INHIBITION OF REPLICATION OF BACTERIOPHAGE T2 BY PHENETHYL ALCOHOL*

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Berrah and Konetzka (1962) reported that the bacteriostatic action of phenethyl alcohol can be attributed to the selective and reversible inhibition of DNA synthesis in susceptible bacteria. This communication deals with the effect of phenethyl alcohol on the replication of the bacteriophage T2 in Escherichia coli H.

METHODS

Phenethyl alcohol, at a concentration of 0.34%, was added to an exponentially growing TSB culture of E. coli H at 5×10^7 cell/ml. The culture was incubated for 2 hrs, during which time there was no significant increase in cell numbers nor any net synthesis of DNA. However, RNA and protein increased approximately 3-fold. The methods employed for these determinations have been described previously (Berrah and Konetzka, 1962). After the 2-hr incubation period, 20 ml of the culture were filtered through a Millipore HA filter (47 mm), and the collected cells were resuspended in 5 ml of TSB + 0.34% PEA. One-tenth ml of a suspension of T2 at 5×10^{10} PFU/ml was added and the mixture aerated for 5 min. Two and one-half ml samples of the suspension were then filtered through a Millipore HA filter. One sample was washed on the filter with 3 separate

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Abbreviations: DNA - deoxyribonucleic acid; RNA - ribonucleic acid; PEA - phenethyl alcohol; TSF - Trypticase soy broth; PFU - plaque forming units; dCMP - deoxycytidine-5'-phosphate.

40 ml volumes of TSB + 0.34% PEA, and the cells were finally resuspended in 10 ml of the same medium; another sample was treated in an identical manner, except that the last 40 ml volume of TSB did not contain PEA, and the cells were resuspended in 10 ml of TSB without PEA. This procedure removed over 99.9% of the unadsorbed phage. The infected cells were appropriately diluted into TSB + 0.34% and TSB without PEA, respectively, incubated at 37 C, and samples were withdrawn at intervals and plated in the usual manner for the determination of a single step growth cruve (Adams, 1959). Samples were also treated with chloroform before plating to determine the intracellular phage (Séchaud and Kellenberger, 1956).

The methods for the single cell bursts were identical to those employed for the single step growth curve except that the infected cells were diluted immediately after the 5 min adsorption period to contain 0.7 infected cells/ml of broth with and without 0.34% PEA. The diluted samples were incubated at 37 C for 1 1/2 hrs before being plated. The addition of 2.5 ml of the seeded top agar to the PEA-containing samples diluted the compound sufficiently so that it was not inhibitory to the plating cells.

Bacterial cell counts were determined by means of a Petroff Hausser counting chamber.

RESULTS

A normal burst of approximately 200 PFU was observed in the control cells (Table 1), although these cells were in the presence of PEA for 2 hrs prior to infection and for the 5 min interval during the adsorption period. Despite the fact that DNA synthesis in the cells had been prevented for 2 hrs, removal of the PEA allowed normal replication of T2. However, in the presence of PEA there was a marked inhibition of T2 replication, as determined by the single step growth curve experiment. The burst size of the cells in the presence of PEA was approximately 4. The infected bacteria in the presence of PEA lysed to the same extent as the control cells, but there was no substantial increase in PFU. The number of morphologically identi-

Table 1

Effect of PEA on T2 Replication in E. coli H

Minutes after Adsorption	PEA Removed after Infection		PEA Present after Infection	
	PFU x 10^7/ml	Bacterial Cell Count x 10^7/ml	PFU x 10^7/ml	Bacterial Cell Count x 10^7/ml
10	4.8 (<0.01)*	5.6	5.1 (<0.01)	4.7
15	4.5		4.9	
20	5.1 (<0.01)		4.8 (<0.01)	
25	5.1		5.0	
30	4.6 (0.16)		4.9 (0.16)	
35	4.7		12.1	
40	1070.0 (1030.0)		19.3 (17.6)	
45	1070.0		16.2	
50	942.0 (976.0)		18.8 (15.9)	
55	960.0		22.6	
60	978.0 (954.0)	<0.5	18.8 (19.4)	<0.5

* The figures in parentheses represent PFU in the equivalent chloroformed samples.

fiable bacteria decreased over 90% in each case. The inhibition of T2 replication was further substantiated by the results of the single cell burst experiment (Table 2). The control cells released a normal number of PFU, but the PEA-treated cells released only 1-9PFU/cell.

DISCUSSION

The selective and reversible inhibition of DNA synthesis by PEA is strikingly demonstrated by the effect of this compound on T2 replication. According to the data obtained from the single step growth curve experiment, synthesis of T2 is inhibited approximately 98%. However, this inhibition may indeed be 100%. With the high multiplicity of infection employed and the percent infection attained, the cells are probably multiply infected and, consequently, the infective units released from the PEA-treated cells may simply represent the "protein coating" of the input T2 DNA. The results also imply that phage proteins are being synthesized

Table 2

Effect of PEA on Single Cell Bursts of T2

Control Cells (PEA removed after infection):	
Number of Plates with plaques:	6
Plaque counts:	124, 182, 251, 315, 352, 366
Number of Plates without plaques:	42
PEA-inhibited Cells:	
Number of plates with plaques:	8
Plaque counts:	1, 1, 2, 5, 6, 7, 7, 9
Number of Plates without plaques:	40

in the PEA-treated T2-infected cells, for not only do the infected bacteria lyse (Table 1), but they also form phage-induced dCMP deaminase at about the same rate as untreated infected cells (Keck et al., 1960). An increase in phage proteins can also be detected by complement-fixation procedures. A detailed report of these findings is in preparation.

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