

ADSORPTION OF Vi BACTERIOPHAGES BY TYPHOID BACILLI AND PARATYPHOID C STRAINS

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It has been stated by d'Herelle that in general there are two varieties of bacteriophages, viz. specific phages that act only on one type and non-specific phages that appear to show affinity for further types of bacteria.

Sonnenschein (1925) employed specific bacteriophages as diagnostic bacteriophages, also for the bacteriological typhoid diagnosis.

Marcuse (1934) found in examining 180 typhoid strains that the typhoid-diagnostic-phage of Sonnenschein at first attacked 70 per cent of his strains and after increase of its virulence 99 per cent. He did not indicate any serological difference between sensitive and non-sensitive strains. The serological difference between strains attacked by typhoid-specific bacteriophages and strains that are insensitive to these bacteriophages was found only after Felix had shown the presence of Vi antigen. Early in 1936 it was reported by workers in three different countries that typhoid specific bacteriophages merely attack typhoid bacilli containing Vi antigen (Craigie & Brandon, 1936, 1936*a, b*; Scholtens, 1936; Sertic & Boulgakov, 1936).

As typhoid-specific bacteriophages attack typhoid bacilli in the Vi antigen, they are called Vi bacteriophages.

Kauffmann (1935) found that certain paratyphoid C strains contain the Vi antigen of the typhoid bacillus.

I examined a few of these strains (viz. East Africa, Witts Blood, Baghdad) and arrived at the same conclusion as Kauffmann (1936), viz. that these strains are insensitive to Vi bacteriophages, both on solid and in fluid culture media.

Strains of bacteria exist that are proof against some bacteriophages in spite of adsorbing them. In the case of the above paratyphoid C strains and Vi bacteriophages I obtained similar results.

For the adsorption experiments I used the following technique: The bacteriophage suspension was diluted to $10^5 \times$ or $10^6 \times$. To 1 c.c. of this I added 1 c.c. bacterial suspension obtained by suspending 3 agar-cultures in 10 c.c. physiological saline solution and heating this for two and a half hours at 56°C . The mixtures were kept in a refrigerator overnight. For counting purposes it is not necessary to remove the bacteria with the adsorbed

bacteriophage corpuscles by centrifugation because Kimura (1935) stated that the adsorption of the bacteriophage by the bacteria is irreversible.

Levine (1926) made use of this phenomenon in the same manner with his adsorption experiments on the bacteriophage.

In order to count the number of bacteriophage corpuscles according to the technique suggested by Gratia, 3 c.c. of fresh broth culture of a phago-sensitive strain and 1 c.c. of the suspension to be examined were added to 5 c.c. melted agar of 2 per cent at 50° C., after which this mixture was poured into a plate 10 cm. diameter. At times it was necessary to dilute the suspension 5 times as otherwise the number of plaques¹ would be too large to admit of easy numbering. On the following day the plaques appear as transparent spots.

The result of an adsorption experiment with paratyphoid C strains is given in Table I, Exp. A. For purposes of comparison the adsorbing capacity of V forms of strains Ty 871 and 860 and of the W forms obtained by the action of Vi bacteriophages from these V forms have also been determined.

Table I

Experiment A		Experiment B	
Adsorption of Vi bacteriophage Ty 1 by paratyphoid C strains containing Vi antigen and by V and W forms of typhoid strains		Adsorption of Vi bacteriophage Ty 1 by heated suspensions of typhoid bacilli and by suspensions of coli bacilli	
Dilution 10 ⁶ × of bacteriophage Ty 1 absorbed with	Plaques numbered	Dilution 10 ⁵ × of bacteriophage Ty 1, adsorbed by heated typhoid bacilli and by suspensions of coli bacilli	Plaques numbered
Ty 871 V form	24	Ty 3007 heated	24
Ty 871 V form	31	Ty 923 heated	40
Ty 871 W form a	100	Ty 897 heated	17
Ty 871 W form b	107	Ty 972 heated	18
Ty 871 W form c	103	<i>Coli</i> 811	194
Ty 860 V form	34	<i>Coli</i> S ₁	223
Ty 860 V form	33	<i>Coli</i> S ₂	176
Ty 860 W form a	123	<i>Coli</i> S ₃	198
Ty 860 W form b	119	<i>Coli</i> S ₄	191
Ty 860 W form c	91	<i>Coli</i> S ₅	256
Ty 860 W form d	124	<i>Coli</i> S ₆	268
Paratyphoid C, East Africa	1	<i>Coli</i> S ₇	218
Paratyphoid C, Witts Blood	18	<i>Coli</i> S ₈	212
Paratyphoid C, Baghdad	19	<i>Coli</i> S ₉	227
Control 1	125	Control. Bacteriophage only	237
Control 2	110		
Control 3	134		
Control 4	109		
Control 5	97		

Adsorbing power of suspensions containing Vi antigen. In Exp. A the plaques were numbered in 1 c.c. of the undiluted mixture of bacteriophage and bacteria. In Exp. B this mixture was diluted five times before numbering.

As expected, the W forms did not adsorb the bacteriophage. Further, the thermostability of the adsorbing capacity of typhoid bacilli for Vi bacteriophages was tested, following the same technique. Suspensions of typhoid

¹ The term "plaques" denotes the clear areas due to action of the bacteriophage.

strains (V type) were heated for one hour in a boiling water-bath, whereupon their adsorption capacity for Vi bacteriophages was examined. One of these experiments is described under B in Table I. Suspensions of ten different *coli* strains were taken for purposes of comparison. From these experiments the adsorbing capacity of typhoid bacilli for Vi bacteriophages appears to be thermostable.

It will be known that the adsorbing capacity for Vi agglutinins is thermostable. In this the affinity for Vi bacteriophages corresponds with the affinity for Vi agglutinins.

SUMMARY

Evidence has been obtained that where the adsorbing capacity for Vi agglutinins is known to be present it is also possible to show affinity for Vi bacteriophages. However, from the conduct of paratyphoid C strains containing Vi antigen it appears that the presence of Vi antigen causes only the adsorption of Vi bacteriophages, but that in the production of lysis by these bacteriophages other factors play a part.

CONCLUSIONS

1. The paratyphoid C strains described by Kauffmann and containing Vi antigen are insensitive to Vi bacteriophages notwithstanding the adsorption.
2. The adsorbing capacity of typhoid strains for Vi bacteriophages is thermostable.
3. Parallelism exists between the adsorbing capacity for Vi bacteriophages and for Vi agglutinins.

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