A SEARCH FOR BACTERIOPHAGES ACTIVE UPON BACTERIA OF THE *BRUCELLA* GENUS

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Most species of bacteria have been found to be susceptible to the action of bacteriophages. No bacteriophages, however, have been found that are lytic for members of the *Brucella* genus. Gwatkin (1931) examined nine samples of faeces, thirteen milk samples, three foetuses, six foetal membranes and three specimens of blood obtained from cows, some of which were suffering from *Br. abortus* infection. None was shown to contain bacteriophages active on strains of *Br. abortus*.

Since a method of typing strains of *Brucella* would be of considerable value in the epidemiological study of *Brucella* infection in man and animals, it was decided to continue the search for bacteriophages that might be of use for this purpose. Although none was found, a short account of the methods employed may be of some value to any future workers in this field.

METHODS

Forty-eight strains of *Br. abortus*, ten of *Br. melitensis* and seven of *Br. suis* were used in these studies.

(1) A search for lysogenic strains was first carried out, as this is the method most generally used at the present time for the isolation of bacteriophages. All the strains were cross-cultured by the method of Wilson & Atkinson (1945), using different combinations of strains on different occasions. The basal and superimposed strains were used as 48 hr. liver-broth cultures and also as saline suspensions of different densities. The solid media used in the cross-culturing process included nutrient agar, liver agar, serum agar, blood agar and Bordet-Gengou medium. Incubation was carried out in air and also in 10% carbon dioxide. Different incubation temperatures were used including 37° C. for 48 hr. or longer, 37° C. for 16 hr. followed by 22° C. for 2 days, 22° C. for 24 hr. followed by 37 and 22° C. alone. Other experiments were carried out using centrifugates of liver-broth cultures for 'spotting' in the cross-culture technique. Tubes of liver broth were also seeded with very small inocula of five different strains and incubated under differing conditions. The tubes were then centrifuged and the supernatant fluid used for 'spotting'.

(2) Efforts were made to 'adapt' phages lytic for other species of bacteria, to make them lyse *Brucella* strains. The phages used were two *Salmonella typhi* Vi phages (types G and J), two *Bacterium coli* phages, one shigella phage (C16), phage C16 after 'adaptation' on a strain of *Pasteurella pseudotuberculosis rodentium* and five *Streptococcus lactis* phages. Where necessary these phages were first propagated until they were active in high titre upon their propagating strains. They were 'spotted' on to plates previously spread with liquid cultures of the *Brucella* strains. The plates were then incubated and examined. As these phage preparations were in broth, a number of them were seeded lightly with cultures of some of the brucellas, incubated, and examined for evidence of lysis. If visible growths occurred, they were spread on liver agar plates, incubated and observed for the presence of plaques. They were also centrifuged and the supernatant fluid removed and used for further passage.

(3) Seitz filtrates of thirty samples of faeces, mainly from cows, four sewage samples, and twelve specimens of uterine fluid from cows that had aborted were examined by the 'spotting' technique and also by adding to young broth cultures of brucellas, incubating and then centrifuging. The centrifugates were used for further passage and, where possible, as culture media in which the original culture was again grown. These experiments were then repeated using Seitz filtrates of 24–48 hr. liver-broth cultures made from the faeces, the sewage and the uterine fluid samples.

(4) As *Br. abortus* can usually be demonstrated in the large rail tanks of milk entering London, it was considered that this source would be a likely place to search for phages active on *Br. abortus*. Samples of raw milk from twenty of these tanks were treated with rennet and the whey subjected to Seitz filtration after preliminary centrifugation. The filtrates were then used for 'spotting' and as culture media for the *Br. abortus* strains in 20–30 ml. amounts. These were incubated under differing conditions. They were observed for any indication of lysis and, after bacterial growth had become apparent, they

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https://doi.org/10.1017/S0022172400014741 Published online by Cambridge University Press

were seeded on to liver agar plates, incubated and examined for evidence of plaques. Subsequently they were centrifuged and the supernatant fluid used for further passage.

In all the above experiments, where possible, controls, including heated filtrates, were kept. In no case was there any evidence of bacteriophage activity or any form of inhibition.

In view of this work and the previous report by Gwatkin (1931) it would appear that bacteriophages active upon brucellas do not exist, or are very rare, or the methods used are not suitable for their demonstration.

SUMMARY

1. None of forty-eight strains of *Brucella abortus*, ten of *Br. melitensis* and seven of *Br. suis* were shown to be lysogenic.

2. It was not possible to 'adapt' bacteriophages that were active upon other species of bacteria to lyse brucellas.

3. Thirty samples of faeces, mainly from cows, four sewage samples, twelve specimens of uterine fluid from cows that had aborted, and twenty samples of bulk milk failed to yield bacteriophages active upon brucellas.

REFERENCES

GWATKIN, R. (1931). *J. Infect. Dis.* **48**, 404.
WILSON, G. S. & ATKINSON, J. D. (1945). *Lancet*, **1**, 647.

(*MS. received for publication* 19. x. 49.—Ed.)