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Meso-tartrate resistance and phylogenetic relationships of biotypes of *Salmonella typhimurium*

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SUMMARY

Meso-tartrate utilizing and resistant (mT^+) recombinants were produced readily in transductions from an mT^+ donor strain to 42 naturally occurring *meso*-tartrate non-utilizing and sensitive (mT^-) recipient strains of *Salmonella typhimurium*, and were produced, sometimes as frequently, in interbiotype crosses between different pairs of strains from the mT^- biotypes 2, 18, 26 and of the FIRN group (biotypes 30 and 32). These findings suggested that the sites of the *mta* mutations were independent in strains from the different mT^- biotypes and were in agreement with their probable different lines of descent. Intrabiotype crosses indicated genetic homogeneity among strains of biotype 26 and among strains of biotype 30; on the other hand, they suggested that in each of the biotypes 2, 18 and 32, a minority of strains had *mta* mutations present at different intragenic sites from those of the majority of strains. The derivations of the strains of the minority types are discussed.

1. INTRODUCTION

Reactions with the tartaric acid isomers provided useful information in biotyping strains of *Salmonella typhimurium*; thus, among 2030 naturally occurring strains of diverse origin, the inabilities to utilize *dextro*-tartrate (24 % of strains), or *laevo*-tartrate (5 %), or *meso*-tartrate (18 %), were minority characters (Duguid *et al.* 1975).

Whereas the growth of bacteria unable to utilize *dextro*- or *laevo*-tartrate was generally not adversely affected by the presence of these isomers in the growth medium (Alfredsson *et al.* 1972; Duguid *et al.* 1975), *meso*-tartrate severely inhibited the growth of all *meso*-tartrate non-utilizing (mT^-) strains in peptone and mineral-salts media, i.e. they were *meso*-tartrate sensitive (Alfredsson *et al.* 1972; Alfredsson & Old, 1973; Duguid *et al.* 1975). Experiments with cell-free extracts of such *meso*-tartrate sensitive bacteria showed that *meso*-tartrate was a competitive inhibitor of isocitrate dehydrogenase and isocitrate lyase activities and, thus, indicated why *meso*-tartrate sensitivity was most readily demonstrated under conditions in which the tricarboxylic acid cycle was operative for growth, e.g. mineral-salts media with citrate or glycerol as carbon source (Old, Alfredsson & Brown, 1980). *Meso*-tartrate is also a competitive inhibitor of the isocitrate

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enzymes of *meso*-tartrate utilizing (mT^+) strains, which, however, are usually resistant to *meso*-tartrate except for that period prior to induction of the enzymes required for the utilization, and removal, of the inhibitor (Old, Alfredsson & Brown, 1980).

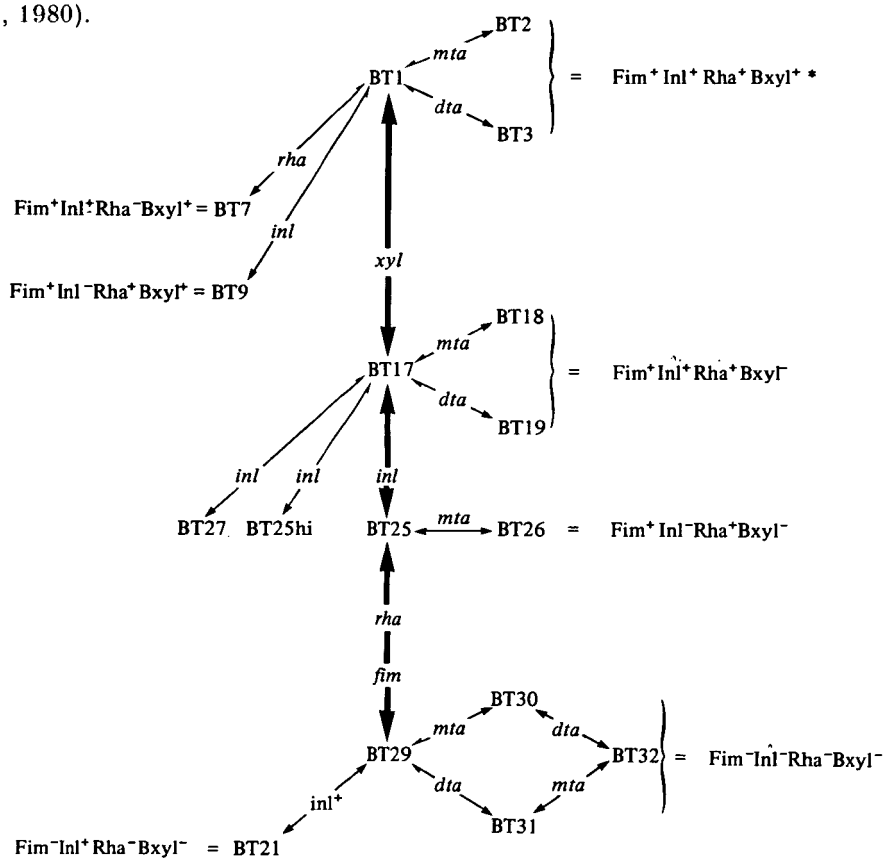

Fig. 1. Proposed relationships between the primary biotypes of *Salmonella typhimurium*. To the left of the main line of derivation (BT1 ↔ BT17 ↔ BT25 ↔ BT29), are routes that have been previously established (see text and references); and, to the right, proposed routes whereby the *meso*-tartrate negative biotypes (2, 18, 26, 30 and 32) and the *dextro*-tartrate negative biotypes (3, 19, 31 and 32) may have been derived from the *meso*- and *dextro*-tartrate positive primary biotypes (1, 17, 25 and 29).* +/− = positive/negative, with respect to the biotype characters: Fim, production of type-1 fimbriae; Inl, fermentation of *meso*-inositol at 37 °C; Rha, fermentation of L-rhamnose; Bxyl, utilization of D-xylose as sole carbon source.

Previous transductional studies (Duguid, Old & Hume, 1962; Old, 1963; Morgenroth & Duguid, 1968; Old & Duguid, 1979; Old, Dawes & Barker, 1980) suggested that all naturally occurring strains of *S. typhimurium* of the FIRN biotypes 29, 30, 31 and 32 (see Fig. 1) had been derived from a single FIRN ancestral clone that diversified in secondary biotype characters and phage types (Duguid *et al.* 1975; Anderson *et al.* 1978) and that the FIRN biogroup had descended by mutations in *rha* and *fim* from biotype 25 (Old, 1972; Old, Dawes & Barker, 1980).

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The probable descent of biotype 25 strains from an ancestral biotype 17 strain by a mutation in *inl* (Mortlock & Old, 1979; Old & Mortlock, 1979) and that of biotype 17 strains from an ancestral biotype 1 strain by a *xyl* mutation that rendered the biotype 17 strains (and their descendants) unable to utilize D-xylose as sole carbon source (Duguid *et al.* 1975; Old, Dawes & Barker, 1980) have been discussed. These studies have indicated that the relationships between the different known primary biotypes of naturally occurring strains of *S. typhimurium* are essentially as shown in Fig. 1.

Thus, although the probable routes of derivation of most of the biotypes have been indicated, some questions remain unanswered, among them the derivation and interrelationships of the different mT$^-$ biotypes, all 368 strains of which – in biotypes 2 (69 strains), 18 (7), 26 (252), 30 (10) and 32 (30) – were *meso*-tartrate sensitive regardless of their phage type or source (Duguid *et al.* 1975).

The aim of the present study, which describes the transduction to mT$^-$ strains of the ability to utilize *meso*-tartrate (the position of this locus, for which we suggest the symbol *mta*, has not been described; Sanderson & Hartman, 1978) was to investigate whether the sites of the *mta* mutations in a phenotypically homogeneous group of naturally occurring mT$^-$ strains of *S. typhimurium* were identical or different and, hence, to establish whether or not they had common ancestors.

2. MATERIALS AND METHODS

(i) *Bacteria.* We tested 42 of the naturally occurring *meso*-tartrate sensitive strains of *S. typhimurium* from the series biotyped by Duguid *et al.* (1975), and their biotype (BT), phage type (PT), source, place and date of isolation are shown in Table 1. The definitive phage types (Anderson *et al.* 1977) were determined at the Division of Enteric Pathogens, Colindale Avenue, London. The majority of the 42 strains were tested both as donors and recipients in the transduction experiments (see, however, footnote Table 2). The mT$^+$ donor strain, which utilized and was resistant to *meso*-tartrate, was LT2-497, a cysteine-requiring, streptomycin-resistant laboratory mutant line derived from Lilleengen's (1948) type-2 strain, LT2, and was received from Professor B. A. D. Stocker (see Old & Duguid, 1979).

(ii) *Culture media.* Nutrient Broth No. 2 and Nutrient Agar were Oxoid preparations. *Meso*-tartrate mineral salts agar (MTA) was prepared as previously described (Duguid *et al.* 1975). In experiments with the four auxotrophic strains from biotypes 30 and 32 (symbol 'y'), MTA contained cysteine (10 μg/ml).

(iii) *Donor phages.* Propagation of phage P22 on donor strains and titration of lysates have been described (Old & Duguid, 1971, 1979).

(iv) *Transduction experiments.* Nutrient broth (50 ml in a 500-ml flask) received an inoculum of *c.* 10^5 recipient bacteria from an overnight broth culture, and was aerated at 37 °C until the viable count was *c.* $2{\cdot}5 \times 10^8$ bacteria/ml. To 1-ml aliquots of the recipient broth culture, donor phages were added (0·1 ml) at a multiplicity of 5–10, and the mixture held at 37 °C for 10 min to allow phage adsorption to occur. Two 0·2-ml volumes of each donor-recipient pair (i.e. *c.* 5×10^7 bacteria of the original mixture) were spread until dry on each of two plates of

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Table 1. *Biotype, phage type, source, place and date of isolation of 42* meso-*tartrate non-utilizing and sensitive strains of* Salmonella typhimurium *used in transduction experiments*

Strain no.	Full biotype	Phage type	Source	Place of origin	Date
S527	2a	111	Cattle	U.K.	1960
S537	2a	14	Man	U.K.	1960
S571	2a	6	Man	U.K.	1960
S723	2a	73	Un	Denmark	1955
S752	2a	15a	Un	Sweden	Un
S1459	2a	1	Pig	U.K.	1962
S2295	2a	11	Environment	U.K.	1963
S2644	2a	42	Turtle	Vietnam	1956
S2681	2a	4	Dog	France	1959
S2778	2a	83	Man	Poland	1959
S2995	2a	135	Man	Eire	1967
S3324	2a	104	Man	U.K.	1962
S3393	2a	173	Mouse	U.K.	1961
S2619	2f	89	Man	Tunisia	1955
S2652	2h	74	Monkey	Senegal	1957
S1441	18a	12a	Man	U.K.	1962
S1573	18a	14	Bird	U.S.A.	1962
S1589	18a	2	Bird	U.S.A.	1962
S1590	18a	2	Bird	U.S.A.	1962
S2591	18e	155	Man	Zaire	1956
S2643	18e	70	Man	Zaire	1956
S1545	26a	2	Cattle	U.S.A.	1962
S1546	26a	6	Man	U.S.A.	1962
S1566	26a	49	Pig	U.S.A.	1962
S1727	26a	NRT	Environment	Sri Lanka	1962
S1837	26a	16	Man	Netherlands	1962
S1931	26a	114	Man	Australia	1964
S3205	26a	135	Man	Finland	1968
S3337	26a	117	Environment	U.K.	1961
S3431	26a	29	Man	U.K.	1964
S3459	26d	29	Cattle	U.K.	1969
S3411	26gi	29	Man	U.K.	1960
S2204	30by	80	Environment	U.K.	1962
S2266	30by	80	Cattle	U.K.	1963
S2364	30by	80	Cattle	U.K.	1964
S735	32b	1	Un	Un	Un
S2565	32b	160	Man	U.K.	1965
S3381	32b	161	Man	U.K.	1967
S6724	32b	14	Man	U.K.	1958
S2805	32bef	146	Man	U.S.A.	1962
S2327	32bi	40	Bird	U.K.	1963
S2366	32by	80	Man	U.K.	1964

Un = unspecified.

MTA. Control samples of the recipient bacteria and donor phage from LT2-497, and of the recipient bacteria alone (i.e. 'no-phage' controls) were inoculated similarly on plates of MTA to demonstrate (i) that the recipient strains were susceptible to transduction of *mta* (i.e. ability to utilize *meso*-tartrate) and (ii) the frequency of mutation to mT^+.

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After incubation of the MTA plates at 37 °C for 48–72 h, plates were scored for recombinant or mutant mT^+ colonies. The average number of mT^+ colonies obtained per two plates in experiments with the same donor–recipient pair is recorded as the result for the cross of that pair of strains (see footnote, Table 2).

(v) *Characterization of mT^+ transductants.* From each donor-recipient mixture that yielded colonies on MTA, subculture to fresh MTA was made and purified colonies were (*a*) serotyped as *S. typhimurium* by slide agglutination tests with Salmonella agglutinating sera – *O* 4, *H* i and *H* 1, 2 – from Burroughs Wellcome Ltd, Beckenham, Kent, and, if the serological characters were correct, (*b*) biotyped by the primary tests of Duguid *et al.* (1975).

3. RESULTS

The aggregated results of the *meso*-tartrate transduction experiments with 42 *meso*-tartrate sensitive strains from five different mT^- biotypes are shown in Table 2, along with the results of their 'no-phage' control tests. Evidence that the mT^+ bacteria arising from platings of different donor-recipient mixtures on MTA were recombinants was provided by the demonstration that the recipient strain either did not mutate to the mT^+ state in control platings without phage, or did so at a rate much lower than that which could account for the number of colonies obtained in transduction platings; and, furthermore, from the absence of mT^+ colonies in significant numbers from control platings of these recipient strains with phage P22 lysates propagated on the homologous strain, i.e. in 'self-crosses'.

In individual strains that mutated to mT^+, the number of mT^+ mutant bacteria produced varied over the range from 3×10^{-7} to 1×10^{-8}, and in platings of all 42 strains in all of the reported experiments, the average mutant frequency – expressed as the number of mT^+ colonies per bacterium plated – was $5{\cdot}5 \times 10^{-8}$.

(i) *Transduction from an mT^+ donor to mT^- recipients*

The results show that phage propagated on the mT^+ (resistant) donor strain LT2-497 from biotype 1y produced mT^+ recombinants from each of the 42 naturally occurring mT^- recipient strains, and that the majority of mT^- strains from the different mT^- biotypes were equally susceptible to transduction of *mta*; the FIRN strains S2364 and S6724 were slightly less competent as recipients. The frequency of production of mT^+ bacteria per phage-infected bacterium plated from the original mixture was, with all strains considered, of the order of not less than $1{\cdot}5 \times 10^{-5}$, i.e. about 50 times higher than the highest frequency of production of mT^+ mutants in control platings of the recipient strains alone. It is concluded, therefore, that most of the mT^+ bacteria were recombinants and not spontaneous mutants.

(ii) *Transduction from mT^- donors to mT^- recipients*

Table 2 indicates that mT^+ recombinants were obtained from many crosses among naturally occurring mT^- strains from different mT^- biotypes, and, to a lesser extent, among mT^- strains within three of the mT^- biotypes.

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Table 2. *Transduction of ability to utilize* meso*-tartrate between donor and recipient strains of different biotypes of* Salmonella typhimurium

mT+ colonies obtained* in crosses with recipient† strains from the mT− biotype:

Donor strain		2														18						26												30			32						
Bio-type	No.	S527	S537	S571	S723	S752	S2295	S2619	S2644	S2652	S2681	S2778	S2995	S3324	S3393	S1459	S1441	S1573	S1589	S1590	S2591	S2643	S1545	S1546	S1566	S1727	S1837	S1931	S3205	S3337	S3411	S3431	S3459	S2204	S2266	S2364	S2327	S2366	S2565	S2805	S3381	S735	S6724
2	S527	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	S537	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	3	3	3	0	0	2	1	.	4	.	.	4	3	3	3	2	3	2	0	0	3	.	.	.	.	.	.
	S571	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	1	.	0	.	.	3	0	1	0	0	0	0	0	0	0	.	.	.	.	.	.
	S723	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	S2295	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	S2619	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	3	4	4	1	1	3	2	.	4	.	.	4	3	4	4	4	2	1	0	1	1	.	.	.	.	.	.
	S2644	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	.	1	.	.	4	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.
	S2652	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	4	4	4	0	0	4	3	.	4	.	.	4	3	4	3	4	4	2	1	2	3	.	.	.	.	.	.
	S2681	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	4	4	4	1	1	4	4	.	4	.	.	4	4	4	2	4	4	4	1	3	3	.	.	.	.	.	.
	S2778	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	4	4	4	0	0	4	3	.	4	.	.	4	4	4	4	4	4	4	1	2	4	.	.	.	.	.	.
	S2995	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	2	2	0	0	2	1	.	4	.	.	3	1	3	2	1	1	1	1	2	4	.	.	.	.	.	.
	S3324	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	S3393	0	1	0	0	1	0	0	0	0	0	0	4	0	0	4	4	4	4	4	0	0	4	2	.	4	.	.	4	4	4	4	4	4	3	1	2	3	.	.	.	.	.	.
	S1459	4	3	4	4	0	4	4	0	1	4	4	4	3	2	0	2	2	4	1	0	0	4	4	.	4	.	.	4	4	3	3	4	4	4	1	4	3	.	.	.	.	.	.
18	S1441	.	1	1	.	.	.	1	0	0	2	1	2	.	1	3	0	0	0	0	1	1	0	0	.	0	.	.	0	1	0	0	0	0	1	0	1	0	.	.	.	.	.	.
	S1573	.	0	2	.	.	.	1	0	1	1	2	1	.	1	2	0	0	0	0	1	1	0	1	.	1	.	.	1	1	0	0	0	4	2	1	2	4	.	.	.	.	.	.
	S1589	.	0	0	.	.	.	0	0	0	0	1	1	.	0	0	0	0	0	0	1	1	0	.	.	0	.	.	1	1	0	0	0	0	0	1	1	1	.	.	.	.	.	.
	S1590	.	0	1	.	.	.	1	0	1	2	2	3	.	1	2	0	0	0	0	1	1	0	1	.	1	.	.	1	0	0	0	0	3	2	1	1	3	.	.	.	.	.	.
	S2591	.	0	.	.	.	.	0	0	0	0	0	0	.	0	0	2	2	3	2	0	0	2	1	.	3	.	.	3	1	3	3	1	0	0	0	0	0	.	.	.	.	.	.
	S2643	.	0	.	.	.	.	0	0	0	1	0	1	.	0	2	2	2	3	2	0	0	2	1	.	3	.	.	3	1	3	3	1	0	0	0	0	0	.	.	.	.	.	.
26	S1545	.	0	0	.	.	.	0	0	0	0	0	1	.	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	.	.	.	.	.
	S1546	.	0	0	.	.	.	0	0	0	1	1	3	.	1	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	.	.	.	.	.
	S1566	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.	.	.
	S1727	.	1	4	.	.	.	3	0	1	4	4	4	.	2	4	0	1	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	4	3	1	4	2	.	.	.	.	.
	S1837	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.	.	.
	S3205	.	3	4	.	.	.	4	0	3	4	4	4	.	3	3	0	1	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	4	4	1	4	4	.	.	.	.	.
	S3337	.	0	.	.	.	.	0	0	0	0	1	0	.	0	0	0	1	0	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	.	.	.	.

30	S2204	.	0	0	.	.	.	0	2	0	0	1	1	.	0	1	1	1	0	0	0	0	1	1	.	1	.	.	1	1	1	1	0	0	0	0	0	0	.	.	.	.	.
	S2266	.	0	1	.	.	.	0	0	0	0	1	2	.	0	1	1	1	1	1	0	0	1	1	.	4	.	.	2	2	2	3	1	0	0	0	0	0	.	.	.	.	.
	S2364	.	0	2	.	.	.	1	0	1	3	3	1	.	1	3	3	4	4	4	1	1	4	2	.	4	.	.	4	3	4	4	4	0	0	0	0	0	.	.	.	.	.
32	S2327	.	1	1	.	.	.	1	0	0	2	1	4	.	1	3	4	4	4	4	0	0	4	2	.	4	.	.	4	3	4	4	4	0	0	0	0	0	0	0	0	1	1
	S2366	.	0	1	.	.	.	1	0	0	1	1	1	.	1	1	2	3	1	2	3	1	2	2	.	3	.	.	3	3	2	3	1	0	0	0	0	0	0	0	0	1	1
	S2565	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0	0	0	2	1
	S2805	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0	0	0	2	1
	S3381	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0	0	0	2	1
	S735	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	1	1	1	0	1
	S6724	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	3	3	3	2	1	0
1	LT2-497	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	3
None (no-phage control)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* The results are expressed as numbers of mT^+ colonies obtained per 5×10^7 bacteria plated from the original phage-bacterium mixture and, for ease of presentation, symbols have been used to indicate the following ranges of numbers of mT^+ recombinants per cross: 0 = < 10; 1 = 10–49; 2 = 50–99; 3 = 100–249; 4 = 250– > 1000.

† Strain S752 (BT 2) and strain S1931 (BT 26) were tested as recipients but not as donors.

. = cross not performed.

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(*a*) *Intrabiotype 2 crosses.* In biotype 2, some important biotype/phage types (BT–PT's) were represented by multiple strains of diverse origin, e.g. BT 2a/PT 1 (6 U.K. strains), 2a/4 (5), 2a/6 (3), 2a/11 (2), 2a/74 (9), 2a/89 (7), 2a/104 (3), 2a/135 (2) and 2a/173 (3). Before proceeding to the experiments outlined in Table 2, we performed a series of crosses between different pairs of strains within each of these BT/PT groups, none of which, however, yielded mT^+ recombinants. Accordingly, in the experiments reported in Table 2, these nine BT/PT's were represented by single strains (Table 1). The other six strains – S527, S537, S723, S752, S2644 and S2778 (Table 1) – were the sole representatives of rarer BT/PT's among the biotype 2 strains in the larger series of strains (Anderson *et al.* 1978). By such a choice, we included representatives of many minor and major clones among strains of biotype 2.

Table 2 shows that of the many crosses performed among biotype 2 strains, tests among 90 different donor–recipient pairs of 13 of them did not yield mT^+ recombinants at a rate higher than that obtained from platings of these same recipients without phage. Since these strains were known effective recipients of *mta* from LT2-497, the absence of mT^+ recombinants in intrabiotype crosses of the 13 strains was of interest. On the other hand, S3393 and S1459 were fertile in crosses with each other and in crosses with, respectively, few and many of the other mT^- strains of biotype 2 (Table 2). Another three strains of BT 2 (namely 2a, 2f and 2h) and PT 1, isolated in Africa and Europe several years prior to the U.K. series of strains of BT 2/PT 1 in 1960–2 (of which S1459 was representative), gave mT^+ recombinants in crosses with S1459, despite their similarity of BT/PT, but were not included in the intrabiotype 2 crosses, however, for the experiments that revealed their fertility with S1459 were performed subsequent to those reported in Table 2.

(*b*) *Intrabiotype 18 crosses.* The transduction experiments with strains of biotype 18 revealed the existence of two genetically distinct groups. Crosses between pairs of strains from the two different groups were fertile, whereas crosses between pairs of strains within each group were not (Table 2).

(*c*) *Intrabiotype 26 crosses.* The 11 strains of biotype 26 included representatives of minor BT/PT's and of major clones of known importance such as 26a/2, 26a/29, 26a/44, 26a/49 and 26a/135 (see Anderson *et al.* 1978). We did not detect mT^+ recombinants in any tests made of the 60 different donor–recipient pair crosses with members of biotype 26 (Table 2).

(*d*) *Intrabiotype 30 crosses.* Crosses with different pairs of the three FIRN strains of type 30by/80 were not fertile (Table 2).

(*e*) *Intrabiotype 32 crosses.* The majority (five) of the tested strains of the FIRN biotype 32, representing major and minor clones, did not yield mT^+ recombinants in crosses with each other. There were, however, two strains, S735 and S6724, the sole representatives of their BT/PT's (Anderson *et al.* 1978), that produced mT^+ recombinants in crosses with each other and with the other five FIRN strains of biotype 32 (Table 2).

(*f*) *Interbiotype crosses.* There was an absence of fertility in crosses between all

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tested strains of the FIRN biotype 30 and most of those of the FIRN biotype 32. That finding apart, however, most strains from any mT$^-$ biotype produced mT$^+$ recombinants in crosses with most strains tested from any other mT$^-$ biotype, sometimes at a frequency as high as that in crosses with an mT$^+$ donor. The results are detailed in Table 2.

(iii) *Characters of the mT$^+$ transductants*

The isolated clones of mT$^+$ recombinants were confirmed as *S. typhimurium* and shown always to retain the other biotype characters of the recipient strain. Thus, the biotypes of the mT$^+$ recombinants isolated from the recipient strains of biotypes 2, 18, 26, 30 and 32 were, respectively, 1, 17, 25, 29 and 31.

4. DISCUSSION

It will be convenient to consider in turn the results of the intrabiotype crosses for each mT$^-$ biotype. The finding that mT$^+$ recombinants were not produced in any of many crosses performed between different pairs of 13 naturally occurring strains of biotype 2 indicated that the site of the *mta* mutation in these 13 strains (in phage types and related phage types that represented *c.* 65% of biotype 2 strains) was identical or overlapping. However, the demonstrable fertility between strains S1459 and S3393 (representing *c.* 15% of biotype 2 strains) was evidence that the intragenic sites of the *mta* mutations in these two strains were different from each other; each was different from that in the above group of 13 strains. Thus, there was good evidence of heterogeneity among biotype 2 strains which, with their different *mta* mutations, probably arose independently from different biotype 1 ancestors.

The genetic evidence for two distinct *mta* mutations among biotype 18 strains was substantiated by other data. Thus, those in the larger of the two groups were of biotype 18a and all had been isolated in 1962 in U.K. or U.S.A.; those in the smaller group had been isolated in Zaire in 1956 and all had additionally the rare secondary biotype marker 'e', i.e. they were unable even to ferment D-xylose in peptone media (Duguid *et al.* 1975; Anderson *et al.* 1978).

In contrast to the diversity of *mta* mutational sites in strains of biotypes 2 and 18, our results suggested that the site of the *mta* mutation was the same in all tested strains of biotype 26 of which we had chosen representative major and minor clones (Anderson *et al.* 1978).

Because of the already established identity of FIRN strains with regard to *fim*, *inl* and *rha*, it was not surprising, therefore, to find similar identity of *mta* in strains from phage types and related phage types representing, respectively, 100 and 95% of the FIRN strains in the mT$^-$ biotypes 30 and 32, suggesting that the principal route of derivation of biotype 32 strains was from an ancestral strain of biotype 30 after a mutation in the ability to utilize *dextro*-tartrate (*dta*: see Fig. 1). It is significant also that in biotypes 30 and 32, respectively, all and 50% of the strains were of phage type 80, most of them cysteine auxotrophs (Anderson *et al.* 1978).

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The atypical strains in biotype 32 were S6724 and S735 which by their ability to recombine to mT+ with representatives of the majority of other mT− FIRN strains were shown to have *mta* mutations different from that in the majority. Strain S6724 was of interest in that of 105 FIRN strains of phage type 14 from the larger series (Anderson *et al.* 1978) it alone belonged to biotype 32 although its source, place and date of isolation corresponded to those of many other strains of BT 31/PT 14. It would seem likely, therefore, that S6724 originated after a mutation in *mta* from an ancestral bacterium of biotype 31 rather than from one of biotype 30. That being so, the site of the *dta* mutation in S6724 would probably be different from that in those biotype 32 strains derived from a biotype 30 ancestor which, in turn, probably originated after a mutation in *mta* from one of biotype 29 (see Fig. 1). In the absence of a suitable selective medium for the isolation of dT+ recombinants – in view of the inability of dT+ strains to utilize *dextro*-tartrate as sole carbon source (Alfredsson *et al.* 1972; Duguid *et al.* 1975) – such genetic data is not yet available. The other strain of biotype 32, for which our results have indicated yet another *mta* mutational site, was S735, Lilleengen's (1948) phage type 1 strain which, however, is of uncertain pedigree.

Although we have been unable to test all possible combinations among mT− strains from our collection of naturally occurring *S. typhimurium*, our careful choice of strains from each mT− biotype allowed representatives of major and minor clones to be included in our studies (Anderson *et al.* 1978) and enabled us to obtain useful information about the relationships between strains within each mT− biotype and about those of strains from different mT− biotypes. The present study has provided useful additional data that indicate further parts of the genealogy of the biotypes of naturally occurring strains of *S. typhimurium*.

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