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Bacteriophage restriction in *Salmonella typhimurium* by R factors and transfer factors

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SUMMARY

A total of 2716 R factors and transfer factors isolated from *Escherichia coli* and salmonellas of human and animal origin were studied for their phage-restrictive effects in *Salmonella typhimurium* phage type 36. All of 1402 wild *fi*$^+$ factors were non-restricting. The F factor of *E. coli* K12 was unique among the F-like factors tested in that it inhibited lysis of type 36 by one typing phage. In contrast, eleven distinct changes in the phage type of 36 were produced by *fi*$^-$ I-like factors. I-like plasmids can thus be subdivided by this method.

I-like R factors and transfer factors from human and animal enterobacteria were categorized by their phage-restrictive effects in type 36. Factors resembling Δ in this respect predominated among *fi*$^-$ I-like factor from human *E. coli* and *S. typhimurium* and from porcine *E. coli*. Δ-like and ColI-like *fi*$^-$ factors were equally distributed in bovine *S. typhimurium*. ColI-like factors were commonest in bovine and avian *E. coli*.

INTRODUCTION

Restriction of bacteriophage lysis by R factors was first demonstrated by Watanabe *et al.* (1964), when *fi*$^-$ R factors introduced into *Escherichia coli* K12 were shown to inhibit the multiplication of externally infecting λ and T1 phages. Anderson & Lewis (1965*b*) and Anderson (1966) found that some *fi*$^-$ R factors restricted lysis by the *Salmonella typhimurium*, *S. paratyphi B* and *S. typhi* typing phages, reducing the sensitivity of the host strains in characteristic fashion, and thereby altering the 'phage type' of the salmonellas concerned. Carriage of the Δ transfer factor and its derivative R factor T-Δ, for example, reduced the sensitivity of *S. typhimurium* phage type 36 (= type 36), which is sensitive to all of the 30 *S. typhimurium* typing phages, to type 6, which is sensitive to only six of the phages.

These results led to the suggestion that the subdivision of R factors and transfer factors into *fi*$^-$ and *fi*$^+$ classes could be supplemented by determination of their bacteriophage restriction in *E. coli* K12 and in standard salmonella hosts (Anderson, 1966). This paper describes the effects of R factors and transfer factors on the sensitivity of type 36 to the *S. typhimurium* typing phages. The material examined

Table 1. *Laboratory strains used as recipients*

ERL No.	Description	Drug resistance*	Designation
19R689	*S. typhimurium* phage type 36	Sensitive	Type 36
14R525	*E. coli* K12F$^-$*lac*$^+$Nx^r†	Nx	K12
13R140	*E. coli* K12F$^-$*lac*$^+$	SSu‡	K12 SSu
16R99	*E. coli* K12F$^-$lac$^+$	K‡	K12K
22R721	*S. typhimurium* phage type 36	SSu	Type 36 SSu
4R914	*S. typhimurium* phage type 36	K	Type 36 K

* K, Neomycin-kanamycin; S, streptomycin; Su, sulphonamides.
† Nalidixic acid-resistant mutant.
‡ Resistance determinants only.

was isolated from human and animal enterobacteria during the 3 year period 1969–72. Detailed descriptions of this survey, and of various aspects of it, are in preparation.

MATERIALS AND METHODS

Transfer of R factors and transfer factors to type 36

The conjugation techniques used were those of Anderson & Lewis (1965*a*, *b*).

Transfer from E. coli

Wild, drug-resistant strains of *E. coli* were crossed with strain 19R689, a nalidixic acid-resistant mutant of *S. typhimurium* type 36. After about 16 hr., mating mixtures were plated out on MacConkey agar plates containing the appropriate drug plus 40 μg/ml. of nalidixic acid to eliminate the drug-resistant donor strains. Transfer factors in drug-sensitive *E. coli* were detected by their ability to mobilize 'standard' streptomycin-sulphonamide (SSu) and neomycin-kanamycin (K) resistance determinants by the triparental cross for determinant mobilization (Anderson, 1965), using *S. typhimurium* type 36 (19R689) as the final recipient. The SSu determinant is most easily mobilized by I-like transfer factors, whereas the K determinant is best mobilized by F-like transfer factors (Anderson, 1968).

Transfer from salmonellas

R factors from drug-resistant salmonellas were first transferred to strain 14R525, a nalidixic acid-resistant mutant of *E. coli* K12F$^-$lac$^+$ (= K12). They were then transferred from 14R525 to type 36, counter-selecting against the K12 donor with colicin E2 (Anderson & Lewis, 1965*a*, *b*). Transfer factors in drug-sensitive salmonellas were again detected by the triparental cross for mobilization of the SSu and K determinants, using K12 as the final recipient.

The characters of the laboratory strains of *S. typhimurium* and K12 used in these investigations are summarized in Table 1.

Phage-typing

Recipient strains of type 36 were phage-typed by the methods of Callow (1959) and Anderson (1964, and in preparation).

Table 2. *Phage restriction in* Salmonella typhimurium *phage type* 36 *by R factors and transfer factors*

A. Type strains

Bacterial strains: Type strains	Source	ERL no.	Drug resistance	Transfer factor type‡	Reactions with routine test dilutions of the typing phages*: 1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Phage type† (Callow, 1959; Anderson, 1964, and in preparation)
Type 36	Bovine	8M677	Sensitive	-	SCL	CL	CL	CL	CL	CL	CL	CL	CL	CL	SCL	CL	CL	CL	CL	SCL	CL	CL	CL	CL	CL	CL	CL	CL	SCL	CL	OL	CL	SCL	OL	36
Type 125	Unknown	4M2563	Sensitive	*fi*⁻ I-like (ColI)	+ + +	SCL	+ + +	SCL	CL	+ + +	SCL	SCL	SCL	OL	–	–	CL	CL	OL	+ + +	CL	OL	SCL	SCL	SCL	CL	SCL	SCL	SCL	CL	CL	CL	SCL	OL	125
Type 6	Human	M736	Sensitive	*fi*⁻ I-like (Δ)	–	–	–	–	+	CL	–	–	–	–	–	–	–	–	CL	+	–	–	–	–	–	–	–	–	–	SCL	–	OL	–	SCL	6
Type 29	Human	M3878	Sensitive	*fi*⁻ I-like (Δ)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+ + +	–	CL	–	–	29
Type 29	Bovine	RT1	ASSuTFu	*fi*⁻ I-like (Δ)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+ + +	–	CL	–	–	29

B. Type 36 carrying R factors and transfer factors from wild strains

Origin of R factors and transfer factors	Source	ERL no.	Drug resistance§	Resistance transferred to type 36	Transfer factor type	Reactions with routine test dilutions of the typing phages: 1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
E. coli	Human	27R301	CSSu (ColI)	CSSu (ColI)	*fi*⁻ I-like	+ + +	CL	SCL	CL	CL	OL	OL	CL	SCL	OL	–	–	CL	OL	OL	+ + +	CL	CL	CL	CL	OL	CL	CL	CL	SCL	CL	OL	CL	CL	OL	125
S. typhimurium type 6	Human	12R1373	T	T	*fi*⁻ I-like	–	–	–	–	–	SCL	–	–	–	–	–	–	–	–	SCL	–	–	–	–	–	–	–	–	–	–	+	–	+ + +	–	+	6
E. coli	Human	27R379	AKS	AKS	*fi*⁻ I-like	–	–	–	–	–	SCL	–	–	–	–	–	–	–	–	SCL	–	–	–	–	–	–	–	–	–	–	–	+	–	–	145
E. coli	Human	27R867	ASSuT	AS	*fi*⁻ I-like	–	+	–	–	SCL	+	+	–	–	+	–	–	CL	CL	+ + +	+	–	+ + +	+	+	+	+	+	+	CL	SCL	CL	+	OL	NC 1
E. coli	Human	27R696	ASSuT	ASSu	*fi*⁻ I-like	–	–	–	–	–	–	–	CL	–	+ + +	SCL	CL	–	+ + +	OL	+	CL	+ + +	–	–	+ +	+ + +	–	SCL	–	+	OL	SCL	+ +	+	NC 2
E. coli	Human	27R1	ASSu	A	*fi*⁻ I-like	–	–	–	–	–	–	–	+ + +	–	OL	+	+ +	OL	–	+ +	–	+ + +	–	–	–	–	–	–	–	–	+ + +	SCL	+ + +	OL	–	OL	85
E. coli	Human	27R315	ACKSSuT	ACKSSuT	*fi*⁻ I-like	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	–	–	–	–	–	–	–	–	–	21
S. paratyphi B (Beccles)	Human	27R39	AT	AT	*fi*⁻ I-like	–	–	–	–	+	SCL	–	–	–	–	CL	CL	+ + +	+	SCL	–	–	+	–	CL	–	–	+	–	–	–	+	+ + +	–	+	NC 3
E. coli	Human	29R59	ACT	ACT	*fi*⁻ I-like	–	–	+	+	+ +	OL	–	–	–	+	SCL	CL	OL	+ + +	OL	+	–	+	–	CL	–	–	+	–	–	+ +	+	+ + +	–	SCL	NC 4
S. paratyphi B (Taunton var. 1)	Human	23R8	T	T	*fi*⁻ I-like	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	U
S. typhimurium type 145	Human	24R816	Sensitive, TF+‖	TF only	*fi*⁻ I-like	+	+ + +	+ +	–	–	–	+ + +	–	SCL	SCL	–	–	CL	–	CL	+	–	SCL	SCL	SCL	+ +	SCL	SCL	SCL	+	+	+	CL	+ +	+ + +	NC 5
S. typhimurium type 29	¶		A	A-Δ	*fi*⁻ I-like	+	+	+	+ + +	+ + +	SCL	+ + +	SCL	+	+ + +	–	–	CL	SCL	CL	+ +	SCL	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+	CL	CL	CL	+	OL	NC 6
F factor (F-*lac*)	*E. coli* K12		Sensitive, TF+	TF only	F factor	SCL	CL	CL	CL	CL	CL	–	CL	CL	CL	SCL	CL	CL	CL	CL	SCL	CL	CL	CL	CL	CL	CL	CL	CL	SCL	CL	OL	CL	SCL	OL	NC 7

* Reactions with typing phages: CL, confluent lysis; SCL, semi-confluent lysis, OL, confluent lysis with a heavy central opacity due to secondary growth; + to + + +, increasing number of discrete plaques.
† U = does not react with typing phages; NC (1–7) = react with typing phages but do not conform to a designated type.
‡ *fi* = fertility inhibition; I-like (see Meynell, Meynell & Datta, 1968); (ColI) = colicin I-producing; (Δ^+) = carrying the Δ transfer factor (Anderson & Lewis, 1965*b*).
§ Resistance symbols: A, ampicillin; C, chloramphenicol; K, neomycin-kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines; Fu, furazolidone.
‖ TF+ = carrying transfer factor only.
¶ Artificially made in the laboratory (see Anderson, 1969).

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Determination of the fi *character and* I *specificity of* R *factors and transfer factors*

The *fi* character of R factors and transfer factors was examined by the method of Pitton & Anderson (1970). The I-like character was determined by testing the ability of the host strains to support growth of phage If1 (Meynell & Lawn, 1968).

RESULTS

Phage restriction in S. typhimurium *phage type* 36 *by* R *factors and transfer factors*

Thirteen different patterns of typing phage restriction were produced by various R factors and transfer factors in type 36 of *S. typhimurium*. These patterns, and the control reactions of types 36, 125, 6, and 29 of *S. typhimurium* with the thirty *S. typhimurium* typing phages, are summarized in Table 2.

Ten R factors and one transfer factor producing the phage restriction patterns in *S. typhimurium*, shown in Table 2, were isolated from wild strains of *S. typhimurium*, *S. paratyphi B* and *E. coli* of human origin. The F factor of *E. coli* K12F$^+$, and the A-Δ R factor artificially produced in the laboratory (Anderson, 1969), also caused phage restriction. With the exception of the F factor, all phage-restricting factors were *fi*$^-$ and I-like.

Five patterns of typing phage restriction, produced by R factors from the *E. coli* strain 27R301, the *S. typhimurium* type 6 strain 12R1373, and the *E. coli* strains 27R379, 27R1 and 27R315, corresponded to known phage types of *S. typhimurium*. These were types 125, 6, 145, 85 and 21 respectively. The phage restriction in *S. typhimurium* resulting from introduction of R factors from *E. coli* 27R867, 27R696, 29R59, and from *S. paratyphi B* strain 27R39, did not correspond to known phage types of *S. typhimurium*. Nor did those produced by the A-Δ factor, by the factor from *S. typhimurium* strain 24R816, and by the F factor of K12. These seven restriction patterns were designated 'non-conforming' (NC) 1–7. Finally, one R factor, isolated from *S. paratyphi B* strain 23R8, restricted all the *S. typhimurium* typing phages. Type 36 carrying this R factor was therefore designated untypable (U).

The drug-sensitive type strains of *S. typhimurium* types 125, 6, 29 and 145, proved to be carrying *fi*$^-$ I-like transfer factors. The factor from 4M2563, the type strain of 125, also carried the genetic determinant for the production of colicin I (ColI$^+$). M736, the type strain of type 6, isolated in 1945, carries a transfer factor yielding restriction identical with that of Δ, which converts type 36 into type 6 (Anderson & Lewis, 1965*b*). The R factor from the wild *S. typhimurium* type 6 strain 12R1373, isolated in 1971, was also Δ-like. The R factor from the wild strain of *E. coli* 27R301 restricted phages 12 and 13 of the *S. typhimurium* typing scheme, thereby producing type 125; it was also ColI$^+$. The *fi*$^-$ I-like factor from 5M4750, the type strain of type 145, isolated in 1965, converted type 36 to type 145, as did the R factor from the wild *E. coli* strain 27R379. Although the differences between types 6 and 145 are small, they are nevertheless constant.

Table 3. *Source of* fi⁻ *I-like R factors and transfer factors from human strains of enterobacteria*, 1969–1972

Phage type produced in type 36	Source of factor				Total	%
	E. coli	*S. typhimurium*	*S. enteritidis*	*S. paratyphi B*		
125 ColI⁺	8 (2)	2 (2)	9 (3)	0	19 (7)	3·8
125 Col⁻	15 (5)	2 (2)	0	0	17 (7)	3·4
Total	23 (7)	4 (4)	9 (3)	0	36 (14)	7·1
6	104 (11)	141 (5)	0	2 (1)	247 (17)	48·8
145	13 (2)	2 (1)	0	0	15 (3)	3·0
NC 1	12 (1)	0	0	0	12 (1)	2·4
NC 2	12 (1)	1 (1)	0	0	13 (2)	2·6
85	1 (1)	0	0	0	1 (1)	0·2
21	0	1 (1)	0	0	1 (1)	0·2
NC 3	0	0	0	4 (2)	4 (2)	0·8
NC 4	8 (1)	0	0	0	8 (1)	1·6
U	11 (3)	1 (1)	0	1 (1)	13 (5)	2·6
NC 5	0	1 (1)	0	0	1 (1)	0·2
fi⁻nr I-like	86 (10)	37 (3)	10 (5)	22 (3)	155 (21)	30·6
Total	270 (37)	188 (17)	19 (8)	29 (7)	506 (69)	
% of overall total	53·4	37·1	3·8	5·7		

SYMBOLS. See Table 2.
ColI⁺ = transfer factors with I, Ia or Ib colicinogeny determinants.
Col⁻ = non-colicinogenic.
fi⁻nr = fertility inhibition minus, non-restricting for *S. typhimurium* typing phages.
All of 69 factors tested, shown in parentheses, were I-like.

Sources of fi⁻ *I-like R factors and transfer factors from human enterobacterial strains*

A total of 1060 R factors and transfer factors from human strains of *E. coli*, *S. typhimurium*, *S. paratyphi B* and *S. enteritidis* were examined for restriction of the *S. typhimurium* typing phages. These transfer systems will be described in detail in later articles. Of 554 factors that were *fi*⁺ and non-restricting, 503 (90·8 %) were R⁺.* The remaining 506 factors were *fi*⁻ and included 463 (91·5 %) R⁺ lines. All of 69 *fi*⁻ factors (62 = 90 % R⁺) investigated promoted multiplication of the If1 phage, and were therefore I-like.

The enterobacterial sources of *fi*⁻ factors, both restricting and non-restricting for *S. typhimurium* typing phages, are shown in Table 3, and the proportion carrying resistance in Table 4.

Of 506 of these factors examined, 270 (234 = 86·7 % R⁺) were isolated from wild *E. coli* strains, 188 (181 = 96·3 % R⁺) from *S. typhimurium*, 19 R factors from *S. enteritidis* and 29 R factors from *S. paratyphi B*.

155 factors (134 = 86 % R⁺) were *fi*⁻ and non-restricting (= *fi*⁻nr) for the *S. typhimurium* typing phages; all of 21 (18 R⁺) of this group examined were

* The symbol R⁺ is used here to indicate transfer factors associated with R determinants; that is, R factors.

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Table 4. fi⁻ *I-like R factors from human strains of enterobacteria*, 1969–1972

Phage type produced in type 36	Source of factor: *E. coli*	*S. typhimurium*	*S. enteritidis*	*S. paratyphi B*	Total
125 ColI⁺	8	2	9	0	19
125 Col⁻	15 (12)	2	0	0	17 (14)
Total	23 (20)	4	9	0	36 (33)
6	104 (92)	141 (134)	0	2	247 (228)
145	13	2	0	0	15
NC 1	12	0	0	0	12
NC 2	12	1	0	0	13
85	1	0	0	0	1
21	0	1	0	0	1
NC 3	0	0	0	4	4
NC 4	8	0	0	0	8
U	11	1	0	1	13
NC 5	0	1	0	0	1
fi⁻nr I-like	86 (65)	37	10	22	155 (134)
Total	270 (234 = 86·7 %)	188 (181 = 96·3 %)	19	29	506 (463 = 91·5 %)

Symbols. See Tables 2 and 3.

When all transfer systems were R⁺, the figure alone is given. When some transfer systems were R⁻, figures in parentheses indicate the numbers that were R⁺.

Percentage resistance is shown at foot of Table.

I-like. Factors producing the Δ type of phage restriction (= Δ-like) were predominant amongst the *fi*⁻ factors tested; 247 (228 = 92·3 % R⁺) of 506 factors (48·8 %) converted type 36 to type 6; all of 17 (15 R⁺) Δ-like *fi*⁻ factors examined were I-like.

Surprisingly few R factors isolated from human strains carried the determinant for colicin I production; only 19 (3·8 %) were ColI⁺ and converted type 36 to type 125. However, a further 17 non-colicinogenic factors (14 R⁺) restricted only phages 12 and 13 of the *S. typhimurium* typing scheme. This restriction yields type 125 and is characteristic of most ColI factors. All of 7 R factors of this class tested were I-like; their transfer factors, which produced the phage restriction, were evidently similar to that of ColI factors.

Nine phage types were produced by the remaining 68 *fi*⁻ R factors from human enterobacterial strains. Only the types designated NC 6 and NC 7 (see Table 2), which had arisen from infection of type 36 with the 'synthetic' R factor A-Δ and the F factor respectively, were not produced by factors from wild human strains. After the Δ-like and ColI-like factors, the commonest phage-restricting factor found was that which converted type 36 to type 145. Fifteen R factors gave rise to this phage type, and all of 3 tested were I-like.

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Factors from human E. coli. *Total* 270. 234R$^+$ (86·7 %)

Eight different patterns of phage restriction of *S. typhimurium* type 36 were produced by the 270 *fi*$^-$ factors from human *E. coli*. 104 *fi*$^-$ factors (92 = 88·5 % R$^+$) were Δ-like, 86 (65 = 75·6 % R$^+$) were non-restricting for the typing phages, and 23 (20 R$^+$) gave rise to type 125. However, 57 R factors yielded phage types other than 6 and 125. Eleven R factors from *E. coli* completely inhibited lysis by all the *S. typhimurium* typing phages.

Factors from human S. typhimurium. *Total* 188. 181 R$^+$ (96·3 %)

The majority of *fi*$^-$ factors from human strains were Δ-like: 141 (74·5 %) of 188 strains from 5 phage types of *S. typhimurium* carried this class of factor; 134 (95 %) of the Δ-like factors were R$^+$. Thirty-seven of the 188 strains (19·7 %) carried *fi*$^-$ I-like R factors which did not restrict the typing phages, and 10 carried R factors giving rise to 6 other distinctive patterns of phage restriction: 21, 125, 145, NC 2, NC 5 and U. Only 4 R factors, 2 of which were ColI$^+$, produced type 125.

Factors from human S. enteritidis. *Total* 19. *All* R$^+$

Nineteen *fi*$^-$ R factors were isolated from drug-resistant *S. enteritidis* strains. Ten were non-restricting for typing phages, and all of 5 tested were I-like. Drug resistance transfer in the remaining 9 strains was mediated by a ColI$^+$ factor, which converted type 36 of *S. typhimurium* into type 125.

Factors from human S. paratyphi *B*. *Total* 29. *All R*$^+$

Twenty-two of 29 resistance factors from *S. paratyphi B* strains did not cause restriction in type 36; all of 7 factors of this group examined were I-like. However, 7 *fi*$^-$ R factors, of which all of 4 examined were I-like, produced phage restriction in type 36: two R factors were Δ-like, 4 gave rise to the NC 3 phage type, and 1 restricted lysis by all the *S. typhimurium* typing phages.

Sources of fi$^-$ *I-like R factors and transfer factors from animal enterobacterial strains*

A total of 1656 *fi*$^-$ and *fi*$^+$ factors from bovine, porcine and avian strains of *E. coli* and *S. typhimurium* were examined. All avian strains were isolated from broiler fowls. 848 factors (51·2 %), of which 566 were from bovine, 198 from porcine and 84 from avian enterobacteria, were *fi*$^+$; 512 *fi*$^+$ factors (90·5 %) from bovine strains and all *fi*$^+$ factors from porcine and avian enterobacteria were R$^+$. Of 126 *fi*$^+$ R factors tested, 86 bovine, 29 porcine and 11 avian in origin, all were non-restricting (= *fi*$^+$nr) for the *S. typhimurium* typing phages.

The sources of the *fi*$^-$ I-like factors from the bovine, porcine, and avian enterobacteria, and the phage restriction produced by these factors in type 36, are presented in Table 5; the proportion carrying resistance in Table 6.

Of 808 *fi*$^-$ factors (750 = 92·8 % R$^+$) tested for phage restriction, 271 (240 = 88·6 % R$^+$) were from strains of bovine *E. coli* and 61, all R$^+$, from bovine *S. typhimurium*; 211 *fi*$^-$ factors (201 = 95·3 % R$^+$) came from porcine *E. coli*; 2, both R$^+$,

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Table 5. *Source of* fi⁻ *I-like R factors and transfer factors from animal strains of enterobacteria*, 1969–1972

Phage type produced in type 36	Source of factor: Bovine *S. typhimurium*	Bovine *E. coli*	Porcine *S. typhimurium*	Porcine *E. coli*	Avian *S. typhimurium*	Avian *E. coli*	Total
125 ColI⁺	30 (3)	127 (6)	0	21 (2)	3 (3)	98 (4)	279 (18)
125 Col⁻	0	82 (2)	0	1 (1)	0	102 (16)	185 (19)
Total	30 (3)	209 (8)	0	22 (3)	3 (3)	200 (20)	464 (37)
6	30 (5)	3 (3)	2 (2)	157 (3)	0	47 (5)	239 (18)
NC 1	0	2 (1)	0	0	0	0	2 (1)
U	0	2 (1)	0	0	0	0	2 (1)
fi⁻nr I-like	1 (1)	55 (6)	0	32 (1)	0	13 (2)	101 (10)
Total	61 (9)	271 (19)	2 (2)	211 (7)	3 (3)	260 (27)	808 (67)
% of overall total	7·5	33·5	0·2	26·1	0·4	32·2	

SYMBOLS. See Tables 2 and 3.
All of 67 factors tested, shown in parentheses, were I-like.

Table 6. fi⁻ *I-like R factors from animal strains of enterobacteria*, 1969–1972

Phage type produced in type 36	Source of factor: Bovine *S. typhimurium*	Bovine *E. coli*	Porcine *S. typhimurium*	Porcine *E. coli*	Avian *S. typhimurium*	Avian *E. coli*	Total
125 ColI⁺	30	127 (112)	0	21	3	98 (90)	279 (256)
125 Col⁻	0	82 (72)	0	1	0	102 (93)	185 (166)
Total	30	209 (184)	0	22	3	200 (183)	464 (422)
6	30	3	2	157 (150)	0	47	239 (232)
NC 1	0	2	0	0	0	0	2
U	0	2	0	0	0	0	2
fi⁻nr I-like	1	55 (49)	0	32 (29)	0	13	101 (92)
Total	61	271 (240 = 88·6 %)	2	211 (201 = 95·3 %)	3	260 (243 = 93·5 %)	808 (750 = 92·8 %)

SYMBOLS: see Tables 2 and 3.
When all transfer systems were R⁺, the figure alone is given. When some transfer systems were R⁻, figures in parentheses indicate the numbers that were R⁺. Percentage resistance is shown at foot of table.

from porcine *S. typhimurium*; 260 (243 = 93·5 % R⁺) were from avian *E. coli*, and 3, all R⁺, from avian *S. typhimurium*.

A hundred and one *fi*⁻ factors (92 = 91·1 % R⁺) were non-restricting for the *S. typhimurium* typing phages. The predominant phage-restricting effect was to change type 36 into type 125; 464 factors (422 = 90·9 % R⁺), 279 of which were ColI⁺, and 256 (91·8 %) of these R⁺, gave rise to this phage type. 239 factors

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(232 = 97·1 % R^+) were Δ-like, converting type 36 into type 6. All of 18 factors (16 R^+) of this class tested were I-like. Only 4 R factors produced other restriction in type 36: 2 completely inhibited lysis (U), and 2 gave rise to the NC 1 type (see Table 2).

Factors from bovine enterobacteria. Total 332. 321 R^+ (96·7 %)

S. typhimurium. *Total* 61. *All* R^+. Sixty-one fi^- R factors from bovine *S. typhimurium* strains were examined; 30 were ColI$^+$ and converted type 36 to type 125; 30 were Δ-like and changed type 36 into type 6. Only one fi^- I-like factor was non-restricting for the *S. typhimurium* typing phages.

E. coli. *Total* 271. 240 R^+ (88·6 %). Restriction in type 36 with fi^- factors from bovine *E. coli* was rather different from that with factors from the *S. typhimurium* strains. Whereas 30 of 61 factors (49·2 %) from bovine *S. typhimurium* were Δ-like, only 3 R factors of 271 fi^- factors (1·1 %) from *E. coli* belonged to that category. The predominant type of factor from bovine *E. coli* was that which converted type 36 into type 125; 209 (184 = 88·1 % R^+) of 271 factors (77·1 %) gave rise to this phage type, and 127 (60·8 %) were ColI$^+$. Eighty-eight of the 127 ColI$^+$ factors (69·2 %) carried a K determinant. The transfer factor with which the ColI determinant is usually associated is evidently common in bovine *E. coli*. The two fi^- R factors resulting in the NC 1 type, and the two blocking lysis by all the typing phages, originated in bovine *E. coli*.

Factors from porcine enterobacteria. Total 213. 203 R^+ (95·3 %)

S. typhimurium. *Total* 2. *Both* R^+. Only three strains of porcine *S. typhimurium* were examined for R factors or transfer factors in this survey. Two strains, both belonging to type 6, carried Δ-like R factors; the third strain tested, type 12a, was drug-sensitive and did not carry a transfer factor.

E. coli. *Total* 211. 201 R^+ (95·3 %). In contrast to the factors from bovine *E. coli*, 157 (150 = 95·5 % R^+) of 211 fi^- factors (74·4 %) from porcine *E. coli* were Δ-like. Twenty-two fi^- R factors, of which 21 were ColI$^+$, yielded type 125, and 32 (29 R^+) were non-restricting for the typing phages. No other restriction patterns were encountered.

Factors from avian enterobacteria. Total 263. 246 R^+ (93·5 %)

S. typhimurium. *Total* 3. *All* R^+. Only 3 strains of avian *S. typhimurium* carrying fi^- R factors were examined in this survey. All 3 factors from these strains were ColI$^+$, and changed type 36 into type 125.

E. coli. *Total* 260. 243 R^+ (93·5 %). Two hundred (183 = 91·5 % R^+) of 260 fi^- factors (77·0 %) from avian *E. coli* yielded type 125 when introduced into type 36; of these, 98 (90 = 91·8 % R^+) were ColI$^+$, and 102 (93 = 91·2 % R^+) were non-colicinogenic. As can be expected, all of 20 factors (16 R^+) of this class examined were I-like. Δ-like R factors were present in 47 *E. coli*, and fi^- R factors non-restricting for typing phages in a further 13 strains.

Table 7. *Main categories and origins of R factors and transfer factors*

Source		Total no. of factors	Type of factor: No.			Type of factor: Percentage		
Species	Organism		Δ-like	ColI-like	*fi*⁻nr	Δ-like	ColI-like	*fi*⁻nr
Human	*E. coli*	270	104	23	86	38·5	8·5	31·9
Human	*S. typhimurium*	188	141	4	37	75·0	2·1	19·7
Bovine	*E. coli*	271	3	209	55	1·2	77·1	20·3
Bovine	*S. typhimurium*	61	30	30	1	49·2	49·2	1·6
Porcine	*E. coli*	211	157	22	32	74·4	10·4	15·2
Avian	*E. coli*	260	47	200	13	18·1	76·9	5·0

DISCUSSION

Of the 2716 transfer systems from human and animal enterobacteria studied, 1402 (51·6 %) were *fi*⁺ and caused no restriction in type 36 of *S. typhimurium*. The remaining 1314 factors were *fi*⁻, and all of 136 factors of this group tested were I-like. With the exception of the F-factor of *E. coli* K12, all phage-restricting factors were *fi*⁻ and I-like. Restriction patterns in type 36 may therefore be added to the criteria available for the characterization, and thus the classification, of transfer systems (Anderson, 1966).

Eleven distinct changes in *S. typhimurium* type 36 were produced by infection with *fi*⁻ I-like factors from wild *E. coli* and salmonellas. The F-factor was unique among F-like factors tested in that it restricted one of the *S. typhimurium* typing phages. However, all other F-like factors tested were compatible with the F-factor of K12, an indication that they are different from F.

Three *fi*⁻ factors, Δ, ColI and the factor from 5M4750, determined the phage types of the *S. typhimurium* strains in which they were first encountered. Although all are 'I-like', their phage-restricting effects on strains of *S. typhimurium* are quite different. The Δ-like factors, the prototype of which was described by Anderson & Lewis (1965*b*), inhibit lysis by 24 of the 30 *S. typhimurium* typing phages, to produce phage type 6. The ColI-like factors inhibit lysis by only two typing phages, to give rise to type 125 (Anderson, 1966), and the factor from *S. typhimurium* 5M4750 inhibits lysis by 27 typing phages, to produce type 145.

The three main groups of factors, and their origins, are shown in Table 7.

Among the *fi*⁻ I-like factors the Δ-like group predominated in human *E. coli* and *S. typhimurium*: 104 out of 270 factors (38·5 %) from human *E. coli* belonged to this category. In human *S. typhimurium* infection the preponderance of Δ-like factors was more marked: 141 of 188 *fi*⁻ factors (75 %) were of this type. All of 118 strains of phage type 6, of which 111 were R⁺, carried this class of factor; a significant number when it is recalled that the phage-restricting effect of the Δ group of plasmids on *S. typhimurium* type 36 is to change it into type 6, and that the drug-sensitive type strain of type 6, which was identified almost 30 years ago, carries a Δ-like factor. It is thus probable that the phage type-determining agent in all strains of type 6 is a transfer factor of the Δ group. This has proved to be

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true in every strain of type 6 so far examined. Transfer of R factors of this group would also be expected to occur in other types of *S. typhimurium*, and this was found, for example, in the 23 strains belonging to types other than 6 among the 141 Δ-carrying lines of *S. typhimurium* identified.

Bovine *S. typhimurium* yielded 23 type 6 strains, all of which were drug-resistant, and seven strains belonging to other phage types also carried Δ-like factors.

The most important strain of *S. typhimurium* in man and bovines which carries a Δ transfer system is that of phage type 29, which caused a prolonged epidemic of bovine and human infection between 1964 and 1970. It has been postulated elsewhere that this outbreak was caused by a single line, in fact a clone, of type 29 (Anderson, 1968, 1969, 1971). It so happened that in the original group of drug-resistant *S. typhimurium* cultures isolated from November 1964 onwards on a farm in Devon, 54 belonged to phage type 29 and 35 to phage type 44. A recent scrutiny of these cultures has shown that the type 44 cultures, all of which are multi-resistant, carry a transfer factor of the ColI-like group – that is, a factor which converts type 36 into type 125. All these cultures are ColI$^-$.

Type 44 had a long history of bovine association before the appearance of transferable resistance, and its pattern of typing phage sensitivity is unchanged by the introduction of a ColI-like transfer factor. Type 29, in contrast, had been uncommon before the widespread outbreak it caused in bovines and man. The first strain of this type identified in that outbreak has been intensively studied in the Enteric Reference Laboratory, and is designated RT1 (Anderson & Lewis, 1965*a*, *b*; Anderson, 1966, 1968, 1969). It was the source of Δ, the prototype of the Δ-like group of transfer factors. Δ is incompatible with the ColI-like factors. Introduction of a ColI factor into RT1 resulted in displacement of the Δ factor (the resistance determinants were unaffected), and the strain simultaneously changed into phage type 44 (E. S. Anderson & H. R. Smith, unpublished observations). This revealed that the extensive type 29 outbreak in bovines and man had been caused by one strain of a type that had long been associated with bovines, and not by a newly introduced type of *S. typhimurium*. We do not yet know why the Δ-carrying line should have persisted and spread while that carrying the ColI-like (but Col$^-$) transfer factor disappeared. But we now know that all strains of type 29, whether or not they are drug-resistant, carry a Δ-like factor. By good fortune, *S. typhimurium* 4M5235, the drug-sensitive 'type 29' line on which the original experiments were carried out to examine its ability to accept transferable resistance descended from RT1 (Anderson & Lewis, 1965*a*, *b*), was later found to belong, in fact, to type 16, which does not carry a transfer factor, although its sensitivity to the typing phages closely resembles that of type 29. Had the sensitive recipient really belonged to type 29, difficulties would have occurred because a Δ-like transfer factor would already have been present, so that the resistance transfer findings could have been confused.

In the study reported here, factors yielding phage type 125 in *S. typhimurium* were of two types; those carrying a colicin I determinant (= ColI$^+$), and those which were Col$^-$; each of these types showed R$^+$ and R$^-$ representatives. Colicin I determinants alone are non-transferring, and require association with a transfer

factor to become transferable. Why they should be associated particularly with this class of transfer factor is not clear, since their transfer is easily mediated by Δ. Perhaps the '125' type of transfer factor has the greatest long-term stable linkage with ColI determinants.

Relatively few factors from human salmonellas gave rise to type 125, but factors of this class were particularly common in bovine and avian *E. coli*, and to a lesser extent in porcine *E. coli*. Animal *E. coli* probably constitute a reservoir of this type of factor, which, under suitable conditions, can enhance the spread of drug resistance to pathogenic bacteria. A relatively common R factor in *S. typhimurium* and *E. coli* is KColI, in which both the neomycin–kanamycin R determinant and a ColI determinant are linked to the same transfer factor. The use of neomycin or kanamycin against infection with *S. typhimurium* carrying KColI therefore selects for colicinogeny as well as kanamycin resistance. The spread of drug resistance by a ColI factor has recently been particularly evident in enterobacteria from broiler fowls (E. S. Anderson & L. G. Savoy, in preparation).

Although many *fi*$^-$ I-like R factors and transfer factors could be distinguished by their phage restriction, a considerable proportion were non-restricting for *S. typhimurium* typing phages, and therefore could not be differentiated further on this basis. Supplementary subdivision of Δ-like factors, and of factors giving rise to type 125, would be advantageous. Differences between the phage-restricting effects of Δ and those of a modified form of Δ, designated Δm, were apparent in Vi-type A of *S. typhi* (Anderson, 1966). This host, or others, may therefore be useful for the subdivision of R factors and transfer factors that produce identical phage restriction, or lack of it, in *S. typhimurium*.

Phage restriction in *E. coli* and salmonellas may also be useful in the classification of F-like transfer systems, although the 680 tested in this survey produced no *S. typhimurium* phage restriction. However, the F factor itself has been shown here to restrict one of the *S. typhimurium* typing phages, and we have an F-like R factor that also restricts lysis by these phages in a manner different from that of F.

From the taxonomic point of view, the designation of transfer systems as I-like (Meynell, Meynell & Datta, 1968) is unfortunate. The letter I was introduced because the respective sex fimbriae ('I' fimbriae) were first observed in a strain carrying the classical ColI-P9 plasmid (Fredericq, 1956). The If1 phage, for which I fimbriae are the receptor, was isolated by Meynell & Lawn (1968), on a derivative of a strain (*S. typhimurium* ERL ref. 4M91) carrying the ColI$^+$-kanamycin R factor R144, and on a derivative of a line (*S. typhimurium* ERL ref. 3M2318) carrying the Col$^-$R$^+$ factor R64. R64 is a Δ-like factor. The transfer of ColI is mediated by a transfer factor (TF) with which the genetic determinant of I colicinogeny is covalently linked. We have found that the 'ColI' system can exist in the following states: ColI$^+$TF$^+$, ColI$^+$TF$^-$ and ColI$^-$TF$^+$. ColI$^+$TF$^-$ strains produce colicin I but do not support the growth of phage If1 because they lack 'I' fimbriae. ColI$^-$TF$^+$ strains, in contrast, are non-colicinogenic, but synthesize 'I' fimbriae and therefore support the growth of phage If1; they produce the 125 type of restriction in type 36 of *S. typhimurium*. Since the 'I' designation of the transfer factor they carry was derived from that of a ColI$^+$TF$^+$ R factor, it is misleading to attach the I symbol

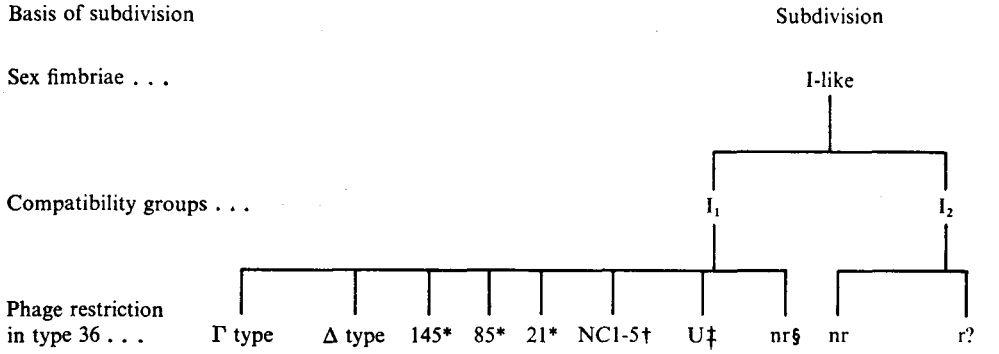

Fig. 1. Subdivision of I-like plasmids. * The phage type numbers indicate the restriction pattern and the provisional plasmid type designation. † NC1–5: specific restriction patterns, each indicating a distinct plasmid type (see Table 2 and text). ‡ Restriction of all *S. typhimurium* typing phages and provisional plasmid type designation. § Non-restricting in *S. typhimurium*. r ? No representatives yet available.

to ColI$^-$TF$^+$ lines. Moreover, additional confusion arises from the fact that the Δ group of transfer factors also produce so-called 'I' sex fimbriae, and support growth of phage If1. They are therefore categorized as I-like, although they are rarely found in association with a ColI determinant. The same applies to all the other *fi*$^-$ I-like factors identified.

The male-specific If1 phage does not distinguish between the Δ-group and the 'ColI' group of transfer systems. Subdivision can be effected by the laborious demonstration of serological differences between the respective sex fimbriae. But the factors can be easily distinguished from each other by the clear-cut differences in phage restriction produced in type 36 of *S. typhimurium* by the Δ group of transfer factors on the one hand, and the 'ColI' group of transfer factors on the other (Anderson, 1966).

Unfortunately, the I designation has been widely accepted and it may now be difficult to abandon. Moreover, we have recently subdivided factors that stimulate synthesis of I fimbriae into groups I_1 and I_2 (Grindley, Grindley & Anderson, 1972; Grindley, Humphreys & Anderson, 1973). The I_2 group is I-like and codes for I fimbriae, but is compatible with, and therefore distinct from, Group I_1 plasmids, which are incompatible with each other. Group I_2 is at present represented by only one plasmid, TP114 (Grindley *et al.* 1972), and although it is non-restricting in *S. typhimurium*, its further characterization is as yet incomplete. Group I_1, however, is common. The Δ type of plasmids are well known, and it seems logical to designate the other common ('125') restriction type Γ(gamma), to indicate its distinctness from Δ. The other phage-restricting factors can also be provisionally regarded as representing distinct types. The non-restricting I_1 plasmids are designated 'nr', and although they are probably heterogeneous, their present designation can be retained until more is known about them. We can therefore subdivide these plasmids on the basis of sex fimbrial synthesis, compatibility and phage restriction in *S. typhimurium*, as shown in Fig. 1.

Fig. 1 indicates that a considerable heterogeneity of *fi*$^-$ I-like plasmids can be demonstrated by their patterns of phage restriction in *S. typhimurium* type 36. The

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heterogeneity may be by no means fully exposed in this serotype because it may extend further than can be shown by restriction in *S. typhimurium*, or indeed by that in any host, as we have already indicated. This applies particularly to the 'U' and 'non-restricting' groups of plasmids. Moreover, it must be recalled that I-like plasmids may be fi^+ (Grindley & Anderson, 1971), and this offers further possibilities of subdivision of these factors.

The fact that most of the factors described are found in both animal and human enterobacteria suggests a common origin. And it is evident from earlier studies (Anderson & Lewis, 1965*a*, *b*; Anderson, 1968, 1969, 1971) that salmonellas carrying resistance transfer systems pass from animals to man. But the extent to which animal non-pathogens contribute to the drug-resistant enterobacterial population of the human intestine is not yet clear, although it may be substantial.

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