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Inactivation of Intracellular Phage Precursor by Iodoacetic Acid.*

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There exists experimental evidence for the view that phage production involves the reaction: Inactive intracellular phage precursor + phage ⟶ phage.[1] The phage precursor has been shown to develop whenever the environmental conditions favor active bacterial metabolism and growth.[2] We term cells which contain a store of precursor "activated bacteria", and have found that the precursor content of the bacteria is very thermolabile, being destroyed at rapid rates when the cells are heated to 45°-50°C.[3] At these temperatures destruction of the precursor takes place long before sufficient damage is done to the cells to destroy their reproductive mechanism. The critical thermal increment for heat inactivation is 90,000, suggesting that the precursor either is a protein or contains a protein.

In the present paper we wish to report experiments in which destruction of precursor was brought about by concentrations of iodoacetic acid that were not lethal for the bacteria used.

Activated staphylococci were prepared by growing the organisms in a heavily oxygenated medium.[2] The activated cells were separated from the broth, resuspended in phosphate buffer solution of pH 6.1 and were stored for 1 hour at 5°C before using. Four ml aliquots of activated cell suspension were added to 1 ml amounts of varying concentrations of iodoacetic acid. The mixtures were kept at 5°C for given periods of time after which aliquots were tested for the presence of phage precursor and for the numbers of viable bacteria per ml. The precursor test was done by adding 4 ml amounts of cell suspension to 1 ml of phage solution containing 1×10^9 activity units/ml.[4] The test suspensions were kept at 5°C for 6 minutes to allow interaction of phage and residual activated cells after which they were diluted for titration. Controls consisted of: (a) Activated bacteria

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maintained at 5°C and pH 6.1 in phosphate buffer without iodoacetic acid for 30- and 60-minute periods. When subsequently tested by the addition of phage it was found that the precursor content of the cells had not diminished. (b) Known amounts of phage were exposed to concentrations of iodoacetic acid varying from M/1,000 to M/100,000 in phosphate buffer pH 6.1 at 5°C for varying periods. These concentrations of iodoacetic acid did not diminish phage titers during periods of exposure up to 1 hour.

The results of these experiments are summarized in Table I and show that appreciable inactivation of phage precursor is produced by as little as M/10,000 iodoacetic acid acting on activated bacteria for 15 minutes. During this time no measurable number of cell deaths occurred as determined by the plate count.

Since phage precursor is readily destroyed by concentrations of iodoacetic acid insufficient to kill staphylococci, it was thought that in a mixture of bacteria and phage a dilution might be found which would serve to completely inhibit phage production while permitting bacterial growth. In 15 experiments the rates of phage production and bacterial growth were followed in the presence of concentrations of iodoacetic acid ranging from M/4,000 to M/20,000 with $[\text{Bacteria}]_o = 2 \times 10^7$ $[\text{Phage}]_o = 1 \times 10^8$, at pH 7.2 and 6.1 and temperature 36°C. The rates of phage production were materially lowered in the iodoacetic acid mixtures and it took longer for lysis to occur. The end titers likewise were lower, averaging 25% of the

TABLE I.

Inactivation of intracellular phage precursor by CH_2I COOH. Activated Staphylococci were exposed to different concentrations of CH_2I COOH for varying periods of time. Samples were tested for phage precursor and for the number of viable bacteria per ml.

Molarity CH_2I COOH	Exposure (minutes)	Bacteria $\times 10^9$/ml	$[\text{Phage}]_o$	[Phage] Final
Control	0	4.6	2×10^8	1.4×10^9
.0001	1.0		"	1.0 "
.0001	3.0		"	1.0 "
.0001	5.0		"	1.0 "
.0001	15.0	2.5	"	4.0×10^8
.0001	30.0	3.8	"	3.7 "
.0001	60.0	4.1	"	2.8 "
Control	0	1.3	"	1.0×10^9
.00025	5.0		"	1.0 "
.00025	15.0	1.0	"	3.3×10^8
.00025	30.0	3.2	"	2.6 "
Control	0	6.0	"	1.1×10^9
.0005	5.0		"	4.5×10^8
.0005	15.0	8.2	"	3.0 "
.0005	30.0	4.7	"	2.2 "

control titers. Unfortunately the rate of bacterial growth likewise was reduced by the iodoacetic acid and it is therefore not possible to interpret the results as being due to the inactivation of precursor by iodoacetic acid. They could equally well arise from the effect of iodoacetic acid on bacterial growth with resultant lowering of the number of precursor-producing units available.

To summarize, it has been shown that concentrations of iodoacetic acid ranging from M/2,000 to M/10,000 serve to abolish the phage-augmenting capacity of activated staphylococci (organisms containing phage precursor) at 5°C and pH 6.1, without killing them.

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Quantitative Determination of Pneumococcal Capsular Polysaccharide by Photronreflectometric Titration of Precipitation with Excess Antibody.*

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Several recent publications[1, 2] have again dealt with the occurrence of capsular polysaccharide (SSS) in the blood of pneumococcal pneumonia patients and the serious prognosis usually associated with this finding. A number of observers in the past[3–10] have called attention to the relationship of SSS to the virulence of pneumococci and

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