

THE INACTIVATION OF BACTERIOPHAGE BY MERCURY BICHLORIDE; THE REACTIVATION OF BICHLORIDE-INACTIVATED PHAGE

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(Accepted for publication, July 16, 1933)

There are at the present time two schools of thought with reference to the nature of bacteriophage. One group, championed chiefly by d'Herelle and Burnet, considers phage to consist of living "corpuscles," while the other group views the lytic principle as partaking of all the major characteristics of an enzyme.

A study of the kinetics of the bacterium-bacteriophage reaction by Krueger and Northrop (1, 2), together with additional data reported by Krueger concerning first, the sorption of bacteriophage by living and by dead bacteria (3) and second, the heat inactivation of bacteriophage (4), gave indirect information tending to support the enzyme concept of phage. During further experimental work one of us (A. P. K.) on several occasions noted apparent significant reversals of phage inactivation. These data were interpreted as indicating either the independent multiplication of residual active phage in the absence of growing bacteria—a phenomenon not hitherto proven to occur—or the reactivation of inactivated phage, a reaction common to many enzymes. The present paper details experiments undertaken to investigate the inactivation of antistaphylococcus bacteriophage by HgCl_2 and the reversal of the process.

Methods

An antistaphylococcus phage and a single strain of *S. aureus* described in previous papers were used (1-6). The medium was beef infusion broth containing 1 per cent peptone, 0.5 per cent sodium chloride, adjusted to pH 7.6. All phage titrations were performed according to the method described by Krueger (5, 6).

The titration procedure rests upon the observation that the time of lysis in a standard bacterial suspension under standard conditions is a function of $[P]$,

the initial concentration of phage. Since the initiation of lysis depends upon the attainment of a critical P/B ratio and since also $dP/dt \propto C \times dB/dt$, it is clear that any possible inhibition of bacterial growth by $HgCl_2$ in the titration set-up must be ruled out. As a means of determining the optimal dilutions for phage titration the growth of the bacterium in broth to which had been added various amounts of $HgCl_2$ was followed. It was found that a concentration of 1:10,000,000 of $HgCl_2$ exerted no measurable inhibition on the growth of the organism. Consequently throughout the experiments the phage determinations were carried out with dilutions containing 1:10,000,000 or less of $HgCl_2$.

1. *The Inactivation of Phage by $HgCl_2$.*—5 ml. of 1:5,000 $HgCl_2$ were added to an equal volume of standard phage containing 1×10^{10} activity units per ml. The mixture was maintained at 22°C. and samples for phage titration were removed at intervals; they were at once diluted 1:1,000 with broth.

2. *The Reversal of Phage Inactivation by $HgCl_2$.*—5 ml. of standard phage were mixed with 5 ml. of 1:5,000 $HgCl_2$. The mixture was kept at 22°C. for 0.5 hour at which time samples were secured for titration of the residual active phage and a 1 ml. sample was taken for the reversal procedure. This last was mixed immediately with 1 ml. of a saturated solution of H_2S in water and was set aside for 12 minutes. It was then centrifuged at high speed to remove the fine precipitate of HgS . The supernatant was pipetted off, thoroughly aerated until free from H_2S , and diluted for the phage titration.

Controls consisted of: (a) phage diluted with 1:10,000,000 $HgCl_2$ to check any possible effect of this concentration on the titration results, and (b) phage exposed to the H_2S and aerated as was done with the reversed fraction.

EXPERIMENTAL RESULTS

1. *Inactivation of Phage.*—Fig. 1 is a composite plot of the results obtained in a series of experiments on the inactivation of phage by $HgCl_2$ at 22°C. The inactivation proceeds logarithmically with time and is therefore a pseudomonomolecular reaction with one reactant ($HgCl_2$) greatly in excess of the other, so that its concentration remains practically constant throughout the experiment.

The reaction may then be expressed as $dP/dt = k [HgCl_2] [P_0 - P_t]$ where

P = bacteriophage in activity units,
 P_0 = initial phage concentration,
 P_t = phage inactivated in time t ,
 and $[HgCl_2]$ = concentration of bichloride of mercury.

This, on integration, gives $k = 1/t [HgCl_2] \cdot \ln P_0/[P_0 - P_t]$. It will be noted that in the plot of experimental data the curve does not

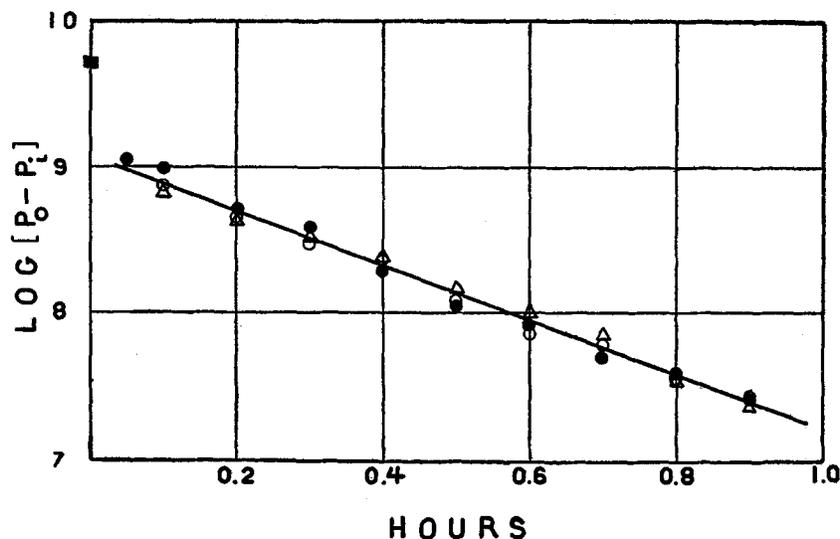


FIG. 1. Composite plot of logarithms of residual active phage units against time during HgCl_2 inactivation of phage at pH 7.6 and 22°C . (three experiments). ■ represents the common origin at $t = 0$ for all three initial phage concentrations.

TABLE I

Summary of Four Separate Experiments in All of Which Phage Was Inactivated with HgCl_2

The initial $[P]$ was 5×10^9 . In three cases reactivation following removal of Hg^{++} with H_2S restored the $[P]$ to its original titre (100 per cent reversal).

A	B	C	D	E	
				Inactivation	Reactivation
Inactivated phage 1:10,000 HgCl_2 0.5 hour	Reactivated phage As A. Followed by H_2S . Centri- fuged. Aerated till H_2S -free	Control 1×10^{10} phage units + H_2S . Aerated till H_2S -free	Control 1×10^{10} phage units titrated in presence of 1:10,000,000 HgCl_2	per cent	per cent
(1) 2.2×10^8	5.0×10^9	9.0×10^9	Not included in this experiment	4.4	100
(2) 1.3×10^8	2.1×10^9	8.9×10^9	Not included in this experiment	2.6	42
(3) 6.3×10^8	5.0×10^9	1.0×10^{10}	1.0×10^{10}	12.6	100
(4) 2.5×10^8	5.0×10^9	1.0×10^{10}	1.0×10^{10}	5.0	100

originate in the actual $[P]_0$ used. We have observed in the broth suspension of phage a faint, fine precipitate appearing at once upon the addition of HgCl_2 . This precipitate develops in an identical fashion in plain broth and it seems likely that a certain amount of phage is carried down with it accounting for the immediate precipitous drop in $[P]$.

2. *Reactivation of Inactivated Phage.*—Table I is a summary of four experiments in which standard phage was subjected to the action of HgCl_2 for 0.5 hour. The Hg^{++} ion was then removed with H_2S . In three of the four experiments reactivation was complete; that is, the final concentration of active phage was 100 per cent of that originally present before inactivation. In the fourth experiment the final phage titre was 50 per cent of $[P]_0$.

DISCUSSION

The data given above show first that the inactivation of phage by HgCl_2 follows the course of a pseudomonomolecular reaction, and secondly, that when the mercury ion is precipitated from the mixture an increase in $[P]$ occurs. The increase in phage concentration after removal of the Hg ions with H_2S may be explained in several ways.

First, it is possible that in the presence of H_2S , phage actually multiplies. Such is probably not the case, however, for one of the few points concerning phage upon which agreement is general, is that growth of a susceptible organism conditions phage multiplication. There are no recorded critical experiments in the literature indicating that $[P]$ can be increased by any other agency. Further, our controls exposed to H_2S and aerated, as in the reversal series, exhibit, if anything, a slight decrease in $[P]$.

Second, enough H_2S may be left behind after aeration to exert a stimulating effect on bacterial growth, consequently shortening the time of lysis in the titration set-up and thereby effecting an apparent increase in the initial $[P]$. Here again, the H_2S controls rule out such an occurrence for they too would exhibit this effect.

One alternative explanation remains; namely, that HgCl_2 inactivation of phage is actually reversed by precipitation of Hg^{++} ions with H_2S . This fits the observed data.

CONCLUSIONS

1. The inactivation of antistaphylococcus bacteriophage suspended in infusion broth at pH 7.6 and 22°C. by HgCl_2 proceeds according to the equation $dP/dt = k [\text{HgCl}_2] [P_0 - P_t]$ over the range studied.

2. This inactivation can be reversed by precipitation of Hg^{++} with H_2S . In the present experiments the inactivation was carried out until only some 5 per cent of the initial phage remained active. After reactivation the $[P]$ had increased to 100 per cent of the initial $[P]$.

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