A New Vi-phage Type of *Salmonella typhi*; with a Discussion of Methods of Preparation of Typing Phages for New Vi-Types

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SUMMARY: A new Vi-phage type of *Salmonella typhi*, T4904, is described. The homologous typing phage (phage 4904) was prepared from phage D6, a host-range mutant of Vi-phage II. The new type belongs to the E group and consists of type E1 carrying the determining phage d6. The formula E1(d6) has therefore been assigned to it. The host range of phage 4904, the homologous typing phage, is considered in relation to the structural formula of T4904. This formula fully explains the ability of the phage to lyse strains other than T4904. The general aspects of adaptation of Vi-phage II to new Vi-types of *S. typhi* are discussed. It is shown that the older attitudes to this subject require modification in the light of recent work. The applications of the results of study of the temperate type-determining phages to the solution of practical problems in Vi-phage typing are described.

The commoner Vi-phage types of *Salmonella typhi* were described some years ago (Craigie & Yen, 1938; Craigie & Felix, 1947), but strains which represent new, rather rare, types are still encountered occasionally. The type to be described was isolated in October 1952 from the blood and faeces of a Chinese girl of 18 years who was suffering from clinical enteric fever; the strain was numbered T4904. The patient lived with her brother in Bradford, and on investigation the brother, who was free from symptoms, was found to be excreting *S. typhi* in his urine. He was 25 years of age and had come to this country from Hong Kong in 1949. No history of typhoid fever was obtained from this man, but it was assumed that he was a chronic carrier. The strain isolated from him proved to belong to the same Vi-type as T4904. It is not yet known whether this type is endemic to Hong Kong. Reference has been made to T4904 in a previous publication (Anderson & Fraser, 1955a) but a full description of the new type seems worthwhile because the work on it provided opportunities for the practical confirmation of hypotheses recently advanced by the author (Anderson, 1955; Anderson & Fraser, 1955a).

The Vi-types of *Salmonella typhi* and their corresponding typing phages, all of the latter being derived from Vi-phage II of Craigie & Yen (1938), are designated by identical symbols. For example, type D6 is lysed by phage D6. A number of Vi-types of *S. typhi* owe their type specificity in part to the presence of temperate phages which are designated ‘type-determining’ phages (Anderson, 1951; Felix & Anderson, 1951; Anderson & Felix, 1953b). The remainder of the specificity is controlled by the non-lysogenic precursors of the types. The term ‘non-lysogenic’ is used only in relation to the absence of type-determining phages. Many temperate phages that do not have a type-
determining function are found in *S. typhi*; the presence or absence of these is not considered here. The type-determining phages are designated by small letters corresponding to the capitals used for the respective lysogenic Vi-types, and by a number with a superscript prime sign when the Vi-type from which they are isolated is designated numerically. Thus, type D6 carries phage d6 and type 25 phage 25'. The determining phages are unrelated to Vi-phage II, from which the typing phages are derived. The recent suggestions concerning the use of structural formulae for the lysogenically determined Vi-types (Anderson, 1955; Anderson & Fraser, 1955a) will be adopted at relevant points in this paper. The formulae consist of the symbol of the non-lysogenic precursor type followed by that of the temperate type-determining phage in parenthesis. Thus, type D6, which can be prepared artificially by lysogenizing type A with phage d6, is designated A(d6). Although they appear to be distinct, phages f2 and 30' possess identical type-determining characters and one only may be specified in the formulae of types which may be determined by either. For example, type 29 can have the alternative formulae of A(f2) or A(30'); for convenience, only one of these formulae may be given.

**EXPERIMENTAL**

**Media**

Bacto dehydrated nutrient broth (Difco Laboratories), in a strength of 2%, was the nutrient basis of all media used in the experiments described in this paper. For the preparation of solid media 1.3% New Zealand powdered agar was added.

**Examination of T4904**

Tests with the Vi-typing phages showed that T4904 was resistant to all the available 'adapted' preparations of Vi-phage II in routine test dilution (R.T.D.). Two other Vi-phages described by Craigie & Yen (1938) are used in this laboratory in routine typing tests. These are known as Vi-phages I and IV. T4904 was sensitive to the former but not to the latter of these phages. It was resistant to a non-Vi phage used in this laboratory in routine phage-typing tests.*

Phage A is probably the wild type of Vi-phage II (Anderson & Fraser, 1955a) and has been regarded as the most suitable starting-point for the preparation of typing phages for new Vi-types (Craigie & Felix, 1947; Anderson & Felix, 1953a). Attempts were made, therefore, to adapt phage A to T4904, but these were unsuccessful. Although the strain was resistant to the routine typing adaptations of Vi-phage II, that is, no considerable degree of lysis occurred with any typing phage, phage D6 regularly produced a few plaques

* This phage was mentioned originally by Felix & Callow (1943) and has been previously referred to as an 'O phage' (see, for instance, Anderson & Felix, 1953a). Unpublished observations made by the present writer in 1949 showed that this phage attacks the majority of salmonellas and, in view of this indication of non-specificity in regard to the O complex, and also because it attacks some rough salmonellas lacking the O antigen, the designation 'non-Vi phage' is most suitable.
on T4904 and phage E1 occasionally did the same. A detailed titration of phage D6 on type A and T4904 revealed that its titre on T4904 was 1/1,000 of its titre on type A. Single plaques were cut from agar plates in which phage D6 had been titrated on T4904, together with a small amount of the surrounding culture, transferred to 20 ml. amounts of nutrient broth in screw-capped bottles, and incubated for 5 hr. at 38.5°. The resulting lysates were heated to 57° for 40 min. to kill the host cells, centrifuged to remove the dead bacilli, and titrated on types A and D6 and on T4904. This titration showed that the newly adapted phages had titres approaching 10⁶, that they attacked all three of the indicator strains used equally well, and that they had R.T.D.'s of at least 1/5000. If they exhibited a satisfactory degree of specificity, therefore, they would be suitable for use as typing phages for the new type represented by T4904.

A single line of the adapted phage, which will be designated hereafter 'phage 4904', was chosen for further scrutiny. Since its preparation in 1952 this phage has been tested on a number of occasions on all the available Vi-type strains of Salmonella typhi, and T 4904 has similarly been subjected to many tests with all the available typing phages. The results of this work can be summarized as follows:

(1) Phage 4904 in R.T.D. produces the same degree of lysis on types A, D1, D3, D6, E1, 29 and T4904. It also lyases equally well type E7, which was first prepared artificially and defined by the author in 1951 (see Felix & Anderson, 1951; Anderson & Felix, 1953b) and has recently been discovered in the field by Scholtens. The designation type E7 is recent (see Scholtens, 1955). The author's type E7 has the formula E1(f2), whereas Scholtens's representative of the type appears to be E1(30'); for a further discussion of this subject see Anderson & Fraser, 1955a). Phage 4904 does not attack the remaining specific Vi-types of Salmonella typhi. It is neutralized by anti-Vi-phage II serum prepared by immunizing rabbits with phage A.

(2) T 4904 is fully lysed only by its homologous adapted phage in R.T.D. It is also sensitive to a lesser extent to phage E7.

These findings prove that T 4904 represents a new type. The cross-reactions of its homologous typing phage will be discussed later.

**The structure of the new type**

T 4904 was found to be carrying a temperate phage which was destroyed by heating to 57° for 30 min., formed micro-plaques on types A, C and E1 and did not attack types D6, F2, 29, 30 or T4904. Type A, when lysogenized with this phage, became type D6; the phage converted type C into type C2 (which is also known as type 38), and type E1 into a type identical with that of T 4904. The changes undergone by types A and C in this series of experiments showed that the phage carried by T 4904 belongs to the d6, f2, 30' group of type-determining phages, and it appears to be indistinguishable from phage d6 described in previous publications (Felix & Anderson, 1951; Anderson & Felix, 1953b).

Strains carrying phages of the d6, f2, 30' group tend on storage to lose their
A new Vi-phage type of S. typhi

Determining phage so that non-lysogenic variants can be isolated from them (Anderson, 1951; Felix & Anderson, 1951). When T4904 was stored at room temperature on dry Dorset egg slopes without subculture for about a year it exhibited greatly increased cross-reactions with phages E1 and E2, and a number of single-colony isolations from such a culture proved to belong to type E1 (which is fully sensitive to phages E1 and E2) and were non-lysogenic. These E1 lines, when lysogenized with phage d6 or with the determining phage carried by T4904, were re-converted into strains showing phage sensitivities identical with those of T4904. This work proved that T4904 consists of type E1 carrying phage d6; it thus belongs to the E group of Vi-types and the structural formula E1(d6) has been assigned to it in accordance with the suggestions of Anderson (1955) and Anderson & Fraser (1955a).

The host range of phage 4904

It has been pointed out that the host range of phage 4904 includes types A, D1, D5, D6, E1, E7 and 29 as well as the homologous strain. Type A is lysed by all adaptations of Vi-phage II and can be omitted from the following discussion. The remainder of the host range of phage 4904 can be explained on the principles recently described by Anderson (1955) and Anderson & Fraser (1955a). T4904 carries the determining phage d6. It can only be lysed, therefore, by the host-range mutant of Vi-phage II which is able to overcome the obstacle offered by this determining phage; this mutant is the same as that which can lyse type D6 (structural formula A(d6)) and, when obtained in the pure state, constitutes the typing phage D6. Therefore, the typing phage for T4904 will lyse type D6 and any other types lysed by phage D6. Such types are, in the D group, D1 and D5. The ability of phage 4904 to lyse type E1 is due to the fact that T4904 has the formula E1(d6) and, in adaptation to it, Vi-phage II undergoes a phenotypic change identical with that elicited by type E1. Phages d6, f2 and 30' are closely similar in properties (see Anderson & Felix, 1953a), but strains carrying phage d6 are relatively resistant to Vi-phage II adapted to strains carrying phages f2 and 30'. The converse does not hold, however, for strains carrying phages f2 and 30' are fully sensitive to adaptations of Vi-phage II to strains carrying phage d6. Thus, type 29 (= A(f2) or A(30')) is fully sensitive to phage 4904 because the latter is adapted to a strain of the formula E1(d6). Moreover, as phage 4904 possesses the E1 adaptation, it will also lyse type E7, which has the alternative formulae of E1(f2) or E1(30'), as effectively as it lyses type 29.

DISCUSSION

There has been a tendency to exaggerate the importance of using phage A as the starting phage for the adaptation of Craigie & Yen's Vi-phage II to new Vi-types of the typhoid bacillus (see, for example, Craigie & Felix, 1947; Anderson & Felix, 1953a). This has been due to lack of knowledge of the processes underlying the phenomena of adaptation. It was shown by Anderson & Felix (1952, 1953a, c) that both phenotypic and genotypic changes played
a part in the evolution of the Vi-typing phages. However, it was suggested by
the same authors (1953a, c) that the whole process of adaptation of Vi-phage II
to Salmonella typhi was phenotypic in nature. This suggestion would be
confirmed if a host cell were identified which could precipitate the total rever-
sion of host range of apparently permanently altered preparations to that of
phage A, which is probably the wild type of Vi-phage II. Recent work has
shown this view to be incorrect (Anderson, 1955; Anderson & Fraser, 1955a, b).
It has been shown that Vi-typing phages having stable host ranges which are
different from that of phage A are host-range mutants of Vi-phage II. They
exhibit a clonal distribution in fluctuation tests and pre-exist in concentrated
stocks of phage A. Thus, the adaptation of phage A to Vi-types of S. typhi that
can only be lysed by such host-range mutants is a process of selection of the
mutants concerned.

**Determination of the genotype of Vi-typing phages**

The genotype of a typing adaptation of Vi-phage II can be determined by
growing the phage on type A. Under such conditions the phenotypically
modifiable portion of the phage reverts to the wild state, thus unmasking the
basic genotype. The change of an adapted phage to phage A when grown on
type A indicates that it had undergone a phenotypic change only during its
original adaptation, and that it possesses the wild genotype of Vi-phage II. As
the majority of the lysogenically determined types can be lysed only by host-
range mutants of Vi-phage II (Anderson & Fraser, 1955a), the reversion of an
adapted phage to phage A by growth on type A suggests that the Vi-type of
Salmonella typhi responsible for the earlier adaptation is not lysogenically
determined. Thus, phage E1 changes to phage A during propagation on Type A.
As phage A is the wild genotype of Vi-phage II the change undergone by
phage E1 indicates that it is unlikely that type E1 owes its type specificity to
the presence of a determining phage; no determining phage has hitherto been
isolated from type E1. In contrast to the foregoing example is that of phages
F2, 30 and E7 which correspond to Vi-types of S. typhi having the formulae
F1(f2), C(f2) and E1(f2), respectively. They all attack types A and 29
(type 29 has the formula A(f2)) equally well. When these phages are grown on
type A they all change to a phage corresponding to a Vi-type of the formula
A(f2), that is, to phage 29. This phage lyases only types A and 29 and represents
the basic host-range mutant which is capable of overcoming the block in the
multiplication of the wild genotype of Vi-phage II produced by the presence of
the determining phage f2. It will be seen that an experiment of the type just
described gives valuable information concerning the nature of the determining
phage that may be carried by a Vi-type of which the structural formula is
unknown.

These observations necessitate a change of attitude towards the adaptation
of Vi-phage II to types of Salmonella typhi that can only be lysed by host-range
mutants of the Vi-phage. Many such types owe their specificity to determining
phages. T4904 provides an excellent example of this phenomenon. As its
formula is E1(d6) it demands two changes in Vi-phage II before it will support
A new Vi-phage type of S. typhi

the growth of this phage. Firstly, there is the host-range mutation which enables the Vi phage to overcome the barrier to its multiplication erected by phage d6; this mutant occurs in concentrated populations of phage A with a frequency of about 10^{-6} and, as has been indicated above, is identical with the typing phage D6. Secondly, the selected mutant particle must undergo phenotypic modification to enable it to multiply in the E1 component of the E1(d6) complex; such modifications occur with a frequency of about 10^{-8}. Thus, when a concentrated stock of phage A is applied to a culture of the formula E1(d6) the frequency with which the necessary double event will enable a suitable particle to encounter a cell that it can lyse without obstruction is of the minute order of 10^{-9}. The mutant selected will have the same genotype as that which lyses type D6 (=A(d6)), that is, it will have the genotype of phage D6. The slender chance of detecting such an occurrence is complicated by the possibility that the mutant selected by strain E1(d6) from the parent population of phage A may differ in some details of host range from that of the known phage D6; this will cause confusion when its host range is examined and when the relations of its homologous type strain to other types are being defined.* A rational approach, therefore, when attempting to adapt Vi-phage II to a strain with a formula such as E1(d6), is to commence with a mutant of correct initial host range when possible; the chances of successful adaptation thereby become greatly improved. For example, the adaptation of phage D6 to E1(d6) requires only the phenotypic change to cover the E1 specificity; the frequency of this change is 10^{-9}—an enormous improvement on the 10^{-9} chance indicated above.

The adaptation of Vi-phage II to new types of Salmonella typhi

The practical application of these principles concerns the adaptation of Vi-phage II to new types that carry determining phages. Not all types are lysogenically determined, and a number of apparently non-lysogenic types can by lysed only by host-range mutants of Vi-phage II. In such cases no information at present available suggests that other preparations of Vi-phage II than phage A might be more suitably used as the starting-point for the new typing phages. Naturally, as phage A seems to represent the wild phenotype and genotype of Vi-phage II, it is on general grounds the most satisfactory phage to use initially in attempting adaptations to new types. But if difficulty is experienced in adapting phage A to a new type, attempts should be made to isolate and identify a determining phage from the new type. T4904 can again be used as an example: had attempts to prepare a typing phage for it been confined to phage A, the new type might not have been identified. However,

* Such a situation arose in connexion with the adaptation of Vi-phage II to type C2(=C(d6)). Desranleau, who discovered the type (see Desranleau & Martin, 1950), selected a mutant of Vi-phage II that lyses type D5 very poorly. On the other hand, Dr P. R. Edwards of the Communicable Diseases Center, Chamblee, Georgia, U.S.A., who adapted Vi-phage II to type C2 independently, selected the true D6 mutant which lyses type D5. We have also demonstrated that C2 can select a mutant having the genotype of phage 29 (type 29 = A(f2)); this lyses strains carrying phage d6 less readily than does the D6 mutant.
a determining phage could have been isolated from the strain before its Vi-type was established. The lysogenization of type A with this phage would yield type D6 (=A(d6)), and the identity of the determining phage would thereby be established as phage d6. It would then have been evident that the most satisfactory method of preparing a typing phage for T4904 was to start with phage D6, thus by-passing the step which involves the uncertainty of selecting this host-range mutant from phage A.

An indication has been given in a previous publication (Anderson & Fraser, 1955a) of how the investigation of type-determining phages can help in the preparation of typing phages for Vi-types that have already been defined. The preceding discussion shows that the method could be applied equally well to the preparation of typing phages for lysogenically determined types that have not yet been identified.

It has been suggested (Anderson, 1955; Anderson & Fraser, 1955a) that, for the study of the phenomena underlying the Vi-phage typing method, the use of structural formulae for the lysogenically determined types, and the use of a system of grouping of lysogenic types based on non-lysogenic ancestors possessed in common by certain types, afford considerable help. This can be seen from the work presented above. The types that do not owe their specificity to determining phages remain enigmata at present, but much information is now available about the lysogenically determined types and their respective typing phages. The identification of the determining phage carried by T4904, and of the non-lysogenic precursor of the type, furnishes us with a structural formula for the new type. By the application of the principles outlined recently (Anderson, 1955; Anderson & Fraser, 1955a), it is possible from this formula to deduce the host range of the preparation of Vi-phage II adapted to the type. The formula is E1(d6). The homologous typing preparation is a phenotypically modified mutant of Vi-phage II that, as has been explained earlier, can be expected to attack type D6 (=A(d6)), type E1, type E7 (=E1(f2) or E1(80')) and type 29 (=A(f2) or A(80')) as well as the homologous type. In addition, types D1 and D5 will be lysed by phage 4904 because they are sensitive to phage D6. The formula of T4904 thus fully explains why its adapted typing phage shows cross-reactions in the D and E groups of Vi-types. Finally, a logical reason is provided for placing T4904 in the E group of Vi-types because its non-lysogenic component is type E1, and grouping is preferably based on the nature of non-lysogenic precursor types.

REFERENCES


A new Vi-phage type of S. typhi


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