THE FORMATION OF BACTERIOPHAGES IN MIXED CULTURES BY RECOMBINATION OF GENETIC ELEMENTS; CHARACTERISATION OF PHAGE TYPES OF SALMONELLA PARATYPHI B BY PHAGE REACTIONS AND LYSOGENIC PROPERTIES

by

R. TH. SCHOLTENS

(Received March 31, 1952).

The discovery of the Vi antigen by FELIX and Pitt (8) has given an impulse to the development of knowledge in another field, the field of bacteriophage. CRAIGIE and BRANDON (2), SERTIC and BOULGAKOV (19) and SCHOLTENS (17) demonstrated that specific phages exist attacking only those strains of \textit{S. typhi} that contain the Vi antigen.

A special property of a serological subgroup of these phages allowed CRAIGIE and Yen (5) to develop the method of phage typing. When propagated upon a given strain of \textit{S. typhi} these Vi bacteriophages develop a specific affinity for this very strain. Higher dilutions of the preparations obtained attack this strain, and strains from the same outbreak, and other strains considered therefore to belong to the same type. These higher dilutions are utilised so as to recognise the strains of the same type. The specificity of the preparations is such, that type after type, and preparation after preparation could be added to the system, without affecting the classification of the strains belonging to types already recognised. Up till now 29 types have been found.

It appears that the factors underlying the difference between the types rule also the affinity of the other (Vi) bacteriophages for (typhoid) bacteria. These bacteriophages each attack a different combination of types. Inversely, each bacterial type is attacked
by a different combination of phages out of a given set and can be recognised by these reactions.

It is clear that by the addition of a new phage to such a system not only bacterial strains, which did not react with any of the phages already utilised, can be recognised, but also a further subdivision of types already identified may be found.

Systems consisting of a set of unadaptable phages have been utilised successfully for typing purposes with various bacterial species e.g. *Staphylococcus aureus* [Fisk (10), Fisk and Mordvin (11); Wilson and Atkinson (21)], *S. typhi murium* [Lilleengen (12)].

In most countries for typing *S. paratyphi* B the system of Felix and Callow (7, 9) is used. This system consists partly of preparations obtained by adaptation of a given bacteriophage, and partly of other phages attacking several types. The preparations are distributed by Dr Felix from the Central Reference Laboratory to laboratories in different countries. In this way, in all countries typing is performed with the same dilutions of exactly the same preparations. There is no need to explain here the immense value of this procedure.

A second group of phenomena depending on the mentioned principle underlying sensitivity to bacteriophage is spontaneous lysogenicity. Otto and Munter (14) isolated phages from pure bacterial cultures. Lisbonne and Carrère (13) incubated mixed cultures of two different strains and demonstrated the appearance of bacteriophages. A relation appeared to exist between the phages produced by spontaneous lysogenicity and bacterial types found by phage reactions. Craigie (3) showed strains of type D₁ of *S. typhi* to be lysogenic. Boyd (1) demonstrated that strains of *S. typhi murium* can be subdivided in types characterised by the phages produced spontaneously. For *S. paratyphi* B it was found that a relation existed between "phagetypes" as recognised by phage reactions, and the characteristics of the phage produced by lysogenicity [Scholtens (18)]. This was also shown by Felix and Callow (9) and Nicolle and D’Yves Hamon (15).

Moreover, it could be demonstrated that types of *S. paratyphi* B can be characterised by lysogenicity and phage reactions [Scholtens (18)]. One of these types was a type "Midwoud" (the same strains have been typed "Beccles" by Felix).

In filtrates of type "Midwoud" only a single bacteriophage