GENE TRANSFER BY F′ STRAINS OF *ESCHERICHIA COLI* K-12[1]

II. Interaction Between F-Merogenote and Chromosome During Transfer

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Abstract

PITTARD, JAMES (Yale University, New Haven, Conn.) AND EDWARD A. ADELBERG. Gene transfer by F′ strains of *Escherichia coli* K-12. II. Interaction between F-merogenote and chromosome during transfer. J. Bacteriol. **85**:1402–1408. 1963.—When F′ strains harboring the F-merogenate F_{14} are mated with female recipients, the transfer of the F-merogenote begins, in the majority of cases, before chromosome transfer. The markers on F_{14} are transferred in the sequence *met-1*, *arg-1*, *ilva-16*, and sex-factor, *met-1* being transferred first and sex-factor being transferred last, 9 min after *met-1*. In the class of zygotes that have received both the F-merogenote marker *met-1* and the chromosomal marker *xyl* or *mal*, the gradient of recombination frequencies for the F-merogenote markers *arg-1* and *ilva-16* is much steeper than in the corresponding zygotes that have not received chromosomal markers. In F′ strains which exhibit an increased frequency of transfer of chromosome markers, this gradient of recombination frequencies for merogenote markers is much steeper. An analysis of experiments involving an F′ strain with a much shorter F-merogenote, F_{16}, and of a triparental mating in which F-merogenote and chromosome were transferred from different donor cells reveals that the effect of chromosome transfer on the recovery of distal F-merogenote markers in the zygotes is not due to any form of postzygotic elimination. It is suggested that, when F′ strains which are transferring F-merogenote begin to transfer chromosome, the latter event causes breakage of the F-merogenote. A second consequence of this interaction is a delay of 8 to 10 min in the first appearance of chromosomal markers in the zygotes.

In the accompanying paper (Pittard, Loutit, and Adelberg, 1963), it was shown that a single F′ cell can transfer both F-merogenote and chromosome to a female recipient. In such a conjugation, the initiation of chromosome transfer appears to be delayed 9 min, in comparison with a conjugation involving the parent Hfr strain from which the F′ strain was derived.

One of the F′ strains used in these experiments contains an exceptionally long F-merogenote, designated F_{14}. This element, when present in a first-generation male, requires 9 min for transfer. Since F_{14} carries a large number of detectable loci (Pittard et al., 1963), it has been possible to investigate the kinetics of F-merogenote transfer by cells that are simultaneously transferring chromosome. The present paper reports the results of such experinents, which show that chromosome transfer alters the linkage of markers on the F-merogenote.

Materials and Methods

Organisms. The strains used in this work are described in Table 1. They are all derivatives of *Escherichia coli* K-12.

Media and culture methods. The media and culture methods were those described by Adelberg and Burns (1960).

Mating conditions. The conditions employed for measuring the kinetics of zygote formation were those described by Adelberg and Burns (1960), with the following modifications. Male cells were grown to exponential phase in glucose minimal medium supplemented with the required growth factors. Mating cells were mixed in ratios of ten or more females to one male. Conjugation was interrupted by subjecting 1-ml samples of mating mixture to violent agitation with a Vortex Junior mixer and then killing the males with bacteriophage T_6. In all kinetic experiments, the recipient strain used was AB1450, which carries the mutant alleles *met-1*, *arg-1*, *and ilva-16* (*see* Pittard et al., 1963). For the sake of clarity, we will use capital italicized letters for chromosomal

[1] The experiments reported in this paper form part of the thesis submitted to Yale University by James Pittard, in partial fulfillment of the requirements for the Ph.D. degree.

TABLE 1. *List of strains**

Strain no.	Auxotrophic characters										Energy source utilization		Response to		Sex
	ile	*ilva*	*arg*	*met*	*thi*	*thr*	*leu*	*pro*	*try*	*his*	*xyl*	*mal*	T^6	*str*	
AB1450	+	*16*†	*1*	*1*	*2*	+	+	+	+	*1*	*4*	*1*	R	R	♀
AB1469	+	*7*	*3*	+	*1*	+	+	*2*	+	*4*	+	+	S	S	♀
AB1206	+	+	+	+	*1*	+	+	*2*	+	*4*	+	+	S	R	♂F_{14}
AB1446	+	+	+	+	*1*	+	+	*2*	*15*	*4*	+	+	S	R	♂F_{14}
AB1516	+/+	+/*7*	+/*3*	+/+	*1*	+	+	*2*	+	*4*	+	+	S	S	♂F_{14}
AB1540	+/+	+/*7*	+/*3*	+/+	*1*	+	+	*2*	+	*4*	+	+	S	S	♂F_{14}
AB1528	+/+	+/*7*	*3*	+	*1*	+	+	*2*	+	*4*	+	+	S	S	♂F_{16}
AB313	+	+	+	+	−	*1*	*6*	+	+	+	+	+	S	R	♂Hfr

* The following abbreviations are used: *ile*, isoleucine; *ilva*, isoleucine and valine; *val*, valine; *arg*' arginine; *met*, methionine; *thi*, thiamine; *thr*, threonine; *leu*, leucine; *pro*, proline; *try*, tryptophan; *his*, histidine; *ser*, serine or glycine; *xyl*, xylose; *mtl*, mannitol; *mal*, maltose; *lac*, lactose; *gal*, galactose; T_6, bacteriophage T_6; *str*, streptomycin; R, resistant; S, sensitive; +/−, heterozygous; . . , not tested.

† Numbers refer to allele numbers that have been allotted to mutant strains in these laboratories.

markers (e.g., MAL^+) and lower case italicized letters for F-merogenote markers (e.g., met^+).

Scoring unselected markers. At least 80 recombinants were tested at each time interval by transferring colonies to master plates of the selective medium and then replicating the new growth onto appropriate media.

RESULTS

Isolation and properties of F′ strains. The isolation and properties of F′ strain AB1206 were described in the accompanying paper (Pittard et al., 1963). This strain harbors the F-merogenote F_{14}. When F_{14} is transferred to recipient cells, two types of first-generation F′ males can be isolated. Type I is exemplified by strain AB1540. Type II is exemplified by strain AB1516, which was described in some detail in the accompanying paper. Type II strains differ from type I strains in two respects: (i) the frequency of recombination for chromosomal markers is severalfold higher (Fig. 1), and (ii) the gradient of recombination frequencies for F-merogenote markers (i.e., the relative frequencies obtained after a given time of conjugation) is much steeper (Fig. 2).

Both type I and type II males have been isolated from zygotes obtained in a cross of F′ strain AB1446 (a *try* derivative of AB1206) with a given F^- recipient. Furthermore, cultures of type I males have been observed to change to type II after storage at 5 C for several weeks followed by subculture in minimal medium. The basis for the different behaviors of type I and type II males is not known, nor is it known why type I males change to type II. There seems to be a definite correlation, however, between an increase in the frequency of chromosome transfer and an increase in the steepness of the gradient of recombination frequencies for F-merogenote markers; it is this correlation which is relevant to the present experiments.

A new F′ strain has been produced by the phage-mediated transduction of F-merogenote markers to an F^- recipient. Phage Plkc, grown on strain AB1206, was allowed to infect F^- strain AB1469, after which the recipients were plated on agar selective for $ilva^+$ recombinants. One strain obtained in this manner, AB1528, was found to possess a much-shortened F-merogenote bearing only the (*ile-ilva*) group of genes. This F-merogenote, designated F_{16}, required less than 2 min for transfer, in comparison with 9 min for F_{14}. Strain AB1528 transfers both F_{16} and chromosome.

Influence of chromosome transfer on the transfer of F-merogenote markers. It will be recalled that F′ strain AB1516 required 9 min to transfer F_{14}, and that the initiation of chromosome transfer was delayed 9 to 10 min in this strain. Our first hypothesis was that chromosome transfer to any given zygote is delayed until the F-merogenote has been completely transferred. This hypothesis predicts that if one examines only that class of recombinants having received chromosomal markers such as XYL^+ or MAL^+, they

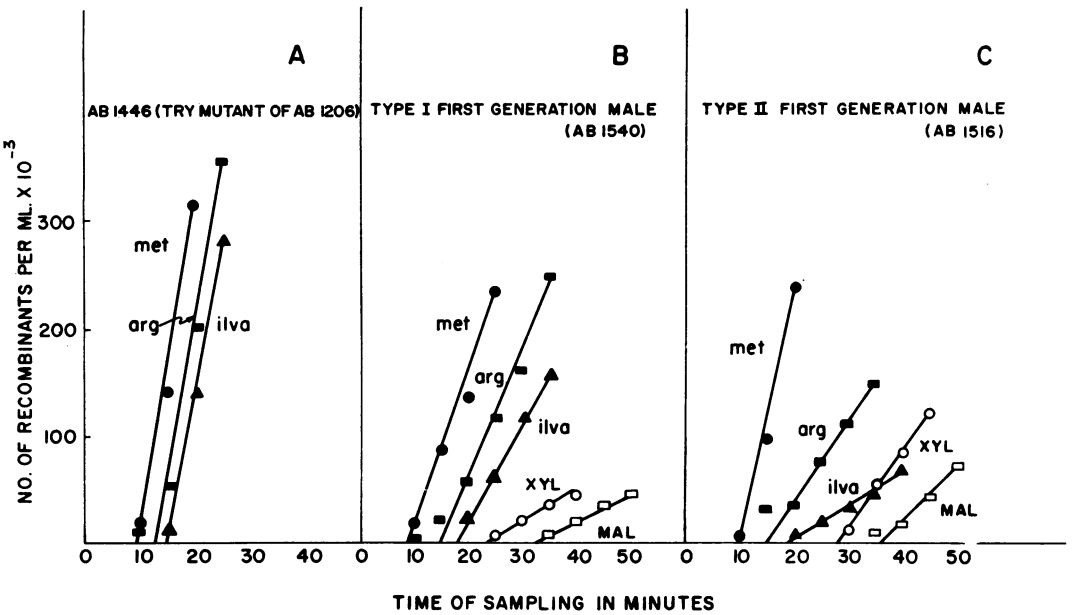

FIG. 1. *Kinetics of zygote formation for F-merogenote and chromosomal markers transferred from F′ strains AB1206, AB1516, and AB1540 to female strain AB1450.*

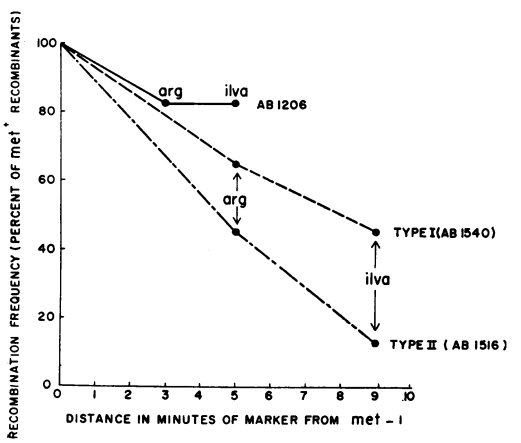

FIG. 2. *Gradient of recombination frequencies of F-merogenote markers. The data represent the numbers of recombinants obtained after a 40-min interrupted mating, expressed as percentages of the values for met⁺ recombinants.*

will contain all the F-merogenote markers at equal and high frequencies; furthermore, these frequencies will not be found to increase with the time at which the conjugation is interrupted. (This is essentially what is observed when one scores a selected class of recombinants for proximal chromosomal markers.)

This prediction was tested for both a type I F′ male (AB1540) and a type II F′ male (AB1516), selecting for XYL^+ and MAL^+ after different times of conjugation. At least 80 recombinants from each selection at each time were scored for the presence of the F-merogenote markers met^+, arg^+, and $ilva^+$. The results (Fig. 3) do not confirm the hypothesis. Although the frequencies of merogenote markers do not increase with time, the three markers are recovered with widely different frequencies. Approximately 70% of the XYL^+ recombinants are met^+, 40% are arg^+, and 20% are $ilva^+$, regardless of the time at which conjugation was interrupted. Figure 3C illustrates a control experiment in which selected MAL^+ recombinants were scored for the unselected proximal marker XYL^+, and selected XYL^+ recombinants were scored for the distal marker MAL^+. As predicted, the proximal chromosomal marker was inherited at a frequency which was constant with time, whereas the distal marker was inherited at a frequency which rose sharply with time.

These observations, together with the earlier-mentioned correlation between frequency of chromosome transfer and gradient of recombination frequencies for F-merogenote markers, suggested that chromosome transfer might be inter-

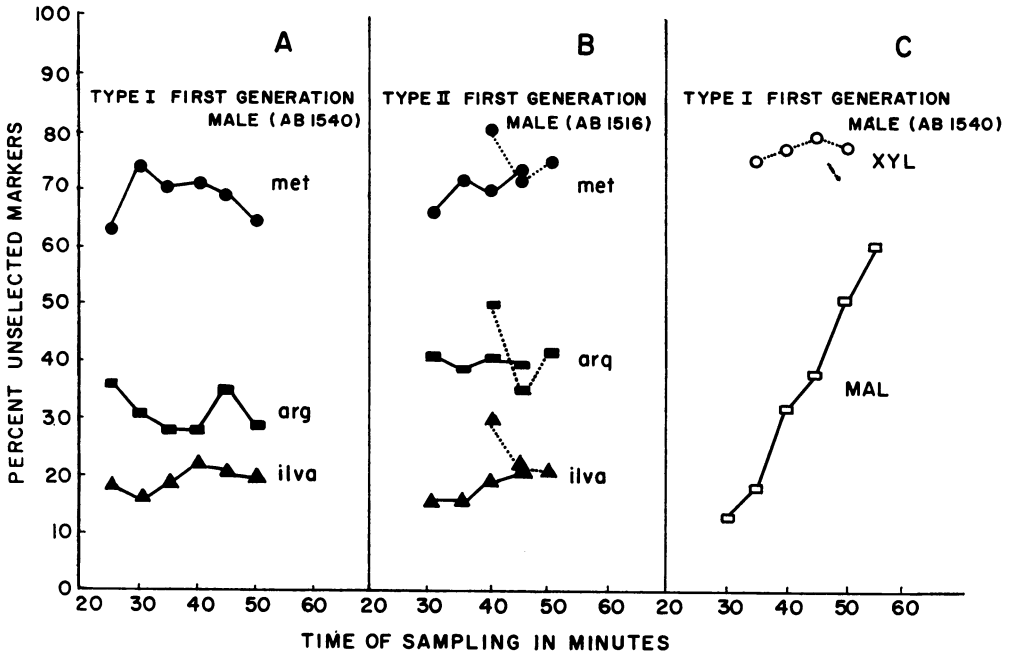

FIG. 3. *Per cent recovery of unselected markers in recombinants selected for chromosomal markers (solid line, XYL selected; broken line, MAL selected).*

fering with the completion of F-merogenote transmission. To test this hypothesis, the inheritance of the markers *arg*$^+$ and *ilva*$^+$ in recombinants selected for *met*$^+$ (most of which received only the F-merogenote) was compared with the inheritance of these markers in recombinants selected for *met*$^+$ and *XYL*$^+$ (all of which thus received both F-merogenote and chromosome). If our hypothesis is correct, the linkage between *met*$^+$ and the two unselected F-merogenote markers would be significantly reduced in the recombinants receiving chromosomal markers. Furthermore, the hypothesis predicts that in the recombinants receiving only F-merogenote the distal merogenote markers *arg*$^+$ and *ilva*$^+$ will be recovered at a frequency which increases with the time of conjugation, whereas in the recombinants receiving both F-merogenote and chromosome the frequencies for *arg*$^+$ and *ilva*$^+$ will remain constant with time.

The results (Fig. 4A) confirm the above prediction. When F′ strain AB1540 was allowed to transfer only F_{14} to F^- strain AB1450 (*met*$^+$ selection), the number of *met*$^+$ recombinants receiving *arg*$^+$ and *ilva*$^+$ rose with time to values of 56 and 44%, respectively, by 40 min. When the class of recombinants receiving *both* F-merogenote and chromosome were scored, however, (*met*$^+$ *XYL*$^+$ selection), the number of *met*$^+$ recombinants receiving *arg*$^+$ and *ilva*$^+$ remained constant at levels averaging 45 and 25%, respectively. A control experiment, using F′ strain AB1206 as donor, is shown in Fig. 4B. Strain AB1206, it will be recalled, does not transfer chromosome to a significant extent.

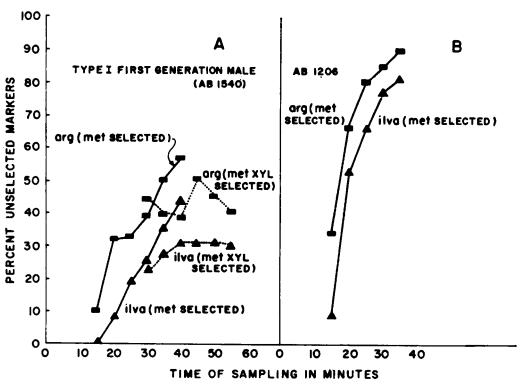

FIG. 4. *Per cent recovery of the F-merogenote markers arg-1 and ilva-16 in recombinants selected on the one hand for F-merogenote marker met-1 and on the other hand for both F-merogenote marker met-1 and chromosomal marker XYL (solid line, met-1 selected; broken line, met-1 and XYL selected).*

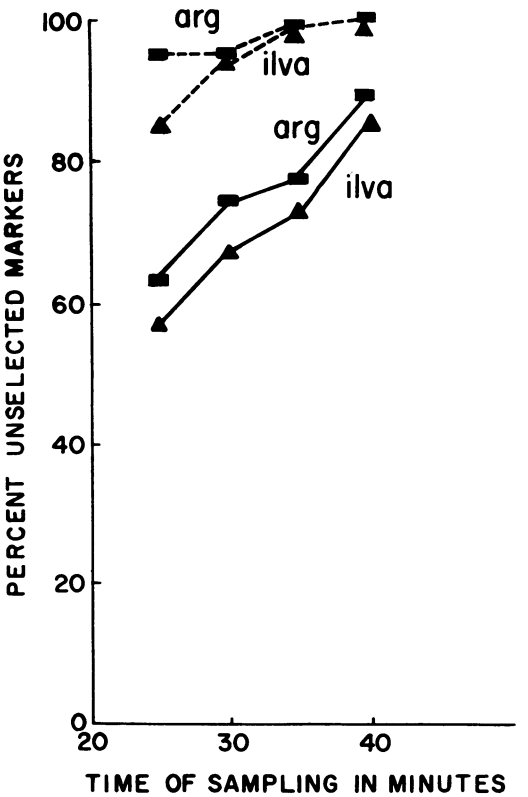

FIG. 5. *Per cent recovery of the F-merogenote markers arg-1 and ilva-16 in recombinants obtained in the triparental cross between F′ strain AB1446, Hfr strain AB313, and female strain AB1450. Recombinants were selected for the F-merogenote marker met-1 in one case and for both F-merogenote marker met-1 and chromosomal marker XYL in the other case (solid line, met-1 selected; broken line, met-1 and XYL selected).*

Thus, chromosome transfer interferes with the recovery of F-merogenote markers in such a way that distal markers are affected more than proximal markers. This could be explained by either of two models. (i) The entire F-merogenote might be transferred to a given zygote before the arrival of the chromosome. The latter occurrence would then intefere with the postzygotic integration or maintenance of F-merogenote markers, such that the last F-merogenote marker to arrive would be affected the most. (ii) Alternatively, chromosome transfer might prevent the transfer of any F-merogenote marker which had not reached the zygote before chromosome transfer began.

These two alternatives were tested by carrying out a triparental mating (Fischer-Fantuzzi and DiGirolamo, 1961) in which an F^- cell simultaneously received F_{14} from F′ strain AB1446 and chromosome from Hfr strain AB313. According to the first model above, chromosome transfer should interfere with the recovery of F-merogenote markers in recombinants; according to the second model above, chromosome transfer should not interfere with the transfer of F-merogenote from a different donor cell.

The results (Fig. 5) are in agreement with model ii. The recovery of the unselected F-merogenote markers *arg*$^+$ and *ilva*$^+$ increased with time in the *met*$^+$ *XYL*$^+$ class and was higher, instead of lower, than in the *met*$^+$ class.

Models i and ii also make different predictions concerning the effect of chromosome transfer on the recovery of the *ilva*$^+$ marker transferred as part of the shortened F-merogenote, F_{16}. According to model i, chromosome transfer should cause postzygotic elimination of the *ilva*$^+$ marker of F_{16} to the same extent that it does for the *ilva*$^+$ marker of F_{14}. On the other hand, according to model ii, the early arrival of the *ilva*$^+$ marker of F_{16} (about 5 min as compared with 19 min for the *ilva* marker of F_{14}) would spare its transfer from chromosomal interference. The results of the appropriate crosses using the F′ strain AB1528 as the donor of F_{16} are shown in Fig. 6 and 7. The data plotted in Fig. 7 show that chromosome transfer does not affect the recovery of the F_{16} *ilva*$^+$ marker, again confirming model ii. In passing, it may be noted that the transfer of F_{16} does not cause a delay in the initiation of chromosome transfer.

DISCUSSION

The experiments reported above suggest the following picture of genetic transfer by F′ strains of *E. coli*. In many conjugating pairs, F-merogenote transfer begins before chromosome transfer. The markers of the merogenote penetrate the zygote sequentially; in the case of F_{14}, *met*$^+$ first appears in zygotes at 10 min, *arg*$^+$ at 15 min, and *ilva*$^+$ at 19 min, F being tightly linked to *ilva*$^+$.

In a certain percentage of the conjugating pairs, chromosome transfer also commences. Entry of the chromosome into the zygote breaks the F-merogenote in transit, so that the only F-merogenote markers to be recovered in chromo-

somal recombinants are those which have preceded the chromosome. Distal markers of the F-merogenote are thus affected more than proximal markers.

The interaction between chromosome and F-merogenote causes further movement of the former to be delayed for approximately 9 min. The reason for this delay is not known. It might represent the time necessary for the conjugation tube to become unblocked, or for a new conjugation tube to be established.

A striking feature of these experiments is seen in the different behaviors of F_{14}-carrying strains AB1206, AB1540, and AB1516. AB1206 transfers only F-merogenote; AB1540 transfers chromosome as well as F-merogenote, the markers of the latter showing a steeper gradient of recombination frequencies as measured at any given iime; and AB1516 transfers chromosome at an tncreased frequency, with a correspondingly greater effect on the gradient of recombination frequencies of F-merogenote markers (Fig. 1 and

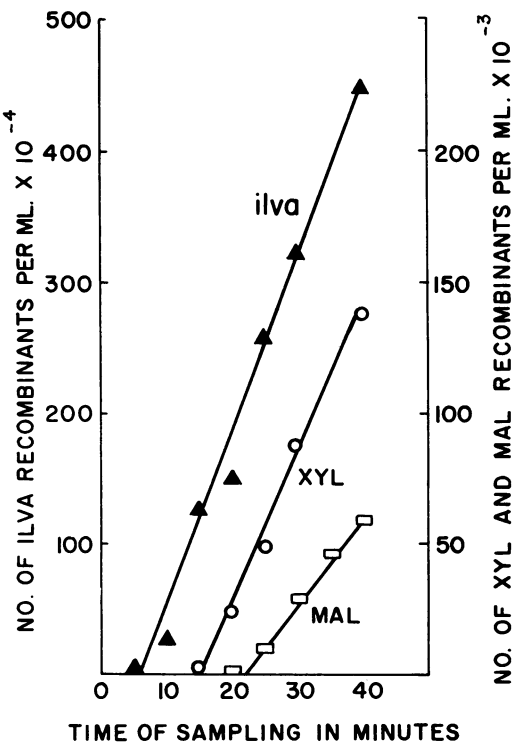

FIG. 6. *Kinetics of zygote formation for chromosomal and F-merogenote markers transferred from F′ strain AB1528 to female strain AB1450.*

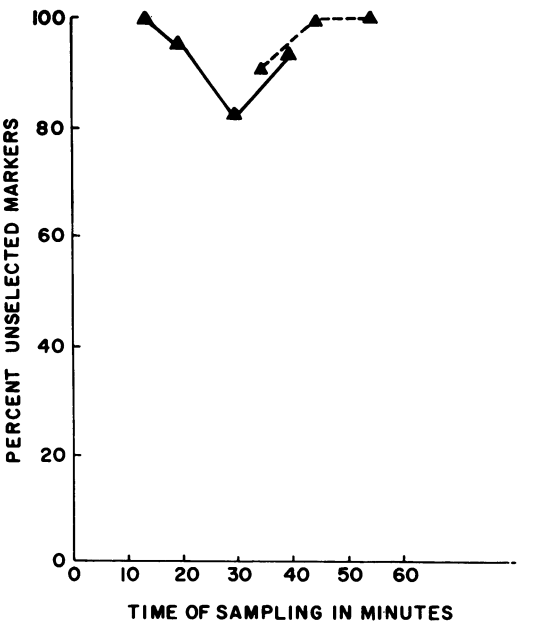

FIG. 7. *Per cent recovery of the F-merogenote marker ilva-16 in recombinants selected for chromosomal markers XYL and MAL (solid line, XYL; broken line, MAL). The male donor is F′ strain AB1528 and the recipient is female strain AB1450.*

2). The reason for these differences is not known; similar variations have been observed in the case of F′ strains carrying F_3 (Loutit and Adelberg, *unpublished data*). The difference between AB1540 and AB1516 is not ascribable to a difference in the number of F_{14} elements per cell, since measurements of one enzyme activity controlled by the *ilva* locus, dihydroxy acid dehydrase (Myers, 1961), suggests that there are two F-merogenotes per nucleus in both strains.

Addendum in Proof

On the basis of their studies on the transfer of chromosomal markers by strains of *E. coli* carrying the F-merogenote *F-lac*, Scaife and Gross propose that chromosome transfer in such F′ strains requires a crossing-over between *F-lac* and its homologous chromosomal region (J. G. Scaife, *personal communication*). The interaction between F_{14} and chromosome described in this paper can be explained in terms of their model: obligatory crossing-over would account for the 9-min delay in chromosome transfer and for the decrease in linkage between F_{14} markers in recombinants receiving chromosomal material. The observed low

recovery (20%) of the late F_{14} marker *ilva*$^+$ compared with the much higher recovery (80%) of the early F_{14} marker *met*$^+$ would, according to this model, be caused by crossing-over events resulting in the transfer of the chromosomal marker *ilva*$^-$ instead of the F_{14} marker *ilva*$^+$. The model predicts, therefore, that if the F′ donor carries *ilva*$^+$ on both F'_4 and the chromosome, recombinants selected for chromosomal markers should now receive *ilva*$^+$ at high frequency. When such a homozygous *ilva*$^+$/$^+$ F′ male was used as donor, 91% of the *MAL*$^+$ recombinants were found to be *ilva*$^+$ as predicted. Further predictions of the crossing-over model are now under test using this F′ system.

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