The Presence of the Transmissible Agent F in Non-Recombining Strains of *Escherichia coli*

BY G. FURNESS AND D. ROWLEY

*The Wright-Fleming Institute of Microbiology, St Mary's
Hospital Medical School, Paddington, London, W. 2*

SUMMARY: A proportion of wild-type strains of *Escherichia coli* unable to recombine with *E. coli* K12 F[−] mutants can 'infect' them with the sex-differentiating character known as F. Of these strains, not all infect the same *E. coli* K12 F[−] mutants and the ease of transfer of F varies among them, suggesting that there are different F agents. This is supported by the finding that there are great variations in the transfer of unselected markers in crosses in which the only known difference is the origin of the F factor possessed by the F⁺ parent.

Colicinogenicity and F are frequently associated and all of nine strains producing colicin I possess F, but they are not identical, since the two properties have been separated by treatment with cobalt chloride.

Recombination between mutants of *Escherichia coli* K12 appears to require cell to cell contact. There are differences between the parent cells in that one donates (F⁺) and one accepts (F[−]) genetic material (Hayes, 1953). The F⁺ character (or Hfr) must be present in at least one of the parent strains for a cross to be fertile (Lederberg, Cavalli & Lederberg, 1952). One of the most important characteristics of this F⁺ character is that of being infective, that is, a high proportion of an F[−] strain can be made F⁺ by growing an F⁺ and F[−] strain together for a short time, i.e. 1–2 hr. (Cavalli, Lederberg & Lederberg, 1953). Once acquired the F⁺ character is inherited. In spite of this infective nature, which suggests a similarity to phage, it has not been possible to demonstrate the F⁺ character in the absence of bacterial cells. The possibility that the F agent might be of phage or prophage nature obviously deserves consideration.

Whilst it is certain that the F character is necessary for recombination to occur in *Escherichia coli* K12, it is by no means certain that possession of a transmissible F agent is sufficient to enable a strain to recombine. Cavalli *et al.* (1953) searched for transmissible F agents in *E. coli* and *Salmonella typhimurium* strains, using its infective property for an *E. coli* K12 F[−] mutant as the criterion of its presence, and failed to find F in any strains infertile with K12 mutants.

Some *Escherichia coli* and other enteric organisms are colicinogenic, that is they produce various colicins (Gratia, 1925; Fredericq, 1948). This property, like the F⁺ character, can often be transferred to other non-colicinogenic strains by growing them with the colicinogenic strain. Using mutants of K12, Fredericq (1954*b*) has shown that F has to be present in either the colicinogenic (donor) or non-colicinogenic (acceptor) strain for the transfer to take place. Moreover, he has reported the transfer of colicinogenicity to an

F- mutant of K12 from a strain of *Shigella sonnei* (Fredericq, 1954a) which so far as we know is not interfertile with K12. This suggested to us that this *Shigella* strain might contain an F agent without itself being fertile.

More recently, further data have been produced by Jacob & Wollman (1955). In order to account for the extreme rapidity and great efficiency of transfer of the F agent they have postulated that it is attached near to that part of the chromosome (O) which first enters the F- parent during the process of chromosome transfer and that there is a point 'R' on the chromosome situated between F and the next known marker where the chromosome preferentially breaks. According to this theory, F transfer is an early and necessary step in the process of recombination.

It would seem likely that cells possessing F and able to transfer it to F- cells might reasonably be expected to undergo recombination for other markers also. For this reason, we have investigated the possibility implied by Fredericq's work that F may occur in strains that cannot be shown to recombine by the usual methods. This supposition was confirmed and extended, in that six out of thirty randomly chosen wild-type strains of *Escherichia coli* were found to carry F agents, though they were not demonstrably fertile with K12.

METHODS

Cultures

All cultures were grown at 37°.

Wild-type strains of Escherichia coli. These were randomly isolated during the routine examination of specimens obtained from patients, no two cultures being obtained from the same person. With the exception of *E. coli* I₄, which was of an 'intermediate' type, the wild strains were indole and methyl-red positive, V.P. negative, and unable to utilize citrate as the sole source of carbon. They were sensitive to 500 µg. streptomycin/ml. With one exception, those transferring F differed from each other either in their biochemical reactions or in the colicins they produced (see Table 1).

Strains of Escherichia coli K12. The following auxotrophic mutants were used: Strain 58.161—a methionine-requiring mutant (M⁻) fermenting lactose, arabinose, galactose, maltose, xylose and mannitol (lac⁺, arab⁺, gal₅⁺, mal⁺, xyl⁺, mtl⁺). Strain W677—auxotrophic for threonine, leucine and thiamine (T⁻, L⁻, B₁⁻), and not fermenting lactose, arabinose, galactose, maltose, xylose or mannitol (lac⁻, arab⁻, gal₅⁻, mal⁻, xyl⁻, mtl⁻).

Both streptomycin-sensitive and -resistant mutants of F⁺ and F⁻ strains were used. Strains 58.161 S^R(1)F⁻ and 58.161 S^R(2)F⁻ were two independent streptomycin-resistant mutants.

Colicin-sensitive indicator strains. *Escherichia coli* B and *Shigella dysenteriae* P15, kindly supplied by Professor P. Fredericq, were used as indicator strains for colicinogenicity; the K12 mutant W677 S^RF⁻ was also used.

Media. The formula of Davis & Mingioli (1950) was used for the preparation of minimal medium and that of Lederberg (1950) for eosin-methylene-blue sugar-containing media. The minimal medium was supplemented with

Table 1. *Differential characters of wild strains of Escherichia coli transferring F*

Culture of <i>E. coli</i>	Colicins produced	Fermentation of					
		Dulcitol	Galactose	Lactose	Salicin	Sucrose	Xylose
A	—	— — ₃	A ₂ — ₃	A ₁ G ₁	— — ₃	— — ₃	A ₂ — ₃
H	I	A ₂ G ₂	A ₂ G ₂	A ₁ G ₁	A ₂ G ₂	— — ₃	A ₁ G ₁
J	V, B	A ₂ G ₂	A ₁ G ₁	A ₁ G ₁	A ₂ G ₂	— — ₄	A ₁ G ₁
L	—	A ₁ G ₁	A ₁ G ₁	A ₁ G ₁	A ₁ G ₁	A ₁ G ₁	A ₁ G ₁
O	B	A ₂ G ₂	A ₁ G ₁	A ₁ G ₁	A ₂ G ₂	— — ₄	A ₁ G ₁
R	I, B	A ₂ G ₂	A ₁ G ₁	A ₁ G ₁	— — ₄	— — ₄	A ₁ G ₁
U	I*	A ₂ G ₂	A ₁ G ₁	A ₁ G ₁	— — ₃	— — ₃	A ₁ G ₁
I ₁	I	A ₂ G ₂	A ₁ G ₁	A ₁ G ₁	A ₂ G ₂	— — ₆	A ₁ G ₁
I ₂	I	A ₁ G ₁	A ₁ G ₁	A ₁ G ₁	A ₂ G ₂	— — ₆	A ₁ G ₁
I ₃	I	A ₁ G ₁	A ₁ G ₁	A ₁ G ₁	A ₂ G ₂	— — ₆	A ₁ G ₁
I ₄ †	I	— — ₃	A ₁ G ₁	A ₂ G ₂	A ₂ G ₂	— — ₃	A ₁ G ₁
I ₅	I	A ₁ G ₁	A ₁ G ₁	A ₁ G ₁	A ₂ G ₂	A ₁ G ₁	A ₁ G ₁
I ₆	I	— — ₄	A ₁ G ₁	A ₁ G ₁	A ₂ G ₂	— — ₄	A ₁ G ₁

A, denotes acid; G, gas; —, negative; numeral suffix, days incubated to obtain result; *, probably two others; †, intermediate type.

All strains ferment arabinose, glucose, maltose, and mannitol within 24 hr. with production of acid and gas.

20 µg. thiamine/ml. when necessary. All streptomycin media contained 500 µg. streptomycin/ml.

Transfer of F. Although transfer of F to *Escherichia coli* B occurs only with difficulty, De Haan (1954) found *E. coli* B could be infected with F from K12 mutants by growing the two strains together in broth. The following adaptation of his method was used. To 100 ml. of nutrient broth were added about 3×10^8 organisms of the wild and the K12 S^RF— tester strains (58.161 and W677). After incubation of the mixture for 24 hr. at 37° it was diluted approximately 1/20,000 into fresh broth and incubated for a further 24 hr. Serial transfer of the mixture was repeated at least three times. The K12 S^R strain was recovered by overnight incubation of 20 ml. of streptomycin broth inoculated with 1/200 ml. of the mixed culture. Since the K12 S^R mutant was originally F— it could not recombine with an alternative F— strain unless it had become F+ during serial transfer with the wild *E. coli* strain. This culture recovered in streptomycin broth was tested for infection with F by attempting recombination with an F— strain as follows: 4 ml. of the streptomycin broth culture was mixed with an equal volume of the K12 S^RF— mutant having complementary auxotrophic characters; for example, when 58.161 S^R was tested for the possession of F it was mixed with W677 S^RF—. The mixture and controls of 5 ml. of each parent separately were washed twice in minimal salts (Davis & Mingioli, 1950), resuspended in 0.2 ml. and spread on plates containing streptomycin minimal medium alone when the F— parent was M[–], and supplemented with thiamine when the F— parent was TLB₁[–]. Since in these experiments both the K12 strain under test and the complementary K12 F— strain were streptomycin-resistant the presence of streptomycin in the minimal medium should not diminish the yield of prototrophic

recombinants; but would ensure that any surviving cells of the wild-type strain under test would not form colonies (since all the wild-type strains were streptomycin-sensitive).

Replica recombination technique. Each F⁺ parent was spread over a circular area about 0.5 cm. in diameter on a nutrient agar plate, which was incubated for 5–6 hr. and then replica-plated by a velveteen pad on to a lawn plate of streptomycin minimal or a streptomycin minimal thiamine medium seeded with about 3×10^9 washed cells of the F[–] parent.

Recombination in broth. 4 ml. of an overnight culture of the wild strain of *Escherichia coli* and the K12 S^RF[–] mutant were mixed and incubated together for 8–24 hr. The mixture, and 5 ml. of each parent, as controls, were washed twice in minimal salts, resuspended in 0.2 ml. and spread on plates containing streptomycin minimal medium alone when the F[–] parent was M[–] and supplemented with thiamine when the F[–] parent was TLB[–].

A representative sample of the colonies appearing from recombination experiments was purified and analysed for the transfer of unselected markers by the technique of Clowes & Rowley (1954) to confirm that they were recombinants.

Testing for colicinogenicity

The strain of *Escherichia coli* to be tested was plated to give single colonies on nutrient agar. After 24 hr. incubation the culture was sterilized by subjecting it to chloroform vapour for 30 min. (Fredericq, 1948) and layered with nutrient agar. The surface was then spread with a suspension of the indicator strain, dried and incubated overnight at 37°.

RESULTS

Transfer of F

Thirty wild-type strains of *Escherichia coli* were screened for their ability to transfer F to the K12 mutants 58.161 S^R(1)F[–] and to W677 S^RF[–] by successive transfer of the mixed culture in broth. Although the K12 mutant 58.161 S^R(1) was recovered in every case by inoculating the mixture into streptomycin broth, W677 S^R was only recovered from 14 of the 30 mixtures. Subsequently, 14 of the 16 wild-type strains contained in mixtures from which W677 S^R could not be recovered were shown to produce a colicin to which W677 S^RF[–] was sensitive, this perhaps explains the elimination of W677 S^R F[–] in some of these mixtures. The ability of 7 of the 30 recovered 58.161 S^R(1) tester strains to recombine with another F[–] strain indicated that 7 of the 30 wild strains of *E. coli*, A, H, J, L, O, R and U were able to transfer F to 58.161 S^R(1)F[–] (see Table 2), but of the 14 mixtures in which the W677 tester strain was recovered only one, strain A, gave a positive result. Control recombination plates seeded with the recovered K12 tester strain alone never grew colonies, confirming that those obtained in the crosses were not due to recombination between the wild and F[–] strains during serial transfer, nor to mutation of the wild strain to streptomycin-resistance during recovery of the *E. coli* K12 S^RF[–] strain from the mixture. The seven *E. coli* strains found to

possess this F+ character were tested for fertility with K12 by the 'recombination in broth' method; *E. coli* strain A alone recombined with 58.161 S^R(1) F⁻. Thus 6 wild strains of *E. coli*, H, J, L, O, R and U, could transfer fertility to a K12 F⁻ mutant without themselves being fertile, with the K12 mutants.

Table 2. *Differences in the number of recombinants obtained under standard conditions from matings of Escherichia coli W677 S^RF⁻ with E. coli 58.161 S^R(1) infected with F by contact with various wild strains of E. coli*

Culture of <i>E. coli</i> used to transfer F to 58.161 S ^R (1)	No. of recombinants
A	250-500
H	100-1000
J	10-50
L	10-50
O	10-50
R	50-100
U	10-50
Control 58.161 S ^R F+	1000
58.161 S ^R (1)F ⁻	0

The wild strains differed significantly in their ability to transfer F to the two K12 F⁻ mutants (see Table 3). Moreover, these K12 mutants have unpredictable differences in their ability to become infected with the F+ character, although they were derived from the same parent strain. Differences in the ability of K12 mutants to become infected with the F+ character were confirmed when attempts were made to infect a third K12 mutant, 58.161 S^R(2)F⁻, with the F of the wild strains (see Table 3).

Table 3. *Differences in the ability of wild strains of Escherichia coli to transfer the F+ character and colicinogenicity to E. coli 58.161 S^R(1)F⁻, 58.161 S^R(2)F⁻ and W677 S^RF⁻*

Wild strains of <i>E. coli</i>	K12 acceptor strain					
	58.161 S ^R (1)F ⁻		58.161 S ^R (2)F ⁻		W677 S ^R F ⁻	
	Character transferred		Character transferred		Character transferred	
	F+	colicino- genesis	F+	colicino- genesis	F+	colicino- genesis
A*	+	NC	-	NC	+	NC
H	+	+	+	+	+	+
J	+	-	-	-	-	-
L	+	NC	-	NC	-	NC
O	+	-	-	-	-	-
R	+	+†	-	-	-	-
U	+	+†	+	-	-	-
I ₁	+	+	-	+	-	+
I ₂	+	-	-	-	-	-
I ₃	+	-	-	+	-	+
I ₄	-	-	+	+	-	+
I ₅	-	+	+	+	-	+
I ₆	-	-	-	+	+	+

* Can recombine with *E. coli* 58.161 S^R(1)F⁻ c. 1 in 10⁸.

-, Negative result; +, positive result; NC, wild strain not colicinogenic; † indirect evidence, see text.

A minimum of three successive transfers was empirically chosen when screening the 30 wild strains for their ability to transfer *F*. It was subsequently found that wild strains varied considerably in the minimal time of mixed growth required to transfer detectable fertility to the K12 tester strains.

Stability of F+ state

The streptomycin-broth cultures of 58.161 S^R(1) which had been recovered from a mixture with *Escherichia coli* H and which presumably contained *F*+ organisms since it recombined with W677 S^RF⁻, was plated out on nutrient agar. Single colonies found to be *F*+ on first isolation lost this character on subculture; indicating that the *F* agent of *E. coli* H was unstable in strain 58.161 S^R(1). Some *F*+ clones were therefore plated out and single colonies tested for fertility by replica recombination. Those clones producing the largest number of recombinants were again plated out, and the colonies again tested by the replica recombination method until a stable fertile strain was obtained. A stable strain of 58.161 S^R(1) infected with *F* from *E. coli* H was thus isolated and labelled 58.161 S^R(1)_{F_H}.

This stable strain was then used as *F*+ donor in further transfer experiments with 58.161 *F*- and W677 *F*-. However, on testing single colonies obtained from *F*+ clones the *F* state of either 58.161 or W677 was found to be unstable which indicated that the stability of the *F*+ state of strain 58.161 S^R(1)_{F_H} did not result from an alteration of the *F* agent obtained from *E. coli* strain H.

In the course of these experiments it had been noted that after exposure to *Escherichia coli* H the colonies of the K12 mutants 58.161 S^R(1), 58.161 *F*- and W677 *F*- varied in size from 1 mm. in diameter to the normal 2-3 mm. Sometimes one or more segments of a small colony were of normal size. This suggests that *F*+ clones when first infected with *F* could be detected by colony size.

The elimination of Escherichia coli K12 F- mutants from mixed cultures by E. coli H

When *Escherichia coli* strain H was grown together with W677 S^RF⁻ it was found that the wild strain rapidly came to predominate. The rate of elimination of both 58.161 S^R(1)*F*- and W677 S^RF⁻ was ascertained by making differential counts of mixtures after varying periods of growth. Mixed cultures of *E. coli* H and W677 S^RF⁻ or 58.161 S^R(1)*F*- were studied and in the course of about fifty generations the ratio of *E. coli* H to W677 S^RF⁻ was found to increase from 1:3 to 2 × 10⁶:1. Similarly, the ratio of *E. coli* H to 58.161 S^R(1)*F*- changed from 1:5 to 680:1.

These results suggested possible antagonism and further investigation revealed that *Escherichia coli* H produced a colicin to which K12 mutants were sensitive. Of the 30 wild strains tested for the *F*+ character 19, including strains J, O, R and U, were found to be colicinogenic; they produced wide zones of inhibition of the K12 mutants W677 and 58.161 after overnight incubation. That is, of the six non-fertile wild strains shown to be able to

infect K12 strains with the F agent five were shown to be colicinogenic; the remaining strain, *E. coli* L, was further tested for colicinogenicity after varying periods of incubation using *E. coli* B, *E. coli* W 677 and *Shigella dysenteriae* P. 15 as indicator strains, without any evidence of the production of a colicin. Professor P. Fredericq kindly identified the colicins produced by the colicinogenic strains which are enumerated in Table 1. Three of these five strains produce more than one colicin. Three strains produce colicin B (Fredericq, 1948) and three colicin I, and one strain, R, produces both B and I.

Transfer of colicinogenicity

As five of the six wild strains able to transfer F were colicinogenic the transfer of colicinogenicity to the K12 S^BF⁻ mutants was examined by the techniques used for the transfer of F. Colonies of the K12 S^B mutants recovered from the mixtures by plating on streptomycin agar were transferred to a second plate by replica plating and the original streptomycin plate was tested for colicin production. Thus transfer of F and colicinogenicity could be compared.

Of the five colicin-producing strains, H, J, O, R and U, only one, *E. coli* H, transferred colicinogenicity to all the three K12 S^BF⁻ mutants (see Table 3). Transfer of both the F⁺ and colicinogenic attributes from strain H to 58.161 S^B(1)F⁻ and 58.161 S^B(2)F⁻ occurred within 24 hr., the majority of the colonies being colicinogenic. In contrast, using W 677 S^BF⁻, colicinogenic colonies were rare although all those possessing this attribute were also F⁺. When colicinogenic colonies of either W 677 S^B or 58.161 S^B(1) were plated out only a small proportion of the colonies obtained were colicinogenic, indicating that their colicinogenic property like the F⁺ state was unstable.

The colicinogenic *Escherichia coli* strains, J, O, R and U could not be shown by the techniques employed to transfer colicinogenicity to the F⁻ tester strains, although they could all transfer F to 58.161 S^B(1)F⁻, and one of them (*E. coli* U), to 58.161 S^B(2)F⁻ also (see Table 3). Some of the recombinants derived from crosses of W 677 S^BF⁻ with 58.161 S^B(1)F⁻ infected with F by contact with *E. coli* R and U were, however, colicinogenic when first isolated, but those in which F was derived from *E. coli* R had lost this property when tested again. As these recombinants were colicinogenic it would be inferred that the F⁺ parents were also colicinogenic, and the fact that this could not be demonstrated directly shows the inadequacy of the technique used.

The relationship between production of colicin I and the F state

Since the F⁺ and colicinogenic properties have been shown to involve similarities in transfer, particularly with respect to time of transfer and stability in the new host, the possibility exists that there may be a connexion between these factors. The most competent F⁺ donor, strain H, produced colicin I only. A search was therefore made amongst a further 70 wild strains for those producing this colicin. Each strain was plated out on two plates of nutrient agar and tested for colicin production by the normal technique using

W677 as a universal indicator strain and 58.161 S^R(1)F_H as the indicator strain which was resistant to colicin I alone. Only those wild strains producing a colicin active on W677 and giving no evidence of action on 58.161 S^R(1)F_H were tested for F. Six such strains were found: *E. coli* I₁, I₂, I₃, I₄, I₅, I₆. All were streptomycin-sensitive and with the exception of *E. coli* I₂ and I₃, were distinguishable from each other by biochemical tests (see Table 1).

These strains were examined for their ability to transfer colicinogenicity and F to K12 mutants. All the six strains could transfer F to one or other of the K12 S^RF⁻ mutants, although there were differences in this respect (see Table 3). Similarly, they varied in their ability to transfer colicinogenicity (see Table 3). With the one exception of *Escherichia coli* I₂ transfer of fertility was shown to be accompanied by the ability to transfer colicinogenicity. Since both attributes (F⁺ and colicinogenicity) however were unstable to varying degrees in their new bacterial host, it was not possible to obtain a clear answer as to whether both factors are always transferred together and a different approach had to be used in order to demonstrate that these factors are clearly separate.

Elimination of F and colicinogenicity

An attempt was made to eliminate colicinogenicity by treating cells of 58.161 S^R(1)F_H suspended in minimal salts with varying doses of UV light. Several thousand colonies derived from these cells were tested and all were colicinogenic. Thereafter, use was made of the observation by Hirota (1956) that treatment of K12 F⁺ mutants with cobalt chloride caused some of the cells to become F⁻.

Peptone water (1% 'Evans' peptone, 0.2% (w/v) glucose, pH 7.4) inoculated with 58.161 S^R(1)F_H, which was stable for both colicinogenicity and F, was incubated for 24 hr; cobalt chloride was added to give a final concentration of 0.02M. The peptone water was re-incubated overnight and plated on nutrient agar containing 0.04M-sodium citrate; single colonies were tested for colicinogenicity. Out of 450 colonies tested for colicinogenicity two were non-colicinogenic; they were found to be F⁻ also. 147 colonies of an untreated culture of 58.161 S^R(1)F_H were tested for the F⁺ character; all were positive, indicating the stability of this character. One of 14 colonies derived from a culture treated with cobalt chloride however was F⁻, but was still colicinogenic. It is therefore possible to separate these two properties.

The effect of F on the transfer of unselected markers

In all recombination experiments involving, as F⁺ parent, a mutant of K12 infected by a wild strain of *Escherichia coli*, some of the recombinants were examined for the transfer of unselected markers. The results for sugar markers, thiamine-requirement and colicinogenicity for nine crosses are given in Table 4. Although the numbers are small it is evident that there were significant differences in the extent of transfer of these characters to the recombinants, depending on the origin of the F carried by the donor strain. The marked differences in transfer between crosses 3, 5 and 6 suggests that the original wild strains may contain different F agents. This difference was most marked

Table 4. *Transfer of unselected markers by mutants of Escherichia coli K12 infected with F by wild strains of E. coli*

Crosses	Selecting medium	Selected markers of F + parent	No. of colonies tested	Percentage unselected markers transferred to the recombinants										Colicinogenesis
				B ₁ *	Fermentation of						Mal	Xyl	Mtd	
(1) *A/58.161 S ^R (1)F + × W677 S ^R F -	MMB ₁	T.L.	241	29.7	Lac	Arab	Gal	Mal	Xyl	Mtd				N.C.
(2) A/58.161 S ^R (1)F + × W677 S ^R F -	MM	T.L.B ₁	72		71	45	42	39	25	27				N.C.
(3) H/58.161 S ^R (1)F + × W677 S ^R F -	MMB ₁	T.L.	206	13.6	85	55	55	60	44	44				N.C.
(4) H/58.161 S ^R (1)F + × W677 S ^R F -	MM	T.L.B ₁	114		41	18	18	18	16	16				97
(5) I ₁ /58.161 S ^R (1)F + × W677 S ^R F -	MMB ₁	T.L.	48	0	54	50	50	39	46	45				N.T.
(6) I ₂ /58.161 S ^R (1)F + × W677 S ^R F -	MMB ₁	T.L.	40	2.5	29.2	0	0	0	0	0				14.6
(7) I ₃ /58.161 S ^R (1)F + × W677 S ^R F -	MMB ₁	T.L.	49	4.1	5.1	0	0	0	0	0				0
					23.4	0	0	0	0	0				0
(8) H/W677 S ^R F + × 58.161 S ^R (1)F -	MM	M	43		44.3	53.3	46.5	67.5	63.7	72				100
(9) A/W677 S ^R F + × 58.161 S ^R (1)F -	MM	M	243		6	23	23	26	28	30				N.C.

* The prefix to the F + parent denotes the wild strain denoting F. N.C. F + parent non-colicinogenic; N.T. recombinant not tested for colicinogenesis.

when using as the F donor the wild strain A, which was itself weakly fertile with 58.161 S^R(1)F⁻. When data from crosses 1 and 3 in Table 4, i.e. A/58.161 S^RF⁺ and H/58.161 S^RF⁺ × W677 S^RF⁻, are analysed in detail by arranging the sugar markers, by trial and error, in the order which accounts for the greatest proportion of recombinants by single cross-overs (Table 5), it

Table 5. Segregation of sugar markers on mating *Escherichia coli* 58.161 S^R(1) infected with F by *E. coli* A or H with *E. coli* W677 S^RF⁻ on minimal medium supplemented with B₁

Recombinant class						Number of recombinants in class			
						T+L+B _I ⁻ recombinants F+ parent		T+L+B _I ⁺ recombinants F+ parent	
Lac	Arab	Gal	Mal	Xyl	Mtl	*A 58.161 S ^R (1)F+	*H 58.161 S ^R (1)F+	*A 58.161 S ^R (1)F+	*H 58.161 S ^R (1)F+
-	-	-	-	-	-	19	89	6	10
+	-	-	-	-	-	44	39	12	1
+	+	-	-	-	-	9	3	2	2
+	+	+	-	-	-	14	3	4	3
+	+	+	+	-	-	10	8	8	1
+	+	+	+	+	-	3	0	3	0
+	+	+	+	+	+	18	13	19	3
-	-	+	+	+	+	.	.	.	1
-	-	-	+	+	+	2	1	.	.
-	-	-	-	+	+	2	4	1	.
+	+	+	-	-	+	1	.	1	.
+	-	-	+	+	+	5	1	1	.
+	-	-	-	+	+	2	.	.	.
+	-	-	-	-	+	1	1	.	.
-	+	-	-	-	-	3	2	.	1
-	-	+	-	-	-	3	1	.	1
-	-	-	+	-	-	4	3	.	.
-	-	-	-	+	-	1	2	1	.
-	+	+	-	-	+	3	.	1	.
-	-	-	-	+	+	2	1	1	.
-	+	+	+	+	+	1	.	.	.
+	-	+	+	+	+	3	2	3	.
+	+	-	+	+	+	1	.	1	.
+	+	+	-	+	+	2	.	.	2
+	+	+	-	-	+	.	.	1	1
+	+	-	+	-	-	6	.	1	.
+	+	-	-	+	-	2	.	1	.
+	-	+	+	-	-	.	.	1	.
+	-	-	+	+	-	2	.	.	.
+	-	+	+	+	-	3	2	1	.
+	-	-	+	-	+	1	.	3	1
-	-	+	-	+	-	1	.	.	.
-	+	+	+	-	+	1	.	.	.
-	+	-	-	+	+	.	1	1	1
-	+	-	-	-	+	.	1	1	.
Total recombinants						170	178	72	28

Markers of parent strains of K12 mutant. 58.161: -M⁻, Lac⁺, Arab⁺, Gal⁺, Mal⁺, Xyl⁺, Mtl⁺. W677: -T-L-B_I⁻, Lac⁻, Arab⁻, Gal⁻, Mal⁻, Xyl⁻, Mtl⁻.

* Prefix letter denotes the wild strain denoting F.

is found that in spite of great differences in the total transfer of sugar markers from the F⁺ parents the same order of markers is obtained, namely, lactose, arabinose, galactose, maltose, xylose and mannitol. These data are insufficient to determine the relative order of xylose and mannitol. This is in partial agreement with the order given by Clowes & Rowley (1954) and in agreement with Cavalli-Sforza & Jinks (1956).

DISCUSSION

Wild cultures of *Escherichia coli* which would cross with K12 F⁻ are known but it had not previously been shown that infertile strains may contain the F character (Cavalli *et al.* 1953) until a strain was reported by Lederberg & Lederberg (1956) which was infertile with K12 F⁻ mutants yet transferred F. It has now been found that a relatively high proportion of wild strains, infertile according to our tests with the F⁻ strains, can infect them with F. Other factors, besides the possession of F, are therefore involved in the ability of a strain to recombine with K12 auxotrophs to give phototrophic (or B₁-exacting) recombinants.

Individual mutants derived from the same parent can differ in their ability to accept F agents and two independently isolated streptomycin-resistant mutants of 58.161 F⁻ are completely different in this respect. Hayes (1953) similarly has reported that M-F⁻ and TLB₁F⁻ mutants of K12 vary in their ability to become infected with the F of K12. A greater proportion of wild strains might have been shown to possess a transmissible F agent if, in the present work, other F⁻ tester strains and more exhaustive techniques had been employed, such as colicin-resistant strains and daily examination for transfer of F.

Differences in the ease of transfer of F from wild strains of *Escherichia coli* to the standard K12 S⁺F⁻ mutants suggests that there is more than one F agent. This is supported by the fact that not every wild strain can infect the same F⁻ mutant. After infection the F state is lost spontaneously, a finding previously noted by Lederberg *et al.* (1952) suggesting that these F agents differ from that of K12. Moreover, this suggestion is further strengthened by the recombination data in which there are great variations in the transfer of unselected markers in crosses which only differ, so far as we know, in the F agent carried by the F⁺ parent. Another F agent which affects gene recombination has also been reported (Bernstein, 1957).

There are several similarities between colicinogenicity and the F⁺ state in K12. Both are transferred rapidly when donor and acceptor strains are incubated together. Colicin production can be increased by UV irradiation and similarly fertility is increased in recombination after irradiating the F⁺ parent (Hayes, 1952). The widely used mutants of K12 have never been shown to produce colicins, although this might be due to lack of a suitable indicator strain. Five of the six wild strains of *Escherichia coli* which transfer F in these experiments are able to produce colicins I or B or both. A search for wild types producing the same colicin, i.e. colicin I, as strain H, which had the most easily transmissible F agent, revealed six more strains, all of which were F⁺ but could not recombine. Thus, in this small series, strains producing colicin I have been shown to be F⁺. On the other hand, although these two properties are similar in many respects, they are different entities, since it was found that treatment with cobalt chloride could eliminate the F agent without loss of colicinogenicity.

We wish to thank Professor P. Fredericq for his gift of cultures and for typing the colicins produced by our strains and Dr C. W. Morley for strains of *Escherichia coli*. We are grateful to Mr P. Harwood for his excellent technical assistance.

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(Received 27 February 1957)