BACTERIOPHAGE FORMATION WITHOUT BACTERIAL GROWTH

III. The Effect of Iodoacetate, Fluoride, Gramicidin, and Azide on the Formation of Bacteriophage

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In recent years it has become increasingly apparent that adenosinetriphosphate is the energy source for many types of endergonic reactions. Proof of this statement is found in the need of energy-rich phosphate for such diverse reactions as carbohydrate synthesis (1), uptake of CO_2 by autotrophic bacteria (2), fat metabolism (3), methylation (4), acetylation (5), muscle contraction (6), and perhaps peptide synthesis (4, 7). Consequently the relationship of adenosinetriphosphate (ATP) to phage formation was investigated with the aid of iodoacetate, fluoride, and azide (NaN_3). These substances share the common property of blocking ATP formation by inhibiting certain reactions in the Embden-Meyerhof carbohydrate cycle.

Iodoacetate prevents reaction 1, (8).

(1) Phosphoglyceraldehyde + coenzyme 1 + inorganic phosphate + adenosinediphosphate
⇅ triosephosphate dehydrogenase
phosphoglyceric acid + reduced coenzyme + ATP

Fluoride inhibits reaction 2, (9).

(2) 2-phosphoglyceric acid
⇅ enolase
phosphopyruvate

The exact mechanism by which azide inhibits ATP synthesis is not known (10).

There is good evidence that staphylococcus forms ATP according to the Embden-Meyerhof cycle and that the usual phosphorylated intermediates are concerned in the metabolism of the organism (11–14).

The system used to study the effect of these inhibitors was the penicillin-treated bacteria described in the previous paper (15). All the inhibitors used prevented multiplication of the bacteria. In the penicillin bacterial system, however, phage formation does not depend on multiplying bacteria; therefore, the inhibitors do not act indirectly on phage formation by inhibiting the multiplication of the bacteria.

RESULTS

The Effect of Iodoacetate, Azide, and Fluoride on Phage Production.—Table I illustrates that iodoacetate, sodium fluoride, and azide completely inhibit the formation of phage and also prevent the synthesis of ATP. This effect is reversible. When the inhibitor was diluted out there was no inhibition of phage production. That ATP was the phosphorous compound actually being

TABLE I

Effect of Iodoacetate, Fluoride, and Azide on Phage Production and ATP Formation

All tubes contained 5.6 cc. Locke's solution + 0.4 cc. nutrient broth + 0.2 cc. M/2 glucose + 1.0 cc. broth containing 160 γ of penicillin and 0.8 cc. phage solution. No phage in sample 5. Inhibitors and penicillin added to bacterial suspension 2 hours before phage. Lysis of control tube took place at 6 hours. No change in turbidity of samples containing inhibitors. ATP analysis based on 18.1 mg. of bacterial protein which was determined by micro-Kjeldahl.

Sample	Addition	ATP	Initial phage units	Final phage units
		γ		
1	0.001 M iodoacetate	2	10^8	10^5
2	0.06 M NaF	4	10^8	10^4
3	0.005 M NaN_3	3	10^8	10^8
4	None		10^8	10^9
5	None	25		

TABLE II

Comparison of Inorganic Phosphate, Adenosinetriphosphate, Ribonucleic Acid, and Desoxyribonucleic Acid of Normal and Infected Cells

All tubes contained 5.0 cc. broth containing 1 per cent glucose and 1.0 ml. of broth containing 180 γ of penicillin. Infected sample received 1 cc. of phage solution having a titer of 10^7 phage units per cc. Samples taken for analysis at 3 hours. This was 15 to 30 minutes before lysis. Ribonucleic acid expressed as γ ribose for total bacterial protein. Colorimeter readings used for desoxyribonucleic acid values. Analyses based on 11.1 mg. bacterial protein which was determined by micro-Kjeldahl.

Sample	Inorganic phosphate	ATP	Ribonucleic	Desoxyribonucleic
	γ	γ	γ	
Infected	21	14	360	30
Control	19	13	355	30

measured was indicated not only by the fact that it had phosphate groups hydrolyzable in N HCl at 100° in 7 minutes, but also that the compound was precipitated by barium at pH 8.2 in 10 per cent alcohol and would transfer one of its acid-labile phosphate groups to glucose to form glucose-6-phosphate in the presence of crystalline yeast hexokinase and magnesium. ATP is the only known substance that can phosphorylate glucose in the above manner (16).

The Effect of Gramicidin on Phage Formation.—Gramicidin was found to prevent the multiplication of phage. It has been found by Hotchkiss (17) and confirmed in the present work that gramicidin completely prevents the uptake of inorganic phosphate from the medium. The significance of this observation will be discussed later in the paper.

Chemical Analysis of Infected Cell.—Inorganic phosphate, ATP, ribonucleic acid, and desoxyribonucleic acid were determined in normal cells and infected cells (Table II). No differences were detected in any of the compounds between the two types of cells under the experimental conditions. It is very possible that conditions may be found where differences could be shown. Cohen (18) has reported an increased synthesis of desoxyribonucleic acid in *E. coli* cells infected with T_2 phage.

DISCUSSION

Iodoacetate, fluoride, and azide prevent the formation of bacteriophage. Since these substances all inhibit the formation of adenosinetriphosphate, it appears that energy-rich phosphate is needed in the formation of bacteriophage. It has been reported that azide prevents that formation of adaptive enzymes (19). Iodoacetate and azide have also been found to prevent the multiplication of vaccinia virus in tissue culture (20).

Gramicidin prevented the production of bacteriophage. This result is of interest in view of Cohen's (18) report that only the phosphate added to the medium is found in the bacteriophage. As gramicidin completely prevents the uptake of phosphate from the medium it would, according to Cohen's results, prevent the formation of phage. If Cohen is correct, it makes the precursor theory of phage formation less likely, for in this case, one would expect to find some bacterial phosphate in the bacteriophage.

Cohen (18) has shown that only extracellular nitrogen is found in the T_2 phage. In the preceding paper (21) it was reported that multiplying bacteria do not form phage unless accessory substances are added. If the precursor is a normal constituent of cells, one would expect that growing bacteria would form phage without such substances being added. It appears that the phage is directly formed from substances in the media.

Methods

Assay of bacterial suspensions and phage was carried out as described previously (15). Bacterial suspensions were prepared in the same manner as described previously (15).

Chemical Determinations.—The bacterial suspension was centrifuged and washed two times with saline at 0°. The cells were then ground and extracted in a mortar with 5 cc. of 10 per cent trichloroacetic acid plus a little alundum. The residue was reextracted with 5 cc. of 5 per cent trichloroacetic acid. The filtrates were combined and analyzed for inorganic phosphate and ATP. Inorganic phosphate was deter-

mined by the method of Fiske and SubbarRow (22). ATP was determined according to Lohmann and Oppenheimer (23).

The trichloroacetic acid–insoluble precipitate was extracted for nucleic acid by the modification of Schneider's method according to Krampitz and Werkman (24). Ribonuclic acid was determined by the method of McRary and Slattery (25). Desoxyribonucleic acid was determined according to Hoagland *et. al.* (26).

SUMMARY

1. Iodoacetate, fluoride, and azide have been found to prevent the formation of phage and to inhibit the synthesis of ATP by *Staphylococcus muscae*. It is suggested that energy-rich phosphate is needed for the synthesis of phage.

2. Gramicidin prevented the formation of phage.

3. No differences were found between normal bacteria and phage-infected bacteria in the inorganic phosphate, adenosinetriphosphate, ribonucleic acid, and desoxyribonucleic acid content of the cells.

4. The mechanism of phage formation is discussed.

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