PATHOLOGY

An Effect of Early Dilution on the Establishment of Lysogeny in *Salmonella typhimurium*

It is known that the frequency of lysogenic response in a culture of *Salmonella typhimurium* strain *LT2*, infected with the temperate bacteriophage *P22*, can be influenced by the ratio of phage to bacterium (multiplicity of infection)[1–3], and by modifying the metabolism of the infected cells soon after infection, for example, by the addition of chloramphenicol[4]. With a high multiplicity of infection a high frequency of lysogenic response is obtained. Using a low multiplicity of infection a low lysogenic response results. If these infected cells are exposed to chloramphenicol (25 μgm./ml.) 5 min. after infection and the chloramphenicol removed by dilution 15 min. later, then practically every cell infected becomes lysogenic.

We have been examining the effect of lower concentrations of chloramphenicol in contact with cells infected with a low multiplicity of infection (2·8) for a shorter period of time. Fig. 1 shows that a concentration of 5 μgm./ml. in contact with the cells for 7 min. is sufficient to reverse the 'decision' of the majority of the cells to lyse. What is more interesting is that the infected control culture shows a high frequency of survivors when sampled and diluted into buffer 5 min after infection. This high frequency of survivors decreases with time until it reaches a minimum at 12 min. In all our previous experiments the control value for lysogenic response was measured at the end of the experiment and thus gave this minimum value.

In view of these results, measurements were made of the frequency of lysogenic and lytic responses of a similarly infected culture when samples were removed and diluted into buffer at room temperature during the first 15 min. after infection. The results are shown in Fig. 2. There is an evolution of the lysogenic response during this period rising to a maximum 6 min. after infection, decreasing to a minimum at 12 min. The lytic response complements the lysogenic response. These results were later found to be true, even when dilutions were made into broth or buffer at room temperature (26·5° C.) or 37° C.

Under the conditions of the experiments above, 80 per cent of the cells would have been infected with at least one phage particle by 2 min. At 6 min., 95 per cent would be infected. This may account for the rise in lysogenic response between 2 and 6 min. after infection.

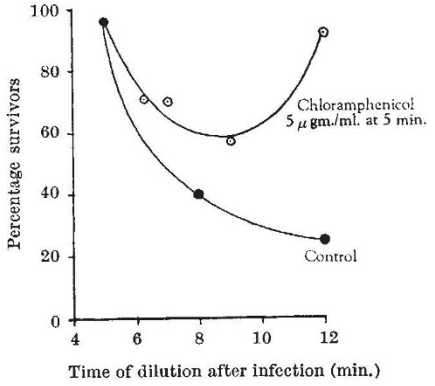

Fig. 1. Effect of dilution on survival of *LT2* cells infected with phage *P22*, with and without chloramphenicol

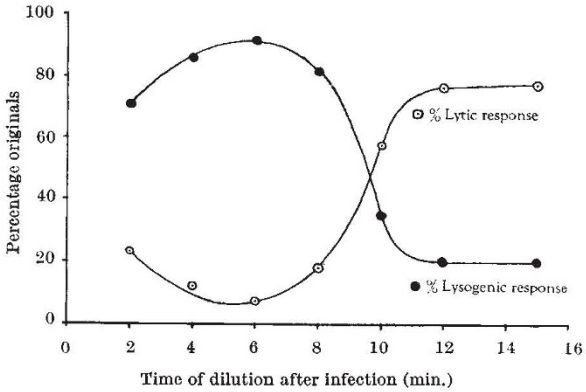

Fig. 2. Effect of dilution on lysogenic and lytic responses in *LT2* cells infected with phage *P22*

Chloramphenicol is said to reverse the 'decision' of a cell to lyse by virtue of its ability to inhibit protein synthesis[4]. The results reported in this communication indicate that dilution into fresh medium is equally effective if carried out before the 'decision' period is reached. This 'decision' period was shown by Lwoff to be 6–10 min. after infection in *S. typhimurium* infected with an *A1* phage[2]. The importance of dilution in a lytic phage system has been reported by Lanni[5], who showed that in the *T5-E. coli F* system dilution of high bacterial concentrations is necessary to stabilize the phage–host complex.

It would be interesting to know whether the effect of early dilution reported here has been found in other lysogenic systems or is restricted to *S. typhimurium*.

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A Filterable Hæmolysin from *Escherichia coli*

Some strains of *Escherichia coli* are hæmolytic when grown on blood-agar, and attempts made to demonstrate a soluble hæmolysin in culture filtrates are usually unsuccessful. We have been able to obtain hæmolytic filtrates with four such strains when grown in a culture medium prepared as follows.

A pound of lean beef is cut into small pieces and boiled in 500 ml. of *N*/20 aqueous sodium hydroxide. After simmering for 15 min. it is strained through a muslin cloth, and excess fluid removed. The meat is dried by pressing between filter paper and placed in a flask to which 500 ml. of infusion broth is added; the *p*H is adjusted to 7·6. The medium is sterilized by steaming for 24 hr. and autoclaving for 20 min. at 120° C.

Three of the strains used (0138 : *K81B*, 0139 : *K82B*, and 0141 : *K85B*) were recovered from diseased