent period, (3) complete lysis in tubes to which inhibitor was added late (Figs. 1 to 4). Lysis occurred at the same time in all tubes in set (3)—with iodoacetate, a few minutes earlier than that of the control. The time interval covered by the samples in set (2) corresponds to the length of time necessary for a control suspension to clear, *i.e.* if it took 10 minutes from the onset to completion of lysis of infected cells in the absence of drug, then there was a 10 minute interval in the middle of the latent period during which the drug caused partial lysis.



FIGS. 1 to 6. Turbidities, black column represents turbidity equal to that at beginning of latent period; white means complete clearing; triple cross-hatching, onset of lysis; double cross-hatching, marked reduction in turbidity; single hatching, almost clear.

FIGS. 1 to 3. Control, samples were withdrawn at intervals during the latent period, and the phage content and turbidity of each sample were determined immediately.

Inhibitor, inhibitor was added to samples at intervals during the latent period; all samples were incubated (37°C.) until lysis of control occurred. At this time, phage content and turbidity of all samples were determined.

FIG. 1. Addition of M/250 iodoacetate during latent period of phage T2 in *E. coli* B.

In the staphylococcal system, occasional, partial lysis occurred even when the drug was added at the onset of infection. This lysis may be entirely un-

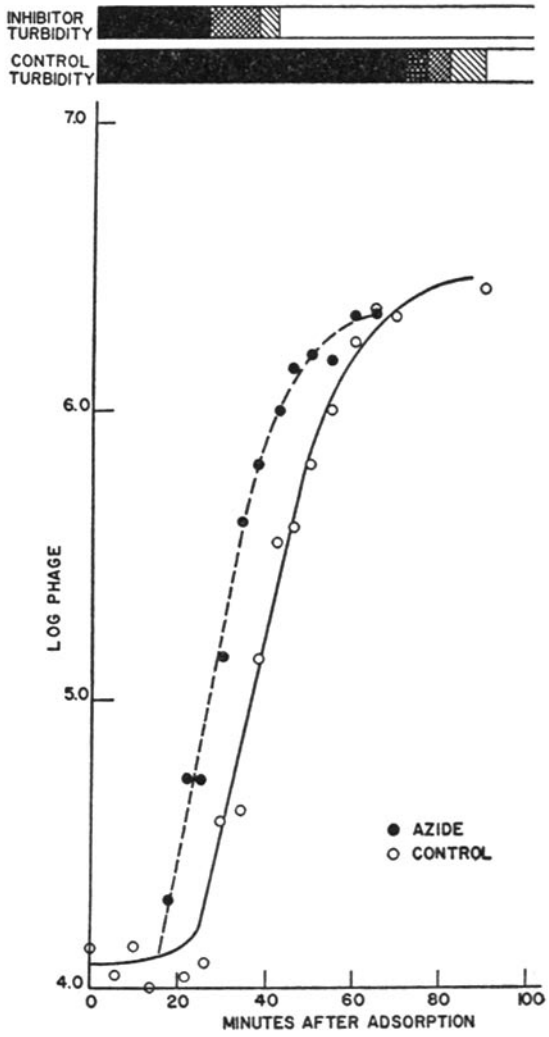


FIG. 2. Addition of M/5 azide during latent period of phage T2 in *E. coli* B.

related to the effect of iodoacetate on phage infection (Ralston, personal communication) and will be discussed elsewhere. In contrast to the other systems, in some experiments, cells of *M. aureus* exposed to iodoacetate quite early (but not at the beginning) in the latent period cleared completely (and also released phage). In other experiments, the amount of lysis was propor-

tional to the time of addition of inhibitor (the later the addition, the greater the amount of final lysis).

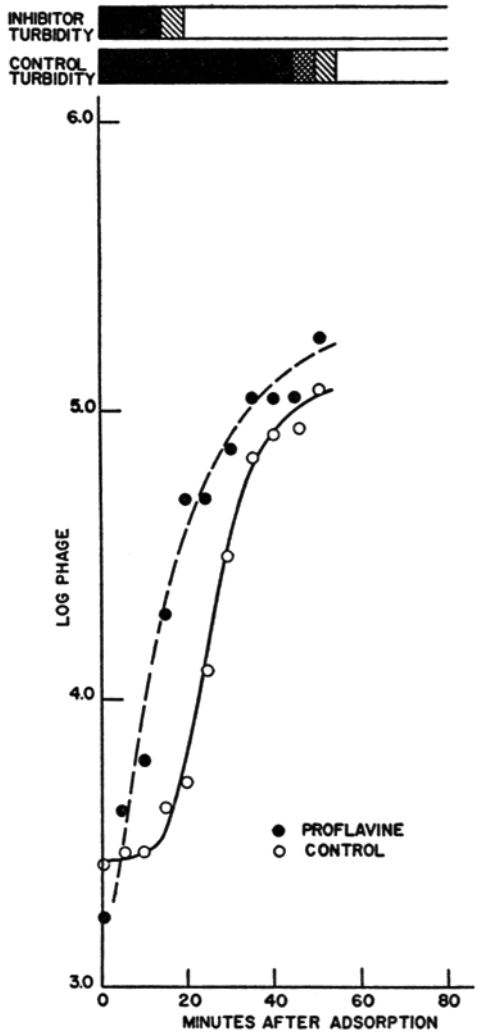
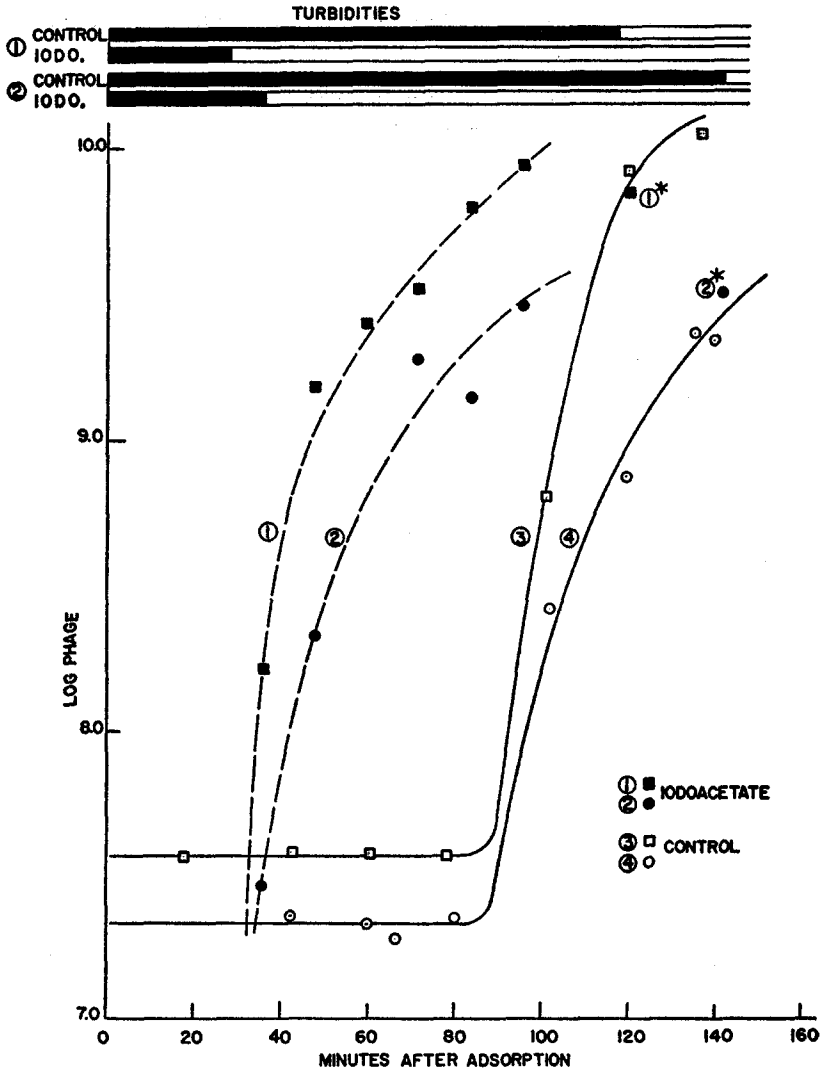


FIG. 3. Addition of 0.0065 mg./ml. proflavine during latent period of phage T2 in *E. coli* B.

These results indicate that the minimal bacteriostatic concentrations of iodoacetate, azide, and proflavine had effects similar to those of chloramphenicol (Bozeman *et al.*, 1954) on free phage, adsorption, on lysis, and final phage yields, when the inhibitors were added at intervals during the latent



FIGS. 4 to 6. Control, sample withdrawn at time indicated. Each sample plated for plaque count at once; turbidity also determined.

Inhibitor, inhibitor added to sample at time indicated. All samples incubated at 37°C. until lysis of control was observed. At this time, phage content and turbidity of all samples were determined.

FIG. 4. Addition of M/1000 iodoacetate at intervals during latent period of phage N in *B. mycoides* N (Experiments 1 to 4 run on different days.).

*① and *② represent control yield at time indicated in Experiments 1 and 2.

period. The effects of these inhibitors were also similar to those reported by Foster (1948) for a subbacteriostatic concentration of proflavine, except that

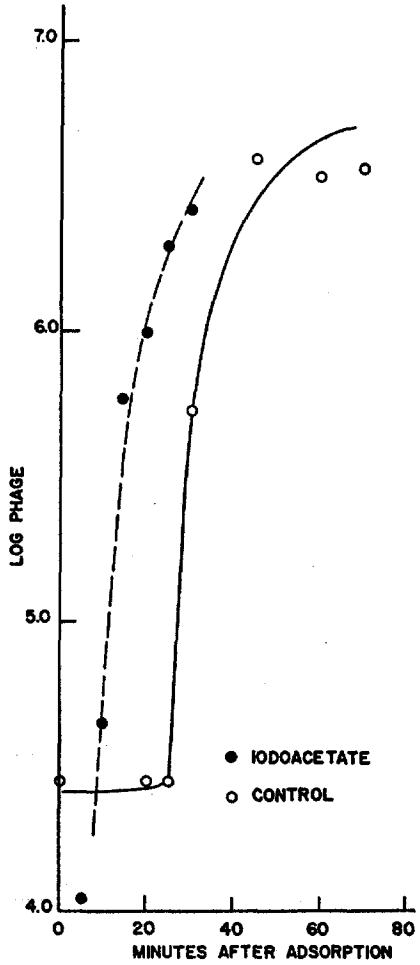


FIG. 5. Addition of M/1000 iodoacetate at intervals during latent period of phage 14 in *M. aureus* 145.

she found proflavine did not inhibit lysis even when added at the beginning of the latent period.

Other points of resemblance between the action of chloramphenicol and of the inhibitors tested by us are the following:

(*a*) *Similar Lack of Bactericidal Action.*—If *E. coli* and *B. mycoides* cells are exposed to minimal bacteriostatic concentrations of chloramphenicol

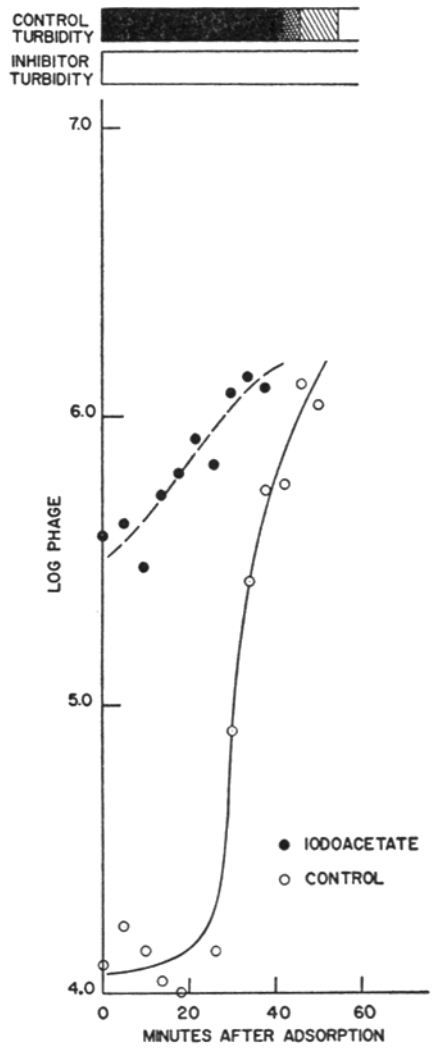


FIG. 6. Addition of M/2500 iodoacetate at intervals during latent period of phage T2 in *E. coli* B.

(Bozeman *et al.*, 1954) and iodoacetate (Baer, 1951) respectively for a time equal to the latent periods of their phages, they form about as many colonies as untreated cells.

(*b*) *Reduced Rate of Phage Synthesis by Subbacteriostatic Concentrations of Inhibitor.*—In the coliphage system, subbacteriostatic concentrations of proflavine, iodoacetate, azide (Fig. 6), and chloramphenicol (Edlinger, 1951)

allow continued phage growth at a reduced rate, when the inhibitor is added at intervals during the latent period.

(*c*) *Similar Effects Following Removal of Drug during Latent Period.*—When drug (iodoacetate in the *mycoides* phage system (Baer, 1951); chloramphenicol with coliphage (Bozeman *et al.*, 1954)) was introduced with the phage and then removed early in the latent period, the burst was delayed and the final phage yields were less than that of an untreated control. When the drug was removed past the middle of the latent period, there was a loss of infective centers (rapid in the *mycoides* system, slower in the *coli* system).

d. Parallel Effects of Increasing Subbacteriostatic Concentrations on Cell Growth and Phage Formation.—"Increasing but still subbacteriostatic levels of chloramphenicol have an essentially parallel inhibitory effect on the multiplication of phage in infected cells and on the growth of uninfected cells" (Bozeman *et al.*, 1954). This is also true of iodoacetate in the *B. mycoides* system (Baer, 1951) and of proflavine, azide, and iodoacetate in the *E. coli* system (unpublished data).

DISCUSSION

Anderson and Doermann (1952) showed that the phage yield of *E. coli* cells broken open prematurely by exposure to ultrasonic vibration at a given time equalled the final yield obtained by adding proflavine at that time. They concluded that proflavine limited the level of mature phage to that present at the time inhibitor was added. In chloramphenicol-inhibited cells, premature sonic disruption failed to show any increase in phage over the final yield obtained when chloramphenicol was added (Bozeman *et al.*, 1954). Therefore, chloramphenicol also served to titrate intracellular phage.

For the following reasons, we suggest that the final phage yields obtained with iodoacetate, azide, and proflavine in the *B. mycoides*, *E. coli*, and *M. aureus* systems also represent the intracellular phage completed prior to treatment with inhibitor:

1. There is a striking identity between the action of these inhibitors and that of chloramphenicol in almost every respect.

2. There is a close resemblance between the effect of proflavine (Foster, 1948) and that of these inhibitors during the very part of the latent period when the formation of mature phage is known to take place in the T2 phage system.

3. The increase in the final phage yields with successively later additions of drug cannot be explained as an interference of the drug with lysis. When the drug was added late, the final lysates obtained were all very clear, especially with iodoacetate, yet they often varied more than tenfold in their phage content (Figs. 1 and 4). This could not be the case if these lysates contained varying proportions of lysed cells which yielded a normal burst and of unlysed

cells which released no phage. (When inhibitor was added during the middle part of the latent period the lysates did show varying degrees of turbidity. The titers of these lysates were usually low; such lysates were interpreted as consisting of cells which had lysed, releasing one or two particles per bacterium and those which did not lyse.)

There is a differential effect of inhibitor on lysis and on phage production at different times during the latent period. If inhibitor is added late, only phage production is affected—not lysis. Therefore, phage synthesis and lysis proceed independently during the last stages of phage infection. Added early, inhibitor stops both lysis and phage production. (In chloramphenicol-inhibited cells, disrupted with ultrasonic waves, no phage was found. In a few experiments in the *B. mycoides* system, cells—exposed to iodoacetate early in the latent period—lysed much later than the control but no phage was found in these lysates.) Hence, lysis does depend on whether phage synthesis reaches a critical stage. This stage in time coincides with the appearance of the first phage particles in the chloramphenicol-, iodoacetate-, and azide-inhibited systems. Once this point is reached, lysis will take place at the normal time regardless of the number of mature particles formed.

Our results differ from Foster's (1948) in two respects. In her work, a subbacteriostatic concentration of proflavine inhibited phage production when inhibitor was introduced at the onset of infection. This concentration had no effect on lysis. In our experiments, only bacteriostatic concentrations of proflavine stopped production of active phage when the drug was added at the beginning of the latent period. These concentrations prevented lysis.

Foster (1948) described proflavine only as "diaminoacridinium sulfate," and she referred to earlier studies of Fitzgerald and Babbit (1946) with this compound. Fitzgerald *et al.*, used the 2,8-diamino compound. For this reason, we also worked with the 2,8-diamino compound. A later paper on proflavine action by De Mars *et al.* (1953) revealed that Foster apparently worked with 2,6-diaminoacridinium sulfate. This may explain the difference in our results.

Also, our T2r phage may be a variant of that used by Foster. Phage adsorption was very slow in the glucose synthetic medium used by Foster. For this reason perhaps, a high initial phage to bacterium ratio of infection was necessary for visible lysis. In addition, when the drug was introduced at the beginning of the latent period, higher concentrations of proflavine were needed to stop phage production in glucose synthetic medium than in nutrient broth. The results, however, were similar in both media and resembled those with iodoacetate and azide in nutrient broth.

The use of an inhibitor such as iodoacetate might prove to be a simple and convenient tool for titrating mature intracellular phage. Inhibitor could be used with any size of sample, and there would be a wide margin of safety between the concentration which stops phage production and that which interferes with lysis. The 2, 8-diaminoacridinium sulfate produced rather

erratic results from day to day, while iodoacetate gave satisfactory, reproducible findings. Minimal bacteriostatic concentrations of all three inhibitors produced variable degrees of lysis when inhibitor was added during the middle of the latent period.

SUMMARY

The minimal bacteriostatic concentration of iodoacetate, azide, or proflavine was added at intervals during the latent periods of virus in three different bacterium-bacteriophage systems (*M. aureus*, *B. mycoides*, *E. coli*). For each interval at which inhibitor was added, the occurrence of lysis and the final yield of phage were determined. In the *B. mycoides* and *E. coli* systems, when added during the first part of the latent period, inhibitor prevented lysis and no phage was released. Introduction of inhibitor during the last part of the latent period resulted in normal lysis and in a linear increase in phage that progressively approached the yield obtained in the absence of inhibitor (the later the introduction, the higher the yield). In the *M. aureus* system, phage production and lysis in the presence of inhibitor followed the same general pattern, except that release of phage and normal lysis occurred in infected cells to which inhibitor had been added quite early in the latent period.

Our results, when compared with those of Foster (1948) with proflavine and Bozeman *et al.* (1954) with chloramphenicol, suggest that (1) the final phage yields represent the amount of mature intracellular virus present at the time of addition of inhibitor and (2) the reactions leading to lysis proceed independently of those leading to the formation of mature virus once phage infection has reached a critical point in time.

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