

THE RESISTANCE DEVELOPED AGAINST BACTERIOPHAGE

BY R. TH. SCHOLTENS

Rijks Instituut voor de Volksgezondheid, Utrecht.
Director Dr W. Aeg. Timmerman

WHEN a given bacterial culture suffers lysis by a particular phage the secondary growth which later develops is resistant to that race of phage and to many others, but not to all. Further it may be found that when two phages, A and B, presumed pure, act on the same culture, one, A, stimulates in the secondary growth resistance to phages A and B while the other, B, stimulates resistance to itself alone, leaving the strain sensitive to A. Such differences d'Hérelle ascribes to the differing potency or virulence of phages for the same organism. The more potent phages, he says, engender resistance to the weaker; the weaker fail to rouse resistance to the stronger. He admits, however, that there are certain exceptions.

The following experiments, with various phages for *Salmonella typhi*, lead me to a very different conclusion and suggest another explanation which applies equally to d'Hérelle's exceptional cases.

The bacteriophages, ϕ^* Ty 5 and ϕ Gä 7, both acting on *S. typhi*, were isolated from canal and ditch water respectively: the first was isolated by aid of the typhoid strain Ty 1460, the second by that of the Gaertner strain Gä 13. Both were purified, during growth on the same cultures, by repeated transfers from single plaques (3 and 9 times respectively).

The secondary cultures, Ty 1460 sec. bact., ϕ Ty 5 and Ty 1460 sec. bact. ϕ Ty-Gä 7, obtained after lysis of Ty 1460 by these phages, were compared with regard to their relative resistance. Tests were made by streaking with the platinum loop an agar plate over which a few drops of broth culture of *S. typhi* had been evenly distributed. If the culture was sensitive no growth occurred during incubation overnight on the area inoculated with the phage.

It was found that ϕ Ty 5 and ϕ Ty-Gä 7 had each rendered the bacilli resistant to itself but not to the other: exposure to ϕ Ty 5 conferred no protection against ϕ Ty-Gä 7; exposure to ϕ Ty-Gä 7 none against ϕ Ty 5.

Using the same bacteriophages this experiment was repeated, with identical results, with three further strains of *S. typhi*.

The experiment was extended. Various other bacteriophages were isolated, by the same means as ϕ Ty 5 and Ty-Gä 7, from surface waters in Holland. All the phages isolated with Ty 1460 acted exclusively on typhoid bacilli.

* The symbol ϕ is used throughout for phage and $\phi\phi$ for phages.

They were three times transplanted from single plaques. Of the phages isolated with G \ddot{a} 13 a number attacked typhoid bacilli also. Such races were preserved and, in the course of twenty serial subcultures on the Gaertner strain, were purified by nine transplantations from single plaques. There is reason, therefore, to believe that the phages were "pure".

Table I. *Affinity of the bacteriophages used for the various types of bacteria*

Bacteriophages for which the affinity or these strains were investigated	Place from which isolated	Bacteria types investigated												
		<i>S. typhi</i>	<i>S. enteritidis</i> Gaertner (4 strains)	<i>S. gallinarum</i> (Sanguinarium)	<i>S. stanley</i>	<i>S. derby</i>	<i>S. abortus equi</i>	<i>S. reading</i>	<i>S. paratyphi B</i> (Schottmüller)	<i>S. paratyphi A</i>	<i>Bact. coli</i> 80	<i>Bact. coli</i> (4 other strains)	<i>Bact. prodigiosum</i>	<i>V. cholerae</i>
γ 1	Utrecht, Oudegracht	+	-	-	-	-	-	-	-	-	-	-	-	-
γ 2	Rotterdam	+	-	-	-	-	-	-	-	-	-	-	-	-
γ 3	Between Rotterdam and Delft	+	-	-	-	-	-	-	-	-	-	-	-	-
γ 4	Delft	+	-	-	-	-	-	-	-	-	-	-	-	-
γ 5	The Hague	+	-	-	-	-	-	-	-	-	-	-	-	-
γ-G \ddot{a} 6	The Hague	+	+	+	-	-	+	+	+	+	+	-	-	-
γ-G \ddot{a} 7	Leiden	+	+	+	-	-	+	+	+	+	+	-	-	-
γ-G \ddot{a} 9	Utrecht, Oudegracht	+	+	+	-	-	+	+	+	+	+	-	-	-
γ-G \ddot{a} 10	Utrecht, Nieuwegracht	+	+	+	-	-	+	-	+	+	+	-	-	-

+ indicates that the strain was attacked by the bacteriophage.
 - indicates that the strain was resistant towards the bacteriophage in question.

Table II. *Resistance of secondary cultures of typhoid strain Ty C, obtained after lysis with nine different bacteriophages, against these nine bacteriophages*

Bacteriophages against which the resistance was examined	Secondary cultures, obtained with the nine different bacteriophages as shown in each column Ty C secondary of bacteriophage									
	Ty 1	Ty 2	Ty 3	Ty 4	Ty 5	Ty-G \ddot{a} 6	Ty-G \ddot{a} 7	Ty-G \ddot{a} 9	Ty-G \ddot{a} 10	
Ty 1	-	-	-	-	-	+	+	+	+	
Ty 2	-	-	-	-	-	+	+	+	+	
Ty 3	-	-	-	-	-	+	+	+	+	
Ty 4	-	-	-	-	-	+	+	+	+	
Ty 5	-	-	-	-	-	+	+	+	+	
Ty-G \ddot{a} 6	+	+	+	+	+	-	-	-	-	
Ty-G \ddot{a} 7	+	+	+	+	+	-	-	-	-	
Ty-G \ddot{a} 9	+	+	+	+	+	-	-	-	-	
Ty-G \ddot{a} 10	+	+	+	+	+	-	-	-	-	

+ indicates lysis by the bacteriophage in question.
 - indicates resistance towards the bacteriophage in question.

Table I indicates the place of origin and the range of lytic action for certain bacteria of the several bacteriophage races. Secondary cultures of a typhoid strain, Ty C, were prepared with the nine phages listed in Table I, and the resistance of each secondary race to each of the nine phages was tested (Table II).

Table I shows that the bacteriophages under discussion fall into two groups: those, φφ Ty 1, 2, 3, 4 and 5, which, of the bacteria studied, attack typhoid bacilli alone, and those, φφ Ty-G \ddot{a} 6, 7, 9 and 10, which lyse also

certain related bacteria. A coincident grouping is again encountered in Table II. Any phage of either group renders Ty C resistant to all members of its own group but leaves the strain fully sensitive to all members of the opposing group.

Now, it is quite clear that the relationship constantly displayed by these two groups of *typhi*-phages cannot be explained along the lines suggested by d'Hérelle of a quantitative difference in virulence, for the difference presented is bilateral. A more reasonable hypothesis would seem to be that the groups of phages have qualitatively different actions and each a distinct point of attack in the substance of the sensitive bacterium. Then phages of the type of φ Ty 5 would render only their own "point of attack" resistant without reducing the susceptibility of that selected by phages of the type of φ Ty-Gä 7; conversely phages of the φ Ty-Gä 7 group in stimulating resistance to themselves would not affect the specific "point of attack" of races like φ Ty 5.

Differences in virulence are discernible within these groups: thus φ Ty 1 is visibly more virulent than φ Ty 3. Yet φ Ty 3 confers resistance against φ Ty 1. Hence small or considerable differences of virulence do not affect the degree of resistance induced.

According to Marcuse the bacteriophage makes its attack on the bacterial receptors. One would therefore believe that $\varphi\varphi$ Ty-Gä 6, 7, 9 and 10 launch their attack on the common receptor (IX) (Kauffmann-White schema, 1934) of *S. typhi* and *S. enteritidis* and this possibility is supported by the fact that they also attack *S. gallinarum*. Admittedly they also lyse certain other bacteria not credited with receptor IX, and it must be assumed that they have affinity for more than one receptor.

Following the same line of argument it is to be presumed that phages like $\varphi\varphi$ Ty 1-5, which only lyse typhoid bacilli, must attack a receptor peculiar to *S. typhi*. The only receptor at present known which fulfils this condition is the typhoid Vi-antigen. According to Felix this factor is absent or poorly represented in those typhoid strains which are freely agglutinable with Gaertner serum (O-agglutinins). I have found typhoid strains which are readily agglutinable by Gaertner antiserum relatively or entirely insensitive to $\varphi\varphi$ Ty 1, 2, 3, 4 and 5, and further that when these phages act on sensitive typhoid cultures, inagglutinable with Gaertner serum (O-inagglutinable forms in the sense of Felix), the resistant secondary cultures are agglutinable with Gaertner serum, i.e. O-agglutinable.

These facts speak very strongly for the special relation of *typhi*-phages of the first group (φ Ty 5 type) to the Vi-antigen. It would seem that such phages attack the Vi-antigen specifically and, altering it, render the typhoid strains sensitive to O-agglutinins.

The resistance which arises in various species of bacteria to members of the second group of *typhi*-phages (φ Ty-Gä 7 type) is also readily explicable on the argument that these phages all attack the same types of bacterial re-

ceptors throughout producing the same specific changes. In the case of typhoid bacilli these changes do not involve the Vi-receptor.

It still remains to explain the type of relationship in which ϕ A stimulates resistance to $\phi\phi$ A and B while ϕ B immunizes the bacterium only to itself, the type of relationship on which d'Hérelle based his hypothesis of quantitatively graded resistance.

I have already indicated the possibility that a single phage may have two—perhaps more—specific points of attack: it seems probable that phages of the ϕ Ty-Gä 7 type attack both receptor IX and other elements. It is also conceivable that *typhi*-phages should exist which attack both the Vi and IX receptors or which have a still wider qualitative polyvalence. Then such phages must stimulate resistance against all phages of lesser range of attack, but these more limited races would fail to immunize against all the diverse activities phages of higher valency, leaving some unchecked.

SUMMARY AND CONCLUSIONS

1. Two groups of *typhi*-phages, one lysing typhoid bacilli only, the other Gaertner bacilli also, have been studied. Each phage stimulated, in *Salmonella typhi*, resistance to all phages of its own group but none to those of the opposing group.

2. It is probable from the range of their activity, that *typhi*-phages of the second group attack the receptor IX (of the Kauffmann-White system of *Salmonella* antigens).

3. There are strong grounds for believing that the phages of the first group, the specific *typhi*-phages, attack the Vi-antigen of Felix, for

(a) typhoid cultures which are readily agglutinated by Gaertner anti-serum are relatively or entirely insensitive to these specific *typhi*-phages,

(b) the specific *typhi*-phages transform O-inagglutinable cultures into O-agglutinable forms, and

(c) the Vi-antigen is the only known element of the typhoid bacillus which is peculiar to that organism and so corresponds in specificity with the phage.

4. The fact that resistance acquired against a particular bacteriophage does not prevail against all other bacteriophages is not to be ascribed to the quantitatively differing potency of phages as suggested by d'Hérelle but to their qualitatively different activities. Each type of phage has its particular "point of attack" in the bacterium and the distinct "points of attack" of different phages develop their resistance independently. It seems that, as Marcuse has urged, the "points of attack" are located in the bacterial receptors.

(MS. received for publication 11. v. 1936.—Ed.)