The Genetic Control of Colicinogenic Factors E_2 , I and V

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SUMMARY

The state of colicinogenic factors E_2 , I and V (*col E₂*, *col I* and *col V*) in *Escherichia coli* K12 was studied. The analysis of the results of conjugation experiments involving different Hfr or F^+ and F^- strains shows that: (1) The frequency of transfer of these colicinogenic factors differs markedly: *col V* is transferred with maximum efficiency, *col E₂* and *col I*, on the contrary, only at low frequency. (2) Each colicinogenic factor is transferred from different types of Hfr or F^+ strains with similar frequencies. (3) They are not linked with chromosomal markers. (4) The non-colicinogenic character of the donor parent is never transmitted to recombinants. (5) Zygotes that have received *col V*, transmit it to all daughter cells. All these results lead to the conclusion that colicinogenic factors E_2 , I and V are in an extrachromosomal state in F^+ and Hfr bacteria. The same conclusion would apply to their state in F^- bacteria, since the results with colicinogenic factor V seem to indicate that this factor replicates in this type of bacteria autonomously and at a faster rate than the chromosome.

INTRODUCTION

Colicines are antibiotic substances produced by certain strains of Enterobacteriaceae that have a lethal action on other strains of the same family. Their synthesis is controlled by hereditary factors called colicinogenic factors.

The genetic behaviour of the colicinogenic factor E_1 in crosses between F^+ and F^- strains of *Escherichia coli* K12 was studied by Fredericq & Betz-Bareau (1953). In crosses involving colicinogenic (*col⁺*) F^+ and non-colicinogenic (*col⁻*) F^- bacteria, the *col⁺* character was transferred to recombinants; in reciprocal crosses F^+ *col⁻* $\times$ F^- *col⁺* recombinants never received the *col⁻* character belonging to the donor parent. From the asymmetric transfer of *col* in these crosses and from the absence of linkage with chromosomal markers they concluded that this factor was of cytoplasmic nature.

The transfer of colicinogenic factor E_1 (*col E₁*) derived from *E. coli* K30 was also studied in Hfr $\times$ F^- matings by Alföldi, Jacob, Wollman & Mazé (1958). Their results showed that the frequency of transfer of the *col⁺* character in crosses

between Hfr *col*⁺ bacteria belonging to different Hfr types and F⁻ *col*⁻ bacteria varied according to the type of Hfr used in the cross and led them to conclude that *col E*₁ is localized in the chromosome of the Hfr between *thr leu* and *met thi*. Similar conclusions were drawn from reciprocal crosses Hfr *col*⁻ × F⁻ *col*⁺. In these crosses *col*⁻ did not appear among recombinants and a lethal zygosis was found to occur with each type of Hfr with a frequency corresponding to that of the transfer of *col*⁺ in the reciprocal cross. This lethality was assumed to be due to the penetration of *col*⁻ into the F⁻ cell.

They also found that once transferred to the zygote, the *col*⁺ character passed to all daughter cells without segregating. This indicated that *col*⁺ can multiply in the F⁻ autonomously and at a greater rate than the chromosome.

Thus, colicinogenic factor E₁, when present in the cell, can exist either in an integrated state (in the chromosome of the Hfr) or in an autonomous state (in F⁺ and F⁻ bacteria). These properties pointed to the inclusion of colicinogenic factor E₁ in the category of episomes (Jacob & Wollman, 1958*b*).

The peculiar behaviour of this factor posed, then, the question of whether it could be considered as representative or characteristic of all colicinogenic factors. The study of other colicinogenic factors was undertaken in an attempt to solve this question.

The present paper is concerned with an analysis of the state of colicinogenic factors E₂, I and V in strains of *E. coli* K12. To make the results comparable to those of Alföldi *et al.* with *col E*₁, similar strains and methods were employed in this study.

MATERIALS

Media. The synthetic medium employed was that of Wollman & Jacob (1959). The minimal medium was supplemented before use with different amino acids, depending on the selective requirements, at a final concentration of 80 µg./ml.

Other media employed were: nutrient broth and nutrient agar (nutrient broth with 2 % of agar).

Streptomycin, when required, was added at a concentration of 200 µg/ml.

Bacterial strains. The strains used in the experiments are described in Table 1 and were kindly supplied by Professor P. Fredericq, Dr E. L. Wollman and Dr E. Balbinder.

Strain 58-161 was utilized as sensitive indicator of colicines, and resistant strains derived from it, as resistant ones.

All strains employed in matings were previously made resistant to the colicine considered, to eliminate the lethal effect of the colicine on sensitive strains.

METHODS

Hfr and F⁺ strains were made colicinogenic by mixed culture with the original F⁺ donor strains: 12-317, CA 53 and 12-94. The mixture was incubated overnight in nutrient broth at 37°. Aliquots were plated on appropriate selective media and *col*⁺ colonies detected by the double agar-layer method (Fredericq, 1954). Colicinogenic factors were transferred to the F⁻ cells by the same method, employing Hfr colicinogenic strains as donors instead of the F⁺ cells to prevent transmission of the F factor.

Table 1. *Bacterial strains*

Name	Characteristics	Origin	References
<i>E. coli</i> CA 53	Prototrophic <i>str-s</i> col I ⁺ F ⁺	*	—
<i>E. coli</i> K12			
12-94	<i>met</i> ⁻ <i>str-r</i> col V ⁺ F ⁺	†	—
12-317	<i>cys</i> ⁻ <i>his</i> ⁻ <i>str-r</i> col E ₂ ⁺ F ⁺	†	—
58-161	<i>met</i> ⁻ <i>str-r</i> F ⁻	—	Tatum & Lederberg (1947)
Hfr H	<i>thi</i> ⁻ <i>str-s</i>	—	Hayes (1953)
Hfr type 4	<i>thr</i> ⁻ <i>leu</i> ⁻ <i>thi</i> ⁻ <i>str-s</i>	C 600	Jacob & Wollman (1957)
Hfr type 2	<i>met</i> ⁻ <i>str-s</i>	58-161	Jacob & Wollman (1957)
Hfr AT IIIA	Prototrophic <i>str-s</i>	AB311	Taylor & Adelberg (1960)
112	<i>cys</i> ⁻ <i>his</i> ⁻ <i>str-s</i> F ⁺	—	Wollman (1953), Wollman & Jacob (1959)
PA 309	<i>thr</i> ⁻ <i>leu</i> ⁻ <i>thi</i> ⁻ <i>his</i> ⁻ <i>try</i> ⁻ <i>arg</i> ⁻ <i>str-r</i> F ⁻	P 678 <i>str-r</i>	Jacob & Wollman (1958 a)

* This strain was sent by Professor P. Fredericq.

† These strains were received from Cold Spr. Harbor and belonged to Professor P. Fredericq.

Abbreviations: Requirement for *thr*: Threonine; *leu*: Leucine; *try*: Tryptophan; *his*: Histidine; *arg*: Arginine; *met*: Methionine; *cys*: Cysteine; *pro*: Proline; *thi*: Vitamin B₁; *str-r*: Resistance to streptomycin; *str-s*: Sensitivity to streptomycin; *lac*: Fermentation of lactose.

Fig. 1. Genetic map of *E. coli* K12. Symbols are defined in Table 1. The genetic markers employed are indicated on the outer circle (Wollman & Jacob, 1959; Taylor & Adelberg, 1960). The origin and direction of each Hfr is indicated on the inner ones.

Matings were performed by mixing exponentially growing cultures of donor (Hfr or F⁺) and recipient (F⁻) cells in titre of about 2×10^8 bacteria per ml., in proportion of 1 donor:20 recipient cells or 20 donors:1 recipient cell. The mixture, of a total volume of 4 ml., was incubated at 37° in 200 ml. Erlenmeyer flasks. Samples were withdrawn at different intervals, diluted and plated on selective media.

For interruption of mating, samples were treated in Waring Blendor (Wollman & Jacob, 1955) during 1 min.

Recombinants, such as *thr*⁺ *leu*⁺ *str-r*, *try*⁺ *str-r*, *his*⁺ *str-r*, etc., receiving the ability to synthesize an amino acid or vitamin from the donor and the *str-r* character from the recipient, were selected on synthetic medium supplemented with streptomycin and the corresponding amino acids.

In Hfr *col*⁺ × F⁻ *col*⁻ and F⁺ *col*⁺ × F⁻ *col*⁻ crosses, the transfer of the *col*⁺ character was studied by plating a suitable dilution of the mating mixture on agar plates with streptomycin where only F⁻ cells form colonies; the *col*⁺ character of these colonies was determined by the halos formed on a sensitive indicator strain.

Unless otherwise stated, the frequency of recombinants was measured at 120 min. after mixing the strains, and is defined as the ratio of number of recombinants to number of the minority parental strain, multiplied by 100.

The transfer of *col*⁺ was measured, like the frequency of recombinants, by the ratios the number of *col*⁺ F⁻ *str-r* cells that received the *col*⁺ character from the donor parent to the number of the minority parental strain, multiplied by 100.

In every crossing experiment the frequency of transmission to recombinants of an early Hfr marker was measured, as a control of the efficiency of that Hfr as a donor.

Crosses of *col*⁺ × *col*⁻ and *col*⁻ × *col*⁻ were performed as controls of the corresponding *col*⁺ × *col*⁻ and *col*⁻ × *col*⁺ crosses.

RESULTS

Matings were generally performed in proportion of 1 Hfr:20 F⁻ bacteria, except that the proportion of 20 Hfr:1 F⁻ was specially employed in *col*⁻ × *col*⁺ crosses in order to verify if the 'lethal zygosis' observed with *col E*₁ (Alfoldi *et al.* 1958) occurred with *col E*₂, *col I* and *col V*.

In the majority of the experiments where the Hfr was in gross excess over the F⁻ cells (20 Hfr:1 F⁻) the frequency of recombinants was 1.5 to 5 times less than that obtained in the same crosses in proportion of 1 Hfr:20 F⁻, but, no decrease in number of the F⁻ cells was observed, except with Hfr H.

As can be seen from Table 2, in crosses performed with Hfr H as a donor in proportion of 20 Hfr:1 F⁻, the number of the F⁻ (*str-r* cells) showed a decrease ranging from 0 to 92 % referred to the viable count at the time of mixing.

This percentage is not constant in different experiments involving the same strains and sometimes no decrease in number is observed.

The possibility that this decrease in viable count of the F⁻ cell could be due to some type of agglutination in the presence of a large number of Hfr H cells was tested by treating the mating mixture in a Waring Blendor, before plating. No differences were observed between the treated samples and the control ones.

Although the reasons for this decrease in number of the F⁻ cells in these crosses as well as of the variability of the decrease, remain unknown, it must be emphasized that it was not found to have any relation to the transmission of *col*⁺ or *col*⁻, since it occurs also in control crosses *col*⁺ × *col*⁺ and *col*⁻ × *col*⁻ (Table 2).

No decrease in fertility due to colicinogenic factors was observed in crosses involving *col E*₂, *col I* and *col V* (Table 3), unlike the results of Fredericq & Betz-

Table 2. *Decrease in number of the F⁻ bacteria measured at 120 minutes after mixing, in crosses involving HfrH or strains derived from it, as donors*

Crosses	Frequency (in %) <i>thr⁺ leu⁺ str-r</i> recombinants	Decrease (in %) of F ⁻ bacteria
Hfr H × PA 309 <i>str-r</i>	0.7	92
	5	0
Hfr H <i>col I⁺</i> × PA 309 <i>str-r col I⁻</i>	0.6	79
	3.2	21
	3.4	0
Hfr H <i>col I⁺</i> × PA 309 <i>str-r col I⁺</i>	1.1	79
	6	50
Hfr H <i>col I⁻</i> × PA 309 <i>str-r col I⁻</i>	10	42
Hfr H <i>col I⁻</i> × PA 309 <i>str-r col I⁺</i>	10	50
	7.2	0
Hfr H <i>col E₂⁺</i> × PA 309 <i>str-r col E₂⁻</i>	6.2	0
	6	50
	2.6	65
Hfr H <i>col E₂⁺</i> × PA 309 <i>str-r col E₂⁺</i>	6.3	40
	1.6	0
Hfr H <i>col E₂⁻</i> × PA 309 <i>str-r col E₂⁺</i>	0.8	89
	5	0
Hfr H <i>col V⁺</i> × PA 309 <i>str-r col V⁻</i>	31	0

Crosses were performed with a ratio of 20 Hfr:1 F⁻. All strains were previously made resistant to the colicine involved in each case. The decrease of the F⁻ bacteria was calculated from the comparison of the viable count of F⁻ cells at 120 minutes after mixing with the count at the initial time.

Table 3. *Comparison of fertility in crosses involving colicinogenic factors, with control crosses*

Crosses	Frequency (in %) of recombinants for an early Hfr marker (<i>thr⁺ leu⁺ str-r</i>)
Hfr type 2 <i>col E₂⁺</i> × F ⁻ <i>col E₂⁻</i>	25
Hfr type 2 <i>col E₂⁺</i> × F ⁻ <i>col E₂⁺</i>	30 (control)
Hfr type 2 <i>col E₂⁻</i> × F ⁻ <i>col E₂⁺</i>	19
Hfr type 2 <i>col E₂⁻</i> × F ⁻ <i>col E₂⁻</i>	21 (control)
Hfr H <i>col I⁺</i> × F ⁻ <i>col I⁻</i>	32
Hfr H <i>col I⁺</i> × F ⁻ <i>col I⁺</i>	25 (control)
Hfr H <i>col I⁻</i> × F ⁻ <i>col I⁺</i>	32
Hfr H <i>col I⁻</i> × F ⁻ <i>col I⁻</i>	22 (control)
Hfr H <i>col V⁺</i> × F ⁻ <i>col V⁻</i>	40
Hfr H <i>col V⁺</i> × F ⁻ <i>col V⁺</i>	33 (control)
Hfr H <i>col V⁻</i> × F ⁻ <i>col V⁺</i>	33
Hfr H <i>col V⁻</i> × F ⁻ <i>col V⁻</i>	35 (control)

All crosses indicated were performed with a ratio of 1 Hfr:20 F⁻.

Strains were previously made resistant to the colicine employed in each cross.

Bareau (1956) with *col E*₁, *B*, *K* and *S3*. It cannot yet be asserted whether these differences reflect or not true differences in the behaviour of colicinogenic factors, since they could also be assigned to differences in the methods or strains employed.

*Colicinogenic factor E*₂

This factor is transferred by Hfr and F⁻ strains with frequencies of about 1 % (Table 4). Crosses were made in proportion 20:1 and 1:20 donor to recipient cells. No lethal zygosis was observed in crosses involving *col E*₂, regardless of which parent carried it. But in crosses with Hfr H performed with a ratio of 20 Hfr:1 F⁻, a decrease in number of the F⁻ (*str-r*) bacteria took place, but, as was explained before, without any relation to *col E*₂ (Table 2).

Table 4. *Crosses between Hfr col E*₂⁺ or F⁺ col E₂⁺ and PA 309 *str-r col E*₂⁻

	Hfr H		Hfr type 2		Hfr type 4		112 (F ⁺)
	20:1	1:20	20:1	1:20	20:1	1:20	20:1
Frequency (in %) of transfer of <i>col</i> ⁺	0.3	0.3	0.6	1.3	0.5	3	1.3
Frequency (in %) of recombinants for an early Hfr marker	3.6	44	4.2	18	10	40	—

20:1 indicates crosses performed with a ratio of 20 donors:1 recipient cell. 1:20 indicates crosses performed with a ratio of 1 donor:20 recipient cells. These strains were previously made resistant to colicine *E*₂.

Early selected markers from Hfr were, *thr*⁺ *leu*⁺ for Hfr H and Hfr type 2 and *arg*⁺ for Hfr type 4. Selected marker from F⁻, *str-r*.

Table 5. *Percentage of col E*₂⁺ among recombinants of crosses between Hfr col E₂⁺ and PA 309 *str-r col E*₂⁻

		Recombinants			
		<i>thr</i> ⁺ <i>leu</i> ⁺ <i>str-r</i>	<i>try</i> ⁺ <i>str-r</i>	<i>his</i> ⁺ <i>str-r</i>	<i>arg</i> ⁺ <i>str-r</i>
Donors	Hfr H { 20:1	0	0	2	7
	{ 1:20	0	0	0	2
	Hfr type 2 { 20:1	0.7	1.5	1.2	1.3
	{ 1:20	0	3	1	2
	Hfr type 4 { 20:1	—	0	0	0.4
	{ 1:20	—	1.5	0	0.3

100 to 400 colonies of each type of recombinants from crosses performed in proportion 20:1 and 1:20 Hfr to F⁻ cells, were analysed. The order of transfer of the Hfr markers is: Hfr H, *thr leu try his arg*; Hfr type 2, *leu thr arg his try*; Hfr type 4, *arg his try*.

Selected early markers from Hfr were, *thr*⁺ *leu*⁺ for Hfr H and Hfr type 2; *arg*⁺ for Hfr type 4. Selected marker from F⁻ = *str-r*.

The percentage of *col E*₂⁺ among F⁻ (*str-r*) cells at 120 min. after mating in crosses performed with a ratio of 1 Hfr:20 F⁻ cells was less than 0.1 %, with the different donor strains. The percentage of *col E*₂ among F⁻ cells, in matings performed in proportion of 20 Hfr:1 F⁻ cell was: 0.3 % with Hfr H and Hfr type 2 and 0.4 % with type 4 and 112 (F⁺).

Col⁺ × *col*⁻ crosses performed with Hfr strains of different type or with F⁺ as donor cells and the frequency of *col*⁺ *str-r* in each cross is registered in Table 4.

Great differences in frequencies of transfer of *col*⁺ from Hfr and F⁺ cells are not apparent since they vary only within 0.3 and 1.3 % in crosses performed with a ratio of 20 donor:1 recipient cell.

The percentage of *col*⁺ colonies among recombinants selected from 1:20 crosses (Table 5) is also low and reveals no linkage with chromosomal markers. Similar results were found in crosses 20:1. A direct correlation between the frequency of *col*⁺ in a recombinant for a given marker and the distance of this marker to the origin seems to exist, at least for Hfr H, since the percentage of *col*⁺ in a recombinant for a distal marker is greater than that corresponding to a recombinant for a nearer one. In addition the % of *str-r* F⁻ cells found to be *col*⁺ at 120 min. is given at the foot of Tables 5, 6 and 8.

Colicinogenic factor I

Colicinogenic factor I is transferred at even lower frequencies than colicinogenic factor *E*₂. To measure such low frequencies a large number of zygotes had to be analysed and crosses in proportion 20 donor cells:1 acceptor cell were considered most convenient for this purpose.

The crosses performed and the frequency of transfer of *col I*, are shown in Table 6.

Table 6. Crosses between Hfr *col I*⁺ or F⁺ *col I*⁺ and PA 309 *str-r col I*⁻

	Donors		
	Hfr H	Hfr type 4	CA 53 (F ⁺)
Frequency (in %) of transfer of <i>col</i> ⁺	< 0.1	0.1	0.5
Frequency (in %) of recombinants for early Hfr marker	4	15	—

These crosses were performed with a ratio of 20 donor:1 recipient cell. These strains were previously made resistant to colicine I. Early selected markers from Hfr were, *thr*⁺ *leu*⁺ for Hfr H and *arg*⁺ for Hfr type 4. Selected marker from F⁻, *str-r*.

The percentage of *col I*⁺ among F⁻ (*str-r*) cells at 120 min. after mating, in crosses performed with a ratio of 20 Hfr:1 F⁻ cell was 0.2 % with Hfr H and Hfr type 4 and 0.3 % with CA 53 (F⁺).

In crosses with Hfr H, a decrease in number of the F⁻ cells occurred both in *col*⁺ × *col*⁻ and *col*⁻ × *col*⁺ crosses as well as in *col*⁺ × *col*⁺ and *col*⁻ × *col*⁻ ones (Table 2).

The low frequency of transmission of the *col*⁺ character in a cross such as Hfr H *col I*⁺ × F⁻ *col I*⁻ could have been assumed to be due to the localization of *col*⁺ at the distal end of the Hfr chromosome. But, the fact that such low frequency is also observed with Hfr type 4, where a distal marker of Hfr H is proximal, shows that this is not the case here.

Colicinogenic factor V

The transfer of *col*⁺ in Hfr *col V*⁺ × F⁻ *col V*⁻ or F⁺ *col V*⁺ × F⁻ *col V*⁻ crosses performed with a ratio of 1 donor:20 recipient cells and measured at 120 min. after mixing the strains, is reported in Table 7.

In contrast to *col E*₂ and *col I*, colicinogenic factor V is transferred with *very high efficiency*, since its frequency greatly exceeds 100 % (of input of minority parent, in this case the Hfr) at the time considered (Table 7).

The time at which the *col*⁺ factor begins to appear in the F⁻ cell was determined in conjugations interrupted by Waring Blendor. Similar times of penetration of this factor were found employing different donor parents (Table 7).

The kinetics of transfer of an early Hfr marker and of the *col*⁺ character of the Hfr was studied in crosses where mating was interrupted. When the number of

col⁺ *str-r* cells (*F*⁻ cells that became *col*⁺) and *thr*⁺ *leu*⁺ *str-r* recombinants is plotted against time in Hfr type 2 *col V*⁺ × *F*⁻ *col V*⁻ cross, performed with ratio 1 donor:20 recipient cells (Fig. 2), it is observed that: (1) The slope of *col*⁺ *str-r* cells rises rapidly and linearly, soon after mating, similarly to *thr*⁺ *leu*⁺ *str-r* recombinants, till about 50 min. after mating, when *col*⁺ *str-r* cells reach a frequency of 100 % (of input of minority parent). (2) Then, they continue increasing at the same rate as the *F*⁻ cells of the population, thus attaining the frequency of 480 % measured at 120 min. (Table 7).

Fig. 2

Fig. 3

Fig. 2. The kinetics of transfer of *col*⁺ and *thr*⁺ *leu*⁺ in Hfr type 2 *col V*⁺ × PA 309 *str-r col V*⁻ cross, performed with a proportion of 1 donor:20 recipient cells. At different time intervals samples were taken, treated in the Waring Blendor, diluted and plated on selective media. To maintain the mixture in logarithmic phase, an aliquot was diluted 1/10 in nutrient broth at 100 min. after mixing and samples were withdrawn from it after 120 min. ×, *thr*⁺ *leu*⁺ *str-r* recombinants; Δ, *str-r* bacteria; □, *col*⁺ *str-r* bacteria.

Fig. 3. The kinetics of transfer of *col*⁺ in 112 (*F*⁺) *col V*⁺ × PA 309 *str-r col V*⁻ cross, performed with a proportion of 1 donor:20 recipient cells. Samples taken at different times were treated in a Waring Blendor, diluted and plated on nutrient agar plates with streptomycin. □, *col*⁺ *str-r* bacteria; Δ, *str-r* bacteria.

Table 7. Crosses between Hfr *col V*⁺ or *F*⁺ *col V*⁺ and PA 309 *str-r col V*⁻

	Donors			
	Hfr H	Hfr type 2	Hfr AT IIIA	112 (<i>F</i> ⁺)
Frequency (in %) of transfer of <i>col</i> ⁺	850	480	2100	1100
Frequency (in %) of a recombinant for an early Hfr marker	24	25	41	—
Time (in minutes after mixing) or penetration of <i>col</i> ⁺ in the recipient cell	6	13	5-10	8

These crosses were performed with a ratio of 1 donor:20 recipient cells.

The strains were previously made resistant to colicine V.

The time at which the *col*⁺ character first appears in the *F*⁻ cell (time of penetration) was determined in conjugations interrupted by Waring Blendor.

On the contrary, *thr*⁺ *leu*⁺ *str-r* recombinants after their initial rise reach a plateau, between 60 and 120 min., and only then begin to multiply at the same rate as the other cells.

In the other crosses reported in Table 7, *col*⁺ *str-r* cells also reach a frequency of 100 % about 50 min. after mating, that is at the time when the maximum frequency of conjugation for chromosomal recombinants is achieved.

In the 112 F⁺ *col V*⁺ × F⁻ *col V*⁻ cross (Fig. 3) the slope of *col*⁺ *str-r* bacteria during the first 50 min. is the same as that of this type of bacteria in the Hfr type 2 *col V*⁺ × F⁻ *col V*⁻ cross. Later, the slope decreases, although remaining steeper than that corresponding to the division of *str-r* cells. This suggests that the increase of *col*⁺ *str-r* cells between 60 and 120 min. is due to some additional reason besides that corresponding to cell division.

The analysis of the percentage of *col*⁺ in recombinants selected in Hfr *col V*⁺ × F⁻ *col V*⁻ crosses (Table 8) indicates that: (1) there is no linkage with chromosomal markers; (2) there is a slight increase of the percentage of *col*⁺ in recombinants for distal markers of the Hfr cells over those for proximal ones.

Table 8. Percentage of *col V*⁺ among recombinants of crosses between Hfr *col V*⁺ and PA 309 *str-r col V*⁻

Donors	Recombinants			
	<i>thr</i> ⁺ <i>leu</i> ⁺ <i>str-r</i>	<i>try</i> ⁺ <i>str-r</i>	<i>his</i> ⁺ <i>str-r</i>	<i>arg</i> ⁺ <i>str-r</i>
Hfr H	79	91	100	100
Hfr type 2	80	100	100	84
Hfr AT IIIA	100	98	92	100

Crosses indicated were performed with a proportion of 1 donor:20 recipient cells.

100 colonies of each type of recombinants were analysed.

The order of transfer of the Hfr markers is: Hfr H, *thr leu try his arg*; Hfr type 2, *leu thr arg his try*; Hfr AT IIIA, *his try leu thr arg*. (The percentage of *col V*⁺ among F⁻ (*str-r*) cells at 120 min. after mating, in crosses performed with a ratio of 1 Hfr:20 F⁻ cells was: 6.6 % with Hfr H; 5.2 % with Hfr type 2; 18 % with Hfr AT-III A and 12.5 % with 112 (F⁺).)

All recombinants selected from reciprocal crosses Hfr *col V*⁻ × F⁻ *col V*⁺ are *col*⁺. The frequency of recombinants in these crosses is the same as that in control crosses *col*⁻ × *col*⁻.

DISCUSSION AND CONCLUSIONS

From the analysis of conjugation experiments involving colicinogenic factors E₂, I and V, it follows that:

(1) Each colicinogenic factor is transferred at about the same frequencies from F⁺ and from Hfr bacteria injecting their chromosomes in various orders. In agreement with this result, similar times of penetration from these different donors were determined for *col V*.

The percentage of *col*⁺ among recombinants from crosses Hfr *col*⁺ × F⁻ *col*⁻ indicates that *col*⁺ is not linked with chromosomal markers. Moreover, with *col E*₂ and *col V* the transfer of *col*⁺ seems to be proportional to the duration of conjugation,

since recombinants for distal markers present a higher percentage of *col*⁺ than those for proximal ones.

From all these results it may be concluded that these colicinogenic factors are not localized on the chromosome in donor cells.

(2) No recombinants have been observed which have received the *col*⁻ character of the Hfr parent. With the colicinogenic factors studied no lethal zygosis was detected in Hfr or F⁺ *col*⁻ × F⁻ *col*⁺ crosses, unlike the findings of Alföldi *et al.* (1958) with *col E*₁; nor was a decrease in fertility due to colicinogenic factors observed.

Therefore, there are no reasons to consider the non-appearance of *col*⁻ in recombinants as the consequence of some type of lethal zygosis masking its transfer.

(3) The kinetics of transfer of *col V*⁺ shows that it is transferred in Hfr *col*⁺ × F⁻ *col*⁻ crosses with an efficiency of 100 % of input of the minority parent within the first 50 min. after mixing (Fig. 2). Later, the number of *col*⁺ *str-r* colonies corresponding to zygotes that have received the *col*⁺ character, increases at the same rate as the F⁻ cells of the population. This means that, once transferred to zygotes with maximum efficiency, *col V*⁺ is capable of multiplying immediately and at a rate that assures its passage to all daughter cells.

The behaviour of *col*⁺ *str-r* colonies is in striking contrast to that of chromosomal recombinants, such as *thr*⁺ *leu*⁺ *str-r*. When chromosomal markers are transferred they must integrate in the genome of the recipient cell in order to reduplicate; the probability of integration being less than $\frac{1}{2}$, the frequency of transmission to recombinants becomes much less than the frequency of transfer to zygotes (Wollman, Jacob & Hayes, 1956). Furthermore, chromosomal recombinants begin to multiply only after integration has been accomplished (Fig. 2).

These results suggest that *col V* divides autonomously in the recipient F⁻ cell, without requiring integration in the recipient genome, and at a faster rate than the chromosome because more than one copy of *col*⁺ per cell must be present in order to assure its transmission to all descendant cells.

(4) Notwithstanding that the initial rise in number of *col*⁺ *str-r* colonies is similar in Hfr type 2 *col V*⁺ × F⁻ *col V*⁻ (Fig. 2) and F⁺ *col V*⁺ × F⁻ *col V*⁻ cross (Fig. 3) the analysis of their slopes between 60 and 120 min. after mixing reveals some differences. Thus, *col*⁺ *str-r* colonies in the cross involving the F⁺, unlike the Hfr type 2 *col V*⁺ × F⁻ *col V*⁻ cross, increase more rapidly than the F⁻ cells of the population, which suggests that some other factor intervenes, in addition to that corresponding to cell division. As one possible explanation of this added increase it can be assumed that new *col*⁺ *str-r* cells continue forming in F⁺ × F⁻ crosses as the consequence of the transfer of *col*⁺ from F⁻ cells that became donors of *col*⁺ from simultaneous reception of F⁺ and *col*⁺ during the first minutes of conjugation. Another assumption would be that a fraction of the original F⁺ cells are able to re-conjugate and so transfer more *col* particles.

Summarizing, the ensemble of results shows that *col E*₂, *col I* and *col V* are in an autonomous state in Hfr and F⁺ cells. Furthermore, analysis of transfer of *col V* suggests that this same interpretation would be valid also for F⁻ cells.

Quite striking are the differences in frequency of transfer between colicinogenic factor V, on one hand, which is transferred with an efficiency of 100 % or even higher and colicinogenic factors E₂ and I, on the other, since they are only poorly transferred (1 and 0.1 %) respectively. Nothing is known about the nature of the

forces governing the transfer of genetic material during conjugation; nevertheless, various hypotheses accounting for the differences of transfer of these colicinogenic particles can be considered, such as: (1) different number of particles per cell, (2) different size of the particles, (3) special localization favouring their passage through the conjugation bridge, and (4) capacity of becoming temporarily fixed at any point of the chromosome. The last point seems least probable in view of the fact that the same frequency of transfer was found with F^+ cells which transfer the chromosome at low frequency and with Hfr cells which transfer it at a high one.

All these considerations are concerned with factors that would affect the transfer of these colicinogenic particles; factors affecting the establishment of *col E*₂ and *col I* in a fraction of the recipient population might also be considered.

These colicinogenic factors, like episomes, are not essential constituents of cells and they may be either present or absent from the cell (Jacob & Wollman, 1958*b*). Even though the possibility of their fixation on the chromosome cannot yet be excluded, the evidence obtained so far with these studies indicates their extra-chromosomal state and leads one to consider them as plasmids (Lederberg, 1952; Jacob, Schaffer & Wollman, 1960).

These factors would be among the few recorded in *Escherichia coli* for which chromosomal localization is lacking. Another published example is the transducing phage 363, which is not transferred in matings involving different types of Hfr (Jacob & Wollman, 1959).

Finally, from the studies with colicinogenic factors it would seem that differences in the state of different factors exist, since *col E*₁ behaves as an episome while *col E*₂, *I* and *V* would lack the integrated state, at least for conditions under which *col E*₁ apparently fixes on the chromosome.

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