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Inhibition of DNA Injection from Mycobacteriophage I3 by Tween-80

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Tween-80 (polyoxyethylene sorbitan mono-oleate), routinely used to prevent clumping of mycobacteria during growth, interferes with the propagation of mycobacteriophage I3. This is brought about by the inhibition of phage DNA injection.

Mycobacteria, when grown in liquid culture, are known to clump and grow as a mat on the surface of the medium. Water-soluble, dispersible lipids such as Tween-80 (polyoxyethylene sorbitan mono-oleate) are used in low concentrations (0.05–0.2%, v/v) to depress the surface tension of the medium, thus facilitating submerged growth of the bacteria (*1, 2*). Tween-80, however, inhibits the propagation of mycobacteriophage D29 on both *Mycobacterium tuberculosis* ATCC 607 (*3*) and *M. ranae* (*4*). This inhibition has been shown to be at the level of phage adsorption in both cases (*4, 5*). A similar but less drastic effect has been observed with the mycobacteriophage D4 (*5*).

The propagation of the transducing mycobacteriophage I3 (*6, 7*) is also inhibited by Tween-80. However, in this case the adsorption of the phage to the bacteria, measured either by the number of infective centers formed or by the number of free phages disappearing from the adsorption medium, is not significantly affected at 37° or at 0°. Furthermore, Tween-80 does not inhibit the growth of the phage per se, for a normal burst size is obtained if adsorption is carried out for 30 min in Tween-80-free medium at 37° (but not at 0°) and Tween-80 is added later. Curiously, a normal burst size is also obtained if phage-bacteria complexes formed in the presence of Tween-80 are suspended in Tween-80-free medium for growth. Thus, the inhibition is relieved if the phage-bacteria complexes are incubated even for a short time of 30 min (as compared to 180 min, the latent period of phage I3) in the absence of Tween-80 at 37° (but not at 0°). This suggests that Tween-80 inhibits an early step in infection subsequent to the attachment of the phage to the bacteria. This can be interpreted to mean that DNA injection is the real target of action of Tween-80 and that DNA injection does not occur efficiently at 0°.

Such a hypothesis is borne out by a direct assay of DNA injection. It can be seen from Table 1 that Tween-80 indeed inhibits DNA injection by 78.4% as measured by the ^{32}P-phage and 66.6% as judged by the activity measurement. At 0°, DNA injection occurs with 23.5% less efficiency than 37° as measured by the ^{32}P-phage and 39.0% less efficiency as judged by activity measurement. The experiments with ^{14}C-phage where the phage protein coat is labeled serve as controls to show that the results obtained are not due to discrepancies in the washing procedure because in no case more than 7% of the adsorbed protein label is retained on the bacteria after washing.

Tokunaga *et al.* (*5*) have shown that Tween-80 overcomes the inactivation of D29 by the ether-ethanol extract of the host bacteria which is a reflection of the capacity of Tween-80 to inhibit adsorption. Commenting on the reduced efficiency of Tween-80 to do the same in the case of D4, the authors have suggested that this might reflect differences in the adsorption mechanism of D29 and D4. The fact that the effect of Tween-80 is so completely different in the case of I3 might well suggest more profound differences in the mechanism of phage-host attachment and subse-

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TABLE 1

EFFECT OF TWEEN-80 AND TEMPERATURE ON DNA INJECTION FROM MYCOBACTERIOPHAGE I3[a]

	Tween-80								
	—, 37°C			0.2% (v/v), 37°C			—, 0°C		
	^{32}P-labeled phage (cpm)	^{14}C-labeled phage (cpm)	Unlabeled phage (PFU)	^{32}P-labeled phage (cpm)	^{14}C-labeled phage (cpm)	Unlabeled phage (PFU)	^{32}P-labeled phage (cpm)	^{14}C-labeled phage (cpm)	Unlabeled phage (PFU)
Input	47,492	15,150	48×10^7	47,492	15,150	48×10^7	47,492	15,150	48×10^7
Unabsorbed	36,933	9,300	7×10^7	38,812	10,900	7.5×10^7	39,000	9,850	5×10^7
Adsorbed	10,587	4,900	45×10^7	8,487	4,300	42×10^7	8,750	4,750	46×10^7
% adsorbed	22.3	32.3	93.8	17.9	28.4	87.5	19.4	31.4	95.8
Uninjected	6,037	3,150	30×10^7	7,575	3,400	38×10^7	5,000	3,450	32×10^7
Injected	4,112	250	16×10^7	715	300	5×10^7	2,600	200	10×10^7
% injected	38.8	5.1	35.6	8.4	7.0	11.9	28.7	4.2	21.7
% inhibition				78.4		66.6	23.5		39.0

[a] DNA injection was assayed by a slight modification of the method described by Newbold and Sinsheimer (*9*). After adsorption, free phages were separated from the bacteria by centrifugation. The supernatant and pellet were called the unadsorbed and adsorbed fractions, respectively. The adsorbed fraction was washed with sodium tetraborate, vortexed for 2 min, and centrifuged again. This supernatant was called the uninjected fraction and the pellet the injected fraction. Subsequent washings did not remove any appreciable quantities of radioactivity. Various fractions were quantitated either by counting aliquots in an LS-100 liquid scintillation spectrometer (in the case of radioactive phage) or titrating with indicator bacteria (in the case of cold phage). The percentage adsorbed was calculated with respect to the input and percentage injected with respect to the amount adsorbed. The percentage inhibition was calculated from the percentage injected taking the control (37° without Tween-80) as zero inhibition. The absolute quantity adsorbed turns out to be much less in the radioactive experiments because there is considerable loss of infectivity during purification of the phage.

quent interaction. This appears all the more likely in light of the fact that I3 is the only mycobacteriophage known to have a contractile tail (*8*).

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