Transfer of the Colicin I Factor in *Escherichia coli* K12 and its Interaction with the *F* Fertility Factor

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SUMMARY

A series of *Escherichia coli* K12 strains of various mating types was made resistant to colicin I (*colI*-r) and colicinogenic for this character (*colI*⁺). In the absence of the *F* sex factor, the number of 'competent donors' of *colI* in broth cultures of colicinogenic strains was about 1 %. Transfer of *colI* to non-colicinogenic strains reached about 7–10 % within 2 hr of mixing, and between 50 and 100 % after incubation overnight. Cells newly infected with *colI* transfer more efficiently, and can infect over 90 % of a recipient strain with *colI* within 1 hr of mixing (high-frequency colicinogeny transfer, HFC). When the *F* factor was present in the colicinogenic donor strain of a mixture, *colI* transfer was reduced; when *F* was present in the non-colicinogenic recipient, *colI* transfer was increased. The colicin I factor appeared to be transferred by a series of Hfr strains as an extra-chromosomal element.

INTRODUCTION

Many strains of Enterobacteriaceae produce the protein-like antibiotics known as colicins, which have been subdivided by Frédéricq (1957) into a number of types. The production of each colicin is presumed to be controlled by a stable genetic determinant called a colicin factor (*col* factor; thus *colI* determines the production of colicin I), cells harbouring such factors being termed colicinogenic. Colicinogeny shows many similarities with lysogeny (the ability to produce a temperate bacteriophage; see Frédéricq, 1958, 1963*a, b*). Probably the most striking is the demonstration by Ozeki, Stocker & de Margerie (1959) that colicin production is due to a lethal synthesis. Colicinogenic bacteria carry a potentiality to synthesize colicin but this is normally expressed only by a small random proportion of cells in the population, which are killed in the process.

Certain strains can transfer their colicinogenic property to non-colicinogenic strains during mixed cultivation (Frédéricq, 1954). This transfer requires cell-to-cell contact, and takes place in the absence of any observable transfer of chromosomal genes. The colicin factors (whose transfer is inferred under these conditions) were thus suggested to exist as extra-chromosomal or cytoplasmic elements (Frédéricq & Betz-Bareau, 1953).

The transfer of colicin factors responsible for the maintenance of several distinct colicins has been investigated in *Salmonella typhimurium* (Ozeki, Stocker & Smith, 1962; Smith & Stocker, 1962; Stocker, Smith & Ozeki, 1963; Smith, Ozeki & Stocker, 1963). The factors *colI* and *colB* were found to be transferred readily, whereas *colK*, *colE1* and *colE2* were transferred very infrequently or not at all. The

infective factors *colI* and *colB* were also capable of mediating the transfer of normally non-infective factors such as *colE1* and *colE2*. Moreover, cells to which the *colI* factor had been recently transferred were found to have a greatly enhanced efficiency of transfer ('high-frequency colicinogeny transfer', HFC) giving rise to an 'epidemic spread' of *colI* within newly infected cultures (Stocker *et al.* 1963).

In *Escherichia coli* $\kappa 12$, *colI* is also transferred, in the absence of the *F* sex factor (Clowes, 1961). The present paper reports experiments on the kinetics of this transfer, on the effect of the presence of the *F* factor, either in the autonomous (F^+) or in the integrated (Hfr) state, on this process.

METHODS

Bacterial strains used. The strains are specified in Table 1. The 'origines' and directions of transfer of the Hfr strains are shown in Fig. 1. The *colI* factor in all strains originates from *Shigella sonnei* P9, transmitted to *Salmonella typhimurium* LT2 (Ozeki *et al.* 1962), and is thus identical in origin with that used in previous investigations (Ozeki & Howarth, 1961; Clowes, 1961; Ozeki *et al.* 1962; Smith & Stocker, 1962; Stocker *et al.* 1963; Smith *et al.* 1963).

Fig. 1. The circular linkage group of *Escherichia coli* $\kappa 12$. The relative positions of the genetic markers are drawn on the outside of the circle. The arrows on the circle represent the leading extremity and direction of transfer of the chromosome in the various Hfr strains denoted on the inside of the circle.

Media. Nutrient broth was prepared from Oxoid No. 2 broth powder at 25 g/l. adjusted to pH 7.2; nutrient agar was made by the incorporation of Davis agar powder at 12 g/l. Minimal agar was, as described by Tatum & Lederberg (1947) with the omission of asparagine, solidified with Davis agar at 15 g/l. and supplemented with D-glucose at 2 g/l. Amino acid supplements were added at 20 mg./l. and vitamin B1 (thiamin) at 5 mg./l. Soft agar used in overlays was made up as Difco agar at 6 g/l. without nutrients or salts and held molten at 45°. Streptomycin was added at 200 mg./l., and azide as sodium azide at m/500 in nutrient agar or m/1600 in minimal agar. Eosin methylene blue (EMB) and tetrazolium media were prepared according to Lederberg (1950*b*), supplemented with either lactose or maltose to score for sugar fermentation.

Table 1. *Bacterial strains*

Strain no.	Genotype	Origin
(a) Strains derived from <i>Escherichia coli</i> K12, 58-161 (<i>met</i>) Tatum & Lederberg (1947)		
501	F ⁺	Original strain, 58-161 (Hayes, 1952)
518	F ⁻	501 by loss of F (Hayes, 1953)
519	F ⁻ <i>colI</i> ⁺	518 infected with <i>colI</i> from 902
524	F ⁻ <i>colI</i> ⁺ <i>str-r</i>	519 made resistant to streptomycin
535	F ⁺ <i>colI-r</i>	501 resistant to colicin I from 902
536	F ⁺ <i>colI</i> ⁺	501 infected with <i>colI</i> from 902
544	F ⁺ <i>colI-r str-r</i>	535 made resistant to streptomycin
547	F ⁻ <i>colI-r</i>	518 resistant to colicin I of 902
550	F ⁻ <i>colI-r str-r</i>	547 made resistant to streptomycin
559	F ⁻ <i>colI</i> ⁺ <i>colI-r</i>	547 infected with <i>colI</i> of 519
(b) Strains derived from <i>E. coli</i> K12, Hfr Hayes (<i>met str-r azi-r T1-r</i>)		
715	Hfr H*	Original strain (Hayes, 1953)
730	Hfr H <i>colI-r</i>	715 made resistant to colicin I of 519
732	Hfr H prototrophic <i>mal- gal- ara- str-s</i>	<i>str-s</i> Hfr recombinant. Otherwise known as A'Hfr (Hayes, personal communication)
733	Hfr H prototrophic <i>colI</i> ⁺ <i>mal- gal- ara- str-s</i>	732 infected with <i>colI</i> of 519
735	Hfr H prototrophic <i>colI-r mal- gal- ara- str-s</i>	732 made resistant to colicin I of 559
(c) Strains derived from <i>E. coli</i> K12 Hfr _{AB} 311 (<i>thr leu str-r T6-s</i>)		
741	Hfr _{AB} 311*	Original strain (Taylor & Adelberg, 1960)
763	Hfr _{AB} 311 <i>colI</i> ⁺	741 infected with <i>colI</i> from 519
(d) Strains derived from <i>E. coli</i> K12, Hfr _{P10} (<i>thr leu thi mal-lac- gal- T1-r T6-s</i>)		
749	Hfr _{P10} *	Original strain Hfr _{P10} , equivalent to Hfr(J)4. (Jacob & Wollman, 1958;
764	Hfr _{P10} <i>colI</i> ⁺	749 infected with <i>colI</i> of 519
(e) Strains derived from <i>E. coli</i> K12, J6-2 (<i>pro his try str-s</i>)		
710	F ⁺	Original strain (Clowes & Rowley, 1954)
711	F ⁻	710 cured by acridine orange†
712	F ⁻ <i>str-r</i>	711 made resistant to streptomycin
707	F ⁻ <i>str-r colI-r</i>	712 made resistant to colicin I from 902
765	F ⁻ <i>str-r colI-r T6r</i>	707 made resistant to coliphage T6
(f) Strains derived from <i>E. coli</i> K12, w677 (<i>thr leu thi mal- lac- gal- xyl- mtl- ara-</i>) Lederberg, 1950a)		
27	F ⁺ <i>str-r colI-r</i>	28 infected with F from 501
28	F ⁻ <i>str-r colI-r</i>	30 made resistant to streptomycin, and to colicin I from 902
30	F ⁻	Original strain w677 (Hayes, 1952)
46	F ⁻ <i>colI</i> ⁺	30 infected with <i>colI</i> from 519
(g) Strains other than <i>E. coli</i>		
902	<i>Salmonella typhimurium</i> LT2 <i>cys D-36 colI</i> ⁺	LT2 <i>cys D-36</i> infected with <i>colI</i> from <i>Shigella sonnei</i> P9 (Ozeki <i>et al.</i> 1962).

Growth requirements: *met*, methionine; *thr*, threonine; *leu*, leucine; *thi*, thiamin; *pro*, proline, *his*, histidine; *try*, tryptophan.

Resistance markers; *str-r*, resistant to streptomycin: 200 µg./ml.; *azi-r*, resistant to sodium azide; *colI-r*, resistant to colicin I; *T1-r*, *T6-r*, resistant to the coliphages T1, T6; *colI*⁺ = colicinogenic for colicin I.

Sugar fermentations; *mal*, maltose; *lac*, lactose; *gal*, galactose; *ara*, arabinose.

* See Fig. 1 for 'origine' and orientation of transfer of these strains.

† Cured by method of Hirota (1960).

Techniques

Colicinogeny was tested by one of several methods. In the stab method (Frédéricq, 1957), colonies (including control *col*⁺) were inoculated by straight wire into nutrient agar. After overnight incubation, the plate was sterilized by exposure to chloroform vapour, and then overlayed with 2.5 ml. soft agar, seeded with 0.1 ml. of an overnight broth culture of a strain sensitive to the colicin (indicator strain). After further overnight incubation, the indicator cells grow as a confluent lawn except around the site of inoculation of a *col*⁺ colony, where a zone of inhibition is observed. In the triple overlay (or sandwich plate) technique, the culture to be tested was diluted to give 10³ to 10⁴ cells/ml., 0.1 ml. of this dilution being plated in 2.5 ml. of soft agar overlay. When this overlay had set, a further 2.5 ml. of unseeded soft agar was plated, to prevent emergence of any of the seeded bacteria as surface colonies. After overnight incubation, the identification of those colonies appearing within the agar overlay which are *col*⁺ was achieved by a third overlay containing indicator bacteria, followed by a second period of incubation. A colony at the centre of the circular inhibition zone may then be 'fished' from the soft agar, and propagated as a stable *col*⁺ strain.

Free colicin was assayed to give a measure of the colicin titre in cultures of known colicinogenic strains. Drops from a series of doubling dilutions of a culture filtrate or supernatant fluid from centrifugation were spotted on a nutrient agar plate, previously overlayed with an indicator strain. When the *col*⁺ strain was *str*-s, a *str*-r indicator was used on streptomycin nutrient agar. Alternatively the culture was freed from viable bacteria by incubation for about 30 min. at 37° after the addition of a few drops of chloroform.

Colicin resistant (col-r) strains were produced from a parental *col*-s strain by its use as an indicator overlay to a chloroformed *col*⁺ stab plate. After incubation for 48 hr, several isolated colonies usually appeared within each inhibition zone. Cultures from these colonies no longer produced inhibition zones when used as indicators, and were now colicin-resistant.

Isolation of colicinogenic strains was achieved by inoculating 0.1 ml. of an overnight broth culture of the strain to be made colicinogenic (recipient) into 5 ml. nutrient broth, together with 1 loopful of a colicinogenic strain (donor), to achieve a ratio of donor to recipient of about 1:20. After overnight incubation, the recipient was re-isolated by the use of either a differentiating (EMB or tetrazolium) medium, or a selective medium (streptomycin or azide nutrient medium, or appropriately supplemented minimal medium). Individual colonies were then tested for the acquisition of colicinogeny by either the stab or sandwich plate techniques.

Kinetics of transfer of colicinogeny at low frequencies (LFC; see Ozeki et al. 1962). Overnight cultures of a *col*⁺ *str*-s donor strain, and a non-colicinogenic (*col*⁻) recipient strain (previously made resistant to both colicin I and streptomycin) were each diluted 1/20 into broth and re-incubated for 90 min. to produce logarithmic (log) cultures at about 10⁸ organisms/ml. Equal volumes were then mixed and re-incubated without shaking or aeration. At various times, samples were removed, gently but rapidly diluted (at early times 10⁻⁴, at later times 10⁻⁵) and 0.1 ml. volumes plated in soft agar on streptomycin nutrient agar plates and further overlayed with unseeded soft agar. After overnight incubation only the *str*-r recipient strain

produced colonies within the agar. Overlay with a *col-s str-r* indicator strain allowed the colonies which have acquired colicinogeny to be easily recognized by their surrounding inhibition zones. Between 300 and 1000 colonies were tested for the acquisition of *colI* at each time of sampling.

Kinetics of high frequency colicinogeny transfer (HFC) was followed from a HFC 'donor mixture' to a recipient, by the method of Stocker *et al.* (1963). Five ml. nutrient broth were inoculated with about 5×10^4 cells from a *colI*⁺ *str-s* strain (donor) and about 10^8 cells of a *col*⁻ *colI-r str-s* (intermediate) strain. After overnight incubation, the mixture was diluted 1/20 in fresh broth and re-incubated a further 90 min. with aeration to about 10^8 cells/ml. Almost 100 % of the intermediate cells in such an HFC 'donor mixture' were *colI*⁺ and since a high proportion of them had recently acquired colicinogeny, they showed a high efficiency of transfer when mixed with the recipient, a *colI*⁻ *colI-r str-r* strain. Log. phase cells of an HFC donor mixture and a recipient, both at 10^8 organisms/ml. were mixed in broth and the transfer of *colI* to the recipient was followed by plating samples at intervals on streptomycin agar and assaying for *colI*⁺ colonies.

The number of 'competent donors' in a *col*⁺ population was determined according to Ozeki *et al.* (1962). 0.2 ml. samples of a culture of a *col*⁻ *str-r* strain were added to a series of tubes containing 2 ml. pre-warmed broth. A culture of the *colI*⁺ *str-s* strain to be assayed (at about 5×10^8 organisms/ml.) was diluted serially tenfold, and from dilutions containing about 50,000, 5000 and 500 cells/ml., 0.1 ml. samples were added to each of 15 (or 25) tubes containing the *col*⁻ inoculum. The tubes were incubated 15 min., streptomycin (to 200 µg./ml.) was added to each, and incubation continued overnight; 0.1 ml. of a 10^{-5} dilution from each tube was then plated in soft agar and tested for the presence of *col*⁺ colonies in the usual way.

Cell to cell contact was observed in mixture of two strains, one of which had been stained with tetrazolium. An efficient method of staining is due to Drs E. & G. Kellenberger (personal communication). To an actively growing culture at a density of about 10^8 organisms/ml., vigorously aerated in broth (pH 7-7.5), a solution of tetrazolium (2,3,5-triphenyltetrazolium chloride) is added to a final concentration of 0.1 % and aeration immediately stopped. After further incubation for 5 min. the culture turns bright red, and the great majority of cells can be seen under the microscope to show 1 or 2 sites ('formazan granules') which are intensely stained. Specific pairing or clumping between cells may thus be observed in mixtures in which only one of the strains is so labelled.

F⁺ testing was achieved by a modified screening method, making use of the male specific phage μ (Dettori, Maccacaro & Piccinin, 1961). Inocula from each of 50 to 100 presumptive F⁺ colonies were taken into tubes containing 2 ml. broth, which were then incubated overnight. One loopful from each tube was spread over an area of about 2 cm. diameter on a nutrient agar plate (about 18 spread areas/plate, including F⁺ and F⁻ controls). By using a sterile glass syringe with a fine needle, one drop (0.01 ml.) of a 10^{10} particles/ml. suspension of μ phage was then placed at the centre of each spread area. After incubation for 6-8 hr, only the F infected strains showed a region of phage lysis within the surrounding area of bacterial growth.

F⁺ and Hfr mediated recombination. Logarithmic phase cultures of donor and recipient strains at about 10^8 cells/ml. were mixed in equal volumes and incubated together for 2 hr, and 0.1 ml. samples were then plated (after suitable dilution

from Hfr crosses) on appropriately supplemented minimal agar to yield several hundred recombinant colonies after incubation for 48 hr.

Interrupted mating in Hfr crosses was achieved by removal of 0.1 ml. samples from donor + recipient mixtures during the 2 hr incubation period into 1 ml. of 10^{10} particles T6 phage/ml. Incubation was continued for a further 15 min. when the samples were vigorously agitated for 1 min. at top speed in a 'Microid' flask shaker (Griffin & George, London), rapidly diluted, and plated on selective minimal media for recombinants as above.

RESULTS

Isolation and characteristics of K12 strains colicinogenic for colicin I

In general, the *colI* factor was readily transferred to a variety of K12 strains during mixed incubation of *colI*⁺ and non-colicinogenic (*col*⁻) strains. The *col*⁻ strain was not killed by free colicin I in the culture, which permitted the isolation of drug-sensitive (*str-s* or *azi-s*) *colI*⁺ strains on differentiating medium. After overnight mixed cultivation, usually between 50 and 100 % of the surviving *col*⁻ colonies acquired *colI*, irrespective of whether *Salmonella* or *Escherichia* strains were used as colicinogenic donors.

From early experiments, the sizes of the inhibition zones produced by *colI*⁺ strains of *Escherichia coli* (3 mm. from an 18 hr 'stab', or 2 mm. from a 24 hr deep-agar colony) were found to be smaller than those produced by *Salmonella* under similar conditions (10 and 6 mm., respectively). This difference was ascribed to the adsorption of colicin by the non-producing cells of the colonies of *E. coli*. Thus, when the *E. coli* strain made *colI*⁺ had previously been made resistant to the colicin (*colI-r*) its inhibition zones were larger, and were in fact similar in size to those produced by *Salmonella* which has been shown to be naturally colicin-resistant (Ozeki *et al.* 1962). That the zone sizes were due entirely to the presence or absence of the colicin-resistance marker, and not to an alteration of the colicin I factor, was shown by the fact that transfer of *colI* to a colicin-sensitive strain from either a resistant (*colI*⁺ *colI-r*) or a sensitive (*colI*⁺ *colI-s*) donor produced a strain (*colI*⁺ *colI-s*) which gave small inhibition zones, whereas infection of a colicin-resistant strain from either donor gave a *colI*⁺ *colI-r* strain which produced the larger inhibition zones. Further experiments showed that the amount of free colicin in cell-free filtrates of overnight *colI*⁺ *colI-r* cultures allowed a sixteenfold dilution whilst still producing inhibition when drops were plated on a lawn of *col-s* indicator cells, whereas a parallel *colI*⁺ *colI-s* culture filtrate produced only very slight inhibition when similarly plated without dilution, and did not do so after even two-fold dilution. The majority of cells in a culture of a colicinogenic strain do not produce colicin (Ozeki *et al.* 1959), and it appeared likely that these cells in a *colI*⁺ *colI-s* culture could absorb the liberated colicin, whereas cells of a *colI*⁺ *colI-r* culture have lost this ability with their mutation to colicin resistance. This conclusion is supported by the finding that the end-point dilution of inhibition from 1 + 1 mixtures of overnight cultures of *colI*⁺ *colI-r* and *colI*⁺ *colI-s* strains was decreased to that of the *colI*⁺ *colI-s* cells after mixing for 3 hr. at 37°.

Number of 'competent donors' of colI in K12F⁻ strains

The proportion of colicinogenic cells which are able at any one time to transmit their *col* factor (competent donors) was determined by Ozeki *et al.* (1962) by measur-

ing the number of *colI*⁺ cells able to initiate an epidemic spread of colicinogeny within a large number of non-colicinogenic cells. A similar estimation was made for *Escherichia coli colI*⁺ strains (Table 2). After overnight incubation of serial inocula of *colI*⁺ *str-s* cells with a large excess of *colI*⁻ *str-r* cells, samples were plated from the tubes and assayed for the presence of *colI*⁺ *str-r* cells, and were found to be made up almost entirely of either *colI*⁺ (>75 %) or *colI*⁻ (<0.01 % *colI*⁺) cells. The proportion of tubes for each size of donor inoculum which did not show any *colI*⁺ cells

Table 2. 'Competent' donors in broth cultures of *Escherichia coli* K12 *colI*⁺ F⁻ strains

Samples (0.2 ml.) of serial tenfold broth dilutions of either log phase (log) or overnight (O/N) cultures of a *colI*⁺ *str-s* donor strain (519, 559 or 46) were mixed with 0.2 ml. of overnight cultures (3×10^8 cells/ml.) of a non-colicinogenic *colI*⁻ *str-r* recipient strain (550), 15 (or 25) tubes being mixed at each broth dilution. After 15 min. incubation, 2 ml. of streptomycin nutrient broth was added to each tube and incubation continued overnight. 0.1 ml. of a 10^{-6} dilution from each tube was then plated on streptomycin agar and the resulting colonies tested for acquisition of *colI*. The mean number of competent donors/tube was calculated from the proportion of tubes in which there were no *colI*⁺ bacteria after overnight incubation, using this value as the zero term of the Poisson equation, e^{-m} , where m = mean number of competent donors/tube.

Strain no.	No. of <i>colI</i> ⁺ donor bacteria added per tube	Nos. of tubes giving negative or positive test for presence of <i>colI</i> ⁺ recipient bacteria		Mean nos. of 'competent donors' per tube	Average nos. of <i>colI</i> ⁺ donor bacteria per 'competent donor'
		-	+		
519 (log)	9.2	14	1	0.07	132
	92	2	13	2.03	45
	921	0	15	> 2.71	< 364
519 (O/N)	2.9	24	1	0.04	72
	28.6	20	5	0.22	130
	286	1	19	2.95	95
559 (O/N)	2.9	25	0	< 0.05	> 59
	29.2	19	6	0.27	108
	292	0	20	> 3.0	< 98
46 (O/N)	2.6	24	1	0.04	64
	25.6	21	3	0.13	197
	256	4	14	1.5	170

was used as the zero term of the Poisson equation to estimate the average numbers of competent donors added to each tube for this donor inoculum. From Table 2 it can be seen that from either log-phase or overnight cultures of both 58-161 and w677 strains, the proportion of competent donor cells was probably between 1 in 50 and 1 in 200.

Kinetics of low frequency colicin transfer (LFC) of colI in the absence of the F factor

In preliminary experiments, the transfer of *colI* from a *colI*⁺ *str-s* to a *colI*⁻ *str-r* strain after mixed growth for 18 hr varied between 20 and 100 %. These fluctuations were evidently due to variations in population density and input ratios of the two strains as shown in Fig. 2. Transfer of *colI* began within 2 hr of mixing and was most rapid at the highest donor to recipient ratio (curve A). With ratios of 2:1 or less (curves, B, C, D), transfer, although initially lower, increased after the first few

hours, the increase now being most marked in mixtures of the lowest donor:recipient ratio curves (C and D), where the value reached was 70–90 % after incubation for 18 hr. With a 1:1 input at about 10^8 cells/ml., transfer was about 7–10 % after the first 4 hr, attaining 80 % after 18 hr. This input ratio and cell concentration was used in all succeeding experiments.

Fig. 2

Fig. 2. The effect of donor to recipient ratios on *coli* transfer under LFC. Log. phase donor strain 519 ($F^- coli^+ str-s$) and recipient 550 ($F^- coli-r str-r$) were diluted and mixed at the following population densities/ml.

Strain 519	Strain 550	Ratio	Curve	
1.2×10^8	2.7×10^6	40:1	A	$\triangle-\triangle$
1.2×10^8	6.7×10^7	2:1	B	$\times-\times$
3.2×10^7	6.7×10^7	1:2	C	$\square-\square$
3.4×10^6	6.7×10^7	1:20	D	$\circ-\circ$

The mixtures of organisms were incubated and at various times samples were removed, diluted and plated on streptomycin agar. After incubation the resulting recipient clones were tested for colicinogeny by overlay with a colicin-sensitive indicator.

Fig. 3. Low and high frequency *coli* transfer. Lower curve ($\circ-\circ$) LFC. Log. phase cultures of donor 519 ($coli^+ str-s$) and recipient 550 ($coli^- str-r$) at about 10^8 organisms/ml. each were mixed in equal volumes and incubated. At various times samples were plated on streptomycin agar and the resulting colonies tested for *coli*⁺.

Upper curve ($\bullet-\bullet$) HFC. Five ml. nutrient broth were inoculated with 5×10^4 cells of 519 donor and 10^6 cells of an intermediate ($coli^- str-s$) strain 547. After overnight incubation the mixture was diluted 1 to 20 in 5 ml. fresh broth and incubated with aeration for 90 min. (to about 10^8 cells/ml.). This HFC 'donor mixture' was mixed with a log. phase culture (at 10^8 cells/ml.) of the recipient strain 550 and the mixture incubated. At various times samples were withdrawn and the recipient selected and tested for *coli*⁺ as above.

Fig. 3

The transfer of *coli* in cultures of *Escherichia coli* thus appears to be a result of two distinct processes, a low initial rate of transfer and a rapid subsequent rate of transfer. In this way it resembles the 'epidemic spread' of *coli* in *Salmonella typhimurium* described by Stocker *et al.* (1963) which was found to be due to the greatly enhanced ability of cells newly infected with *coli* to transfer this factor.

On this interpretation, the initial transfer shown in Fig. 2 would be due to a high donor:recipient ratio, producing an initially high rate of transfer. This is followed by transfer from newly infected recipient cells (recipient to recipient transfer) which is more extensive in cultures of low donor:recipient input, where the probability of recipient to recipient contacts is increased. The occurrence of high frequency colicinogeny transfer (HFC) in *E. coli*, analogous to that found in *S. typhimurium* by Stocker *et al.* (1963) was therefore investigated.

Kinetics of high frequency colicinogeny transfer (HFC) of colI in the absence of the F factor

Equal volumes of an 'HFC donor mixture' prepared according to Stocker *et al.* (1963) and a log phase culture of a non-colicinogenic *colI-r str-s* recipient strain, both at about 10^8 cells/ml. were mixed and incubated without aeration. At various times, diluted samples were plated in soft agar overlays on streptomycin agar and the colonies formed after incubation for 24 hr tested for the acquisition of *colI*. The kinetics of *colI* transfer are shown in Fig. 3, together with normal (LFC) transfer for comparison. Transfer from the HFC donor mixture occurred at a very rapid rate, about 60–70 % of the recipient cells having acquired *colI* within the first 15 min. after mixing, and more than 90 % after 1 hr.

The time taken for transfer of the colI factor by HFC cultures was estimated by mixing an HFC donor mixture and a recipient strain, diluting after 1 min. and then rapidly agitating in a 'Microid' shaker to separate cell pairs; under these conditions 30–40 % of the recipient cells acquired colicinogeny, and within 5 min. after dilution, the great majority (>90 %) of the recipient cells were colicinogenic.

The duration of high infectivity after acquisition of colI was determined from an HFC mixture prepared in the usual way, but in which incubation was continued beyond the normal 90 min. period, with dilutions into fresh warm broth at 30 min. intervals to maintain the concentration of organisms approximately constant. At various times, samples were removed, assayed for viable count, and then mixed with about 10^8 cells of a recipient culture. After 10 min. to permit cell contact, each mixture was gently diluted 10^{-5} into buffer and after incubation for 1 hr was plated on streptomycin agar. The transfer of *colI*⁺ to the recipient was assayed in the usual manner. Table 3 shows that an increased transfer efficiency persisted for 2–3 hr although during this period a progressive decay in the degree of transfer took place.

The effect of the F sex factor on colI transfer under LFC conditions

The effect of *F* on *colI* transfer under LFC conditions was investigated by using strains in which the *F* factor was either autonomous (*F*⁺) or integrated (*Hfr*). In the classical fertility cross 58–161 *F*⁺ × w677 *F*[–] where *F* is autonomous (Hayes, 1953), *F* transfer is high and reaches close to 100 % in 1 or 2 hr. In contrast, *F* transfer between the strains 58–161 *F*⁺ × 58–161 *F*[–] is much less efficient and attains a value of only 10–20 % in the first 5 hr and usually no more than 30 % after overnight mixed cultivation (similarly, low efficiencies of *F* transfer are also found in the mixtures w677 *F*⁺ × 58–161 *F*[–] and w677 *F*⁺ × w677 *F*[–] (Clowes, unpublished)).

(i) *Transfer of colI from 58–161 to 58–161* is shown in Fig. 4, where transfer was

followed from a *colI*⁺ strain to a *col*⁻ strain, using four possible permutations of F⁺ and *colI*⁺ in the mating strains, mixed in equal concentrations. The control F⁻ × F⁻ curve (curve A), has the same shape as that shown for LFC in Figs. 2 and 3. The presence of *F* in the *colI*⁺ strain (curve B) initially decreases the rate of transfer. After about 2 hr the rate increased to the value of the F⁻ × F⁻ control. In contrast, when *F* was present in the *col*⁻ strain (curve C), there was an initial enhancement of transfer which progressively increased. Finally, when *F* was present in both strains (curve D), the initial rate paralleled that of the F⁻ × F⁻ control, but increased more rapidly, and after 2 or 3 hr was equivalent to the enhanced rate of transfer in the F⁻ × F⁺ mixture.

Table 3. *Duration of enhanced infectivity in HFC mixtures*

An HFC donor mixture was prepared by inoculation of 5×10^4 cells of a *colI*⁺ *str-s* (519) with 10^6 cells of a *colI-r str-s* strain (547) into 5 ml. nutrient broth, incubating overnight, then diluting 1/20 in fresh broth and re-incubating for a further 90 min. (time 0). Incubation of this mixture was continued with a 1:1 dilution into fresh warm broth every 30 min. to maintain the population at about 10^8 /ml. A recipient strain *colI-r str-r* (550) was grown overnight in broth, diluted 1 in 5 in fresh nutrient broth (10^8 cells/ml.) and held at 4°. At given times 1 ml. of HFC donor mixture was added to 1 ml. pre-warmed recipient culture and incubated for 10 min. at 37°. The mixture was then gently diluted 10^{-5} into buffer, incubated at 37° for 1 hr, and then recipient cells assayed for acquisition of *colI*⁺.

Time of pre-incubation of HFC donor mixture (hr)	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2
Fraction of recipient colonies becoming <i>colI</i> ⁺	161/604	295/1475	194/1314	181/1306	78/1238
Degree of <i>colI</i> ⁺ transfer (%)	26.5	20.0	12.8	10.0	6.3
Time of pre-incubation of HFC donor mixture (hr)	3	4	5	6	
Fraction of recipient colonies becoming <i>colI</i> ⁺	20/934	61/1179	57/1476	42/1374	
Degree of <i>colI</i> ⁺ transfer (%)	2.1	5.2	3.9	3.0	

(ii) *Transfer of colI in 58-161 × w677 mixtures* occurred at a much slower rate in the absence of *F* than in the previous strain system. When *colI* was present in 58-161 F⁻, transfer to w677 F⁻ was only 1-3 %, even after 8 hr of mixed incubation, and did not exceed 20 % after overnight incubation. When *F* was present in either or both strains, *colI* transfer remained low and no significant differences were noted in either the rate of transfer or the final overnight value achieved. Nevertheless, in the 58-161 F⁺ *colI*⁺ × w677 F⁻ *colI*⁻ mixture, *F* transfer was normal, reaching 80-100 % after contact for 1 hr, by which time *colI* transfer was less than 0.1 %.

(iii) *Transfer of colI in Hfr × F⁻ mixtures* was investigated by using Hfr Hayes (and A⁺Hfr) × 58-161 F⁻. The effects of integrated *F* in either strain on *colI* transfer observed in this system (Fig. 5) were similar to those found for autonomous *F* shown in Fig. 4. When an Hfr strain was used as a *colI* donor, *colI* transfer was initially repressed (curve B), but after 4-8 hr increased to the value shown by the F⁻ × F⁻ mixture in curve A. The opposite effect was again observed in transfer of *colI* from F⁻ *colI*⁺ to Hfr *col*⁻ (curve C), when enhanced transfer ensued.

The effect of the F sex factor on colI transfer under HFC conditions

Under LFC conditions, *colI* transfer from either 58-161 F⁻ *colI*⁺ or w677 F⁻ *colI*⁺ to w677 F⁻ *col*⁻ was less than 1 % even after contact for 5 hr, making observation of the effect of *F* on this transfer tedious and inaccurate in these systems. However

with the enhanced efficiency of HFC, the effect of *F* on *colI* transfer can be investigated in these mixtures. The kinetics of such transfer in a system using 58-161 newly infected with *colI* is shown in Fig. 6. In all mixtures, HFC conditions prevailed, and by 30 min. at least 60 % of the recipient w677 population had acquired *colI*. However the presence of *F* in the newly infected *colI*⁺ strain diminished transfer within this period from 80 to 60 %, whereas when *F* was present in the recipient, transfer was increased to 85 % during this period. Very similar results were found with parallel mixtures of 58-161 strains used as both donor and recipient under HFC conditions, and in this system the overall transfer in all mixtures was higher, reaching 50-80 % after 15 min. and 75-90 % after 30 min.

Fig. 4

Fig. 5

Fig. 4. The effect of autonomous *F* on LFC transfer of *colI*. The curves show the transfer of *colI* in the following strains:

Curve A	$F^- \times F^-$; $F^- \text{ colI}^+ \text{ str-s (519)} \times F^- \text{ colI-r str-r (550)}$	$\bigcirc - \bigcirc$
Curve B	$F^+ \times F^-$; $F^+ \text{ colI}^+ \text{ str-s (536)} \times F^- \text{ colI-r str-r (550)}$	$\bullet - \bullet$
Curve C	$F^- \times F^+$; $F^- \text{ colI}^+ \text{ str-s (519)} \times F^+ \text{ colI-r str-r (544)}$	$\triangle - \triangle$
Curve D	$F^+ \times F^+$; $F^+ \text{ colI}^+ \text{ str-s (536)} \times F^+ \text{ colI-r str-r (544)}$	$\times - \times$

For details, see legend to LFC, Fig. 3. The transfer at each time is based on scoring between 400 and 1000 recipient clones for colicinogeny.

Fig. 5. The effect of integrated *F* (Hfr) on LFC transfer of *colI*. The curves show the transfer of *colI* in the following strains:

Curve A	$F^- \times F^-$; $F^- \text{ colI}^+ \text{ str-s (519)} \times F^- \text{ colI-r str-r (450)}$	$\times - \times$
Curve B	$\text{Hfr} \times F^-$; $\text{Hfr colI}^+ \text{ str-s (733)} \times F^- \text{ colI-r str-r (550)}$	$\bigcirc - \bigcirc$
Curve C	$F^- \times \text{Hfr}$; $F^- \text{ colI}^+ \text{ str-s (519)} \times \text{Hfr colI-r str-r (730)}$	$\triangle - \triangle$

For details see Fig. 3. Points on curve are based on 400 to 1000 recipient colonies scored for *colI*⁺.

The effect of *colI* on *F* transfer

(i) The effect of *colI* transfer under LFC conditions on *F* transfer when *colI* was present in the cross, 58-161 *colI*⁺*F*⁺ $\times$ w677 *F*⁻ was negligible. Similarly, when *colI* was present in either strain of the mixture 58-161 *F*⁺ $\times$ 58-161 *F*⁻, *F* transfer was little affected, and was in all cases about 15 % (± 1.5 %) after incubation for 3 hr, reaching about 25 % (± 2.5 %) after 18 hr. Moreover, there appeared to be little linked transfer of the two factors from a *F*⁺ *colI*⁺ strain, some 15 % of the recipients

becoming F^+ after 3 hr and about 10 % $colI^+$, whereas only 2 % had acquired both characters to become $F^+ colI^+$. To investigate whether this co-transfer involved associated structures carrying both the F and $colI$ factors, a second round of transfer was studied from several of these newly infected 58-161 $F^+ colI^+$ strains to

Fig. 6. The effect of autonomous F on HFC transfer of $colI$. The curves show the transfer of $colI$ in the following strains:

	Donor	Intermediate	Recipient	
Curve A	$F^- \times F^-$; $F^- colI^+ (519)$	$F^- (547)$	$F^- colI^- str-r (28)$	○—○
Curve B	$F^+ \times F^-$; $F^+ colI^+ (536)$	$F^+ (535)$	$F^- colI^- str-r (28)$	●—●
Curve C	$F^- \times F^+$; $F^- colI^+ (519)$	$F^- (547)$	$F^+ colI^- str-r (27)$	△—△
Curve D	$F^+ \times F^+$; $F^+ colI^+ (536)$	$F^+ (535)$	$F^+ colI^- str-r (27)$	×—×

HFC cultures were prepared in the usual way and mixed at 1:1 ratio with 10^8 recipient cells/ml. Between 800 and 1000 recipient clones were tested for acquisition of colicinogeny at each time.

Table 4. F transfer under HFC conditions

In F^+ donor or F^- recipient mixtures, usual HFC conditions were used. Log. phase cultures (10^8 cell/ml.) of F^+ donor and F^- recipient were mixed 1:1 and incubated for 2 hr. Clones from the recipient cells were then isolated on streptomycin agar and tested for F and $colI$.

F ⁺ donor (preparation)		F ⁻ recipient (preparation)	% F transfer to recipient	% colI transfer to recipient
Donor	Intermediate			
F ⁺ (535)	—	F ⁻ str-r (550)	19.6	—
F ⁺ colI ⁺ (536)	F ⁺ (535)	F ⁻ str-r (550)	32.4	100
F ⁺ colI ⁺ (536)	F ⁻ (547)	F ⁻ str-r (550)	1	95
F ⁺ (535)	—	HFC system: F ⁻ colI ⁺ str-s (519) + F ⁻ str-r (550)	40.5	100

w677 $F^- col^-$ strains. In all crosses, F transfer achieved the expected value of 100 % in 3 hr, whereas $colI$ transfer remained at 5-6 % and was similar to the transfer found with 58-161 $F^+ colI^+$ donors, in which the two factors had been introduced independently into the 58-161 strain.

(ii) *The effect of HFC on F transfer* was investigated by using F⁺ and F⁻ strains newly infected with *colI*. Table 4 shows that F transfer from such a newly *colI* infected strain (line 2) was increased from about 20 to about 32 %, and a similar increase obtained when an F⁻ strain newly infected with *colI* was mixed with an F⁺ *col*⁻ strain (line 4).

The effect of colI on F⁺ and Hfr fertility

The presence of F, either in the autonomous (F⁺) or integrated (Hfr) state, in *Escherichia coli* K12 strains is shown by the ability of such strains to act as genetic donors, resulting in chromosomal transfer to a recipient strain. In addition, such genetic donors of either F⁺ or Hfr type adsorb and plate a male-specific phage which is not adsorbed by F⁻ strains (Loeb, 1960; Dettori *et al.* 1961). F⁺ *colI*⁺ and Hfr *colI*⁺ strains were found to plate the phage μ of Dettori *et al.* as efficiently as their non-colicinogenic counterparts. F⁻ strains did not plate this phage to any extent (<1 in 10¹¹), and F⁻ *colI*⁺ strains were equally insensitive. Similar results were found whether the *colI* factor had been newly introduced into these strains, or when established colicinogenic strains were used as indicators.

The effect of *colI*, either as a newly introduced or as a stable factor, on the ability of F⁺ and Hfr strains to act as chromosomal donors was studied in crosses with 58-161 F⁺ and Hfr Hayes. In these crosses, transfer of the chromosomal markers *thr* and *leu* to the recipient w677 F⁻ was investigated (Table 5). In the F⁺ cross, there was no apparent effect on *thr leu* transfer, and in the Hfr cross there was a threefold increase when *colI*⁺ was present in the Hfr strain (this may, however, have been within the limits of experimental variation).

Table 5. *The effect of the presence, or new infection of colI on the transfer of the chromosomal markers thr and leu from Hfr and F⁺ strains*

Five ml. of log.-phase cultures of F⁺ (methionineless) or Hfr (prototrophic) strains, both *str*⁻s, were mixed with 5 ml. of log.-phase recipient cultures *thr leu str-r colI-r* (28) and incubated for 2 hr. Samples selected on streptomycin nutrient agar (recipient F⁻) were then tested for colicinogeny. Prototrophic *str-r* recombinants were selected on streptomycin minimal medium plates: 100 recombinants from each cross being tested for *colI*⁺.

Donor (preparation)		No. of <i>thr</i> ⁺ <i>leu</i> ⁺ recombinants ($\times 10^{-3}$)*	Fraction of <i>colI</i> ⁺ among recipient F ⁻ (%)	Fraction of <i>colI</i> ⁺ among <i>thr</i> ⁺ <i>leu</i> ⁺ recombinants (%)
Donor	Intermediate			
F ⁺ (535)	—	3.12	—	—
F ⁺ <i>colI</i> ⁺ (536)	—	2.41	0.8	51.2
F ⁺ <i>colI</i> ⁺ (536),	F ⁺ (535)	3.52	44	100
Hfr (735)	—	1010	—	—
Hfr <i>colI</i> ⁺ (733)	—	3880	2.6	10
Hfr <i>colI</i> ⁺ (733)	Hfr (735)	3140	4	15

* Average of 3 plates.

Transfer of colI to recombinants

Table 5 shows that in the two F⁺ mediated crosses, *colI* transfer to the recombinants was much increased as compared to the population as a whole. The possibility that *colI* was transferred to these recombinants as a chromosomal marker was therefore examined in a series of crosses with Hfr *colI*⁺ strains which differed in

their 'origines' and directions of transfer, selecting in each instance for the same three classes of recombinants *pro*⁺, *his*⁺ and *try*⁺. Table 6 suggests that irrespective of the marker selected, or the donor Hfr strain used, the transfer of *colI* was higher, the later the time of entry of the selected marker.

Table 6. *colI* transfer in crosses of Hfr *colI*⁺ strains with the same recipient strain J62 (*try*, *pro*, *his*, *str-r colI-r F*⁻)

Log.-phase cultures of the Hfr strains (*colI*⁺ *str-s*) and of the recipient 765 (*pro*, *his*, *try*, *T6-r*, *str-r*, *colI-r*), both at about 10⁸ cells/ml., were mixed in equal volumes and incubated for 2 hr. At various times, samples were removed, infected with T6, 'blended', and recombinants selected, from which the time of entry of the marker was determined (Jacob & Wollman, 1961). After 2 hr, unblended samples were plated to select for recombinants which were tested for the acquisition of *colI*⁺.

Hfr strain	Fraction of <i>colI</i> ⁺ among F ⁻ recipients (<i>str-r</i>) (%)	Selected markers								
		histidine (<i>his</i> ⁺)			tryptophan (<i>try</i> ⁺)			proline (<i>pro</i> ⁺)		
		Time of entry (<i>his</i>) (min.)	% rec		Time of entry (<i>try</i>) (min.)	% rec		Time of entry (<i>pro</i>) (min.)	% rec	
			% <i>colI</i> ⁺	% <i>colI</i> ⁺		% <i>colI</i> ⁺	% <i>colI</i> ⁺		% <i>colI</i> ⁺	% <i>colI</i> ⁺
AB 811 (763)	1.4	11	50	6	36	5	7	56	0.016	41
P 10 (764)	3.2	58	0.8	11	83	0.03	16	103	0.003	55
Δ Hfr (733)	7.7	59	0.03	27	34	0.9	17	14	40	14

DISCUSSION

Readsorption of colicin in colicinogenic cultures

The indifference of a bacterial strain to the lethal action of a colicin can arise as a result of two distinct phenomena. The first, a mutation which involves the loss of a cell-wall receptor, is usually termed 'resistance', and is denoted genetically by the transition *col-s* to *col-r*, two allelic markers which have been mapped chromosomally with certain colicins (Frédéricq & Betz-Bareau, 1953; Jenkin & Rowley, 1955). The second is by acquisition of the colicin factor to produce a state of colicinogeny in the strain. Such colicinogenic (*col*⁺) strains are assumed to adsorb colicin as readily as their non-colicinogenic (*col*⁻) counterparts, but in some unknown way its lethal action is impaired, and the cell is said to be 'immune' (Frédéricq, 1956). The characteristics of resistance and immunity are independent, so that although a strain which is sensitive to colicin action must be *col*⁻ *col-s*, an indifferent strain may be either resistant (*col*⁻ *col-r*), immune (*col*⁺ *col-s*), or both (*col*⁺ *col-r*).

The amount of free colicin liberated by a culture of a colicinogenic strain appears, in the case of colicin I, to depend on whether or not this strain is genetically resistant to the colicin. In general, only a minority (10⁻³–10⁻⁵) of the cells of a colicinogenic culture release colicin (Ozeki *et al.* 1959). It is assumed that much of this colicin is adsorbed by the non-producing cells in a non-resistant *colI*⁺ culture (*colI*⁺ *col-s*), and is responsible for decreasing the amount of free colicin in culture filtrates as compared with that found in colicin-resistant (*colI*⁺ *colI-r*) strains. Plate 1 shows this phenomenon, where a number of *colI*⁺ cells of both *col-s* and *col-r* genotype were plated to form surface colonies, and after sterilization with chloroform vapour were overlaid with a sensitive (*col*⁻ *col-s*) indicator. Small and large inhibition zones characteristic of the *colI*⁺ *colI-s* and *colI*⁺ *colI-r* strains respectively are visible.

However, where a *colI*⁺ *colI*-s colony has grown within the normal inhibition zone of a *colI*⁺ *colI*-r colony, the zone was less extensive, presumably due to adsorption of colicin by the *colI*⁺ *colI*-s colony.

Sex factor activities of colI

Since the colicin I factor can effect its own transfer in the absence of the *F* factor, it may be regarded as a sex-factor, and this conclusion is implicit in its ability to mediate chromosomal transfer in *Salmonella* (Ozeki & Howarth, 1961) and in *Escherichia coli* (Clowes, 1961, 1963*b*). In *F*⁺ and Hfr cells, the presence of the *F* factor results in the formation of a specific cell-surface component which has been revealed by its sensitivity to periodate treatment (Sneath & Lederberg, 1961), by its ability to adsorb and plate a specific phage (Loeb, 1960; Dettori *et al.* 1961) and by immunological methods (Ørskov & Ørskov, 1960). These activities may be due to the presence of a single surface component known either as 'mating substance' (Sneath & Lederberg), 'male-specific phage receptor' (Dettori *et al.*) or 'f' antigen (Ørskov & Ørskov), although a complete loss of one activity, such as phage adsorption, may not necessarily be accompanied by the loss of another, such as the ability to produce mating substance and to form recombinants (Clowes, 1963*b*). A similar mating substance may also be produced, in at least a small proportion of cells of a culture carrying the *colI* factor, permitting such cells to aggregate with cells of the complementary *colI*⁻ surface. Such contacts may be seen as cell clumps when *colI*⁺ and *colI*⁻ cultures of *E. coli* are mixed, although no aggregation of cells is seen in pure cultures of either of the individual strains. However, this postulated *colI* mating substance does not appear to mask, and is distinct from, the *F* mating substance. This is shown by the ability of male-specific phage (μ) to plate on cells harbouring *F*, irrespective of whether these cells also carry *colI*, and by the inability of cells carrying *colI*, but not *F*, to plate this phage. These features are also seen in HFC cultures when most cells are able to conjugate and presumably to produce the specific *colI* surface. Furthermore, the surface change produced by *colI* differs from a similar change induced in *E. coli* cells by the presence of RTF (resistance transfer factor) which confers infectious transfer of multiple drug resistance (see Watanabe, 1963). The ability to plate male-specific phage is lost by both *F*⁺ and Hfr strains when they also harbour RTF (Watanabe, Fukasawa & Takano, 1962).

The numbers of 'competent *colI* donors' of about 1 % found in *Escherichia coli* strains appears to be much greater than in *Salmonella*, where the corresponding value is about 1 in 6000 (Ozeki *et al.* 1962). This agrees with the finding of cell clumps in mixtures of *colI*⁺ and *colI*⁻ cultures of *E. coli*, whereas cell clumps are not observed in *Salmonella* except under HFC conditions (in *E. coli* also, a greatly enhanced aggregation of cells is seen in HFC cultures). Moreover, the rate of *colI* transfer in *E. coli* was higher, attaining between 7 and 10 % after contact for 2 hr, whereas in *Salmonella* at this time, less than 0.01 % transfer was observed (Ozeki *et al.* 1962). This difference probably accounts for the ability of stably *colI*⁺ strains of *E. coli* to mediate chromosomal transfer (Clowes, 1961), whereas in *Salmonella*, chromosomal transfer is mediated only by cells newly infected with *colI* (Ozeki & Howarth, 1961).

Such newly infected cells become more efficient donors of *colI* (Stocker *et al.* 1963). The curves shown in Fig. 2 are best interpreted on the assumption that *Escherichia*

coli cells which have recently acquired colicinogeny are also enhanced as donors of *colI*. The transfer of *colI* may thus be regarded as a two part process: a first phase largely due to transfer from the original donor to the recipient, and a second phase predominating after this time, in which transfer occurs from newly infected recipients to non-infected recipients, leading to an exponential increase in the numbers of cells which acquire colicinogeny and termed 'epidemic spread' (Ozeki *et al.* 1962). Under these circumstances optimal transfer obtains when the donor:recipient ratio is low, so that recipient to recipient contacts will be frequent and therefore favour the rapid initiation of this epidemic spread. The transfer of *colI* under high-frequency conditions (HFC) shown in Fig. 3, supports the conclusion that cells newly infected become highly efficient as donors of *colI*. The transfer appears more rapid in *E. coli* than in *Salmonella*, and transfer of *colI* to as many as 90 % of the recipients may occur within the first hour. The duration of this high infectivity appears to be about 4 to 6 generations, and is similar to *Salmonella* (Stocker *et al.* 1963).

The effect of F on colI transfer

The effect of *F* on *colI* transfer is readily interpretable when the kinetic curves shown in Fig. 4 are considered as a two phase process made up of an initial period during which donor:recipient transfer occurs, after which the recipient:recipient transfer predominates. The presence of *F* in the *colI*⁺ donor decreases this early transfer, from which it is concluded that the *F* factor is epistatic to the *colI* factor and in some way suppresses its activity. This epistasis could be due to suppression or masking of the proposed *colI* specific mating surface by that of the *F* mating surface. Furthermore, *colI* transfer does not occur through *F*-mediated contacts, since *F* transfer itself may occur at a rapid rate in the absence of significant *colI* transfer. Transfer of *colI* at a decreased rate is suggested to occur only from those *F*⁺ cells which do not produce the *F* mating surface, and which are known to appear as *F*⁻ phenocopies (Lederberg, Cavalli & Lederberg, 1952) as cells pass from the logarithmic phase to the stationary phase of growth. After the first few hours, recipient:recipient, *F*⁻*colI*⁺ × *F*⁻*colI*⁻ transfer seems likely to account for the shape of the curve, which is similar to that of the control curve, the recipient *col*⁻ strain being in both instances *F*⁻.

In contrast, the presence of *F* in the recipient strain (*F*⁻ × *F*⁺) appears to enhance early transfer (Fig. 4, curve C). Here the *col*⁻ strain produces the *F* mating substance, which may form *F* mediated contacts with the *F*⁻*colI*⁺ strain. Such contacts would be likely to hold cells of the two populations together, so as to either stabilize any specific *colI*-mediated contacts made by the *F*⁻*colI*⁺ cells or increase the probability that such contacts would be formed. The enhancement of transfer found in the early stages is preserved in the later recipient:recipient phase, from which it is inferred that the epistasis of *colI* by *F*, suggested to exist in established *colI*⁺ strains, does not occur to the same extent in cells newly infected with *colI*. In *F*⁺ × *F*⁺ mixtures (Fig. 4, curve D), initial transfer appears to be enhanced, but the increase is not so marked as in curve C. Here the putative *F*⁻ phenocopies in which *colI* is expressed have an increased probability of forming specific *F*-mediated contacts with the *col*⁻ strain which is also *F*⁺. The effect of this *F* directed mating is suggested to outweigh the reduction in the total numbers of *colI*⁺ donors due to the presence of *F*, since this early transfer is generally greater than that in the *F*⁺

col⁺ × *F*⁻ *col*⁻ cross. The latter part of the transfer kinetics is likely to resemble that in the *F*⁻ × *F*⁺ mixture due to the emergence of a similar *F*⁺ *col*⁺ × *F*⁺ *col*⁻, recipient:recipient transfer process.

In Hfr strains (Fig. 5), similar interpretations hold, although there are indications that epistasis is more marked (Fig. 5, curve B). In curve C there is an enhanced early transfer of *colI* from an *F*⁻ to an Hfr strain, which occurs under conditions in which a presumptive chromosomal transfer is proceeding in the majority of cells in the opposite direction. There is however no evidence to support the idea that the same pair of cells may at the same time be able to transfer chromosome in one direction through one contact, and colicin factor through another.

The results in HFC systems (Fig. 6) support the idea that the *F* epistasis of *colI* is largely overcome in newly-infected cells. The effect persists to a small extent in decreasing the efficiency of *colI* transfer from *F*⁺ strains, although the majority of cells are still able to transfer *colI* very rapidly. Similarly, when *F* is present in the recipient, enhanced transfer obtains, so that *F* contacts appear to be able to supplement even this high degree of *colI*-mediated contacts.

The effects of colI on F-mediated activities

No effects of *colI* on *F*-mediated functions are found under LFC conditions; the expression of the *F* mating surface is little affected as judged either by the plating efficiency of male-specific phage, or the fertility of these strains. The apparent inhibition of *colI*-mediated conjugation by *F* thus appears to be true epistasis, since there is no suppression in the opposite sense. However, under HFC conditions *F* transfer may actually be *increased* after new infection with *colI* of either the *F*⁺ or the *F*⁻ cell. In the latter case a high degree of *colI*-mediated contacts made by *F*⁻ *col*⁺ cells is likely to assist and stabilize the formation of *F*-mediated contacts by the *F*⁺ *col*⁻ strain. For the enhancement of *F* transfer by *F*⁺ cells newly infected with *colI*, the possibility exists that either the *F* factor can be transferred through a *colI*-mediated contact, or what seems more likely in view of the fact that judged by phage μ plating the *F* surface is unimpaired even in HFC mixtures, that the sites of both the *colI* and *F* specific mating surfaces are restricted to only part of the cell surface, and are topologically distinct. Similar conclusions have been drawn in previous publications (Jacob, Brenner & Cuzin, 1963; Clowes, 1963*b*).

Genetic classification of colicin factors

The transfer of *colI* to recombinants arising from both *F*⁺ and Hfr crosses is greater than to the recipient population at large (Tables 5, 6). Selection for recombinants selects those cells which have been in contact for longer than average periods to allow time for chromosomal transfer; this is slow, the complete transfer taking 110–120 min. (Jacob & Wollman, 1961). It is suggested that there is a greater probability of *colI*-mediated contact and transfer in cells united over these long periods.

This idea is supported by the results of the three Hfr crosses shown in Table 6. After 2 hr, transfer of *colI* to the *F*⁻ recipients varied from 1.4 to 7.7 %, depending on the Hfr strain used. At this time, transfer to all recombinant classes was higher, even though some markers were proximal and entered early. Moreover, in each

cross, irrespective of the marker in question, or the Hfr donor strain, the transfer to recombinants was greater as the time of entry of the selected marker was later. Thus, in selecting for *his*⁺, *try*⁺ and *pro*⁺ recombinants from Hfr_{AB311}, where the respective times of marker entry were around 11, 36 and 56 min. (such times representing the minimum time that the donor and recipient cells have been in contact) transfer of *colI* was 6, 7 and 41 %, respectively. With Hfr_{P10}, where this particular order of entry was preserved, but where the entry of each marker occurred about 50 min. later, these frequencies were, respectively, 11, 16, and 55 %. In contrast, with Hfr Hayes, where the order of entry is reversed, *pro*⁺ entering at 14, *try*⁺ at 34, and *his* at 59 min., the proportion of each of these recombinant classes acquiring *colI* were 15, 17 and 27%. These results show no correlation of *colI* transfer with any particular chromosomal segment, but support the idea that selection for distal (late) markers selects for cell pairs held together by Hfr to F⁻ surface interactions for periods longer than the time of entry of the marker in question, so increasing the period over which extra-chromosomal *colI* transfer may take place, presumably through topologically distinct *colI*-mediated contacts. Alternatively, it is possible that *colI* is attached together with *F* to the terminal chromosomal segment, in which case selection for later markers would increase the probability that the distal end of the chromosome will enter. However 'interrupted' matings, when donor and recipient are separated after periods of contact of 30 min. or less, invariably give rise to some *col*⁺ recombinants among the early markers selected. This alternative would therefore require that, in addition to the transfer of *colI* on the tail of the chromosome, it is also transferred extra-chromosomally, and for this reason seems unlikely. Moreover, there is no indication of *F* and *colI* linkage in transfer experiments from F⁺ *colI*⁺ cells. Both factors are therefore suggested to exist as independent extra-chromosomal elements in F⁺ cells, the *colI* factor maintaining this situation when the *F* factor is chromosomally integrated in Hfr strains.

Since these experiments were initiated, experiments reported by Nagel de Zwaig, Anton & Puig (1962), suggest that *colI*, *colV* and *colE2* may be transferred as extra-chromosomal elements. *ColE1* is the only colicin factor for which a chromosomal site has been claimed (Alfoldi, Jacob, Wollman & Mazé, 1958) from which it was suggested that all colicin factors were episomes (Jacob, Schaeffer & Wollman, 1960). This was re-investigated by Clowes (1963*a*), and from more extensive experimental evidence it was concluded that *colE1* was in fact transferred extra-chromosomally, with the suggestion that all colicin factors are best considered as plasmids (Lederberg, 1952) having only a cytoplasmic existence, until a direct demonstration of chromosomal location permits their recognition as episomes.

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EXPLANATION OF PLATE

Fig. 1. Relative inhibition zone sizes of *colI*⁺.*colI*-s and *colI*⁺.*colI*-r strains. A mixture of *colI*⁺.*colI*-s (519) and *colI*⁺.*colI*-r (554) was diluted and plated on nutrient agar to produce isolated surface colonies. After overnight incubation, the plate was treated with chloroform vapour, then over-layered with about 10⁸ cells of a *colI*-s indicator (511) in 2.5 ml. soft agar and re-incubated over-night.