The Genetic Behaviour of Colicinogenic Factor E_1

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SUMMARY

Analysis of the genetic behaviour of the colicinogenic factor E_1 ($colE_1$) in crosses involving Hfr or F^+ and F^- strains of *Escherichia coli* $\kappa 12$ shows: (1) this factor is transferred with very high frequency from different donor bacteria; (2) its transfer begins to take place early whatever the type of donor cell; (3) it is not linked with chromosomal markers; (4) it multiplies autonomously in the zygote at such a rate that its passage to all descendent cells is assured; (5) the non-colicinogenic character of the donor parent does not appear among recombinant or recipient cells; (6) no lethal zygosis occurs related to the transfer of a presumptive $colE_1^-$ locus.

All these facts suggest that $colE_1$ exists in an extra-chromosomal state in Hfr, F^+ and F^- bacteria.

No transfer of $colE_1$ from F^- to F^- or Hfr bacteria was observed.

INTRODUCTION

It is well known that the hereditary ability of colicinogenic bacteria to synthesize colicin can be transmitted by cell contact among strains of the family Enterobacteriaceae (Fredericq, 1954*a*, 1956; Hamon, 1956; Ozeki, Stocker & Smith 1962).

Studies on the transmission of colicinogenic factor E_1 ($colE_1$)—controlling the synthesis of colicin E_1 or ER—from F^+ to F^- strains of *Escherichia coli* $\kappa 12$ were carried out by Fredericq & Betz-Bareau (1953*a, b*). They showed that $colE_1$ was transferred efficiently to recombinants in crosses between colicinogenic (col^+) F^+ donors and non-colicinogenic (col^-) F^- recipients, and that in reciprocal crosses recombinants never acquired the non-colicinogenic character of the donor parent. From these results, and also from the lack of linkage of $colE_1$ with chromosomal markers, they considered this factor as cytoplasmic.

Moreover, the behaviour of $colE_1$ in crosses between Hfr of different types and F^- bacteria (Alfoldi, Jacob & Wollman, 1957; Alfoldi, Jacob, Wollman & Mazé, 1958) suggested that it possessed the properties assigned to episomal particles (Jacob & Wollman, 1958). Certain experimental evidence, such as lack of linkage between this factor and chromosomal markers and its transmission from the zygote to all descendent cells, led to the conclusion that it could multiply autonomously in the recipient cell at a faster rate than the chromosome.

On the other hand, the results also showed that the frequency of transfer of $colE_1$, in crosses involving different Hfr col^+ strains and $F^- col^-$ bacteria, varied with the type of Hfr employed. In reciprocal crosses, Hfr $col^- \times F^- col^+$, no transfer of the col^- character was detected and death of the zygotes was found to occur with each

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type of Hfr with a magnitude comparable to the transfer of col^+ in the corresponding reciprocal cross. The apparent correlation observed between the transfer of col^+ and the 'lethal zygotis' in both series of crosses was consistent with a chromosomal location of $colE_1$ in Hfr cells.

No indication of a chromosomal state has been found with colicinogenic factors E_2 , I and V in crosses involving F^+ and different Hfr strains as donors (Nagel de Zwaig, Antón & Puig, 1962). Analysis revealed that, even though these factors were transferred with quite different frequencies ($colE_2$ and $colI$ at low frequency and $colV$ with maximum efficiency), each of them was transferred with similar frequencies from different types of donor cells (F^+ or Hfr), it was not linked to chromosomal markers, and in no case did the col^- character of the donor appear among recombinants.

The number of the F^- bacteria decreased in some matings when the number of the Hfr bacteria greatly exceeded that of the recipient, suggesting some type of lethal zygotis. This lethality could not be related to transfer of the col^+ or the col^- character since it appeared also in control crosses. Subsequently, this effect was confirmed and further analysed by Clowes (1963) in his research on $colE_1$.

Studies on the genetic behaviour of colicinogenic factors E_2 , I and V have been now extended, under the same conditions, to $colE_1$. Evidence is given which demonstrates the autonomous state of this factor in strains of *Escherichia coli* $\kappa 12$, in agreement with the conclusions reached simultaneously by Clowes (1963).

METHODS

Media. The minimal medium (*m.m.*) employed was that described by Davis & Mingioli (1950). Salts, glucose and agar were autoclaved separately and mixed before pouring on to plates. Amino acids, when required, were added at a concentration of 80 $\mu\text{g./ml.}$ and thiamine at a concentration of 5 $\mu\text{g./ml.}$

Nutrient broth and nutrient agar (nutrient broth with 1.5 %, w/v, of Bacto-Agar) and L broth and L broth agar (Lennox, 1955) were also employed.

Streptomycin, when necessary, was added at a concentration of 200 $\mu\text{g./ml.}$

Nutrient broth with 0.7 % (w/v) of Bacto-Agar was used as top agar for the plating of bacteria.

Strains. Bacterial strains mentioned in this paper are listed in Table 1. They were obtained from Dr E. L. Wollman. (For detailed references of these strains see Nagel de Zwaig *et al.* 1962). Strains derived from those listed in Table 1, employed in the present study, are described in the text.

Strain 58-161 was used as a sensitive indicator for colicin E_1 .

The origin and direction of the Hfr strains employed and the location of some essential characters on the linkage group of *Escherichia coli* $\kappa 12$ are shown in Fig. 1.

Phage P_1 used in transduction experiments was kindly provided by Professor S. E. Luria.

Resistant strains. All strains employed in matings were previously made resistant to colicin E_1 to avoid distortion of results due to the lethal action of the colicin on sensitive strains.

For the isolation of these resistant cells, a colicinogenic strain was stabbed on an agar plate and incubated for 24 hr. The colonies thus developed were sterilized by

Table 1. *Bacterial strains*

Name	Relevant characteristics
<i>E. coli</i> K 12	
58	$F^+ \text{met}^- \text{str-s} \text{colE}_1^+$
112	$F^+ \text{cys}^- \text{his}^- \text{str-s}$
Hfr H	$\text{thi}^- \text{str-s}$
Hfr 4	$\text{thr}^- \text{leu}^- \text{thi}^- \text{str-s}$
Hfr 2	$\text{met}^- \text{str-s}$
Hfr AT IIIA	prot. str-s
58-161	$F^+ \text{met}^- \text{str-r}$
PA309	$F^- \text{thr}^- \text{leu}^- \text{thi}^- \text{try}^- \text{arg}^- \text{str-r}$
c600	$F^- \text{thr}^- \text{leu}^- \text{thi}^- \text{str-r}$

The following abbreviations are used: arginine: *arg*; cysteine: *cys*; galactose: *gal*; histidine: *his*; lactose: *lac*; leucine: *leu*; methionine: *met*; thiamine: *thi*; threonine: *thr*; tryptophan: *try*; valine: *val*; resistance to streptomycin: *str-r*; sensitivity to streptomycin: *str-s*; prototrophic: *prot*.

Requirement for a given amino acid or vitamin is indicated by the minus sign.

Fig. 1. Linkage map of *Escherichia coli* K 12 (Jacob & Wollman, 1961) with indication of the origin and direction of the Hfr employed. Symbols are specified under Table 1.

chloroform vapour, and melted top agar seeded with the sensitive strain poured on the surface of the plate (Fredericq, 1958). Resistant colonies were isolated from the halos formed by the action of the colicin on the sensitive strain.

Transfer of colicinogenic factor E_1 . Factor colE_1 was first transferred from strain 58 colE_1^+ to strain PA309/ E_1 by: (a) *Mixed culture*, of both strains, incubated overnight in nutrient broth at 37° . Samples of the mixture were diluted in buffer and plated on nutrient agar plates containing streptomycin, where only strain PA309 could grow. Col^+ colonies were detected by the halos formed on an indicator strain using the double-agar layer method (Fredericq, 1954). (b) *By transduction* with phage P_1 prepared by lytic growth on 58 colE_1^+ . Col^+ colonies were also detected by the double-agar layer technique.

ColE_1 was transferred from PA309 to strain 112 F^+ in mixed culture, as explained

above, and from the latter strain to different Hfr strains, by the same procedure. When $colE_1$ had to be transferred to Hfr or F^+ strains, cultures in the stationary phase of growth were employed in order to make them phenotypically F^- (Lederberg, Cavalli & Lederberg, 1952).

Matings. Details of $Hfr \times F^-$ or $F^+ \times F^-$ crosses, interrupted matings and detection of chromosomal recombinants (those receiving the capacity to synthesize an amino acid from the donor parent and the *str-r* character from the recipient) and of $col^+ str-r$ cells (which combine the col^+ character of the donor and the *str-r* one of the recipient) are described in a previous communication (Nagel de Zwaig *et al.* 1962).

The frequency of chromosomal recombinants or transfer of col^+ is expressed as the ratio of the number of recombinants or $col^+ str-r$ cells, respectively, to 100 initial bacteria of the minority parental strain.

Unless otherwise stated, mating mixtures were sampled and plated for recombinants and $col^+ str-r$ cells 90 min. after mixing the strains.

When the kinetics of recombinant formation was followed during a long period of time, the mating mixture was diluted 1/10 in fresh nutrient broth every 100 min. to maintain the strains in the logarithmic phase of growth.

To measure the frequency of col^+ colonies among chromosomal recombinants or recipients, individual colonies growing on the original plates were transferred to the surface of plates containing the same selective medium; colicinogenic bacteria were then detected by the agar layer method.

For the selection of chromosomal recombinants in $F^- \times F^-$ crosses, the mixture of strains was centrifuged and resuspended in buffer before plating.

When studying the transfer of $colE_1$ from F^- to Hfr cells, platings were done on *m.m.* supplemented with the corresponding amino acids and valine to contraselect the valine-sensitive F^- strain (Manten & Rowley, 1953).

Transduction. For the preparation of stock of phage P_1 and for transduction experiments, the techniques indicated by Lennox (1955) and Arber (1960) were followed.

RESULTS

Crosses between colicinogenic donors and non-colicinogenic recipients

Transfer of colicinogeny

Frequency of transfer. The frequency of transfer of $colE_1$ was studied in Hfr $colE_1^+ \times F^- colE_1^-$ crosses involving different types of Hfr strains, and $F^+ colE_1^+ \times F^- colE_1^-$ crosses, all performed in the proportion of 1 donor to 20 recipient cells.

In each cross the frequency of recombinants for an early Hfr marker was determined in order to verify its efficiency as donor.

Table 2 shows that $colE_1$ is not only transferred from F^+ and different Hfr bacteria with similar frequencies but also that its frequency of transfer greatly exceeds 100 % of input of donor cells, at 90 min. after the beginning of mating.

No differences in the transfer of col^+ were found in matings involving strains made colicinogenic by transduction or by mixed culture (See Methods).

Kinetics of transfer. The time of entry of $colE_1$ in the recipient cells was determined in matings interrupted by a Waring Blender. In this way it was established that $colE_1$ begins to appear among recipient cells within 5 min. of the onset of mating in

Table 2. Frequency of transfer of $colE_1$ in crosses between Hfr $colE_1^+$ or $F^+ colE_1^+$ and PA309 $colE_1^-$

Donor strains	Frequency of transfer of $colE_1$ (in %)
Hfr H $colE_1^+$	780
Hfr 4 $colE_1^+$	1000
Hfr 2 $colE_1^+$	600
Hfr AT IIIA $colE_1^+$	900
112 (F^+) $colE_1^+$	600

Crosses indicated were performed in the proportion of 1 donor to 20 recipient cells. The frequency of transfer was measured at 90 min. after mixing the parental strain and expresses the ratio (in %) of the viable count of recipients becoming col^+ to the initial viable count of donor strains.

Fig. 2. The kinetics of transfer of $colE_1^+$ and thr^+leu^+ in Hfr H $colE_1^+ \times$ PA309 $colE_1^-$ cross, performed in the proportion of 1 donor to 20 recipient cells. Samples were taken at different times, treated in a Waring Blendor, diluted, and plated on selective media. A sample of the mating mixture was diluted 1/10 in fresh nutrient broth every 100 min. to maintain it in the logarithmic phase of growth. Δ , thr^+leu^+str-r recombinants; $\bullet$, col^+str-r bacteria; $\circ$, $str-r$ (F^-) bacteria.

crosses using the donor strains Hfr H $colE_1^+$, Hfr 4 $colE_1^+$, Hfr AT IIIA $colE_1^+$ and 112 $F^+ colE_1^+$.

The kinetics of transfer of $colE_1$ from Hfr H $colE_1^+$ strain to PA309 $colE_1^-$ was followed in interrupted matings using 1 donor to 20 recipient cells and was compared with the kinetics of formation of thr^+leu^+str-r recombinants (Fig. 2).

From Fig. 2, it is clear that: (a) chromosomal recombinants rapidly increase in number soon after mating; later, this increase diminishes gradually attaining at about 80 min. a 'plateau' which extends till 120–140 min. After 140 min. recombinants begin to divide at the same rate as other F^- ($str-r$) cells. (b) The slope of the

curve for col^+ *str-r* cells also shows an initial rapid increase till about 80–100 min. Then, the slope of the curve diminishes and becomes parallel to that for other F^- cells.

At 120 min. after the beginning of mating frequencies of transfer of col^+ of 800–1000 % were attained, in contrast to frequencies of about 40–50 % measured for chromosomal recombinants.

Kinetics of transfer of col^+ similar to the one shown in Fig. 2 were observed in crosses involving Hfr 2 $colE_1^+$ and 112 F^+ $colE_1^+$ strains as donors.

When matings were performed in the proportion of 1 donor to 1 recipient cell, the differences between the kinetics of transfer of col^+ and the formation of chromosomal recombinants become more evident, since under these conditions the plateau of recombinants was attained much earlier.

Formation of recombinants

Colicinogeny among recombinants. Different types of recombinants from Hfr $colE_1^+ \times F^- colE_1^-$ crosses were selected and their col^+ or col^- character determined. As can be seen in Table 3, $colE_1$ appeared in almost the same proportion among recombinants corresponding to proximal or distal markers of any of the different Hfr strains employed. These results show that $colE_1$ is not linked to chromosomal markers localized on different sites on the linkage group of *E. coli* K12.

Table 3. *Percentage of $colE_1^+$ among recombinants of crosses between Hfr $colE_1^+$ and PA309 $colE_1^-$*

Donor strains	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 $colE_1^+$	65	67	64	68
Hfr 4 $colE_1^+$	—	73	57	58
Hfr 2 $colE_1^+$	57	68	75	59
Hfr AT IIIA $colE_1^+$	79	86	74	77

One hundred or more recombinants of each type of recombinant were analysed. All crosses were performed in the proportion of 1 donor to 20 recipient cells.

The order of transfer of the Hfr markers is: Hfr H: *thr leu try his arg*; Hfr 4: *arg his try*; Hfr 2: *leu thr arg his try*; Hfr AT IIIA: *his try leu thr arg*.

The effect of colicinogeny on the frequency of recombinants. The frequency of recombinants for an early Hfr marker was determined in $col^+ \times col^-$ crosses and in the corresponding control $col^+ \times col^+$ crosses. As shown in Table 4, no significant variation or decrease in fertility becomes apparent when the frequency of recombination in both types of crosses was compared.

Crosses between non-colicinogenic donors and colicinogenic recipients

Absence of transfer of the non-colicinogenic character to recipients or recombinants

Hfr $colE_1^- \times F^- colE_1^+$ crosses involving different Hfr strains were performed in order to determine the transfer of the non-colicinogenic character.

In crosses performed in the proportion of 20 donors to 1 recipient cell, no col^- bacteria were detected among the *str-r* resulting zygotes analysed (approximately 1000); nor was the col^- character of the donor parent observed among 500

recombinants from the same crosses using either 1 to 20 or 20 to 1 donor to recipient cells.

Non-appearance of the col^- character among recipients or recombinants cannot be explained by assuming death of zygotes which receive this character. As shown in Table 5, no decrease in number of the F^- cells was observed at 90 min. after the onset of mating in crosses using 20 donors per recipient, except when the strain HfrH was involved. In this case the number of F^- cells decreased but without any relation to the transfer of col^- , since it also occurred with similar frequency in $col^- \times col^-$ control crosses.

Table 4. *Comparison of fertility in $Hfr\ colE_1^+ \times F^- \ colE_1^-$ and $Hfr\ colE_1^+ \times F^- \ colE_1^+$ crosses*

Donors	Type of recombinant selected	Recipient strain	
		PA 309 $colE_1^-$	PA 309 $colE_1^+$
Hfr H $colE_1^+$	$thr^+ leu^+ str-r$	48	52
Hfr 4 $colE_1^+$	$arg^+ str-r$	27	25
Hfr 2 $colE_1^+$	$thr^+ leu^+ str-r$	12	18

Crosses were performed in the proportion of 1 donor to 20 recipients. The frequency of recombination corresponds to 90 min. after the onset of mating.

Table 5. *Variation in number of F^- bacteria determined at 90 min. after mixing in $Hfr\ colE_1^- \times PA\ 309\ colE_1^+$ and $Hfr\ colE_1^- \times PA\ 309\ colE_1^-$ crosses*

Donor cells	Time in min. after the onset of mating	Recipient cells	
		PA 309 $colE_1^-$	PA 309 $colE_1^+$
Hfr H $colE_1^-$	0	1.4×10^7	1.6×10^6
	90	5×10^6	5×10^6
Hfr 4 $colE_1^-$	0	1.4×10^7	1×10^7
	90	3.2×10^7	3×10^7
Hfr 2 $colE_1^-$	0	1×10^7	1×10^7
	90	1.8×10^7	1.7×10^7

All non-colicinogenic strains were previously made resistant to colicin E_1 . Crosses were performed with a ratio of 20 Hfr to 1 F^- . Recipient cells were selected on streptomycin nutrient agar.

As far as this effect is concerned no differences were observed among crosses involving F^- strains made colicinogenic by transduction or conjugation.

As in $col^+ \times col^-$ crosses, no decrease in fertility occurred in reciprocal $col^- \times col^+$; it can be seen in Table 5 that similar frequencies of recombination are registered in $col^- \times col^+$ and $col^- \times col^-$ crosses.

Absence of transfer of col^- from the recipient to the donor

Matings between an F^- strain $thr^+ leu^+ arg^+ val-s\ colE_1^+$ derived from PA 309 and Hfr H $colE_1^- val-r$ bacteria were performed using a 1:1 ratio of donor to recipient cells to determine if $colE_1$ could be transferred from the F^- to the Hfr cell. Although under these conditions, transfer of chromosome from the Hfr to the F^- cell took place, no col^- bacteria were detected among about 5000 colonies growing on minimal

medium supplemented with thiamine and valine, which selects the Hfr non-colicinogenic strain.

This result shows that although conjugation bridges which permit the passage of genetic material are actually formed, *colE*₁ is not transferred from the F⁻ to the Hfr cell, or at least not with a magnitude comparable to that of its transfer in the opposite direction, that is, from the Hfr to the F⁻ cell.

Absence of transfer of colE₁ from colicinogenic F⁻ to non-colicinogenic F⁻ bacteria

Logarithmic cultures of an F⁻ strain, *thr*⁺*leu*⁺*arg*⁺*colE*₁⁺ derived from PA 309 and the F⁻ strain c600 *colE*₁⁻ were mixed together in the proportions of 1:20 and 20:1, during 120 min.

No recombination between chromosomal markers of both strains took place as shown by the fact that no *thr*⁺*leu*⁺*try*⁺*his*⁺ recombinants appeared on the corresponding selective medium. Under these conditions no *col*⁺ cells were detected among the large number of colonies which developed on a medium permitting only growth of the non-colicinogenic F⁻ strain. This indicates that no transfer of *colE*₁ from F⁻ to F⁻ strain occurs.

Table 6. *Comparison of fertility in Hfr colE₁⁻ × F⁻ colE₁⁺ and Hfr colE₁⁻ × F⁻ colE₁⁻ crosses*

Donors	Type of recombinant selected	Recipient strain	
		PA 309 <i>colE</i> ₁ ⁺	PA 309 <i>colE</i> ₁ ⁻
Hfr H <i>colE</i> ₁ ⁻	<i>thr</i> ⁺ <i>leu</i> ⁺ <i>str-r</i>	50	38
Hfr 4 <i>colE</i> ₁ ⁻	<i>arg</i> ⁺ <i>str-r</i>	17	23
Hfr 2 <i>colE</i> ₁ ⁻	<i>thr</i> ⁺ <i>leu</i> ⁺ <i>str-r</i>	20	12

Crosses were performed in the proportion of 1 donor to 20 recipients. The frequency of recombination was measured at 90 min. after the onset of mating.

DISCUSSION AND CONCLUSIONS

Analysis of crosses between Hfr, or F⁻, and F⁻ strains of *Escherichia coli* K12 involving colicinogenic factor E₁ (*colE*₁) indicates that:

(1) *ColE*₁ is transferred very efficiently and with similar frequencies from F⁺ cells, which transfer the chromosome at low frequency, and from different Hfr cells, which transfer it at high frequency and with different origins and polarities.

(2) It begins to be transferred by these different donor strains within the first 5 min. of mating.

(3) It shows no linkage to chromosomal markers localized on different sites on the linkage map of *Escherichia coli* K12.

(4) The non-colicinogenic character of the donor parent is not transferred to the recipient or to recombinants.

All these facts can be explained on the basis of an extra-chromosomal state of *colE*₁ in F⁺ and Hfr bacteria.

Evidence from kinetic experiments also suggests the autonomous state of *colE*₁. Kinetics of transfer of *colE*₁ from Hfr or F⁺ strains shows that this factor is transferred with maximum efficiency during the first 80 min. of conjugation; later on, the

number of col^+str-r cells, in contrast to chromosomal recombinants, continue increasing at the same rate as other F^- cells. It is known that after a chromosomal marker is transferred to a zygote it must become integrated in the recipient genome in order to reduplicate. Only when the integration is completed, that is at about 120–140 min. after the onset of mating, chromosomal recombinants increase in number by cell division (Wollman, Jacob & Hayes, 1956). The fact that col^+str-r do not present a plateau suggests that $colE_1$ is capable of multiplying autonomously in the zygote and at such a rate that enables its passage to all daughter cells of the zygote.

This result agrees with the conclusion of Alföldi *et al.* (1958) based on the analysis by micromanipulation of individual zygotes which received $colE_1$ factor.

The kinetics of transfer obtained with colicinogenic factor V (Nagel de Zwaig *et al.* 1962) are comparable to those presented here.

Similar conclusions as to the cytoplasmic nature of $colE_1$ were reached by Fredericq & Betz-Bureau (1953*a,b*) and Clowes (1963). The results of Alföldi *et al.* (1957, 1958), although leading to a different conclusion, are not in disagreement with those presented here. Their observations on the apparent correlation between the transfer of col^+ and lethal zygosis, on which the hypothesis of the episomal nature of $colE_1$ was mainly based, might have been purely coincidental. Similar lethal effects as those described by them are now known to occur to a variable extent in matings involving certain strains, when the number of the donor bacteria greatly exceeds that of the female ones, but without having any relation to the transfer of a presumptive col^- locus (Nagel de Zwaig *et al.* 1962; Clowes, 1963).

That the efficient transfer of $colE_1$ does not interfere with chromosomal transfer or integration in *Escherichia coli* $\kappa 12$ is shown by the fact that similar frequencies of recombination are observed in crosses involving the col^+ character and in controls. This is not the case with colicinogenic factors B or K (Fredericq & Betz-Bureau, 1956; Puig, 1963), whose presence in the donor cell drastically reduces the frequency of recombinant formation.

Results showing that colicinogenic factors, such as $colE_1$ or $colV$, $colE_2$ and $colI$ (Nagel de Zwaig *et al.* 1962) and $colB$ (Puig, 1963), can be transferred from Hfr cells as efficiently as from F^+ ones do not support the view suggested by Fisher (1962) that a mechanical obstruction of the conjugation canal by the chromosome might prevent the passage of all non-chromosomal material.

No transfer of $colE_1$ from F^- to F^- cells was observed, in agreement with previous observations by Fredericq (1954*b*). Neither was it found to be transferred, at least at high frequency, from F^- to Hfr cells. These results suggest that the presence of the F factor in a bacterial cell promotes the efficient transfer of $colE_1$ not only by determining the formation of conjugation bridges, but also by conferring on it a polarity of transfer of the same direction as that of the chromosome.

These observations on the lack of transfer of $colE_1$ among F^- cells might not apply to all colicinogenic factors; studies on the transfer of $colI$ and $colB$ factors in *Salmonella typhimurium* (Ozeki *et al.* 1962) indicate that they may act as fertility factors, since their presence in the cell promotes not only their own transfer, but also that of other colicinogenic factors and, in the case of $colI$, even that of the chromosome (Ozeki & Howarth, 1961; Clowes, 1961), probably by enabling the cell to conjugate. Preliminary results with $colV$ and $colI$ factors in *Escherichia coli* $\kappa 12$

(Nagel de Zwaig, unpublished) suggest a similar situation as far as the transfer among F^- cells.

The conclusions obtained from this investigation regarding the state of *colE*₁ agree closely with those based on the genetic analysis of *colE*₂, *colI*, *colV* (Nagel de Zwaig *et al.* 1962) and *colB* (Puig, 1963). Since all the evidence so far obtained is consistent in pointing to the non-chromosomal state of these particles in *Escherichia coli* κ 12, they should be considered as plasmids (Lederberg, 1952).

Nevertheless, some colicinogenic factors might become fixed on the chromosome under certain conditions. One may speculate that chromosomal attachment would be more likely for colicinogenic factors resembling episomes, for which chromosomal location is well established. In this connexion, the study of colicinogenic factors having *F*-like functions or interacting with *F* particles, such as *colI* (Ozeki & Howarth, 1961; Clowes, 1961), *colB* (Ozeki *et al.* 1962; Fredericq, 1963; Puig, 1963) and *colV-colI* (Nagel de Zwaig, 1963) seems more promising. According to these assumptions, the isolation of an Hfr strain carrying *colB* fixed on the distal end of the chromosome as a result of a close association with the *F* factor has been reported by Fredericq (1963), though this type of evidence has not so far been obtained with a similar system (Puig, 1963).

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