Transduction of Fimbriation Demonstrating Common Ancestry in FIRN Strains of *Salmonella typhimurium*

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The production of fimbriate (Fim⁺) recombinants was observed in transductional crosses between different pairs of wild-type strains of different biotypes of *Salmonella typhimurium*. Fim⁺ recombinants were readily produced in transductions from Fim⁺ donor strains to Fim⁻ recipient strains and, less frequently, between some pairs of Fim⁻ strains, for example, between almost any strain of the FIRN biogroup (Fim⁻ Inl⁻ Rha⁻ Bxyl⁻) and many strains of the non-FIRN Fim⁻ biogroup. None of numerous crosses between different pairs of FIRN strains gave Fim⁺ recombinants, suggesting that the *fim* mutation was present at the same intragenic site in all FIRN strains. FIRN strains are thought to have descended from a single ancestral FIRN bacterium which originated by a series of mutations from a strain of the common biotype 1a (Fim⁺ Inl⁺ Rha⁺ Bxyl⁺). Two FIRN-like (Fim⁻ Inl⁺ Rha⁻ Bxyl⁻) strains that did not yield Fim⁺ recombinants in crosses with FIRN strains were probably wild-type Inl⁺ mutants from FIRN strains.

INTRODUCTION

Most wild-type strains of *Salmonella typhimurium* form type-1 fimbriae, the filamentous appendages that confer mannose-sensitive haemagglutinating properties on bacteria (Duguid *et al.*, 1966). In this serotype, the possession of the fimbriation character (Fim⁺) was associated with that of the ability to ferment inositol (Inl⁺) and/or rhamnose (Rha⁺) in peptone water within 24 h at 37 °C. The association was confirmed by Duguid *et al.* (1975) in 2030 strains from a variety of sources in 54 countries, including representatives of 19 primary and 144 full biotypes; 275 strains of a Fim⁻ Inl⁻ Rha⁻ (FIRN) biogroup (Morgenroth & Duguid, 1968), all of which were also Bitter's xylose negative (Bxyl⁻), belonged to the primary biotypes 29 to 32.

None of the FIRN strains gave spontaneous Fim⁺ mutants on serial culture in aerobic static broth; all gave Rha⁺ mutants, but only by the two-step procedure described by Morgenroth & Duguid (1968); and none readily gave Inl⁺ mutants on prolonged culture at 37 °C in inositol peptone water (Duguid *et al.*, 1975). Thus, all FIRN strains were alike in the behaviour of their *fim*, *inl* and *rha* mutations.

Following preliminary observations by Duguid *et al.* (1962), we now describe transduction studies which suggest that the site of the *fim* mutation is the same in all FIRN strains and is different from that in many Fim⁻ strains not of the FIRN group. The principle of these studies, which were made on naturally occurring mutant strains, was derived from the observations of Demerec *et al.* (1954) and Hartman (1956) on laboratory mutants. They found that mutations produced independently in the same gene generally gave rise to non-identical alleles, and that phenotypically similar mutants with non-identical alleles were usually distinguishable from one another because they gave wild-type recombinants when crossed by transduction. Thus, the absence of recombination between two mutant

Table 1. *Biogroup, phage type/biotype and source of strains of Salmonella typhimurium most commonly tested as donors of fim*

Biogroup	Strain no.	Phage type/ biotype	Source or derivation
Fim ⁺	LT2	4/1f	Lilleengen's (1948) type-2 strain
	LT2-497	4/1y	Mutant from strain LT2
	S844	1/1a	Man, Malawi, 1960
	S375	1/3a	Boyd's (1956) strain Q1
	S12	135/25f	Man, Scotland, 1955
	S1404	44/26i	Guinea-pig, England, 1961
	S745	49/26y	Lilleengen's (1948) type-9 strain
FIRN	S1577	13/29b	Man, Maine, U.S.A., 1962
	S635	13/29bc	Fowl, Scotland, 1959
	S580	13/29bcf	Man, U.S.A., Friewer & Leifson (1952)
	S2599	13/29bf	Man, Egypt, 1959
	S2584	40/29bf	Bone, India, 1963
	S2266	80/30by	Cattle, England, 1963
	S1298	1/31b	Man, England, 1962
	S7471	1/31b	Man, England, 1958
	S1290	13/31b	Fowl, Scotland, 1962
	S2322	40/31b	Turkey, England, 1963
	S654	92/31b	Chinchilla, England, 1958
	S706	58/31bg	Guinea-pig, Sweden, 1959
	S2716	13/31bgz	Man, England, 1959
	S735	1/32b	Lilleengen's (1948) type-1 strain
	S3381	161/32b	Man, England, 1967
	S704	80/32bf	Parrot, Sweden, 1959
Non-FIRN Fim ⁻	See * strains, Table 2		
FIRN-like	S2338	160/21b	Man, England, 1963
	S6925	13/23by	Dog, Scotland, 1958
Induced Fim ⁻ mutant	LT2-D461	36/7bcz	Mutant from strain LT2
	LT2-D464	36/7bcz	Mutant from strain LT2

strains suggests that they have descended from the same mutant bacterium and not from separately produced mutants.

The gene determining the production of type-1 fimbriae in *S. typhimurium* is located on the chromosome at map position 23 min (see Sanderson, 1972) and is transmissible by transduction with phage P22 (Old & Duguid, 1971). The fimbriate transductants are readily selected from motile recipients by culture of the transduction mixture in aerobic static broth, where aerotactic migration combined with the ability of fimbriate bacteria to grow in a surface pellicle with access to atmospheric oxygen enables the Fim⁺ transductants to outgrow the parental Fim⁻ bacteria confined within the broth (Old & Duguid, 1970).

METHODS

Bacteria. We tested 320 of the wild-type strains of *S. typhimurium* biotyped by Duguid *et al.* (1975) and 4 variants from them. Most of the wild-type strains had been isolated from widely separate sources and epidemic foci. Their 'definitive phage types' (Anderson *et al.*, 1977) were determined at the Enteric Reference Laboratory, Public Health Laboratory Service, London. They were classified by their Fim, Inl and Rha characters into four 'biogroups'.

(i) Fim⁺ biogroup. These were 21 wild-type strains isolated from man and five animal species in seven countries between 1940 and 1963. They represented 14 different full biotypes and 12 different phage types. Strain LT2-497, supplied by Professor B. A. D. Stocker, was a streptomycin-resistant, cysteine-requiring laboratory mutant from strain LT2 (Table 1) and was wild-type in its Fim⁺ character. The results of tests in which it was used as a donor have been aggregated with those of the 21 wild-type Fim⁺ strains.

(ii) FIRN biogroup. These were 258 of the 275 wild-type FIRN strains of Duguid *et al.* (1975). The 4 phage-untypable and 13 of the 16 non-motile strains in the series of 275 were not tested because it is difficult

Table 2. *Phage type/biotype, source, and place and date of isolation of non-fimbriate strains of Salmonella typhimurium in generally fimbriate biotypes (non-FIRN Fim⁻ biogroup)*

Strain no.*	Phage type/biotype	Source	Place	Date
S2365	19/1b	Drain	Wales	1964
S2231*	25/1b	Fowl's egg	Argentina	1962
S519*	69/1bc	Man	England	1934
S2787	5/1bf	Mouse	Tennessee, U.S.A.	1962
S3476	29/1bf	Man	Scotland	1971
S3477	29/1bf	Man	Scotland	1971
S3146	12/3b	Man	Mexico	1969
S2506*	41/3b	Cattle	England	1963
S2334	106/3b	Man	England	1963
S3350	130/3b	Man	New Zealand	1970
S3403*	183/7b	Man	England	1955
S2696	22/9b	Man	Venezuela	1956
S3285	65/12bdhx	Cattle	England	1963
S2700*	3/17b	Turtle	Vietnam	1956
S3104	9/17b	Fertilizer	New Zealand	1969
S3105	9/17b	Fertilizer	New Zealand	1969
S3164	9/17b	Man	New Zealand	1969
S3168	9/17b	Man	New Zealand	1969
S3170	9/17b	Man	New Zealand	1969
S3280	60/17b	Unknown	Scotland	1959
S2549	60/17b	Man	New Zealand	1964
S3106	61/17b	Fertilizer	New Zealand	1969
S1436	64/17b	Sheep	New Zealand	1962
S749	117/17be	Unknown	Sweden	Unknown
S750	117/17be	Unknown	Sweden	Unknown
S3117	3/17bf	Man	Mexico	1969
S2626*	10/17bf	Man	Tunisia	1956
S2656	9/17bg	Man	France	1957
S2802	3/17bj	Duck	S. Carolina, U.S.A.	1962
S6631*	10/25b	Man	England	1958
S3180	66/25b	Man	Germany	1969
S3181*	66/25b	Man	Germany	1969
S2519*	67/25b	Cattle	England	1964
S3308	88/25b	Man	England	1963
S3311	91/25b	Man	England	1958
S3258*	38/25bcg	Man	Canada	1964
S2784*	99/25bhi	Horse	Kentucky, U.S.A.	1962
S1566*	49/26b	Pig	Indiana, U.S.A.	1962
S3144	49/26b	Man	Mexico	1969

* Strains most commonly tested as donors of *fim*.

both to transduce untypable strains and to select Fim⁺ transductants from non-motile recipients (Old & Duguid, 1971). The 258 strains had been isolated between 1947 and 1973 from man, nine other species of mammals, 13 species of birds and some inanimate sources in 19 countries on five continents. They included representatives of 4 primary and 22 full biotypes and 27 phage types (see Anderson *et al.*, 1978). Three non-motile strains, S580, S635 and S1723, were tested as donors; their motile variants, S580M, S6356 and S1723M (Old & Duguid, 1971), were tested as recipients.

(iii) Non-FIRN Fim⁻ biogroup. We tested 39 of the 44 wild-type non-FIRN Fim⁻ strains of Duguid *et al.* (1975), excluding one strain that was phage-untypable, two that gave rise to spontaneous Fim⁺ mutants and two now reclassified as FIRN-like (Table 2).

(iv) FIRN-like biogroup. The two wild-type Fim⁻ Inl⁺ Rha⁻ Bxy⁺ strains (Table 1) differed from FIRN only by being Inl⁺.

Induced Fim⁻ mutants. The Fim⁻ character was not wild-type in two Fim⁻ Rha⁻ Fla⁻ cultures, LT2-D461 and LT2-D464, derived by Dr Jean Dubnau from an *ade pro ilv* streptomycin-resistant line of strain LT2 after treatment with ethyl methanesulphonate (Table 1). Because they were non-motile (Fla⁻), they were used only as donors. Both behaved similarly and their results are given as for a single strain.

Culture media. Nutrient broth (pH 7.3) was 1 % (w/v) Oxoid bacteriological peptone, 1 % (w/v) Oxoid

Lab-Lemco beef extract and 0.5 % (w/v) NaCl, or Oxoid Nutrient Broth no. 2, used in 8 to 10 ml volumes in test tubes (150 × 15 mm) stoppered with cotton-wool. Nutrient agar plates and slants contained Oxoid Nutrient Agar (pH 7.0).

Biotyping and the determination of transduced characters. These were done by the methods of Duguid *et al.* (1975).

Examination for fimbriae. Strains were examined for their ability to form type-1 fimbriae in serial broth cultures incubated for 48 to 96 h at 37 °C under aerobic and static conditions (Duguid *et al.*, 1966). One culture of each series was examined with the electron microscope and all cultures were tested for haem-agglutinating activity in a rocked tile test in which the centrifuged deposit of bacteria was mixed for 20 min with 2 % (v/v) guinea-pig or horse red blood cells in saline. Agglutination of the red blood cells, and its inhibition in a parallel test containing 0.5 % (w/v) D-mannose, indicated the presence of type-1 fimbriae. The Fim⁻ status of strains was proved in two series of six successive cultures. Scanty non-haemagglutinating (type-2) fimbriae in eight non-FIRN Fim⁻ strains (Duguid *et al.*, 1975) did not affect the results.

Donor phages. Phage P22 (Zinder & Lederberg, 1952) was propagated on the donor strains of *S. typhimurium*. The strains most commonly used as donors are detailed in Table 1. The lysates (usually 5×10^8 to 50×10^8 phages ml⁻¹) were titrated and preserved by the methods of Old & Duguid (1971) and their freedom from viable donor bacteria was confirmed before each experiment.

Of the 258 FIRN strains, 93 belonged to phage type 14. They carried a phage related to P22 and generally did not support the free propagation of P22. Lysates of moderate titre (10^8 or 10^9 phages ml⁻¹) were obtained from seven of these strains, perhaps because of variation after phage typing, and were used occasionally as donor phages. Some of the other 32 FIRN strains tested as donors are listed in Table 1.

Transduction of Fim. The methods of Old & Duguid (1971) were used. Mixtures containing 1 to 5 phages per recipient bacterium (about 10^8 bacteria ml⁻¹) were cultured and subcultured once for 48 to 96 h at 37 °C in aerobic and static tubes of broth. Any culture that was haemagglutinating was plated on nutrient agar and the resulting single colonies were subcultured for 48 h in separate tubes of broth to isolate pure haemagglutinating cultures. One haemagglutinating clone from each transduction was tested for agglutination by *S. typhimurium* (O4) antiserum and for fermentation of inositol and rhamnose. Some clones were fully biotyped and examined with the electron microscope.

In each experiment, the recipient bacteria were tested in 'no-phage' control cultures to confirm that they did not give spontaneous Fim⁺ mutants. 'Homologous-phage' tests confirmed that self-crosses of the recipient strain did not generate Fim⁺ progeny. Control mixtures of recipient bacteria and donor phage from the Fim⁺ strain LT2-497 were included in all experiments in which Fim⁻ recipients were crossed with Fim⁻ donors. All the Fim⁻ strains that were motile and phage-typable were tested as recipients.

RESULTS

The aggregated results of the transduction and 'no-phage' control tests with the different biogroups of donor and recipient strains of *S. typhimurium* are given in Table 3. The proportion of tests, donor strains, recipient strains and combinations of strains crossed that gave Fim⁺ recombinants is shown for each class of biogroup cross. That the Fim⁺ bacteria were recombinants is clear from the finding that there were no Fim⁺ mutant bacteria in any of the no-phage and homologous-phage control cultures of the recipient Fim⁻ strains. Seven of the 39 non-FIRN Fim⁻ strains had previously given spontaneous Fim⁺ mutants during serial culture in broth (Duguid *et al.*, 1975), but at a frequency much too low to account for the positive results in the present transduction experiments.

Transduction from Fim⁺ donors to Fim⁻ recipients

The results show that phage propagated on Fim⁺ donor strains produced Fim⁺ recombinants from most wild-type Fim⁻ recipient strains. Fim⁺ recombinants were obtained from 32 of 37 motile non-FIRN Fim⁻, 257 of 258 FIRN and both FIRN-like recipient strains (Table 3). Strain LT2-497 gave Fim⁺ recombinants with 289 of the 291 Fim⁻ recipients that were successfully transduced by any Fim⁺ donor and was a very competent donor of *fim*. It gave positive results in 125/552 tests with non-FIRN Fim⁻, 659/2160 with FIRN and 23/38 with FIRN-like recipients. All but one of the other 21 Fim⁺ strains tested as donors were also competent.

Table 3. *Transduction of fimbriation between donor and recipient strains of different biogroups of Salmonella typhimurium*

In tests in which the donor and recipient strains belonged to the same biogroup, different strains were used as donor and recipient. The number of 'pairs' of strains crossed was the number of combinations of different strains, regardless of which was donor and which was recipient, for example, transductions from strain A to strain B and those from strain B to strain A were counted as for a single 'pair' crossed.

Donor strain biogroup	Results given for:	No. of tests, donors, recipients or pairs giving Fim ⁺ bacteria/no. tested, in crosses with Fim ⁻ recipient strains of biogroup:		
		Non-FIRN Fim ⁻	FIRN	FIRN-like
Fim ⁺	Tests	161/797	803/2913	28/54
	Donors	6/11	21/22	2/2
	Recipients	32/37	257/258	2/2
	Pairs	46/79	326/451	4/4
Induced Fim ⁻ mutant	Tests	40/268	217/411	20/42
	Donors	1/1	1/1	1/1
	Recipients	19/37	134/136	2/2
	Pairs	19/37	134/136	2/2
Non-FIRN Fim ⁻	Tests	98/1427	72/2218	8/24
	Donors	16/20	18/33	1/2
	Recipients	15/37	25/75	2/2
	Pairs	41/292	50/755	2/4
FIRN	Tests	66/763	0/1660	0/353
	Donors	16/36	0/39	0/21
	Recipients	11/37	0/258	0/2
	Pairs	26/202	0/1016	0/39
FIRN-like	Tests	18/70	0/232	0/113
	Donors	2/2	0/2	0/2
	Recipients	3/17	0/6	0/2
	Pairs	5/34	0/12	0/1
None (no-phage control)	Tests	0/808	0/1951	0/86
	Recipients	0/37	0/258	0/2

The Fim⁻ recipients differed markedly in their susceptibility to transduction of *fim*. Most gave Fim⁺ recombinants in about half of their tests with good donors but some were less susceptible, for example, FIRN strain S2804 gave Fim⁺ recombinants with LT2-497 in only 1 of 101 tests and the non-FIRN Fim⁻ strain S2334 did so in only 1 of 37 tests. One FIRN strain, S2792, and five non-FIRN Fim⁻ strains did not give a Fim⁺ recombinant in any of many tests with Fim⁺ donors.

It was not possible to determine the proportion of Fim⁺ transductants originally produced in the mixture of donor phage and recipient bacteria, because the Fim⁺ bacteria were detected by haemagglutination only after selective outgrowth in broth for 48 h, by which time they had formed between 5 and 40 % of the bacterial population. Probably, however, the frequency of transduction was low, for negative results were obtained in some tests made with most competent pairs of donor and recipient strains.

Transduction from Fim⁻ donors to Fim⁻ recipients

The wild-type Fim⁻ strains in which the *fim* gene was in a mutated form were less effective donors of *fim* than the Fim⁺ strains. Table 3 shows that Fim⁺ recombinants were obtained in only 66 of 763 tests of FIRN donors with non-FIRN Fim⁻ recipients and 72 of 2218

tests of non-FIRN Fim^- donors with FIRN recipients, as compared with 161 of 797 and 803 of 2913 tests of Fim^+ donors with these two groups of recipients. The induced (Dubnau) Fim^- mutants, by contrast, were nearly as effective as the best Fim^+ donors with recipients of all wild-type Fim^- biogroups (Table 3).

Non-FIRN $\text{Fim}^- \times$ non-FIRN Fim^- crosses. Twenty of the 39 wild-type non-FIRN Fim^- strains, including the two that were non-motile, were tested as donors in crosses with one or more of the 37 motile non-FIRN Fim^- strains as recipients. Fim^+ recombinants were obtained from 16 of the 20 donors, 15 of the 37 recipients and 41 of the 292 different pairs of strains crossed, in a total of 98 out of 1427 tests.

Non-FIRN $\text{Fim}^- \times$ FIRN crosses. Fim^+ recombinants were obtained in 138 of 2981 tests of crosses between strains of these two Fim^- biogroups. They were given by 23 of the 39 non-FIRN Fim^- strains, including 18 of 33 tested as donors and 11 of 37 tested as recipients. Thirteen of the 16 non-FIRN Fim^- strains that failed to give Fim^+ recombinants in crosses with FIRN strains had given Fim^+ recombinants in crosses with Fim^+ donors and thus they were not generally insusceptible to transduction of *fim*.

FIRN $\times$ FIRN crosses. Fim^+ recombinants were not obtained in any of 1660 tests with 39 FIRN strains as donors and all 258 motile FIRN strains and variants as recipients, in which 1016 different combinations of strains were tested as donor-recipient pairs (Table 3). Specially numerous tests were made with those FIRN strains that had given Fim^+ recombinants as donors or recipients with non-FIRN Fim^- strains so that the absence of Fim^+ recombinants in the FIRN $\times$ FIRN crosses was significant.

FIRN-like strains. The two FIRN-like strains gave Fim^+ recombinants when crossed with Fim^+ or non-FIRN Fim^- strains, but not when crossed with one another or with any FIRN strain (Table 3), and so behaved like FIRN strains.

Characters of the Fim^+ transductants

The isolated clones of Fim^+ transductants always retained the other biotype characters of the recipient strain. Thus, the Fim^+ transductants from FIRN recipients crossed with a Fim^+ Inl^+ Rha^+ Bxyl^+ donor had the characters Fim^+ Inl^- Rha^- Bxyl^- , a combination not found in naturally occurring strains (Duguid *et al.*, 1975).

The Fim^+ transductant cultures studied with the electron microscope were seen to possess fimbriae; as their haemagglutinating activity was inhibited by mannose, these fimbriae were type-1. The transduced Fim^+ character was stable and Fim^- revertants were not encountered in numerous subcultures, although, like wild-type Fim^+ strains, the transduced Fim^+ strains temporarily entered a largely non-fimbriate phase when grown on agar instead of in broth (Duguid *et al.*, 1966).

DISCUSSION

Our results show that the ability to produce type-1 fimbriae (Fim^+) can be transduced from most Fim^+ strains of *S. typhimurium* as donors to most wild-type FIRN and non-FIRN Fim^- strains of *S. typhimurium* as recipients.

Non-FIRN Fim^- strains

The finding that Fim^+ recombinants were produced in many crosses between different wild-type strains in the non-FIRN Fim^- biogroup was evidence that the intragenic sites of the *fim* mutations were different in the donor and recipient strains of each fertile cross. We could not determine how many independent *fim* mutations there were among the 39 non-FIRN Fim^- strains, because we were unable to make a large number of tests on every possible combination of these strains. The positive results showed that there were at least four different *fim* mutations in different strains of the biogroup. These were represented in

strains S1566, S2784, S3403 and S6631, each of which gave *Fim*⁺ recombinants in crosses with each of the other three. Probably, however, there were other *fim* mutations in the biogroup, for most of the 39 strains differed from the others in phage type, biotype and source (Table 2) and probably arose independently by *fim* mutations in different ancestral *Fim*⁺ bacteria.

The only non-FIRN *Fim*⁻ strains that appeared to have a common origin with some other members of the biogroup were five of phage type 9 biotype 17b and one of phage type 61 biotype 17b isolated in New Zealand in 1969, two of phage type 29 biotype 1bf isolated in Scotland in 1971, and two of phage type 66 biotype 25b isolated in Germany in 1969. *Fim*⁺ recombinants were not obtained in crosses between pairs of strains within any of these three groups. The rarity of strains in the non-FIRN *Fim*⁻ biogroup suggests that none established itself as an epidemic strain.

The sites of the *fim* mutations in the FIRN strains and the induced (Dubnau) *Fim*⁻ mutants, which differed from one another, also differed from those in the transducible non-FIRN strains, for *Fim*⁺ recombinants were commonly obtained in crosses between members of these three different groups.

FIRN strains

Although FIRN strains gave *Fim*⁺ recombinants in crosses with many strains of the non-FIRN *Fim*⁻ biogroup and *Fim*⁻ Dubnau strains, showing that the sites of their *fim* mutations differed from those of the latter groups, they did not give *Fim*⁺ recombinants in crosses among themselves. This observation suggests that their *fim* mutations are located at identical sites. The possibility that they are located at different sites that overlap, or are too close for recombination at a detectable frequency, is unlikely in view of the finding that independently originating *fim* mutations in the small non-FIRN *Fim*⁻ group are not overlapping or closely situated. The most likely explanation of this apparent genetic homogeneity of FIRN strains is that all have descended from the same ancestral *Fim*⁻ mutant bacterium.

Further evidence that the FIRN strains have a common evolutionary origin is provided by the observations suggesting that they are also homogeneous in the sites of their *rha* and *inl* mutations. Morgenroth & Duguid (1968) examined 23 FIRN strains for transduction of *rha* and found that *Rha*⁺ recombinants were not produced in any cross between different FIRN strains, although they were produced in most crosses between FIRN strains and non-FIRN *Rha*⁻ strains of primary biotype 7. Similar tests demonstrated the absence of *Rha*⁺ recombinants in crosses between different pairs of a further 110 FIRN strains (Old, 1963). Preliminary experiments on the transduction of *inl* have yielded *Inl*⁺ recombinants from most crosses of FIRN strains as recipients with non-FIRN *Inl*⁻ strains of biotype 9 as donors but not in any of many crosses of different pairs of FIRN strains (D. C. Old, unpublished results).

Since its origin, the FIRN clone must have spread throughout the world and diversified by genetic variation into the 22 full biotypes and 27 phage types now recognized. Most biotype characters occasionally undergo spontaneous change in the laboratory and during epidemic spread of the bacteria in their hosts (Duguid *et al.*, 1975; Anderson *et al.*, 1978) so that the diversification of FIRN biotypes could have been fairly rapid.

The archetypal *S. typhimurium* was probably a *Fim*⁺ *Inl*⁺ *Rha*⁺ *Bxyl*⁺ strain of biotype 1a, the commonest biotype and one in which the genes for all 15 biotyping characters are in their unmutated forms. This archetypal strain probably gave rise by a *xyl* mutation to a strain of biotype 17a, which gave rise by an *inl* mutation to a strain of biotype 25a, which in turn gave rise by successive *rha* and *fim* mutations to the ancestral FIRN strain of biotype 29b. It is from this proposed archetypal FIRN strain that all the other FIRN strains probably descended. The suggested origin of these common biotypes is shown in Fig. 1, modified from Duguid *et al.* (1975).

Fig. 1. Possible mutational routes whereby the known primary biotypes of *Salmonella typhimurium* evolved from the presumed archetypal biotype 1. Shown in parentheses are the D-tartrate-negative (3, 19, 23, 27, 31), meso-tartrate-negative (2, 18, 26, 30) and D-tartrate-negative, meso-tartrate-negative (4, 12, 32) biotypes derived from the major biotypes, which are D-tartrate-positive, meso-tartrate-positive.

* Probably there were two successive mutations in *rha*: Rha⁺ to Rha^w and Rha^w to Rha⁻ (Morgenroth & Duguid, 1968).

FIRN strains show occasional spontaneous back mutations to Inl⁺, Rha⁺ or Bxyl⁺ forms and, after mutagenesis, to a Fim⁺ form (Duguid *et al.*, 1975). Moreover, from a D-tartrate-negative, meso-tartrate-negative strain of biotype 32b, we have derived a culture of biotype 1b by the isolation of spontaneous mutants that were, successively, D-tartrate-positive, meso-tartrate-positive, and capable of utilizing as sole carbon sources, rhamnose, inositol and xylose. From this biotype 1b culture, we derived after mutagenesis a fimbriate line of biotype 1a. Thus, by artificial reversal of the presumed evolutionary pathway, we have demonstrated that FIRN strains possess in a mutated form, the genes that confer Fim⁺, Inl⁺, Rha⁺, Bxyl⁺ and other characters of the archetypal biotype 1a.

The evolutionary sequence described would explain the results of the transductions of *fim* and *rha*, but further experiments are needed to show whether the *inl* mutation is the same in FIRN and biotype 25a strains, and whether the *xyl* mutation is the same in FIRN, biotype 25a and biotype 17a strains. The observation (Old, 1972) that most strains in primary biotypes 25, 26, 29, 30, 31 and 32 have a temperature-sensitive inositol phenotype enabling them to ferment inositol only at temperatures below 37 °C is at least suggestive that the *inl* mutation is the same in these different biotypes.

FIRN-like strains

The absence of Fim⁺ recombinants and Rha⁺ recombinants in any cross between FIRN and FIRN-like strains suggests that the respective sites of the *fim* and *rha* mutations are the same in the two biogroups and supports the view of Duguid *et al.* (1975) that the rare

FIRN-like strains are Inl⁺ back-mutants from FIRN strains. Further evidence of this proposed relationship is seen from the phage types of the FIRN-like strains, since all our other strains of the phage types 13 (43 strains) and 160 (17 strains) have been FIRN.

The two FIRN-like strains were isolated in Britain in 1958 and 1963. They were the only strains of primary biotypes 21 and 23 in the series of 2030 strains, about half of which were isolated in Britain after 1958. Apparently, therefore, they did not establish themselves as epidemic strains. Whether their disappearance was accidental or due to the Fim⁻ Inl⁺ Rha⁻ Bxyl⁻ combination of characters being in some way detrimental to their parasitic competence is unknown.

The transduction method of determining genetic relationships in wild-type strains has thus been helpful in tracing parts of the genealogy of the biotypes of *S. typhimurium*. It was first used for this purpose in studies of nicotinamide-requiring strains of *S. typhimurium* (phage type 135, biotype 25x) by B. A. D. Stocker and J. B. Edgar in 1959 (see Morgenroth & Duguid, 1968; Anderson *et al.*, 1978). It should also be applicable in elucidating the recent evolutionary history of other groups of bacteria.

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