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References and Notes

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Lysis of Cultures Devoid of Vi Antigen by Vi I Bacteriophage of *Salmonella typhosa*

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The concept that the sites for bacterial receptors involved in antigen-antibody reactions correspond to those that determine phage host interactions was developed by Hadley (*1*) and by Burnet (*2, 3*) for certain *Salmonella* serotypes. Levine and Frisch (*4*) observed that differences in the somatic antigens of *Salmonella* group C could be detected by characteristic phage adsorption behavior. Although there are many examples of striking correlations between phage susceptibility and the distribution of heat-stable agglutinogens in various bacterial groups, it soon was observed that there also were many exceptions. This led Burnet (*3*) to explain these discrepancies in terms of the existence of an intimate relationship between the phage and the antibody surface rather than by the assumption of identical adsorption sites for phage and for antibody.

Craigie and Brandon (*5*) described the isolation of a bacteriophage that was specific for the heat-labile Vi antigen of the typhoid organism. The specificity of certain phages for the Vi form of *Salmonella typhosa* led to the demonstration of phage types of this organism by means of preparations of type II Vi phage (*6*). Several Vi phages have been described that are serologically distinct and differ also in respect to particle size, thermal death point, and lytic activity for Vi forms of *S. typhosa*. They have been tested on a great variety of salmonellae, shigellae, *Escherichia coli*, and on Vi and W forms of many strains of *S. typhosa* (*6, 7*). So far as we are aware, none of the Vi phages has been shown to lyse any culture that does not contain Vi antigen, although they are known to attack cultures other than *S. typhosa*. Nicolle *et al.* (*8*) reported that Vi phages lysed *Salmonella paratyphi C* (East Africa), certain cultures of the Bethesda-Ballerup group of paracolons, and cultures described by Kauffmann as *E. coli* (2624-36 and 5396-38).

The object of this report is to present evidence that the specificity of Vi phage I (*6*) is not dependent on the presence of a bacterial component that can be recognized serologically as Vi antigen. This study was initiated as the result of a chance observation of the lysis by typhoid Vi I phage of a culture of *Escherichia freundii* that did not contain Vi antigen. Examination of additional cultures of this species and of their slow lactose-fermenting analogs (*9*) (Bethesda-Ballerup paracolons) revealed that this was not a rare occurrence. Table 1 shows the relationship that exists in these cultures between susceptibility to typhoid Vi I phage and the presence of Vi antigen. The cultures that contained Vi antigen but were unaffected by the phage must be subjected to a more critical examination before it can be stated unequivocally that they are resistant.

The nature of the host-virus relationship in these cultures that appeared to lack Vi antigen was studied along the following lines: (i) comparison of the specificity and serologic nature of Vi I phage propagated on *E. freundii* and on phage type F_1 of *S. typhosa*, and (ii) demonstration of the absence of Vi antigen in certain cultures of *E. freundii* and paracolon bacteria that were lysed by the typhoid Vi I phage. The former was accomplished by the propagation of typhoid Vi I phage on a Vi negative culture of *E. freundii* through four single plaque passages. The starting material was a preparation of Vi I phage that had been propagated on phage type F_1 of *S. typhosa*. This material had been prepared in the Central Enteric Reference Laboratory under the direction of A. Felix and was dated July 1948. The stock of Vi I phage that had been propagated on *E. freundii* 10 was tested in parallel with the Vi I phage received from England by applying both of these to each of the 32 recognized phage types of *S. typhosa*. The results given by the two phage preparations were identical. Even the weaker lysis of types B3, D4, and M given

Table 1. The relationship between susceptibility to Vi bacteriophage and the presence of Vi antigen in *E. freundii* and related paracolons.

Species	No. of cultures	Action of Vi I phage	Vi antigen	Minimum No.* of O groups represented
E. freundii	9	+	−	4
	5	−	+	5
	16	−	−	9
Bethesda-Ballerup paracolons	5	+	−	3
	2	+	+	1
	6	−	+	2
	41	−	−	28

* Some *E. freundii* cultures possessed O antigens that could not be recognized with the serums available.

by the typhoid Vi I preparation was duplicated by the phage grown on *E. freundii*. Many additional tests of these two phages have failed to produce a single divergent reaction. The serologic identity of these phages was established by reciprocal cross-neutralization tests with standardized serums.

The absence of Vi antigen in a phage-susceptible culture of *E. freundii* and in a paracolon bacterium was adduced from inability to demonstrate its presence in agglutination, immunization, and agglutinin adsorption experiments. Slide-agglutination tests of numerous colonies from each of these cultures, as well as from other similar phage susceptible cultures, were made with typhoid Vi serum and were always negative. One rabbit was immunized with lyophilized organisms of *E. freundii* 10; another received cells of a paracolon culture (92) that had been dried from absolute alcohol. Antigens prepared by either means had been shown previously to be effective in engendering Vi-antibody formation by Vi-containing bacteria. No Vi agglutinin could be demonstrated in these serums either by slide- or tube-agglutination tests using living suspensions of *S. typhosa* (2V and Vi I). Although no Vi antigen could be detected by agglutination and immunization experiments, it was necessary to determine whether the cultures that were susceptible to the Vi I phage had the power to bind Vi agglutinin. For this purpose, three different paracolon strains and one strain of *E. freundii* were employed singly to adsorb a Vi serum prepared with a Bethesda-Ballerup paracolon (107). None of the adsorbing strains was able to reduce the Vi titer of this serum as measured by its ability to agglutinate living broth cultures or *S. typhosa* 2V or Vi I before and after adsorption. The adsorbing strains were fully susceptible to the Vi I phage at the time they were used in the experiment.

The afore-described experiments demonstrate the futility of any attempt to establish an absolute correlation between the presence of any particular antigenic constituent of a bacterial cell and its susceptibility to phage. Phage adsorption is a complex process that may be most easily understood in terms of the complementary ionic atmosphere of the phage particle and the adsorbing surface (*10*). It is quite likely that Vi antigen, represented by the presence of certain chemical groupings at the cell surface, exerts its effect on specificity of Vi phages insofar as it contributes electrostatic configurations favorable for adsorption. Pronounced effects of both Vi and O antigen on the electrophoretic behavior of *S. typhosa* have been reported (*11*). Similar effects were also observed (*12*) in a study of the stability of different forms of the typhoid organism in buffer solutions at various *p*H levels. However, it appears that substances serologically unrelated to Vi antigen may mediate the attachment of Vi phages to the cell. In all cultures of *S. typhosa*, and in at least some cultures of *E. freundii* and related paracolons, phage lysis of Vi+ and phage resistance of Vi– forms in the same culture were observed regularly. Vi antigen appears to condition these cells to lysis by Vi phages.

The present observations have no significance for the routine typing of *S. typhosa* by means of specific Vi phages. They may, however, be quite significant for the problem of distribution in nature of Vi-like substances. There are indications that Vi phages other than phage I may have an affinity for some Vi-negative cultures.

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Effects of Destructive Distillation on the Uranium Associated with Selected Naturally Occurring Carbonaceous Substances*

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Little is known regarding the organo-uranium compounds or complexes that may exist in uraniferous coal, shale, or other naturally occurring carbonaceous substances.

During the course of geochemical studies on uraniferous coals (*1*), yields of oil, water, and char were determined by a Fischer assay method (*2*). Because of the nature of this technique, it was difficult to establish with certainty whether volatile uranium compounds had been formed during the course of the retort assay. To investigate this possibility, a number of naturally occurring carbonaceous substances have been subjected to dry distillation in small-scale glass apparatus. Experimental results were evaluated on the basis of material balances using accurate techniques for determining the uranium content of the original substances and of the chars obtained from them. A preliminary search of the literature has revealed no publications regarding the volatilization of uranium from such substances during dry distillation.

This work is part of a program undertaken by the U.S. Geological Survey on behalf of the Division of Raw Materials of the Atomic Energy Commission.

The following samples were chosen to provide a variety of carbonaceous substances; the uranium con-