PSEUDOINVERSIONS IN THE CHROMOSOME OF *ESCHERICHIA COLI* K-12

N. GLANSDORFF[1]

Laboratoire de Microbiologie, Faculté des Sciences, Université de Bruxelles, et Institut de Recherches du C.E.R.I.A., Bruxelles 7, Belgique

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THE following observations are mainly methodological; they concern the use of bacterial conjugation in genetic topography. However, as will be shown hereafter, they are by-products of investigations on the genetic control of arginine biosynthesis in *Escherichia coli* and have precise (although limited) implications for the treatment of this question.

Four of the eight structural genes of the latter pathway are tightly clustered in the order *argE–C–B–H* (GLANSDORFF 1965); Figure 1 gives the gene-enzyme correspondance, as defined by MAAS, MAAS, WIAME and GLANSDORFF (1964). Both enzymatic determinations and genetic evidence suggest that *argB* and *H* (and probably *C*) form an operon, the fate of *argE* being uncertain (GLANSDORFF and SAND 1965). It was thus expected that chromosomal rearrangements which would modify the spatial relationships of the *arg* loci should also alter the regulation of their expression.

In this last respect, we became interested by the *E. coli* strain AB1206, isolated by PITTARD, LOUTIT and ADELBERG (1963); this organism harbours an episome

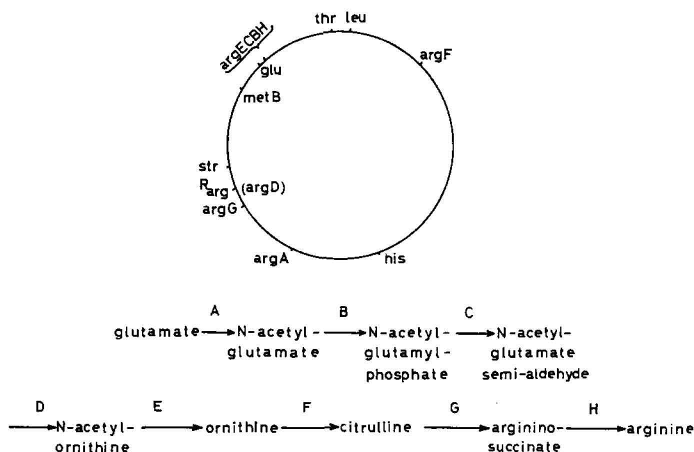

FIGURE 1.—The gene-enzyme relationships for arginine biosynthesis in *E. coli* K-12 (outline of the enzymic steps and map of the corresponding *arg* loci). For abbreviations, see MATERIAL and METHODS. *argD* has been mapped in *E. coli* W only (VOGEL *et al.* 1963).

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of the F′ type (a unit of replication composed of the fertility factor F and a segment of the bacterial chromosome; JACOB and ADELBERG 1959). This episome (F_{14}) carries a segment which represents about 10% of the chromosome (PITTARD and RAMAKRISHNAN 1964); F_{14} can be considered as a small bacterial chromosome, analogous to the genetic material of an Hfr strain. The point which is relevant to our discussion is that F_{14} was said to carry a transposition of the gene *argH* which specifies argininosuccinate lyase, the last enzyme of arginine biosynthesis (PITTARD *et al.* 1963). However, a more recent report stated that the transposition involves the locus responsible for acetylornithinase biosynthesis (*argE*; BAUMBERG, BACON and VOGEL 1965); surprisingly, from the observations of the latter authors as well as from our own (see further), it became clear that the regulation of the whole *arg* cluster was not altered in AB1206.

After an extensive reexamination of the F_{14} episome, we have found that its topography is identical with that of the same genetic region in other strains. However, AB1206, as well as other *E. coli* donor strains, gives rise to pseudotranspositions or pseudoinversions when: (1) The markers investigated are very close to the leading end of the donor chromosome; at 37°C, the transfer of the segment extending from this point to the pseudoinverted markers does not seem to demand more than 1 minute. (2) The order of the genes is deduced solely from the determination of the curves which reflect the kinetics of their transfer (i.e. the kinetics of formation of separated classes of recombinants, from matings interrupted at regular intervals by mechanical means (WOLLMAN and JACOB 1959) or by treatment of the donor strain with a bacteriophage (HAYES 1957).

Possible interpretations of this phenomenon are discussed. A preliminary account of this work has been given previously (GLANSDORFF 1966).

MATERIALS AND METHODS

Genetical and enzymological techniques: The media and the techniques used in the mating and transduction experiments have been described previously (GLANSDORFF 1965). References for measurements of enzyme activities are as follows. The symbol used to represent the genetic determinant of each of these enzymes is indicated in parentheses. ATP: α-N-acetyl-L-glutamate 5-phosphotransferase (*argB*): BAICH and VOGEL (1962). α-N-acetyl-L-glutamate γ-semialdehyde: NADP oxydoreductase (phosphorylating) (*argC*): GLANSDORFF and SAND (1965). L-ornithine α-N-acetylornithine lyase (*argE*): VOGEL and BONNER 1956. L-argininosuccinate arginine lyase (*argH*): RATNER, ANSLOW and PETRACK 1953.

Abbreviations used: arg = arginine; *glu* = glucose (designates a gene involved in phosphoenolpyruvate carboxylation); *his* = histidine; *ilva* = isoleucine and valine; *leu* = leucine; *met* = methionine; *pro* = proline; *pur* = purine; *rha* = rhamnose; *str* = streptomycin; *thi* = thiamine; *thr* = threonine; *T6* = phage T6; *r* = resistance; *s* = sensitivity; F^- = female, recipient; Hfr = male, donor. Capital letter: nomenclature of gene loci according to DEMEREC (1956).

Strains: The basic material of this work is the strain AB1206 (F′, *his, pro, thi,* $T6^s$, str^r), supplied by DR. ADELBERG. PA373 (F^-, *thr, leu, thi, his, metA, argH,* $T6^s$, str^r), PA374 (F^-, *thr, leu, thi, his, metE, argH,* $T6^s$, str^r), P10 (Hfr, *thr, leu, thi,* $T6^s$, str^s), P72 (Hfr, thr^+, leu^+, *thi, metB,* $T6^s$, str^s) and the transducting phage 363 were obtained from DR. F. JACOB. AT11–31 (Hfr, *thr, leu, thi, purD,* $T6^s$, str^r) and AT12–56 (Hfr, *thr, leu, thi,* $T6^s$, str^r) were obtained from R. LAVALLE. The rest of the strains either have been described previously (GLANSDORFF 1965) or have been constructed by transduction experiments from those above.

rha, *arg* and *ilva* mutants isolated in the course of this work were induced by N-methyl-N′-nitro-N-nitrosoguanidine, following the method recommended by ADELBERG, MANDEL and CHEN (1965). Penicillin was used to kill wild-type cells surviving the mutagen treatment, as described by GORINI and KAUFMAN (1960).

EXPERIMENTS

The first section describes the wild-type topography of the genetic markers considered in this study; then follows a recall of the properties of AB1206, with some comments on the use of this strain in transductions. The second section presents determinations of enzyme activities which reflect the activity of the genes involved in the apparent rearrangement undergone by the F_{14} episome. Data concerning enzymes E and H have been reported by BAUMBERG *et al.* (1965), but in a different context and leading to different conclusions, since these authors consider AB1206 as carrying a transposition of the *argE* locus. The third and fourth sections contain the detailed genetic analysis of AB1206, Hfr strains P10 and P72, carried out by matings and transductions.

1. *Topography of the segment investigated—properties of AB1206:* The segment of the *E. coli* chromosome which is relevant to this study is represented in Figure 2. The main part of this map is based on the data recently reviewed by TAYLOR and THOMAN (1964). Additional information for the *met-arg* region has been included (GLANSDORFF 1965). The site of rhamnose mutations was until now uncertain with respect to the closest markers; one rhamnose mutation isolated in the course of this work has been mapped on the following basis (GLANSDORFF 1966): *rha* lies on the left of *metB* (see Figure 3); *rha* is cotransducible with *metB* (30% cotransduction); *rha* is cotransducible with *metE* (1% cotransduction); *metB* and *metE* are not cotransducible (less than 0.01%). *rha* thus lies between *metE* and *metB*.

The episome F_{14}, harboured by the strain AB1206, has originated from the Hfr AB313. It contains about 10% of the bacterial chromosome, and extends from *ilva* to the right of the *arg* region. As shown hereafter, its origin lies between *argH* and *purD*. The episome is represented (Figure 2) after its original description (PITTARD *et al.* 1963), with a transposition of the *argH* locus to the left of

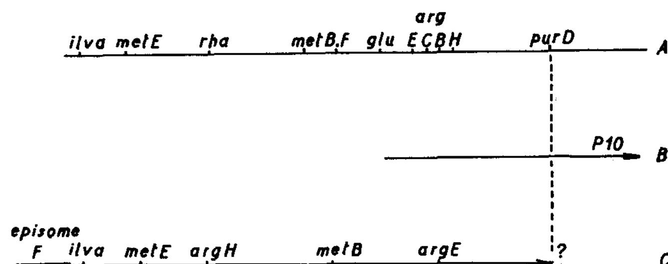

FIGURE 2.—(A) The segment of the *E. coli* chromosome extending from *ilva* to *purD*. (B) The sequence of gene transfer by Hfr P10 (C). The F_{14} episome, original description (see text). Arrangement of gene loci is not precisely to scale.

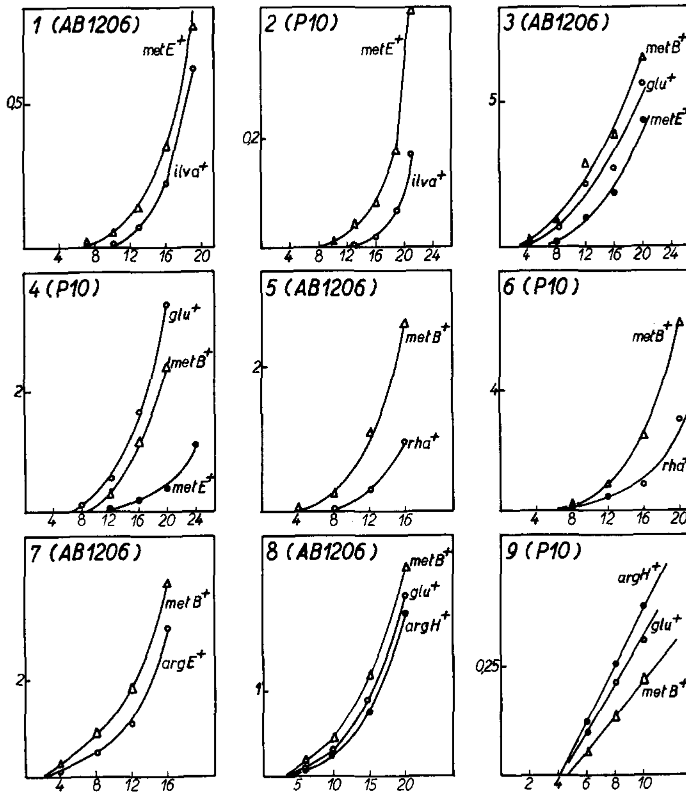

FIGURE 3.—Transfer of *arg*, *glu*, *met*, *rha* and *ilva* alleles by Hfr P10 and F'AB1206. The donor is indicated in parentheses; the genotypes of the recipients are: Crosses (1) and (2), *metE*, *ilva*. Crosses (3) and (4), *glu*, *metB*, *metE*. Cross (7), *argE*, *metB*. Crosses (5) and (6), *metB*, *rha*. Crosses (8) and (9), *argH*, *glu*, *metB*. Abscissa: time (minutes). Ordinate: recombinants (percent input donor strain). Differential supplementation of selective media with homocysteine or vitamin B_{12} was used to distinguish *metE*+ from *metB*+ recombinants, respectively.

metB. The sequence of chromosomal transfer of Hfr P10 is also represented (see section 3).

The interpretation of transduction carried out with AB1206 as donor rests on the assumption, strongly supported by the work of PITTARD and RAMAKRISHNAN (1964), that the genes carried by F_{14} are deleted from the chromosome of AB1206. The origin of the transducing particles produced from AB1206 and carrying *met*, *glu* or *arg* alleles, is thus episomic and does not contain chromosomal material. This was confirmed by three lines of evidence:

(1) The cotransduction of *purD* and *metB*, which amounts to 3% when a (*metB*, *purD*) strain is transduced by a wild-type donor strain, is not observed when AB1206 is used as the donor (less than 0.01%). Moreover, matings performed with the same recipient strain show that AB1206 does not transfer the *purD*+ allele in conditions where 10% of *metB*+ recombinants are formed. The linkage between *metB* and *purD* is thus interrupted in AB1206, leading to the

conclusion that *metB* is on the episome and *purD* on the chromosome. As F_{14} carries the *glu* and *arg* genes, the right limit of the material which is absent from the chromosome and carried by the episome must lie between the *arg* cluster and *purD*. The chromosomal deletion of AB1206 thus seems to correspond exactly to the episomal markers of F_{14}. Although the genetic constitution of AB1206 shows that its formation has involved several events (PITTARD *et al.* 1963), it is conceivable that the chromosomal deletion is an immediate consequence of the formation of F_{14} from the chromosome of the Hfr AB313. This possibility should be considered in relation to the hypothesis proposed by BRODA, BECKWITH and SCAIFE (1964) for the formation of F-prime episomes.

(2) Recessive *ilva* mutations harboured by F_{14} render AB1206 dependent on isoleucine and valine (PITTARD and RAMAKRISHNAN 1964). Similarly, we could isolate *argB* and *rha* mutants from this strain. It was shown, by transfer of the mutated F_{14} to F^- strains, that these mutations are recessive.

(3) When an *argB* mutant of AB1206 is used as donor to transduce heteroallelic *argB* recipients, the yield of arg^+ recombinants is very low: 0.2 to 1.0% of the number of wild-type transductants recovered for a *met* or *glu* allele present in the *argB* recipient. This means that the phage particles obtained from the mutant do not contain a wild-type allele of *argB* which could have been present in the chromosome, although unexpressed.

2. *Enzymatic study of AB1206:* Previous studies suggested that, from the cluster of *arg* loci (*E, C, B, H*) situated on the right side of *glu*, at least two (*B* and *H*) are joined in an operon, with a probable $B \rightarrow H$ polarity (GLANSDORFF and SAND 1965). It was thus essential to examine the expression of the *argH* locus in AB1206, where it was supposed to be transposed (PITTARD *et al.* 1963). Specific activities of argininosuccinate lyase (coded by *argH*) and of the other enzymes specified by the arginine cluster, were determined in AB1206, P4X, and *argG* or *A* derivatives of both strains grown under conditions of derepression in a chemostat. Table 1 shows that there is no significant difference between the two families of strains in a range of variation which goes from maximal re-

TABLE 1

Specific activities of the enzymes specified by the loci argE, C, B *and* H *in P4X and AB1206, under various conditions**

		Enzyme activity (µmoles/hr/mg protein			
Strain	Condition	Acetylornithine lyase (locus *argE*)	Oxydoreductase (locus *argC*)	Phosphotransferase (locus *argB*)	Argininosuccinate lyase (locus *argH*)
P4X*argA*	chemostat	31	2.1	2.5	3.6
P4X	without arginine	13.6	0.7	0.9	1.5
P4X	with arginine	2.4	0.07	0.1	0.15
AB12106*argG*	chemostat	39	2.8	2.9	5.7
AB1206	without arginine	13.8	..	..	1.5
AB1206	with arginine	2.8	0.09	0.1	0.18

* Growth conditions: minimal medium 132 (GLANSDORFF 1965) supplemented with glucose (0.5%) and specific requirements (P4X: 50 µg/ml L-methionine; AB1206: 50 µg/ml L-histidine, 50 µg/ml L-proline, 1 µg/ml thiamine), L-arginine when added: 200 µg/l. The chemostats were limited by L-arginine (10 µg/ml) at a dilution rate of 4 hr^{-1} (AB1206argG) or 2 hr^{-1} (P4XargA). Temperature 37°C, aeration by forced agitation.

pression to nearly complete derepression. Faced with the difficulty of interpreting these results in terms of a transposition of *argH* when considering this locus probably belongs to an *argB* → *H* operon, we were led to an extensive reexamination of AB1206, from a genetical point of view.

3. *The genetic analysis of AB1206 by mating and transduction experiments:* To determine the order of transfer of several genetic markers from AB1206 to F^- recipients, we used HAYES' technique of T6 interrupted matings (1957). $T6^r$ derivatives were thus prepared from the required F^- strains. As AB1206 is *pro*⁻ and all our F^- strains *pro*⁺, we submitted AB1206 to a double counterselection: T6 killing and absence of proline in the different selective media. In crosses performed with Hfr P10, we used T6 and streptomycin for the counterselection of the donor. The various crosses performed are represented in Figure 3.

As numerous workers have found (see TAYLOR and THOMAN 1964, for a review) the kinetics of formation of recombinants is not as a rule a straight line, but a continuous curve which sometimes resembles a broken line, with two successive slopes. The intersection of these curves with the abscissa is not always easy to determine with precision; nevertheless, we conclude from Figure 3 that the sequence of markers injected by Hfr P10 is:

origin–cluster *arg–glu–metB–rha–metE–ilva*

and when AB1206 is used as donor this becomes:

origin–*metB–glu*–cluster *arg–rha–metE–ilva*

Thus, at first sight the *metB–arg* segment seems to have undergone an inversion in AB1206.

This point was examined with particular attention, in view of the theoretical bearing that the occurrence of inversions should have on our representation of the organisation of the bacterial chromosome (see MARGOLIN 1965 and SANDERSON 1965).

From the inversion of the *met–arg* segment in the F_{14} episome we should expect the following consequences: (1) The linkage relationships of the *metB*, *glu* and *arg* alleles should not be modified within the limits of the inversion. (2) The linkage relationships of the same markers with a locus outside the inverted zone should be modified (suggested by R. LAVALLE). (3) The pairing of F_{14} with a complete chromosome within the limits of the inversion should lead to the reversal of the order of chromosomal transfer which this episome usually induces (PITTARD and ADELBERG 1964); thus the kinetic analysis of a cross between a recipient strain and a donor carrying both F_{14} and a normal, F^- chromosome, should give two families of curves, each one related to one of the two possible modes of pairing between F_{14} and the chromosome. (4) Last but not least, the inversion of the *met–arg* segment should appear from an analysis of the genetic constitution of numerous recombinants taken at early times from interrupted matings between recipient strains and AB1206. This type of analysis has in fact legitimized the use of the kinetics of recombinant formation for cartographical

means (WOLLMAN and JACOB 1959) but does not often appear to have been carried out in more recent works; it had not yet been applied to AB1206.

Points (1) and (2) have been tested by transduction.

(a) Had the *argH* locus undergone a transposition leaving a deletion at its normal site, then it should be revealed by transduction of the *metB*, *glu* and *arg* alleles carried by F_{14}, in various recipient strains. For example, a transduction between a (*glu*, *argH*) recipient and AB1206 should give no arg^+ organisms among the glu^+ recombinants, and no or very few arg^+ recombinants, depending of the ability of the transposed fragment to synapse with the *argH* region of the recipient. Were the usual linkage relationships respected (GLANSDORFF 1965), about 90% of the glu^+ recombinants should be arg^+; this is, indeed, the result which is found (see Table 2). With (*glu*, *argC*) or (*glu*, *argB*) recipients, a donor carrying a transposed *argH* locus should give a low number of arg^+ recombinants among the glu^+ class, because *argH*, *B* and *C* are strongly linked in normal strains. The same reasoning would hold if *argE* was the transposed locus. In fact, again 90 to 95% arg^+ recombinants are found among the glu^+ class. Table 2 also shows that in each case, the data are paralleled by the scores of glu^+ recombinants among the arg^+ class.

The fourth transduction shown in Table 2 (*metB*, *glu*) × AB1206, confirms the absence of the arg^- class which should have been contributed by the donor, if carrying a transposed *argH*. This experiment also shows that the cotransduction index normally found for *metB* and *glu* (about 50%) is conserved when AB1206 is the donor.

These results do not show any structural modification within the limits of the *metB–argH* segment carried by AB1206, with respect to the topography which has been worked out with other *E. coli* strains.

(b) Unexpectedly, the results reported in Table 3 show that the distances between *metB*, *arg* and *rha* (outside of the apparent inverted zone) remain roughly the same, whether the cross is performed between "normal" strains, between derivatives of AB1206, or between a "normal" strain and AB 1206. These results contradict the hypothesis of an inversion and give a first indication that the order of the genes could be the same in AB1206 and P10.

(c) The same conclusion arises from the testing of point (3). When the episome F_{14} was introduced in a F^- strain possessing a complete chromosome,

TABLE 2

Transduction analysis of the metB–glu–argH *region, with AB1206 as the donor*

Recipient	Number of unselected versus selected recombinants			
	arg^+/glu^+	glu^+/arg^+	met^+/glu^+	glu^+/met^+
glu-2, argH-2	109/119	37/39	.....	.....
glu-2, argB-1	108/119	32/36	.....	.....
glu-2, argC-1	104/111	28/29	.....	.....
metB-1, glu-1	71/71	.....	30/71	46/79

TABLE 3

Transduction analysis of the arg–metB–rha *region, in different kinds of coupling of "normal" strains and AB1206 derivatives*

Recipient	Donor	Linkage measured		Percent
rha, metB, argE	*rha*+, *met*+, *arg*+	*metB–rha*	43 *met*+/194 *rha*+	22.1
		argE–rha	32 *arg*+/352 *rha*+	9.1
rha, metB, argE	AB1206 *rha*+, *met*+, *arg*+	*metB–rha*	46 *met*+/156 *rha*+	29.5
		argE–rha	13 *arg*+/156 *rha*+	8.3
AB1206 *rha*+, *metB*, *arg*+	AB1206 *rha* , *met*+, *arg*+	*metB–rha*	28 *rha*− /159 *met*+	17.6
AB1206 *rha* , *met*+, *arg*+	AB1206 *rha*+, *met*+, *argB*	*argB–rha*	18 *arg*− /428 *rha*+	4.2

TABLE 4

Genetic analysis of arg+ *and* met+ *recombinants recovered from interrupted matings performed between* (metB *or* E; argH *or* E) *F*− *strains and, either Hfr P10, or AB1206*

Time of interruption (min)	F−(*metE, metB, argE*) × AB1206								F−(*metB, argH*) × AB1206				F−(*metB, argH*) × P10*	
	argE+				*metB*+				*argH*+		*metB*+		*argH*+	*metB*+
	metB+	%	*metE*+	%	*argE*+	%	*metE*+	%	*metB*+	%	*argH*+	%	*metB*+ (%)	*argH*+ (%)
2	.....	...	.....	...	.....	...	.....	...	18/50	36.0	15/53	28.0	..	..
3	24/59	40.6	3/59	5.9	31/48	64.6	4/40	10	.....	...	.....	...	..	..
4	.....	...	.....	...	.....	...	.....	...	60/120	50.0	37/116	33.0	..	..
6	67/120	55.8	9/80	11.3	63/120	52.5	13/80	16.3	72/118	61.0	38/120	32.0	..	..
8	.....	...	.....	...	.....	...	.....	...	.....	...	.....	...	35	63
9	69/120	57.5	17/80	21.3	71/120	59.2	17/80	21.3	.....	...	.....	...	..	..
10	.....	...	.....	...	.....	...	.....	...	.....	...	.....	...	49	65
12	81/120	67.5	19/80	23.8	72/120	60.0	18/80	22.5	93/119	78.0	49/119	38.0	62	72
15	62/78	80.0	.....	...	48/78	62.0	.....	...	.....	...	.....	...	..	..

Percent of unselected donor markers among recombinants is given in the body of the table.
* Experiment from GLANSDORFF (1965).

and the latter transferred to appropriately marked recipient strains, we could not find any reversal of the direction of this transfer.

(d) Conclusive evidence that the order of transfer of AB1206 is indeed the same as that of P10, could be obtained from the analysis of the genetic constitution of recombinants taken at early times during interrupted matings between recipient strains and either AB1206 or P10 (see Table 4). The progressive increase of met^+ among arg^+ recombinants, whether P10 or AB1206 is used as the donor, shows that the apparent inversion of the *met–arg* segment is an artefact of the methodology applied previously.

We have seen that the *arg* cluster is very close to the origin of the F_{14} episome. Indeed, *argH* and *purD* are cotransducible, but F_{14} does not carry the *purD* locus. Formally speaking, the pseudoinversion effect can be attributed to an influence of the origin on the integration of donor genes situated in its immediate vicinity (less than 1 or even 0.5 minute of transfer time). The closer a gene would be to the origin, the less frequently should it appear in the progeny of the cross. The fact that the percent of arg^+ among met^+ recombinants is two times lower when AB1206 is used as the donor rather than P10 gives a direct measurement of this restriction. Conceivable mechanisms of such a phenomenon will be discussed at the end of this paper, together with evidence already available in this topic (Low 1965; Glansdorff 1966).

From a restriction exerted by the origin, we would expect that the curves relating to arg^+ and met^+ recombinants should follow the real order of the genes only at their early beginning. As the restriction effect of the origin does not seem to influence the integration of loci distal to *metB*, the curves should intersect in the very first minutes of mating. This crossing of curves should probably not be demonstrable with certainty by the numeration techniques used in this kind of experiment; its visible consequence should thus consist in pseudotranspositions or pseudoinversions, depending on the number of markers investigated. A tendency for the curves to cross could in fact be observed in experiments where interruptions were performed at very short intervals; it was also more apparent when the speed of the transfer was lowered by a shift from 37°C to 32°C. The results, however, cannot be considered demonstrative.

Our interpretation was strengthened by the occurrence of a second pseudoinversion which could be related to the same restrictive effect. We had mapped the *rha* locus between *metE* and *metB*; the kinetics of transfer of the rha^+ and $argE^+$ alleles to a rha^-, *argE* recipient by the Hfr AT1256 is shown in Figure 4. Figure 4 also represents the transfer of the same alleles by Hfr P72, whose leading end was known to lie between *metE* and *metB* (Hfr No. 5 in Jacob and Wollman 1961). Although the succession of the curves seems to point out the order $\overleftarrow{arg\text{–}rha}$ in this last mating, it is clear from the genetic analysis of arg^+ and rha^+ recombinants that both Hfrs inject the chromosome in the same order, $\overleftarrow{rha\text{–}arg}$; however, a strong restriction operates on the integration of rha^+ from P72 into the recipient strain (see Table 5). The distance separating *rha* and the origin of P72 is of the same order of magnitude as the *argH*–F_{14} origin interval.

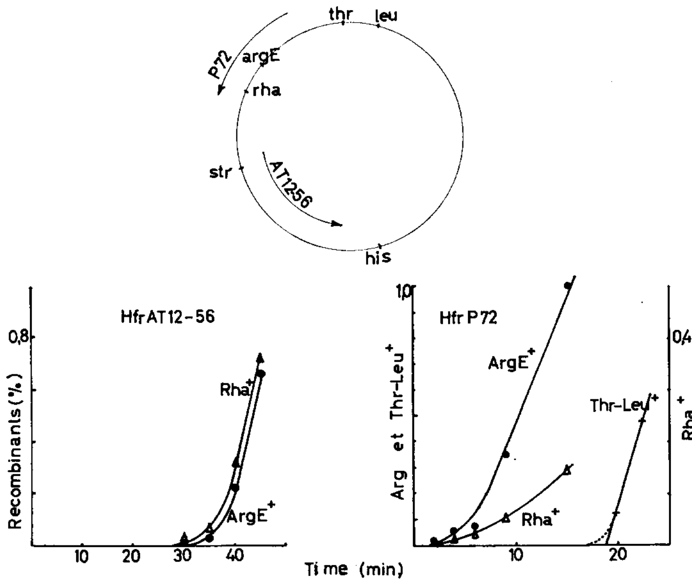

FIGURE 4.—Transfer of *argE, rha* and *thr-leu* alleles by Hfr AT12–56 and Hfr P72. The genotype of the recipient is, in both cases, *rha, argE, thr, leu.*

Indeed, *rha* is cotransducible with *metE* (about 1%), but the latter locus is not injected early by P72. We could show in additional experiments that the restriction operating on *rha* does not affect the locus *metF*; however the cotransduction percent of *metF* and *rha* approaches 20%; *metF* and *argE* are about 40% cotransducible.

DISCUSSION

The main conclusion of this work has to be set forth on a methodological ground. When a genetic marker is very close to the leading end of the chromosome which Hfr and F′ strains transfer during conjugation, the frequency of

TABLE 5

Genetic analysis of rha+ *and* arg+ *recombinants recovered from interrupted matings performed between an* (rha, argE) *F− strain, either Hfr AT12–56 or Hfr P72*

F⁻(*rha, arg*) × AT12-56					F⁻(*rha, arg*) × P72				
Time of interruption (min)	*arg*+		*rha*+		Time of Interruption (min)	*arg*+		*rha*+	
	rha+	%	*arg*+	%		*rha*+	%	*arg*+	%
30	18/23	78	8/36	22	4	7/61	11.4	2/21	9.5
35	47/62	76	32/80	40	6	4/32	12.5	13/49	26.5
40	62/80	78	32/78	41	9	8/119	6.7	21/64	32.8
45	62/80	78	41/76	54	15	6/118	5.1	62/120	51.7
50	68/79	86	45/77	59					

Percent of unselected donor markers among recombinants is given in the body of the table.

integration of this marker in the progeny of the cross is significantly reduced as compared to that of more distal markers. This phenomenon has an important consequence when two or more very proximal markers are investigated. Suppose these markers are *a*, *b*, *c*, injected in that order by the donor strain; we have seen that the determinable parts of their kinetics of transfer will appear in the order *c*, *b*, *a*, giving thus the picture of a pseudoinversion (pseudoinversion or pseudotransposition in the case of two markers). The real order of transfer has to be deduced obligatorily from the analysis of the genetic constitution of several classes of recombinants.

The idea that the leading end of the chromosome might restrict marker integration in its vicinity has already been considered by Low (1965), but without emphasis on pseudoinversion effects; obviously, the latter can only appear when F^- strains carrying several proximal markers are used in the mating experiments, such as those reported by PITTARD *et al.* (1964) and in the present paper. At present, several hypotheses can be advanced to explain the restriction operating on the integration of proximal genes. Low (1965) and ourselves (1966), in a preliminary report of this work, had independently advanced the following explanation: the restriction imposed by the origin should merely reflect a relation between the distance which separates a gene from the origin, and the crossing over frequency within this interval. Indeed, as in bacterial crosses, the genetic material contributed by the donor is limited to a fragment (*a fortiori* when the mating is interrupted for the purpose of gene mapping) the formation of a recombinant requires at least two genetic exchanges, one of them occurring between the selected marker and the chromosomal origin. However, a recent quantitative analysis of linkage between bacterial genes makes such a simple explanation unlikely (VERHOEF and DE HAAN 1966; DE HAAN and VERHOEF 1966). In agreement with the present results, the latter authors have presented convincing evidence that the proximity of the origin has no influence on the integration of markers so close to the origin as to be transferred 1 or 2 minutes after it (the *proA* locus, injected early by Hfr R4). Were the present results ascribable only to the distance (origin–*argH* or *rha*) then one would expect the influence of the origin operating up to markers situated 10 minutes of transfer time from the origin. The model presented by the latter authors may offer an explanation for the short range effect that we observe; the data supporting the model are strongly consistent with the idea that an obligate crossing over occurs between the Hfr and the F^- chromosomes at the level of the origin; it is conceivable that this crossing over could occur not only at the very point of origin, but also in a segment situated in the immediate vicinity of it. The source of another explanation, also compatible with the model of DE HAAN and VERHOEF, can be found in the paper by FULTON (1965) on continuous chromosomal transfer during bacterial conjugation. The low integration frequency of a *pur* marker injected early by HfrC has led FULTON to speculate that the origin of chromosomal transfer of an Hfr strain could vary from one cell to the other; this variation, however, should only take place in a very small proximal segment, adjacent to the F factor inserted in the chromosome. As he has pointed out, the discovery of continuous chromosomal transfer

has until now precluded any simple means of testing this hypothesis by genetical experiments. The question thus remains unanswered. Experiments are now in progress to distinguish between the possibilities mentioned.

Our last conclusion concerns the genetic control of arginine biosynthesis in *E. coli.* A problem had originated from the apparent transposition of the locus *argH* (PITTARD *et al.* 1963) or *argE* (BAUMBERG *et al.* 1965), without any consequence on the control of their expression, nor on the expression of *argC* and *B*, which are their neighbours in the *argECBH* cluster of which at least *argB* and *H* form an operon (GLANSDORFF and SAND 1965). The problem now disappears, since the chromosome rearrangements previously described are methodological artefacts. Moreover, we have yet unpublished evidence that the effective transposition of *argH* a short distance from its site of origin makes the expression of this gene constitutive (SAND and GLANSDORFF). Lastly, we should recall that the locus *argE* might well constitute a unit of expression independent of *argC*, *B* and *H*, although the present work shows that this suspicion can no more be based on the properties of strain AB1206; it has been shown, indeed, that the synthesis of enzyme E does not appear strictly coordinated with that of enzymes C, B and H (see BAUMBERG *et al.* [1965] for enzymes E and H; GLANSDORFF and SAND [1965] for enzymes E, C, B and H).

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SUMMARY

The restriction which the chromosomal origin of *E. coli* males exerts on the integration of very proximal markers in the progeny of crosses, leads to apparent pseudoinversions of the markers investigated in the usual type of kinetic analysis applied to chromosomal transfer. Previously reported chromosomal rearrangements which had been said to disorganize a cluster of arginine loci without altering its regulation, are methodological artefacts of that type.

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