

## Killing of *E. coli* B by phage-free T2 lysates

In a previous investigation, a study has been made of the action of T2 and T4 phages in splitting off a component from isolated and dinitrophenylated membranes of *E. coli* B. The experimental results showed that the active part of the phage tail is able to repeat its action on bacteria membranes, indicating that this action is enzymic. It also appeared that the phage tail enzyme might be the bacteria-killing component of the phage, able to lyse bacteria from the outside<sup>1</sup>. This view is supported by experiments reported here.

Lysates of *E. coli* B infected with T2 are able to split a component from *E. coli* B membranes, even after the lysates have been cleared of phages and ghosts by acid precipitation and high speed centrifugation.

Using analytical procedures already described<sup>1</sup>, no difference could be found between the composition of the split product released by phage-free lysates as compared with that released by phages or phage ghosts.

The residual activity of T2 lysates, however, is reversibly bound to membranes, whereas enzyme which has once been adsorbed to membranes as a part of a whole phage cannot be removed from the membranes by washing.

In contrast to whole T2 phages, T2 lysates are able to split off a component from B/2 membranes as well as from B. This supports the conclusion drawn from earlier experiments, that host range specificity is associated with other parts of the phage tail, rather than with the enzyme<sup>1</sup>.

Like the enzymic part of the phage, the active substance from T2 lysates is able to repeat its splitting action on fresh bacterial membranes. In both cases the reaction is pH-dependent and heat-sensitive, and the split products of the reaction are the same. This indicates that the phage enzyme and the active substance from T2 lysates correspond to the same phage protein structure. It is probable therefore that a phage-specific protein, which is synthesized in surplus by the infected bacteria, is responsible for the enzymic activity of T2 lysates.

The enzymically active protein is of low molecular weight. It passes dialyzing membranes and does not sediment appreciably during one hour at 120,000 g.

We have been able to concentrate this protein and to purify it to some extent. A concentrated solution was found to kill  $1 \cdot 10^{10}$  bacteria to a survival of  $2 \cdot 10^8$  (5', 20'), and  $1 \cdot 10^9$  bacteria to a survival of less than  $1 \cdot 10^3$  (10', 20'). B/2 bacteria are killed at almost the same rate.

Phase contrast microscopy shows characteristic lysis from the outside. The kinetics of lysis of bacteria can be studied by following the decrease in turbidity of the solution. Chloroform-killed bacteria are lysed by preparations which contain only one tenth of the active substance used to lyse normal bacteria. This parallels the findings of BROWN that phage-free T6 lysates are able to lyse chloroform-killed bacteria<sup>2</sup>.

It is not yet certain that the three activities described (the splitting-off of a chemically defined substance from bacterial membranes, the killing of bacteria, and the lysis of bacteria) are functions of the same agent. In the steps used for concentration and purification, however, the three activities always remained together in one fraction.

Further purification of the active fraction and measurement of the kinetics of its killing ability might establish whether the enzymic splitting of the bacterial cell membrane alone is sufficient to kill bacteria.

Details of the experiments will be published at a later date.

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<sup>2</sup> A. BROWN, *J. Bacteriol.*, 71 (1956) 482.

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## The effect of arginylpeptides on the clotting of fibrinogen with thrombin

It has been demonstrated that the enzymic action of thrombin (bovine or human) results in the appearance of N-terminal residues of glycine on fibrinogen, simultaneously with the conversion of the latter to fibrin, and with the release of fibrinopeptides<sup>1-5</sup>. This finding, coupled with the observation<sup>6</sup> that thrombin catalyses rather specifically the hydrolysis of arginine esters and