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Laboratory investigations of a carrier of multiple phage types of *Salmonella paratyphi B*

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INTRODUCTION

Sloan, Wilson & Wright (1960) have described the long-term epidemiological investigations which resulted in the detection of a carrier of *Salmonella paratyphi B* (Mrs X) who was considered to be the source of at least three infections due to this organism. The survey covered a number of years (1954–59) and was hampered by the fact that two different phage types of *Salm. paratyphi B* were responsible for the three cases.

Two cases (Case I, 1954 and Case III, 1958) were due to phage type Taunton, whilst the third was due to phage type Dundee (Case, II, 1958). Examination of the local stream revealed the presence of both these types in the water and of two others, 3aI var. 1 and 3aI var. 4 (see Sloan *et al.* 1960). These findings suggested that more than one excreter was responsible for the stream pollution and for the human cases.

These workers were able to show, however, that there was resident in the neighbourhood one carrier (Mrs X) from whom it was possible to isolate three of the types concerned (3aI var. 4, Dundee & Taunton). They suggest, therefore, that she was the probable origin of the three cases, and of the river contamination.

Infection with multiple phage types of *Salm. paratyphi B* is not unknown, but it is sufficiently rare to warrant special investigation whenever detected.

It has been known for some time that it is possible, *in vitro*, to convert various Vi-phage types of *Salm. typhi* into other types by the use of type-determining temperate phages (Anderson & Felix, 1953; Anderson, 1955). Similar *in vitro* conversions have been demonstrated in some of the phage types of *Salm. paratyphi B* (Hamon & Nicolle, 1951).

Anderson & Williams (1956) quote examples of rare occasions when a possible *in vivo* type conversion has tended to confuse the relationships between epidemiologically related strains. It was thought that the variety of types isolated from Mrs X might be due to such an *in vivo* conversion.

EXPERIMENTAL METHODS

Media

The liquid medium was 2·0% Bacto dehydrated broth (Difco), with 0·85% added NaCl, made up in distilled water. New Zealand powdered agar in a strength of 1·3% was added for the preparation of the solid medium.

Cultures

Representative cultures of the three different phage types isolated from Mrs X were selected from one of the series of single colony isolations referred to by Sloan *et al.* (1960). These are listed in Table 1 and are referred to as test strains. The culture of type 3aI var. 1 isolated from the river was also used, and is referred to as culture B (see Table 1).

As an indicator of possible temperate phages, and also for their propagation, strain B1363 (phage type 1 var. 2 (Anderson & Williams, 1956)) was used. This type has a wide spectrum of phage sensitivity and is not lysogenic in so far as we can determine. It is referred to hereafter as the indicator strain.

Table 1. *List of cultures examined, and of the phages isolated from them*

			Carried phage	
Source	Culture	Phage type	Designation	Stability*
River water	B	3aI var. 1	B′	HS
Mrs X	X1	3aI var. 4	X1′	HS
	X2	Taunton	X2a′	HS
			X2b′	HL
	X3	Dundee	X3′	HS

* HS and HL = heat stable and heat labile, respectively.

Phage typing

The general techniques of phage typing, and the nomenclature of phage types, were those given by Anderson & Williams (1956).

Isolation of temperate phages

Approximately 10^8 log phase organisms of the strain under examination were added to 20 ml. of prewarmed broth. The same amount of indicator culture was also added to the broth, which was then incubated for 5 hr. at 38·5° C.

After growth, the culture was centrifuged and a spot of undiluted supernatant placed on areas of each of the test strains and of the indicator strain. If there was any evidence of phage activity, a portion of the supernatant was heated at 56° C. for 40 min. in a water-bath, in order to kill any remaining bacteria. If heating reduced the degree of phage activity the remainder of the preparation was filtered through sintered glass (A.P.D. = 1·2–1·4μ).

RESULTS

Lysogenicity of cultures

Phages were isolated from each of the four test cultures. The indicator strain was sensitive to all four preparations, but of the test strains only B showed any sensitivity, and this only to the supernatants from X2 and X3.

Three of the preparations were unaffected by heat. The fourth, that obtained from X2, showed a reduced titre after heating when titrated on B but not when

Table 2. *Phage-typing reactions of original and lysogenized cultures*

Culture	Lysogenized by	Paratyphoid B typing phages in R.T.D.* 1	2	3a	3b	Jersey	Beccles	Taunton	B.A.O.R.	Dundee	Worksop	Phage type
B	–	–	–	CL	–	–	CL	CL	+++	CL	–	3aI var. 1
X1	–	–	–	CL	–	–	–	–	+++	CL	–	3aI var. 4
X2	–	–	–	–	–	–	–	CL	–	CL	–	Taunton
X3	–	–	–	–	–	–	–	–	–	CL	–	Dundee
B	X2b′†	–	–	–	–	–	–	CL	–	SCL	–	Taunton
B	X3′‡	–	–	–	–	–	–	CL	–	+++	–	Taunton
B	X3′§	–	–	–	–	–	–	–	–	SCL	–	Dundee

* R.T.D. = routine test dilution (see Anderson & Williams, 1956).
† 6/6 colonies tested.
‡ 2/6 colonies tested.
§ 1/6 colonies tested.
CL = confluent lysis, SCL = semi-confluent lysis, +++ = large numbers of discrete plaques.

titrated on the indicator. Further examination showed that this supernatant contained two different phages. One was heat stable and lysed only the indicator culture, the other was heat labile (100-fold reduction in titre after heating for 40 min. at 56° C.) and lysed both the indicator and B. It was possible to separate the two phages by single plaque propagation on the indicator strain.

The various temperate phages obtained are designated by the addition of a superscript prime sign to the name of the culture from which they were obtained, for example, X 1′ is the phage from strain X 1. The heat stable component of the X 2 supernatant is called X 2a′ and the heat labile X 2b′. The various phages, and their stabilities are indicated in Table 1.

Effect of the temperate phages on phage typing

Since B was the only test culture sensitive to the isolated phages, the effect on it of lysogenization was investigated. Lysogenization could only be effected with phages X 2b′ and X 3′. Single colonies of each of the lysogenized cultures were tested with the routine paratyphoid B typing phages. It was found that a change had taken place in several of the colonies. The phage-typing patterns of the original cultures and of representative lysogenized colonies are shown in Table 2.

It is evident from these results that phage X 2b′ is capable of converting B into type Taunton. Phage X 3′ must consist of two components, one of which is also capable of converting B to type Taunton, whereas the other produces type Dundee.

DISCUSSION

There are two major possible explanations for the carriage of three phage types by Mrs X. It might be a case of true multiple infection as a result of repeated exposure in the primitive sanitary conditions obtaining in the region. On the other hand, it is possible that phage-mediated conversion had occurred in the intestine, or gall bladder, giving rise to this multiplicity of types from an original single infection. It is also possible that a combination of these two factors was at play.

Multiple infection cannot be excluded on the results of these experiments, but a plausible scheme of type conversion can be formulated.

Type 3aI var. 4 is known to be unstable on storage, although the cause of this instability is unknown. A variable proportion of strains of this type will, after an interval of time, give rise to cultures of type 3a I var. 1 (B. R. Callow—unpublished). The culture of type 3aI var. 4 (X 1) from Mrs X is not sensitive to any of the isolated phages. The culture of 3aI var. 1 (B), however, is sensitive to phages from type Taunton (X 2) and from type Dundee (X 3) and is capable of being converted by these phages into types Taunton and Dundee, respectively. It is possible that the original infection of Mrs. X was due to phage type 3aI var. 4. During the course of her carrier state this culture may have given rise to some organisms of type 3aI var. 1, which came into contact with, and were lysogenized by, phages which converted them to types Dundee and Taunton.

No culture of type 3aI var. 1 has been isolated from Mrs X but the explanation given above might account for its presence in the river. If the efficiency of lysogenic

conversion is high in the enteric canal then there would be a selective pressure against type 3aI var. 1. The concomitant presence of the Taunton and Dundee strains might well be sufficient to exert this pressure and to form a self-perpetuating system.

Laboratory experiment cannot decide between the alternative possibilities of multiple infection and *in vivo* conversion. Although repeated exposure to different phage types of *Salm. paratyphi B* is possible, it is not likely that the carrier state would arise from each such infection to give rise to the persistent concurrent excretion of three, or possibly four different phage types. The present experiments do demonstrate, however, that *in vivo* type conversion can be considered as a plausible explanation.

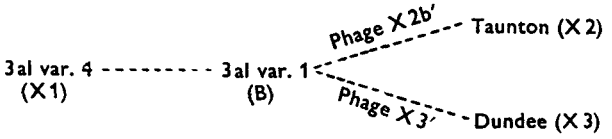
It could also be claimed that although there has been an *in vivo* change of phage types, it has been in the opposite direction to that postulated above. That is, there may have been an original infection with phage type Dundee; this has, by some mechanism, changed to type Taunton, and the latter has given rise to type 3aI var. 1. However, the change from type Dundee to type Taunton has not been detected under laboratory conditions, nor that of type Taunton to type 3aI var. 1.

There is also the possibility that the type 3aI var. 1 organisms did not derive from Mrs X but from another excreter in the neighbourhood. The thorough surveys of Sloan *et al.* (1960), however, did not reveal any such excreter of this type.

The finding of a multiplicity of phage types in a single carrier, and its possible explanation, might give rise to doubts of the long-term reliability of phage typing as an epidemiological tool. Anderson & Williams (1956) and Anderson (1957) mention occasions on which phage-mediated conversion of phage types has led to epidemiological difficulties (see also, Bernstein (1958)). However, during the 19 years in which phage-typing of *Salm. paratyphi B* has been conducted in the Central Enteric Reference Laboratory, over 14,000 cultures have been examined, and only six such incidents have been encountered. Thus, although the possibility of the picture being complicated by the intrusion of type-determining phages is always kept in mind, this rarely occurs in practice and does not reduce the overall validity of the phage-typing method.

SUMMARY

A carrier has been found of at least three phage types of *Salmonella paratyphi B*. Investigation has shown that phage-mediated conversion could account for this multiplicity of types according to the following scheme.



It is not possible, however, to exclude the actual occurrence of repeated infection with different phage types.

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