A NEW PHAGE-TYPING SCHEME FOR *SALMONELLA TYPHI-MURIUM*

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INTRODUCTION

The phage typing of *Salmonella typhi-murium* by the method of Felix & Callow (1943, 1951) has been carried out in the Central Enteric Reference Laboratory (C.E.R.L.) since 1943 and has been successfully used in a number of epidemiological studies of infection due to this organism. Nevertheless, details of this typing scheme were not published until relatively recently (see Felix, 1956). The writer originally undertook the development of a phage-typing method for *Salm. typhi-murium* because it is the commonest salmonella responsible for infection food poisoning, and it seemed that a method of strain identification approaching in efficiency that introduced by Craigie & Yen (1938) for *Salm. typhi* would greatly assist in tracing sources of infection.

The epidemiological study of *Salm. typhi-murium* is complicated by the fact that the organism has multiple indigenous sources in this country. In addition to the rodents traditionally regarded as being responsible for foodstuff contamination, ducks' and hens' eggs, domestic poultry, pigs and cattle may all convey the infection to man. Moreover, the horizon of this salmonellosis, as of most others, is widened by several other factors. Large quantities of foodstuffs are imported from abroad in the form of raw materials or products ready for consumption, and many of these contain *Salm. typhi-murium*. The extension of communal feeding and the fact that raw materials used in the preparation of such foods as trifles, custards, brawns and meat pies may be infected with *Salm. typhi-murium*, or that these foods may be contaminated by being prepared with infected utensils, also contribute to the incidence of this type of food poisoning. In addition, it is important to consider the influence of the food handler who is excreting the organism. With a picture of such complexity, a method of 'fingerprinting' different strains of the organism is clearly indispensable if the epidemiological study of *Salm. typhi-murium* infections is to be effective.

The phage-typing scheme which was introduced in 1943 is shown in Table 1. This system distinguished twelve types and subtypes of *Salm. typhi-murium*. It has proved its worth on many occasions in the study of outbreaks in man, in bacteriological surveys of foodstuffs, and in the investigation of enzootic and epizootic infections in domestic animals. The original phage-typing scheme will be referred to as Scheme 1. Valuable as it proved, it was expected that its value would be enhanced if the type differentiation to which it gave rise could be reliably extended. This belief was based on the high incidence of human infections, the wide distribution of the organism in domestic animals and its probably frequent

Table 1. *Reactions of type strains of* Salmonella typhi-murium *with routine test dilutions of the typing phages* (*Scheme* 1)

Type	1	1*a*	1*b*	2	2*a*	2*b*	2*c*	2*d*	3	3*a*	4
1	OL**	OL	<CL	CL	CL	<CL	CL	OL	OL	OL	–
1*a*	–	OL	<CL	CL	<CL	OL	<CL	OL	OL	OL	–
1*a* var. 1*	–	OL	<CL	–	–	OL	–	OL	OL	<CL	–
1*b*	–	–	<CL	CL	CL	OL	<CL	OL	<CL	<CL	–
2	–	–	–	CL	CL	–	CL	–	–	–	–
2*a*	–	–	–	–	CL	<CL	CL	<CL	–	–	–
2*b*	–	–	–	–	–	OL	–	OL	–	–	–
2*c*	–	–	–	<CL	<CL	<CL	<CL	<CL	–	–	–
2*d*	–	–	–	–	–	–	–	<CL	–	–	–
3	–	–	+ + +	–	–	–	–	–	OL	+ +	–
3*a*	–	–	–	–	–	+ + +	–	SCL	+	<CL	–
4	–	–	–	–	–	–	–	–	–	–	<CL

* Type 1*a* var. 1 was formerly designated type 3*b*. It is now recognized to be a member of Group 1. This variation is always anaerogenic.
** For key to symbols see p. 349.

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intrusion into this country in imported human and animal foods. This, and what we term 'varietal' differences between different strains belonging to certain types of Scheme 1, suggested that the number of epidemiologically valid types was appreciably larger than that recognized hitherto. Work carried out during the past 4 years has confirmed this expectation and has resulted in the evolution of a new and considerably larger phage-typing scheme for *Salm. typhi-murium*. Trials up to the present suggest that this system, to be called Scheme 2, offers advantages over Scheme 1, because of the larger number of types it identifies. It is therefore proposed to substitute the new scheme for the old in the near future. In the meantime, extended parallel tests of the two sets of typing phages on freshly isolated strains of *Salm. typhi-murium* are in progress in order to allot with precision places in the new scheme to types established by the old. Scheme 2 enabled thirty-four types to be recognized in the first instance—a number which, as will be shown in a later publication, has now been increased to about eighty.

EXPERIMENTAL METHODS

(1) *Culture media*

Two media were used in these investigations: tryptic digest broth and Bacto-dehydrated nutrient broth (Difco Laboratories), referred to hereafter as 'Difco broth'. The digest medium was reinforced by the addition of Evans's peptone to give a final concentration of 1 % and the Difco broth was employed at a strength of 2 % with the addition of 0·85 % NaCl.

For solid media 1·3 % New Zealand powdered agar (Davis) was added to the broth preparations. Plates of either medium were freshly poured which, when set, were incubated with closed lids for 24 hr. at 37° C., and then for a further 40 min. with the lids partly off to dry the surface of the agar.

The digest medium was found, on the whole, to be more satisfactory for the propagation and screening of phages, but it showed a greater range of variability from batch to batch than the Difco preparation, the nutrient properties of which appeared to be virtually constant. The Difco medium, although yielding smaller plaques and, generally, less complete lysis was, therefore, more reliable for routine use and was employed throughout for the standardization of phages for the determination of their Routine Test Dilution (R.T.D.).

(2) *The technique of spot titration and routine phage typing*

The host strain was inoculated into broth in a density sufficient to yield a concentration of about 5×10^8 cells/ml. after $2\frac{1}{2}$ hr. incubation with agitation at 37° C. Individual drops from a standard loop, delivering a volume very close to 0·01 ml., were sown on to nutrient agar plates and allowed to dry. Drops of appropriate dilutions of phages were then serially spotted on to the inoculated areas with a similar standard loop, and after they had dried the plates were transferred to the incubator at 37° C.

Readings, made after 5 and 22 hr. incubation, were carried out with a $\times 10$ hand lens by indirect transmitted light. They were recorded as follows.

Individual plaque sizes:

L = large, above 1·5 mm.,
N = normal, about 1·0 mm.,
S = small, 1–0·1 mm.
m = minute, less than 0·1 mm.
μ = micro
} visible only with hand lens,
± to + + + = increasing numbers of discrete plaques (cf. Tables 1 and 2).

Degrees of confluent lysis:

CL = confluent lysis,
SCL = semi-confluent lysis,
< CL
< SCL
} = intermediate degrees of lysis,
OL = confluent lysis with a heavy central opacity due to secondary (lysogenized) growth.

(3) *Agar layer titrations*

These were performed by the methods described by Adams (1950, 1959). They were useful for the screening of temperate phages for clear-plaque mutants and also for the preparation of concentrated phage stocks. Tryptic digest agar was found to be most suitable for the basal layer. Plates to be used for the preparation of stocks were incubated at 37° C. for periods between 6 and 16 hr., the actual time depending on the phage concerned. The surface layer was then harvested to broth. About 6·0 ml. of broth were usually allowed to each plate, and the resulting suspensions were centrifuged at 3000 r.p.m. for 1 hr. before being treated with heat or toluene in order to kill the host cells. It was found that the optimal amount of phage to incorporate in the surface layer to yield high-titre preparations was that producing semi-confluent lysis.

(4) *Isolation of single-plaque lines of phage and preparation of new phage adaptations*

Because it was already known that phage 1 of Scheme 1 and its parent phage 3*b* of the paratyphoid B typing scheme of Felix & Callow (1943, 1951) could undergo host-induced modification, attempts were always made initially to prepare phages for new types from one or other of these two phages. When such attempts proved unsuccessful, efforts were made to carry out the adaptations with other phages.

First attempts at adaptation were usually carried out by titrating the starting phage on solid medium on the strain on which it was to be grown. This enabled discrete plaques of new phage lines to be isolated. Selected plaques, together with a small portion of the surrounding culture, were transferred to 3 ml. of broth, which was then incubated for 3–5 hr. or for as long as lysis continued. This method of titration was also used for subsequent single-plaque purification of newly adapted phages. Single plaques of large-plaque heat-stable phages were suspended in 1 ml. of broth and heated at 57° C. for 40 min. without further incubation, a procedure which frequently yielded high-titre preparations.

A method of phage adaptation frequently employed was to add serial dilutions of the starting phage to about 4×10^7 host bacteria in 4 ml. of broth. The tubes were incubated for an appropriate period which varied with the phage concerned; a range of 5–15 hr. was usual. The suspension which yielded the highest titre of phage of the desired host range was used for the preparation of stocks of the new phage after purification by repeated single-plaque isolation.

The great majority of strains of *Salm. typhi-murium* are lysogenic (see Boyd, 1950, 1951; Boyd, Parker & Mair, 1951) and it is known that many of the typing phages contain one or more contaminating temperate phages. At the R.T.D. of the typing phages, however, the concentration of such temperate phages is too low to cause a significant amount of lysis. When an attempt was made to adapt a given phage to a new strain, control experiments were always carried out to determine whether the phage harvested after the adaptation was basically the same as the starting phage. Thus, when it was necessary to propagate a phage formerly grown on a strain '*a*' on another strain '*b*', the strains were grown alone and with each other in broth, and the supernatants of the resulting suspensions, after centrifugation, were spotted on both strains. Occasionally, '*a*' proved to be carrying a phage able to attack '*b*' and the starting phage was not adapted to '*b*' but was replaced by the temperate phage of '*a*'. Nevertheless, if the resulting phage was useful for typing purposes it was included in the new set after suitable purification.

A number of strains of *Salm. typhi-murium* yielded no temperate phages by our methods, and we have designated these provisionally as 'non-lysogenic', although the term is naturally employed with the reservation that our inability to demonstrate the presence of phages does not establish their absence. The non-lysogenic strains, which are listed later, proved valuable in the production of typing phages, since it was possible to propagate some of these phages on them without risk of contamination or change of specificity.

(5) *Elimination of bacterial cells in phage lysates*

The lysates of all large-plaque, heat-stable phages were sterilized by heating at 57° C. for 40 min. Heat-labile phages were treated with 0·14 % toluene (4 drops from a 50-dropper pipette to 20 ml. of lysate; see Anderson & Felix, 1953).

(6) *The Routine Test Dilution* (R.T.D.)

The reasons for using this dilution of typing phages were discussed by Anderson & Williams (1956). Briefly, it may be said that the application of undiluted phages to cultures on solid media often produces an appearance of lysis which is due to the lethal effect on bacteria of phage which is adsorbed but does not multiply. Serial dilution of such a phage shows the disappearance of this effect between two consecutive dilutions, without resolution of the 'lysis' into discrete plaques. In order to avoid this non-specific bacterial destruction, typing phages are used in dilutions too high for it to occur. The R.T.D. of a phage is the highest dilution in which it will yield satisfactory lysis when applied as a spot to a lawn of its homologous type strain on the surface of a solid medium. In this dilution a typing phage exhibits its highest specificity for routine working purposes, cross-reactions

on heterologous types and non-specific bacterial inhibition being reduced to a minimum. The R.T.D. on Difco medium is usually lower than that on tryptic digest, since the latter is the better nutrient medium; however, whereas the R.T.D. on Difco agar is virtually constant, that on tryptic digest agar prepared in the laboratory varies from batch to batch.

During the experimental stages, phages were grown in volumes of not more than 3–10 ml. Once it was decided that a phage was sufficiently specific to be included in the typing set, larger quantities were prepared for stock purposes, either in liquid medium in volumes of 100 ml. or in agar layer as previously described. For both methods, preliminary titrations were carried out in order to determine the optimal phage:host cell ratio and time of incubation for the production of high-titre lysates.

(7) *Neutralization tests with phage antisera*

The technique of neutralization tests was as follows:

Equal quantities (0·5 ml.) of selected concentrations of phage and serial dilutions of serum were mixed at room temperature. The tubes were then transferred to the refrigerator at 4° C. for 24 hr., after which the mixtures were spotted on to the appropriate indicator strains on the surface of agar plates in order to determine the proportion of particles which had escaped inactivation. The plates were incubated at 37° C. for 16 hr. The end-point was arbitrarily fixed as that dilution of serum which inactivated 90 % of infective centres of the phage concerned. Controls were used in all tests to exclude possible phage inactivating effects of normal rabbit serum and nutrient broth. For the present series, antisera were also prepared against the clear-plaque variants of the new *Salm. typhi-murium* phage 1 and of the *Salm. paratyphi* B typing phage 3*b*. The results of these serological investigations are reported later in this paper.

THE NEW TYPING SCHEME

Scheme 2 is shown in Table 2 (facing p. 352). It has been our custom to arrange the strains and their homologous typing phages of various schemes in such an order that the first phage shown to lyse a given strain is its homologous phage, whatever heterologous sensitivities the strain may exhibit. This method yields an orderly sequence of type reactions. However, reference to Table 2 will indicate that it has not been possible to maintain such an arrangement throughout. Most type identification is done by reaction patterns with a number of phages. Since Scheme 2 was put into routine use a number of new types have been identified, each of which yields an apparently unique reaction pattern. It has not been considered advisable to dovetail these types into the original scheme; they form in effect a supplementary scheme which will be described in a later paper. For the present it suffices to say that a further fifty types have been added to the original thirty-four of Scheme 2, to produce a total of eighty-four.

As has been found in the phage-typing scheme of *Salm. typhi* (Felix & Anderson, 1951; Anderson, 1951; Anderson & Felix, 1953) the characteristic phage-resistance

patterns of the various types of *Salm. typhi-murium* are partly determined by the lysogenicity of the strains concerned. The temperate phages isolated from some of these strains have also contributed typing phages to the scheme and are described in a later section. In the typing scheme of *Salm. typhi*, all the typing phages are adaptations of a single phage—Vi phage II. In the present *Salm. typhi-murium* scheme, on the other hand, adaptation plays a more limited though still considerable part in the preparation of new typing phages.

Comparison of the old and new typing schemes

Scheme 1 was used as the nucleus around which to construct Scheme 2 and the original type strains are thus found under different designations in the new scheme. Table 3 shows the type correspondence of the two systems.

Table 3. *Analysis of type correspondence in original and revised phage-typing schemes for* Salmonella typhi-murium

Old designation	New designation	Old designation	New designation
1	1		7
1*a*	2	2*d*	20
1*a* var. 1	3		20*a*
1*b*	4	3	5
2 (phage 4+)	9	3*a*	6
	10	4	8
2 (phage 4−)	11	Untypable (phage 1*a*+)	24
	12		21
	12*a*		22
2*a*	13		23
	16		25
2*b*	17	Untypable (−ve)	26
	18		27
	19		29
2*c* (phage 4+)	28		30
2*c* poor (phage 4−)*	14		31
2*c* fair (phage 4−)	15		
2*c* good (phage 4−)	15*a*		

Reactions to phages of the old scheme are shown in parentheses.

* 'Poor', 'fair' and 'good' refer to clarity of type reactions.

The epidemiological stability of the old types has been established by years of routine work and Scheme 2 was originally intended to expand rather than to supplant Scheme 1. Table 3 shows that some types, regarded as single in Scheme 1, form groups in Scheme 2. For example, the old type 2*b* is divided into four types: 16, 17, 18 and 19; type 2*c* into 14, 15, 15*a* and 28; type 2*d* into 7, 20 and 20*a*, and so on. In some instances there had been a previous indication that the old types were not uniform, as is shown in the table. Thus, some strains of type 2 were lysed by phage 4 of the old series while others were not; but the strains resistant to phage 4 are shown by Scheme 2 to comprise the four new types: 10, 11, 12 and 12*a*. An analogous feature is exhibited by type 2*c*, as Table 3

Table 2. *Reactions of type strains with routine test dilutions of the new typing phages*

Type strains: Old type	Type strains: New type	Phages in R.T.D. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Type strains: New type	Type strains: Old type
1	1	**CL**	CL	CL	CL	CL	CL	CL	–	–	SCL	SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	SCL	CL	SCL	CL	CL	±	CL	1	1r
1*a*	2	–	**CL**	CL	CL	CL	CL	–	–	±	CL	CL	CL	CL	CL	CL	CL	CL	–	CL	CL	CL	CL	CL	CL	CL	CL	CL	++	CL	2	1*a*
1*a* var. 1	3	–	+++	**CL**	CL	CL	CL	–	–	+	CL	SCL	–	–	CL	CL	CL	CL	–	CL	CL	SCL	SCL	CL	CL	+++	SCL	CL	SCL	CL	3	1*a* var. 1
1*b*	4	–	++	–	**CL**	CL	CL	–	–	±	CL	SCL	+++	CL	+++	CL	CL	++	–	CL	SCL	±	CL	CL	–	CL	+++	CL	+++	CL	4	1*b*
3	5	–	–	–	+++	**CL**	+++	–	–	–	–	++	–	–	+++	+++	+++	–	–	++	±	–	SCL	–	–	–	–	–	–	5	3
3*a*	6	–	++	–	++	±	**CL**	–	–	–	+++	–	–	–	–	–	+++	+++	–	–	–	+++	+++	+++	–	–	–	SCL	++	CL	6	3*a*
2*d*	7	–	–	–	–	–	–	**CL**	±	–	–	–	–	–	–	–	–	–	CL	±	SCL	–	–	–	–	–	–	–	–	–	7	2*d*
4	8	–	–	–	–	–	–	–	**CL**	CL	CL	CL	–	–	–	–	+++	–	–	–	++	±	CL	CL	–	±	++	–	±	CL	8	4
	9	–	–	–	–	–	–	–	CL	**CL**	CL	CL	CL	CL	–	–	SCL	–	–	–	++	–	CL	CL	–	±	+++	–	–	CL	9	
	10	–	–	–	–	–	–	–	–	±	**CL**	SCL	CL	CL	+++	–	SCL	++	–	–	++	++	CL	CL	–	±	++	++	–	CL	10	
2	11	–	–	–	–	–	–	–	–	–	–	**SCL**	SCL	CL	–	–	–	OL	CL	SCL	OL	–	–	–	–	–	–	–	–	–	11	2
	12	–	–	–	–	–	–	–	–	–	–	–	**CL**	CL	–	–	–	+++	–	–	–	–	–	–	–	–	–	–	–	–	12	
	12*a*	–	–	–	–	–	–	–	–	–	–	–	**CL**	CL	–	–	–	–	CL	–	–	+++	–	–	–	–	–	OL	–	–	12*a*	
2*a*	13	–	–	–	–	–	–	–	–	–	–	SCL	–	**CL**	OL	–	++	–	+++	–	–	+++	–	–	–	–	OL	OL	++	OL	13	2*a*
	14	–	–	–	–	–	–	–	–	–	–	SCL	++	SCL	**OL**	–	–	–	–	–	–	–	–	–	–	–	+++	+++	–	OL	14	
2*c*	15	–	–	–	–	–	–	–	–	–	–	–	+++	SCL	–	**SCL**	–	OL	OL	OL	OL	–	–	–	–	–	–	++	+	–	15	2*c*
	15*a*	–	–	–	–	–	–	–	–	–	–	++	CL	CL	–	**OL**	–	OL	–	OL	OL	–	–	–	–	–	–	++	++	–	15*a*	
	16	–	–	–	–	–	–	–	–	–	–	+++	–	–	OL	–	**OL**	+++	+++	–	–	–	±	–	–	SCL	+++	OL	±	OL	16	
2*b*	17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+++	–	**OL**	+++	+++	SCL	–	–	–	–	–	–	+	++	–	17	2*b*
	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	SCL	–	OL	**CL**	SCL	SCL	–	–	–	–	–	–	++	++	–	18	
	19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	SCL	–	–	–	**SCL**	SCL	–	–	–	–	–	–	+++	+++	–	19	
2*d*	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**OL**	–	–	–	–	–	–	–	–	–	20	2*d*
	20*a*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	±	**SCL**	–	–	–	–	–	–	–	–	–	20*a*	
Untypable	21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**OL**	++	–	–	–	–	–	–	–	21	Untypable
Untypable	22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±	**OL**	–	–	–	–	–	–	–	22	Untypable
Untypable	23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**OL**	CL	+++	+++	–	–	++	23	Untypable
Untypable	24	–	–	+++	–	–	–	–	–	–	–	–	–	–	CL	–	–	–	–	–	–	–	–	–	**CL**	–	SCL	–	–	–	24	Untypable
Untypable	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	++	–	–	–	–	–	–	±	+++	**OL**	OL	–	–	+++	25	Untypable
Untypable	26	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+++	+++	**OL**	–	–	–	26	Untypable
Untypable	27	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+++	–	–	–	–	–	**OL**	–	–	27	Untypable
2*c*	28	–	–	–	–	–	–	–	+++	–	–	–	+++	SCL	–	SCL	–	SCL	SCL	SCL	SCL	–	–	–	–	–	–	±	**OL**	–	28	2*c*
Untypable	29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**OL**	29	Untypable
Untypable	30	–	–	–	–	–	–	–	SCL	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	30	Untypable
Untypable	31	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	SCL	+	–	–	+++	31	Untypable
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		

For key to symbols see p. 349.

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Table 5. *Lysogenicity in* Salmonella typhi-murium

New type (1)	Old type (2)	Ref. no. M (3)	Phages carried and plaque size: Thermo-stable (TS) (4)	Phages carried and plaque size: Thermo-labile (TL) (5)	Host range: TS (6)	Host range: TL (7)	Types lysed by TS and not by TL phages (8)	Uniqueness of host range of carried phages: TS (9)	Uniqueness of host range of carried phages: TL (10)
1	1	307	+	+	10; S.g.	4, 11, 12	10; S.g.	+	+
2	1*a*	419	S –	+		1, 4, 7, 8, 9, 11, 12*a*, 13, 14, 15, 18, 19, 20*a*, 22, 30			+
3	1*a* var. 1	1417	–	–					
4	1*b*	1461	+ S	+	S.p.B.3*b*; S.g.	1, 2, 3, 6, 13, 14, 16, 24, 25, 29, 30; S.p.B.1, 3*b*	S.g.	+	+
5	3	298	–	–					
6	3*a*	736	–	–					
7	2*d*	1274	+ S	+	S.p.B.1; S.g.	1, 2, 3, 4, 6, 9, 10, 11, 12, 12*a*, 13, 15*a*, 16, 17, 20, 24	S.p.B.1; S.g.	+	+
8	4	1166	+ LNS	+	9, S.p.B.1, 3*b*; S.g.	S.p.B.1, 3*b*	9; S.g.	+	+
9	2	2130	–	–					
10	2	668	+ N	+	S.g.	1, 4, 6, 7, 8, 9, 11, 12*a*, 13, 14, 15, 18, 19, 20*a*, 24, 29, 30	S.g.	See 15*a*, 24	+
11	2	3770	+ L	+	1, 2, 3, 8, 9, 10, 13, 14, 16; S.g.	4, 5, 7, 8, 9, 10, 13, 14, 15, 16, 18, 19, 20*a*, 24, 25, 26, 30; S.p.B.1, 3*b*	1, 2, 3; S.g.	+	+
12	2	1221	+ LN	+	13, 14, 15*a*, 29; S.g.	1, 2, 3, 4, 7, 8, 9, 10, 11, 12*a*, 13, 14, 15, 15*a*, 18, 19, 20*a*, 24, 25, 26, 29, 30; S.p.B.1	S.g.	+	+
12*a*	2	3889	+ LN	+	1, 2, 3, 4, 13, 14, 15, 15*a*, 16, 17, 18, 19, 30; S.p.B.1, 3*b*; S.g.	1, 2, 3, 4, 7, 13, 14, 15, 15*a*, 16, 17, 20, 20*a*, 29, 30	18, 19; S.p.B.1, 3*b*; S.g.	+	+
13	2*a*	1717	+ LS	+	1, 2, 3, 4, 5, 6; S.p.B.1, 3*b*; S.g.	1, 3, 4, 5, 6, 8, 12, 15*a*, 19, 20*a*; S.p.B.1, 3*b*	2; S.g.	+	+
14	2*c*	1939	+ LN	+	1, 2, 3, 4, 5; S.p.B.1, 3*b*; S.g.	1, 3, 4, 5, 6, 9, 10, 12, 12*a*, 13, 15*a*, 16, 19, 20, 24; S.p.B.1, 3*b*	2; S.g.	+	+
15	2*c*	1184	–	+		11, 16			+
15*a*	2*c*	591	+ S	+	S.g.	1, 4, 7, 8, 9, 11, 12*a*, 13, 14, 15, 16, 18, 19, 20, 20*a*, 30	S.g.	See 10, 24	+
16	2*b*	1664	+ L	+	1, 2, 3, 4, 5, 6, 7, 8, 9, 10; S.p.B.1, 3*b*; S.g.	1, 6, 7, 8, 9, 10, 12, 13, 14, 26; S.p.B.1, 3*b*	2, 3, 4, 5; S.g.	+	+
17	2*b*	1899	+ L	+	1, 2, 3, 5, 6, 16, 26; S.g.	1, 3, 6, 7, 13, 16; S.p.B.1	2, 5, 26; S.g.	+	+
18	2*b*	2540	+ L	+	1, 2, 3, 5, 6, 16; S.g.	1, 3, 4, 6, 7, 9, 10, 11, 12, 12*a*, 13, 15*a*, 16, 17, 24; S.p.B.1	2, 5; S.g.	+	+
19	2*b*	2200	+ LN	+	1, 2, 3, 5, 6, 7, 8, 9, 10, 12*a*, 15, 16, 17, 18, 20*a*; S.g.	1, 3, 4, 6, 7, 9, 10, 11, 12, 13, 15, 15*a*, 16, 17, 18, 20*a*, 24; S.p.B.1	2, 5, 8, 12*a*; S.g.	+	+
20	2*d*	1431	+ L	+	1, 2, 3, 9; S.g.	1, 4, 6, 7, 8, 9, 10, 11, 12*a*, 13, 14, 15, 16, 18, 19, 20*a*, 25, 30; S.p.B.1	2, 3; S.g.	+	+
20*a*	2*d*	2471	+ L	+	1, 2, 3, 6; S.g.	1, 3, 4, 6, 7, 9, 10, 11, 12, 12*a*, 13, 15*a*, 16, 17, 24	2; S.g.	+	+
21	U	3382	–	–					
22	U	4025	+ LN	+ LNm	4, 8, 9; S.g.	1, 2, 4, 7, 8, 9, 10, 13, 16; S.p.B.1	S.g.	+	+
23	U	4201	–	–					
24	U	4205	+ μ	+	S.g.	4, 10, 11, 12, 13, 15	S.g.	See 10, 15*a*	+
25	U	2900	+ NS	+	1, 2, 3, 4, 8, 9, 10; S.p.B.3*b*; S.g.	1, 2, 3, 10, 11, 12, 15; S.g.	4, 8, 9; S.p.B.3*b*	See 26	+
26	U	3662	+ NS	+	1, 2, 3, 4, 8, 9, 10; S.p.B.3*b*; S.g.	1, 2, 3, 4, 7, 8, 10, 11, 12, 12*a*, 13, 14, 15, 18, 20, 25; S.g.	8, 9; S.p.B.3*b*	See 25	+
27	U	4262	+ LN	+	1, 2, 3, 4, 5, 6, 13, 14, 15, 15*a*, 16, 17, 18, 19, 20, 25, 29, 30, 31; S.p.B.1; S.g.	1, 4, 7, 12, 13, 14, 15, 15*a*, 16, 17, 20	2, 3, 5, 6, 18, 19, 25, 29, 30, 31; S.p.B.1; S.g.	+	+
28	U	4260	–	+		8, 9			+
29	U	3878	+ LS	+	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 15*a*, 16, 17, 18, 19, 20, 20*a*, 23, 24, 25, 26, 28, 31; S.p.B.1; S.g.	4, 12*a*; S.p.B.1	1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 13, etc.; S.g.	+	
30	U	3007	+ NS	+	9; S.g.	9, 24; S.p.B.1	S.g.	+	+

U = untypable. Plaque sizes: L = large, above 1·5 mm.; N = normal, about 1 mm.; S = small, 1–0·1 mm.; m = minute, less than 0·1 mm.; μ = micro. Where no size is indicated in TL phages, the plaques are m and μ. Numbers under host range indicate sensitive types of Scheme 2. S.p.B.1 = *Salmonella paratyphi* B phage type 1 var. 2; S.p.B.3*b* = *Salm. paratyphi* B phage type 3*b*; S.g. = *Salm. gallinarum* 780. Spaces are left blank in columns 6, 8, 9 and 10 where phages have not been isolated from the respective strains.

shows. In addition to this expansion of the old types, Scheme 2 enables ten new types to be designated which were untypable by Scheme 1. Nine of these types were totally resistant to the old typing phages. The practical importance of these new type identifications is shown by the fact that the respective type strains were in most instances picked from outbreaks which they caused.

The typing phages of Scheme 2

We have adopted the convention of prefixing *Salm. typhi-murium* typing phages with the letter M; temperate phages are designated with the type number of the strain carrying them followed by a superscript prime sign (see Anderson & Felix, 1953). For example, phage M19 is the typing phage which identifies type 19, and phage 19′ is the temperate phage carried by type 19. The temperate phages of some types have proved useful sources of typing phages for others. Thus, phage M2 was derived from phage 18′; phage M3 from 20*a*′; phage M6 from 13′; phage M12 from 4′; phage 18 from 19′. Two further phages—M8 and M17—were derived from phage 19′ with intermediate adaptation to type 18; phage M13 was prepared by adapting M12 (= phage 4′) to type 13; phage M21 was derived from M2 (= phage 18′) by growth on type 21; and propagation of M21 on type 22 yielded phage M22. Type 31 was contaminated with a temperate phage of unknown origin on first isolation. This phage was found to lyse type 23 and with it we prepared phage M23.

Table 4 summarizes the characteristics of the new typing phages. On initial isolation most of the phages yielded lysis which was masked to a variable extent—sometimes seriously—by secondary lysogenic growth. As this frequently rendered assessment of the amount of lysis difficult, clear-plaque variants of the phages have been isolated where possible. None of these variants seems to be truly virulent, that is, incapable of forming lysogenic complexes with the host cells, but the proportion of cells of a culture that they lysogenize is very much lower than that lysogenized by their parent phages. It has been observed that the lysis of some of the clear-plaque lines is relatively free from secondary growth on some types but obscured by it on others, and it is apparent that the proportion of cells lysogenized by such mutants varies from type to type. Examples of this are found in the reactions of phage 15 on types 1 and 2 (Table 2) where it yields confluent lysis with little secondary growth, and on types 15 and 15*a* where it produces opaque lysis, that is, lysis heavily obscured by lysogenized secondary growth.

It will be recalled that typing phage 1 of Scheme 1 was derived from phage 3*b* of the *Salm. paratyphi* B typing system. It has been shown that phage 3*b* can undergo phenotypic modification of host range as the result of growth on particular strains of bacteria (Bernstein, 1958); this change does not affect the phage genotype and is easily reversed or carried still further by the choice of suitable bacterial host strains. Phage 3*b*, which is carried by a number of types of *Salm. paratyphi* B (Felix & Callow, 1951), has proved useful for typing purposes in both *Salm. paratyphi* B and *Salm. typhi-murium*. By successive adaptation it yielded phages 1, 1*a* and 1*b* of Scheme 1 for *Salm. typhi-murium*. As the original phage 3*b*

Table 4. *The typing phages of Scheme 2*

Phage no.	Starting phage	Propagating strain *Salmonella typhi-murium* type	Lytic reaction	R.T.D.	Neutralized by antiserum to phage M
1	Paratyphoid B typing phage 3*b*	1	Clear	10^{-4}	1
2	Phage 18′*	2	Clear	10^{-5}	1
3	Phage 20*a*′	1	Clear	$0{\cdot}5\times10^{-5}$	1
4	Paratyphoid B typing phage 3*b*	4	Clear	$0{\cdot}5\times10^{-3}$	1
5	M1†	5	Clear	10^{-4}	1
6	Phage 13′	6	Clear	$0{\cdot}5\times10^{-3}$	1
7	M1	7	Clear	10^{-4}	1
8	M18	8	Clear	$0{\cdot}5\times10^{-3}$	None
9	M5	9	Clear	10^{-7}	1 (partial)
10	M9	10	Clear	10^{-6}	1 (partial)
11	M5	11	Semi-clear‡	2×10^{-4}	1 (partial)
12	Phage 4′	9	Clear	10^{-7}	Phage 2 of Scheme 1
13	M12	13	Clear	10^{-6}	Phage 2 of Scheme 1
14	Paratyphoid B typing phage 3*b*	14	Semi-clear	10^{-4}	1
15	M5	15	Semi-clear	10^{-5}	1
16	M5	16	Semi-clear	10^{-4}	1
17	M18	17	Semi-clear	$0{\cdot}5\times10^{-5}$	1
18	Phage 19′	5	Clear	10^{-3}	None
19	M16	19	Semi-clear	2×10^{-4}	1
20	M16	20	Semi-clear	$0{\cdot}5\times10^{-3}$	1
21	M2	21	Semi-clear	$0{\cdot}5\times10^{-3}$	1 (partial)
22	M21	22	Semi-clear	10^{-4}	1 (partial)
23	Contaminant temperate phage of type 31	23	Clear	10^{-4}	1
24	M3	23	Clear	2×10^{-5}	1
25	M4	25	Semi-clear	2×10^{-6}	1
26	M14	26	Semi-clear	2×10^{-6}	1
27	M14	27	Semi-clear	10^{-5}	1
28	M27	28	Turbid	10^{-4}	1
29	Paratyphoid B typing phage 3*b*	29	Semi-clear	$0{\cdot}5\times10^{-3}$	None

* Phage 18′ = temperate phage carried by type 18.
† M1 = typing phage 1.
‡ 'Semi-clear' lysis indicates a moderate degree of lysogenization.

was temperate, the lysis it produced was rapidly clouded by secondary growth. A variant of it was isolated which gave clearer lysis while remaining unchanged serologically and in host range and adaptability. This was used as the immediate starting point for a number of the typing phages of Scheme 2: phages 1, 4 and 14. Indirectly, it produced the following phages, the intermediate host types of which are shown serially in parentheses: phages M 5 (1); 7 (1); 15 (1, 5); 16 (1, 5); 19 (1, 5, 16); 20 (1, 5, 16); 25 (4); 26 (14); 27 (14) and 28 (14, 27).

Thus, thirteen of the twenty-nine phages of this new typing scheme were prepared by adaptation of a single phage which originated from a lysogenic strain of *Salm. paratyphi* B. As far as has been ascertained, the host range is the only changed character in these preparations, and they are all neutralized by an antiserum against paratyphoid B phage 3*b* and *Salm. typhi-murium* phage 1.

Phage M 29 is shown in Table 4 as having been derived directly, and phages M 9, 10 and 11 as having been derived indirectly from phage 3*b*. As this table shows, however, the resulting phages are serologically different from phage 3*b* and we believe that this was due to complete or partial replacement of the starting phage by temperate phages carried by the host strains used for propagation.

Although in general the typing phages were propagated on the types they identified, as Table 4 shows, this practice was not invariably followed; it was sometimes found that other types were more satisfactory propagating strains. Phage M 3, for example, was propagated on type 1, and M 12 on type 9. Specificity was acquired in some phages by growing them initially on the type which they were to identify and subsequently on another type which yielded preparations of higher titre without affecting their host range. Thus, phage 14 was prepared by first adapting *Salm. paratyphi* B phage 3*b* to *Salm. typhi-murium* type 14, and growing the phage so obtained on type 2. When manipulations of this sort are carried out, it is important to ensure that the resulting phage is in fact the product of propagation of the starting phage, and not a contaminant temperate phage carried by the heterologous type now being used for propagation. For this reason, non-lysogenic strains are employed for such 'heterologous' phage propagation when possible.

Phage stocks for routine use were all grown in agar layer as described earlier. The lowest acceptable R.T.D. was 10^{-3}. Some phages yielded very high titres and could be employed in routine dilutions of 10^{-6} or 10^{-7}. Although most phages are stable at 4° C., the temperature of storage, a few deteriorate slowly at that temperature, and a 6-monthly titration of the preparations is carried out in order to adjust the R.T.D. if necessary. The dilutions shown in Table 4 are those currently employed in the C.E.R.L., but small environmental variations in other laboratories may affect lysis so as to necessitate the use of different dilutions, although the adjustment required should be of a minor order if satisfactory media and incubation temperature are used.

The antigenic relations of the new phages

Antisera had been prepared in rabbits against phages 1, 2 and 4 of Scheme 1, and with these the phages of that scheme had been divided into the following

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three serological groups (see Felix, 1956): Group 1: phages 1, 1*a*, 1*b*, 2*b*, 2*d*, 3, 3*a*. Group 2: phages 2, 2*a*, 2*c*. Group 3: phage 4.

The serological findings on the new phages are summarized in column 6 of Table 4. Nineteen out of twenty-nine were serologically indistinguishable from *Salm. paratyphi* B phage 3*b* and *Salm. typhi-murium* phage 1. This number included six phages descended entirely from temperate phages carried by strains of *Salm. typhi-murium*: phages M2, 3, 6, 17, 23 and 24. Phage M17 was originally believed to be derived from M18 which was grown from phage 19′. However, whereas M18 was found to be serologically distinct from M1, M17 was serologically identical with the latter. It was concluded, therefore, that the stock of M18 contained more than one phage and that, in adaptation to type 17, the phage propagated was not that which predominated in the original preparation.

It was interesting to find close antigenic relationships between a temperate phage of *Salm. paratyphi* B (typing phage 3*b*) which has yielded so many typing phages for *Salm. typhi-murium*, and the temperate phages of *Salm. typhi-murium* from which typing phages M2, 3, 6, 17, 23 and 24 were prepared.

Five phages, M9, 10, 11, 21 and 22, showed a partial antigenic relationship to paratyphoid B phage 3*b* and *Salm. typhi-murium* phage 1. Two phages, 12 and 13, could not be distinguished serologically from phage 2 of Scheme 1. Finally, three phages, 8, 18 and 29, were not neutralized by any of the sera prepared in the C.E.R.L.

Phages that were apparently descended directly or indirectly from phage M1 did not always show serological identity with that phage. For example, phages M9, M10 and M11 were prepared by appropriate adaptation of phage M5 which in turn was prepared from M1. Although M5 was serologically indistinguishable from M1, phages 9, 10 and 11 exhibited only a partial relationship to the latter. As we have already suggested, it seemed probable that in such instances the starting phage (M5) had been replaced by a contaminating phage carried by an earlier host strain, for evidence of antigenic modification of phages is flimsy. Phage 29, which was prepared by adaptation of paratyphoid B phage 3*b* to type 29, proved to be antigenically quite different from the starting phage and it was concluded that this was yet another example of phage substitution.

Lysogenicity of the types of Scheme 2

Lysogenicity plays a major role in producing the bacterial phage-resistance patterns that characterize the types of any scheme employing a battery of applied typing phages. Observations on the Vi-phage types of *Salm. typhi* (Felix & Anderson, 1951; Anderson, 1951; Anderson & Felix, 1953) have shown that it is possible to prepare recognized phage types in the laboratory by lysogenizing selected indicator strains with suitable phages, which have been designated 'type-determining phages'. It is clear that the identification of the phages carried by various types could also be used as a method of characterization of strains of the host organism. In one respect this method might be able to detect differences that would be missed by the more usual technique of applying typing phages, for carried phages can be isolated that do not affect the resistance patterns of their

host cells to the available typing phages. Boyd *et al.* (1951), following a suggestion by Boyd (1950), successfully employed temperate phage identification in the epidemiological subdivision of a number of strains of *Salm. typhi-murium*. It has been pointed out by Anderson & Felix (1953), however, that the identity of a bacterial phage type depends on the original host bacterium as well as on the carried phages and, as it was shown by these authors that the lysogenization of different host strains with the same temperate phage produced different phage types, it was apparent that temperate phage identification alone had its limitations in bacterial typing. Moreover, types from which phages cannot be isolated can still be identified by their sensitivity to typing phages.

The most complete method of characterization of a strain would be by the identification of both the phages it carries and its phage sensitivity. Accurate identification of phages is considerably more difficult technically than the application of typing phages, and the latter is the more commonly employed method. However, as we have been aware of the importance of lysogeny in type identification, we isolated and characterized as far as possible the temperate phages of each of the new types. In addition to using all the remaining type strains as indicators for the phages borne by each type, two strains of *Salm. paratyphi* B, phage types 1 var. 2 and 3*b*, and a strain of *Salm. gallinarum*—780—were also employed. Of these, type 1 var. 2 of *Salm. paratyphi* B and *Salm. gallinarum* 780 were known to have wide sensitivity to phages, and type 3*b* is sensitive to paratyphoid B typing phage 3*b*, from which thirteen of the new *Salm. typhi-murium* typing phages originated.

The temperate phages were prepared in the first instance by incubating pure broth cultures of the types from which they were isolated. Indicator strains were not employed for phage enrichment because of the risk of contaminating the phages to be investigated with others contributed by such strains. The phages isolated were tested for heat resistance, for host range as indicated above, for plaque characters and, in some instances, for their ability to be used as typing phages. The results are summarized in Table 5 (facing p. 353).

Type 31 is omitted from this table as its pattern of lysogenicity is not yet clear. Twenty-six out of the thirty-three type strains shown are lysogenic. Multiple lysogenicity is common in *Salm. typhi-murium* and, as Table 5 shows, twenty-three of the twenty-six lysogenic type strains carry both thermolabile and thermostable phages. These phages as a rule could readily be distinguished from each other in any given strain, since the thermostable phages were easily grown in high titre and mostly produced large plaques with a heavy button of central secondary growth, that is, the plaque morphology was that of the 'A' group of phages of Boyd (1950, 1951). The thermolabile phages, on the other hand, showed minute or micro plaques, and usually grew only in low titre. It was generally possible to demonstrate differences between the host ranges of the thermolabile and thermostable phages carried by each strain (see Table 5, col. 6), although a number of indicator strains were frequently lysed in common by both phages.

When a given type carried thermostable phages of different plaque sizes (see Table 5, col. 4) it was usually possible to separate pure lines of each plaque type

and to demonstrate that the respective phages were distinct. In a number of instances, as has been indicated earlier, typing phages could be prepared from the thermostable temperate phages carried by particular types. In addition, propagation of some thermostable temperate phages on suitable strains yielded lines identical in host range with typing phages already present in Scheme 2. The examination of these phages is not yet complete and no further details will be given here.

Columns 9 and 10 of Table 5 show that most of the phages isolated from the type strains exhibited unique host ranges. Had it not been for the large number of indicator strains used, however, it would not have been possible to establish this. These phages have not yet been characterized serologically and, as there is considerable similarity between the plaque morphology of many of them, they are distinguished from each other largely by differences in host range. How far this establishes their separate identity it is at present impossible to say. It must be remembered that phenotypic modification of host range may be impressed on temperate phages by the cells that carry them (Bertani & Weigle, 1953) and it is also possible that host-range mutants of phages may be selected by bacteria destined to become lysogenic. Thus, it cannot yet be stated how many separate phages are represented in the lines isolated from the phage types of the new scheme for *Salm. typhi-murium* and their classification must await further work. At the moment, it seems probable that the thermostable phages belong to the A group (Boyd, 1950, 1951) while those which are thermolabile may belong to the B group of phages.

DISCUSSION

The new typing scheme, containing twenty-nine phages initially defining thirty-four types, is considerably larger than the old, which contained eleven phages and defined twelve types. Because of the greater number of phages employed, Scheme 2 presents increased technical difficulties. Nevertheless, the finer subdivision of *Salm. typhi-murium* which it offers provides an epidemiological tool of considerably greater precision than any so far available. It was indeed with the object of attaining greater accuracy that the development of the new typing scheme was undertaken. Fortunately, bacteriologists are increasingly aware of the help that can be given by phage typing, and many more cultures of *Salm. typhi-murium* are now being examined than previously; in particular, the work has been extended to include animal and other non-human sources. The results obtained hitherto suggest that the new scheme could replace the old with advantage and will be described in detail in a future publication.

SUMMARY

1. A new phage-typing scheme for *Salm. typhi-murium* is described which initially defines thirty-four types of the organism.

2. Types recognizable with the older scheme of Felix and Callow remain distinct with the new system. However, the latter method offers a finer subdivision of the organism than does the former.

3. Thirteen of the twenty-nine typing phages used in the new scheme were prepared by adaptation of *Salm. paratyphi* B typing phage 3*b*, and nineteen are serologically indistinguishable from this phage.

4. The lysogenic properties of the new types are discussed. Twenty-three out of the thirty-three type strains yielded both thermolabile and thermostable phages and a further three yielded thermolabile phages only. No phages could be isolated from the remainder.

5. The distinctive host ranges of the phages carried by most types can be used for type identification.

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REFERENCES

ADAMS, M. H. (1950). Bacterial Viruses. In *Meth. med. Res.* **2**. Chicago: Chicago Year Book Publishers.

ADAMS, M. H. (1959). *Bacteriophages*. New York: Interscience Publishers Inc.

ANDERSON, E. S. (1951). The significance of Vi-phage types F1 and F2 of *Salmonella typhi*. *J. Hyg., Camb.*, **49**, 458.

ANDERSON, E. S. & FELIX, A. (1953). The Vi-type determining phages carried by *Salmonella typhi*. *J. gen. Microbiol.* **9**, 65.

ANDERSON, E. S. & WILLIAMS, R. E. O. (1956). Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *J. clin. Path.* **9**, 94.

BERNSTEIN, A. (1958). A new phage type of *Salmonella paratyphi* B which was responsible for an outbreak of food poisoning. *Mon. Bull. Minist. Hlth Lond.* **18**, 92.

BERTANI, G. & WEIGLE, J. J. (1953). Host controlled variation in bacterial viruses. *J. Bact.* **65**, 113.

BOYD, J. S. K. (1950). The symbiotic bacteriophages of *Salmonella typhi-murium*. *J. Path. Bact.* **62**, 501.

BOYD, J. S. K. (1951). Observations on the relationship of symbiotic and lytic bacteriophage. *J. Path. Bact.* **63**, 445.

BOYD, J. S. K., PARKER, M. T. & MAIR, N. S. (1951). Symbiotic bacteriophage as a 'marker' in the identification of strains of *Salmonella typhi-murium*. *J. Hyg., Camb.*, **49**, 442.

CRAIGIE, J. & YEN, C. H. (1938). The demonstration of types of *B. typhosus* by means of preparations of Type II Vi phage. *Canad. Publ. Hlth. J.* **29**, 448, 484.

FELIX, A. (1956). Phage typing of *Salmonella typhi-murium*: its place in epidemiological and epizootiological investigations. *J. gen. Microbiol.* **14**, 208.

FELIX, A. & ANDERSON, E. S. (1951). Bacteriophages carried by the Vi-phage types of *Salmonella typhi*. *Nature, Lond.*, **167**, 603.

FELIX, A. & CALLOW, B. R. (1943). Typing of paratyphoid B bacilli by means of Vi bacteriophage. *Brit. med. J.* ii, 127.

FELIX, A. & CALLOW, B. R. (1951). Paratyphoid B Vi-phage typing. *Lancet*, ii, 10.

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