VI PHAGE-HOST INTERACTION IN SALMONELLA TYPHOSA

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Since their discovery by Craigie and Yen (1938), the Vi typing phages have been extensively employed in the study of the epidemiology of typhoid fever. Susceptibility to these phages is related to the presence of the Vi antigen, a component found in almost all freshly isolated typhoid cultures. The specific typing preparations, which produce confluent lysis with their homologous typhoid strains, were derived by a process of "phage adaptation" of Vi phage II. Using these phages with a standardized technique for demonstrating lysis, Craigie and Felix (1947) have devised a scheme for the classification of the majority of strains of Salmonella typhosa.

Until recently, little attention was devoted to the mechanism of variation ("phage adaptation") in Vi phage II or to a detailed study of the phages themselves. Anderson and Felix (1953a) examined ten of the standard Vi typing phages by growing them on degraded Vi strains of S. typhosa (i.e., strains lysed by a number of heterologous typing phages). Their results have indicated that the phages can be separated into two groups. The specificity of one group showed a phenotypic modification after propagation on the degraded strains as evidenced by a host controlled variation in lytic range of the resultant phage, while the host range of the other group remained unchanged. Further experiments by Anderson and Felix (1953b) also clarified the part played by the latent phages carried by some of the recognized Vi phage types of S. typhosa. In contrast to the Vi phages, the activity of these latent phages is correlated with the somatic antigen as indicated by their lytic action on Vi negative strains. These lysogenic phages have been implicated by Anderson and Felix as the agents which determine the Vi-type specificity of the strains harboring them. Thus, these workers concluded that the Vi antigen plays a part only in the absorption of Vi phage since phage growth and Vi-type specificity seem to be independent of the Vi antigen.

A further phage-host relationship, the ability of the Vi phage II typing phages to transfer hereditary characteristics from one typhoid strain to another, has been described briefly in a preliminary communication (Baron et al., 1953). The Vi phages appear to act in a manner analogous to the phage PLT-22 employed by Zinder and Lederberg (1952) and Lederberg and Edwards (1953) in their recent experiments. Genetic effects produced by the Vi phages, however, are dependent on the occurrence of the Vi antigen while the action of phage PLT-22 has been correlated with the presence of the XII somatic factor in Salmonella strains.

It is the purpose of the present paper to summarize some of the characteristics of the Vi typing phages as exemplified by the genetic manifestations of phage-host interaction in this system.

MATERIALS AND METHODS

Phages. The specific Vi-phage preparations ("adapted phages") all originated from Vi phage II and represent a series of serologically identical phages which differ in host range. These phages were obtained from Dr. Cora Gunther and Dr. P. R. Edwards and were maintained by propagation on their homologous Vi types of S. typhosa.

Strains. In addition to the Vi-type strains of S. typhosa, a number of other strains and species were employed. A descriptive list of these strains is provided in table 1.

Media. Nutrient broth (Difco) and penassay broth (Difco) were used routinely. Nutrient agar plates were prepared by the addition of 15 g of agar per liter of nutrient broth. Semisolid agar plates were prepared by adding 3.5 g of agar to a liter of nutrient broth. Soft agar for phage assay contained 7 g of agar per liter of distilled water. Eosine-methylene blue agar plates were made using the formula described by Lederberg (1950) for the preparation of a complete EMB medium. Sterile solutions of the appropriate
TABLE 1

| Strain     | Lysotype | Serotype | Biotype
|------------|----------|----------|--------
| S. typhosa 0901 | —        | IX XII, — | + —   |
| S. typhosa Ty2  | Ei       | VI IX XII, d: + | — —   |
| S. typhosa 643  | C4       | VI IX XII, d: — | — —   |
| S. typhosa 58   | degraded | VI IX XII, d: + | — —   |
| S. typhosa A    | degraded | VI IX XII, d: + | — —   |
| S. typhosa E1   | Ei       | VI IX XII, d: + | + +   |
| S. typhosa K    | K        | VI IX XII, d: — | — —   |
| S. typhosa Vi I  | D        | VI (IX), d: — | + —   |
| S. paratyphi C  | C        | VI VI VII, c: + | + +   |
| S. ballerup     | —        | [VI] XXIX, + | + +   |
| E. coli 5396/38 | —        | [VI] XXXI + | + +   |

( ) Not all of this antigen present.
[ ] May or may not be present.
\* Not investigated.

Carbohydrates were added as needed prior to the pouring of plates. A synthetic medium previously described by Formal et al. (1954) was used, with slight modification, in certain experiments.

Standard phage techniques described in detail by Adams (1950) were used for the production of phage lysates. These preparations were filtered through Corning sintered glass filters of ultrafine porosity, checked for sterility, and then assayed by the agar layer method of Gratia (1936). Phage stocks containing $10^8$ to $10^4$ phage particles per ml were prepared routinely either on plates or in aerated broth cultures.

**Experimental Results**

One step growth curves. The single step growth method of Ellis and Delbrück (1939) as outlined by Adams (1950) was applied in order to determine the quantitative characteristics of the phage. By means of this procedure, a comparison can be made of the action of the specific phages on various strains belonging to the same or related types. *S. typhosa* Vi-type strain A is lysed to titer by all of the Vi phages (as compared with the activity of the phage on its homologous strain) and can therefore be used as the plating strain or indicator for any of the phages.

Vi phage E1 produces a confluent lysis on *S. typhosa* type strains A, E1, and E4 as well as on *S. typhosa* Ty2 isolated from a typhoid fever outbreak and classified as an E1 type strain. A series of one step growth curve experiments was performed by adsorbing Vi phage E1 on each of these strains and then plating from the growth tubes on all of the indicator strains. The cultures were grown in nutrient broth at 37 C with aeration for approximately 12 hours and then diluted to a concentration of $5 \times 10^7$ cells per ml. Vi phage E1 was diluted in broth to a titer of $2 \times 10^9$ phage particles per ml. The adsorption tubes contained 0.1 ml of phage to 0.9 ml of the respective cultures. A 1:10 dilution of anti-Vi phage II serum was used to neutralize the free phage. The resultant growth curves are illustrated in figure 1. Vi phage E1, after adsorption on strain A, plated only on strain A, apparently having lost its ability to lyse other strains. The propagation of phage E1 on strain Ty2, considered to be an E1 type strain, yielded a growth curve which differed appreciably from that of phage E1 grown on its homologous strain. The latent period was approximately doubled; the length of the rise period increased somewhat along with a decrease in the burst size and step size. Thus, while Vi phage E1 was adsorbed on the different host cells with approximately the same rates of adsorption, the growth curves revealed both qualitative and quantitative dif-

**Figure 1.** One step growth curves of Vi phage E1 adsorbed on typhoid strains A, E1, and Ty2 and plated on these strains. The phage titer is relative to that in the first growth tube during the latent period. Time of adsorption: 8 minutes.
A somewhat similar situation occurs in the case of the Vi phages, where any of the cells in the plating population devoid of the Vi antigen (W forms) will fail to adsorb and be affected by the phage, thus giving rise to resistant clones. However, in the same population resistant forms are also found which possess the Vi antigen and still absorb Vi phages including the phage which originally lysed the strain. It is generally appreciated that the failure of the lytic reaction in this case and throughout the typing scheme is not due to any inherent change in the Vi antigen (Nicolle et al., 1951).

It might be expected that purified Vi antigen preparations, isolated from typhoid strains and other Salmonella species, would exhibit an antiphage action regardless of whether these strains are lysed by the Vi phages since the phages are adsorbed by the Vi forms of these strains. Preliminary experiments using the supernatant from an 18 hour broth culture of S. typhosa Ty2 disclosed inhibitory effects on both Vi phage E1 and on a Salmonella phage included for control purposes, which lyses both Vi positive and Vi negative strains of S. typhosa. This supernatant, however, failed to show any inhibitory action for the coliphage T1 also employed as a control.

Purified Vi antigens isolated from Salmonella ballerup, Escherichia coli (strain 5396/38), and S. typhosa (strain Ty2) by Webster et al. (1954) were made available for the determination of their activity towards Vi phage E1, the type specific phage for strain Ty2. Dilutions of this phage in broth were made to contain approximately $5 \times 10^6$ phage particles per ml. The purified antigens were dissolved in 0.1 m phosphate buffer, pH 7.0, and were diluted in nutrient broth to give concentrations of 0.01 mg, 0.001 mg, and 0.0001 mg per ml when 0.1 ml aliquots were added to 0.9 ml of diluted phage. All tubes were incubated for 18 hours at 37 C and then assayed for residual phage activity by plating with S. typhosa, strain A. In addition, both a crude and a purified preparation of the somatic antigen of S. typhosa (Webster et al., 1953) were examined using this procedure.

The results of this experiment are presented in table 2 and indicate a specific antiphage action by each of the purified Vi preparations for Vi phage E1. The amount of purified antigen necessary to inhibit 50 per cent of the phage particles is of the order of 0.003 mg for all of these prepara-

**Figure 2.** One step growth curves of Vi phage K adsorbed on typhoid strains K and A and plated on these strains. The phage titer is relative to that in the first growth tube during the latent period. Time of adsorption: 8 minutes.

Inhibition by purified Vi antigen. Many investigators have demonstrated the specific in vitro inactivation of phage by extracts prepared from susceptible cells. Goebel and Jesaitis (1952), however, were the first to show a chemical difference in the purified somatic antigen preparations derived from phage sensitive and phage resistant forms of Shigella sonnet. This difference serves as an explanation for both the lack of antiphage activity of the antigen isolated from the phage resistant variant as well as the inability of this variant to adsorb the phage.
TABLE 2

Inhibition of Vi phage E₁ by purified antigen preparations

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Source</th>
<th>Amount Inhibiting 50 per cent of Phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>7ED Vi antigen</td>
<td>S. ballerup</td>
<td>0.0030 mg</td>
</tr>
<tr>
<td>(purified)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51ED Vi antigen</td>
<td>S. typhosa</td>
<td>0.0035 mg</td>
</tr>
<tr>
<td>(purified)</td>
<td>Ty2</td>
<td></td>
</tr>
<tr>
<td>214ED Vi antigen</td>
<td>E. coli</td>
<td>0.0030 mg</td>
</tr>
<tr>
<td>(purified)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>209 O antigen</td>
<td>S. typhosa</td>
<td>no inhibition 1.0 mg</td>
</tr>
<tr>
<td>(crude)</td>
<td>0901</td>
<td></td>
</tr>
<tr>
<td>210 O antigen</td>
<td>S. typhosa</td>
<td>no inhibition 1.0 mg</td>
</tr>
<tr>
<td>(purified)</td>
<td>0901</td>
<td></td>
</tr>
</tbody>
</table>

Coliphage T1 and a Salmonella phage employed as controls were not inhibited by the highest level of the antigens used.

Landy, Webster, and Sagin (1954) have examined these Vi antigens by a number of immunological tests and have demonstrated that the products derived from E. coli and S. ballerup were significantly more active than that obtained from S. typhosa. Studies on the physicochemical characteristics of these antigens have shown that significant differences exist in their viscosities and acetyl contents (Webster et al., 1954). These tests probably are more sensitive than the phage inhibition procedures which failed to reveal any differences between the Vi preparations. Both the crude and purified somatic antigen preparations from the Vi negative strain, S. typhosa 0901, exhibited no effects towards Vi phage E₁ in amounts exceeding 1 mg.

Transfer of genetic factors by Vi phage II. The competence of the Vi phages in transduction has been previously reported in a preliminary note (Baron et al., 1953) which described the transfer of xylose fermenting ability and streptomycin resistance to typhoid strains lacking these factors by Vi phages E₁ and K. A number of other Vi II phages in addition to types E₁ and K have since been tested, and these have all been found to possess transducing ability. These include phages A₁, B₁, C₁, D₁, E₂, F₁, H₁, and 28. Although all of the Vi II typing phages were not examined, it seems likely that all will act in the same manner. In addition, a number of the type strains which were found to be xylose negative have had this factor transduced by treatment with phage lysates prepared from xylose positive strains.

These strains include S. typhosa types C₅, M, N, O, T, and 25.

1. Fermentative. The transfer of arabinose and rhamnose fermenting ability to typhoid strains lacking these factors was accomplished using phage lysates prepared from mutants positive for these factors, as well as with lysates from strains which were altered for these factors by transduction. The spontaneous mutants occur at a rate of approximately 1 in 10⁶ cells and do not affect the demonstration of the transduction of these factors since a phage treated suspension of 10⁶ cells when plated on EMB plates may show as many as a thousand or more positive clones. Strains altered by the addition of the arabinose factor include the serologically rough culture S. typhosa Vi I which is essentially devoid of the typhoid somatic antigens (table 1). A phage lysate prepared from a xylose positive-arabinose positive strain (Xyl⁺, Ara⁺) will transduce either of these factors to Xyl⁻, Ara⁻, strains, but not both in the same cell. The rate of transduction appears to be considerably higher and more directly related to the number of phage particles adsorbed in the case of the arabinose factor, as opposed to a much lowered frequency of observable transduction in the case of the xylose factor (figure 3). It has not been determined whether the explanation for this phenomenon depends on the inhibitory action of xylose on typhoid strains unable to attack this sugar or is of a more involved genetic nature.

In general, the recipient strains are of different phage types than the phages with which they are treated, allowing transduction to occur in the absence of detectable lysis and in the absence of appreciable cell multiplication as well since a ten minute exposure of the recipient cells to the phage will suffice. The genetic change observed in the altered clones represents a permanent modification of genotype of the cell since the progeny remain positive for the transduced character regardless of the number of transfers made either in the presence or the absence of the selective agent.

2. Nutritional. Recent experiments by Formal et al. (1954) have dealt with a naturally occurring auxotrophic strain of S. typhosa having a nutritional requirement which renders it avirulent for mice. This strain, designated as S. typhosa strain 58 (avirulent), was shown to require a purine in addition to the two amino acids necessary for
the growth of the virulent strain 58. Phage typing of the nutritionally deficient strain has served to classify it as a degraded strain, lysed by any of the typing phages. However, under appropriate conditions the nutritional factor, in this case the ability to synthesize purine, can be transduced to this strain using the “adapted” Vi II phages.

In the demonstration of this genetic factor transfer, as in other experiments, the procedure consists merely of exposing the cell suspension to the phage lysate and then washing the culture before plating on a sufficiently selective medium. In this case, a synthetic medium fortified with cystine and tryptophan but completely deficient in purines was prepared on which carefully washed suspensions of phage treated cells were spread and the plates then incubated at 37°C. Transduction of this factor was evidenced by the appearance of normal sized clones which were observed in considerable number within 72 hours. The spontaneous mutation rate to purine independence is of an extremely low order, and reverted cells were rarely encountered on control plates spread with suspensions containing as many as $10^8$ cells. In the absence of the purine requirement, the cells become virulent for mice as indicated by an LD$_{50}$ of approximately 20 cells in a mucin challenge and in this respect are now similar to virulent typhoid strains. In contrast the LD$_{50}$ as determined for the original culture is of the order of $2 \times 10^4$ cells. The procedures utilized in the determination of the virulence of the cells for mice were essentially those described by Formal et al. (1954).

3. Flagellar. As an example of transduction between Salmonella species, a serotypic combination of Salmonella paratyphi C (Vi VI VII, c:1,5) and S. typhosa Ty2 (Vi IX XII, d: -) was obtained by treatment of the S. paratyphi C strain with a Vi phage lysate prepared from S. typhosa Ty2 using phage E$_1$. Motile forms were isolated by the methods described by Lederberg and Edwards (1953) using a semisolid agar containing flagellar factor “c” and “1,5” antisera, with enough serum added to the semisolid agar to completely immobilize untreated cell suspensions. The motile S. paratyphi C forms re-isolated from the semisolid medium inoculated with the phage treated cell suspension were found to possess the unique serotype: Vi VI VII, d $\leftrightarrow$ 1,5, the “d” phase of the flagellar antigen representing a factor transferred from S. typhosa. This transduction indicates a linking of Group C with Group D of the Kauffmann-White Scheme by employing the Vi antigen as the common factor between the two strains.

**DISCUSSION**

Phage typing procedures are of major epidemiological importance in areas where many
types of *S. typhosa* occur. However, it is apparent that this technique of differentiating cultures loses its value in those localities where one particular phage-type strain predominates. Under these circumstances, the biochemical classification of typhoid strains proposed by Kristensen (1938) may be of use. This classification divides typhoid strains into four categories (biotypes) based on their ability to ferment xylose and arabinose. A number of other investigators have questioned the value of this type of classification because of the possible instability of the fermentative characters. It is, of course, obvious that the value of any single test depends upon the stability of the factor under examination. Felix and Anderson (1951) have discussed precisely this situation and have concluded that it is necessary to employ a number of tests in order to distinguish an epidemic strain from other strains of the same type. Anderson (1951) has also shown that changes in Vi phage type of typhoid strains may be caused by the action of lysogenic non-Vi phages, whose activity is correlated with the presence of somatic components. In addition, it has now become evident that host genetic factors, the expression of which serves to distinguish these strains, can be affected by the Vi phages. While the lytic reaction may be blocked by the presence of the lysogenic type determining phages, strains carrying these phages will nevertheless be altered by the Vi phages. Thus, the observation that the genetic constitution of typhoid strains is quite variable accentuates the hazards inherent in relying on a single criterion in the characterization of these strains for epidemiological purposes.

Pavlatou and Nicolle (1953) have attempted to correlate the differences in fermentative capacity (biotypes) of the strains with their sensitivities to the typing phages (lysotypes). Our experience has indicated that the different biochemical types occur in a fortuitous manner and that by appropriate treatment the various biotypes can easily be produced in the laboratory without a concomitant effect on the phage sensitivity of the strains.

Aside from practical consideration, a number of interesting properties of genetic significance are exhibited by the Vi phages. A combination of host-virus interaction may take place whereby the phage may either cause an alteration in the genetic makeup of the host cell or may itself be altered by the host cell. Thus, phage E, after adsorption on *S. typhosa* strain A, can in turn, either genetically alter or lyse its host cell. If lysis occurs, phage particles are released which show a phenotypic change attributable to host-modification in that they no longer will lyse E, type strains but possess the ability to alter these strains in transduction.

The inhibition of Vi phage by purified Vi antigen is in accordance with previous findings which indicate that the somatic antigen plays no part in the initial adsorption of the phage. Nicolle *et al.* (1951) have demonstrated that Vi containing species other than typhoid, which lack any common somatic factors, can also be lysed by the typhoid Vi phages under certain conditions although whether this occurrence is due to selective mechanisms or to host modification does not seem entirely clear. The demonstration of flagellar transfer between species with only the Vi antigen in common, as well as the transfer of genetic factors to a serologically rough strain of *S. typhosa*, indicates, moreover, that transduction with these phages is compatible with different somatic or rough antigens.

It seems probable that transduction and other related mechanisms involving phage are more prevalent than is at present realized. Iseki and Sakai (1954) have reported on the transduction of biochemical properties in the *Salmonella E* group using a bacteriophage derived from a lysogenic strain of *Salmonella newington*. In addition to the Vi phages and the phage PLT-22 of Zinder and Lederberg (1952), other phages acting as agents of genetic transfer have been encountered in this laboratory and in other laboratories (Lederberg, 1953, personal communication). In any case, the importance of phage as a link in the hereditary mechanisms of microorganisms seems well established and may serve as a model for other viral studies.

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**SUMMARY**

Studies with Vi phage II have been undertaken using the one step growth curve technique. Changes in phage specificity as well as quantitative differences in phage yield were demonstrated when various strains of the same phage type were
employed as host cells. The inhibitory action of purified Vi antigens isolated from three enteric species was examined, and the degree of antiphage activity exhibited by each of the preparations was found to be in close agreement.

Transfer of genetic factors by the Vi phages was examined as a phage-host interaction occurring in this system. The transduction of carbohydrate fermentation factors, as well as of a nutritional factor bearing a relationship to mouse virulence, was demonstrated using typhoid strains and various Vi typing phages. A transfer of flagellar antigen between two Salmonella species was obtained by treatment of Salmonella paratyphi C with a Vi phage lysate prepared from Salmonella typhosa. Possible genetic considerations were discussed, and reference was made to the practical aspects of phage typing of typhoid strains.

REFERENCES


Anderson, E. S. 1951 The significance of Vi-phage types F1 and F2 of Salmonella typhi. J. Hyg., 49, 458.


Kristensen, M. 1938 Studies on type division of typhoid and paratyphoid B bacilli by fermentation. J. Hyg., 38, 688-701.


