EXCHANGE OF GENETIC MATERIAL BETWEEN SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI K-12

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POSITIVE results in attempts to hybridize Salmonella strains with *Escherichia coli* K-12 were first reported by BARON and his colleagues (BARON, SPILMAN and CAREY 1959; BARON, CAREY and SPILMAN 1959), and we have also observed this phenomenon with a *Salmonella typhimurium* wild-type strain LT-7 carrying the mutator gene *mut* (MIYAKE and DEMEREC 1959; MIYAKE 1959, 1960). This paper supplies more detailed information about our studies.

MATERIALS

The *S. typhimurium* mutants used in these experiments were isolated from wild-type strains LT-2, LT-7 *mut*, and LT-7 *mut*$^+$ by a number of investigators in M. DEMEREC's laboratory (Department of Genetics, Carnegie Institution of Washington). Mutation to auxotrophic requirement occurred either spontaneously or after irradiation with ultraviolet light, and the mutants were selected by standard penicillin screening. The strains of *E. coli* K-12 employed are listed in Table 1.

Mutants are referred to by the abbreviations proposed in Microbial Genetics Bulletin No. 16 (1958), namely: *adth*, adenine-plus-thiamine multiple requirement; *arg*, arginine requirement; *asa*, asparatic acid requirement; *azi-r*, azide resistance; *azi-s*, azide sensitivity; *glt*, glutamic acid requirement; *gua*, guanine requirement; *lac*, lactose utilization; *leu*, leucine requirement; *mal*, maltose utilization; *met*, methionine requirement; *mut*, mutator gene; *pan*, pantothenic acid requirement; *pro*, proline requirement; *str-r*, streptomycin resistance; *str-s*,

TABLE 1

Strains of Escherichia coli *K-12 employed in experiments. 0 = starting point of injection*

Strain	Source	Markers	Order of injection
HfrH	W. HAYES	*str-r, azi-r, T1-s, T6-s, met*	0–*thr–leu–pan–pro–lac* . . . .
HfrCS-101	L. L. CAVALLI	*str-s, T2-r, T6-s, lac*$^+$, *mal*$^+$, *met*	0–*lac–pro–pan–leu–thr* . . . .
HfrP4X$_{fi}$	F. JACOB	*met*	0–*pro–pan–leu–thr* . . . *lac*
RT-18 (F+)	J. LEDERBERG	*str-s, T2-r, T6-r, met*	. . . . .
Row (F—)	D. ROWLEY	*str-r, met*	. . . . .

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streptomycin sensitivity; *thi*, thiamine requirement; *thr*, threonine requirement; *try*, tryptophan requirement; *tyr*, tyrosine requirement.

The Salmonella phages generally employed were temperate phage PLT-22 H1, and its virulent mutant H5. In the timing experiments, Hfr bacteria were eliminated by phage T6 (HAYES 1957).

The minimal agar medium contained K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgSO_4$, 0.05 g; $(NH_4)_2SO_4$, 1 g; sodium citrate, 0.97 g; glucose, 2 g; minimal agar, 15 g; demineralized water, 1000 ml. When *lac*$^+$ was the selective marker, sodium citrate was omitted from the medium. To supplement the minimal medium with an amino acid (or a vitamin), 20 mg (or 2 mg) was added to 1000 ml of medium. The EMB agar medium for testing sugar-fermentation ability of bacteria was composed of tryptone, 10 g; yeast extract, 1 g; NaCl, 5 g; K_2HPO_4, 2 g; eosin Y, 0.4 g; methylene blue, 0.065 g; agar, 15 g; sugar, 10 g; demineralized water, 1000 ml. The nutrient agar medium contained NaCl, 5 g; nutrient agar, 23 g; demineralized water, 1000 ml.

METHODS AND RESULTS

Detection of recombinants: As has been previously reported (MIYAKE and DEMEREC 1959), genetic recombination between *E. coli* and *S. typhimurium* was observed only when K-12 Hfr was the *coli* parent and LT-7 *mut* was the Salmonella parent. The procedure for detecting recombinants was as follows. One volume of an overnight broth culture of *E. coli* K-12 Hfr was added to nine volumes of fresh nutrient broth (8 g nutrient broth and 4 g NaCl per 1000 ml H_2O). A culture of *S. typhimurium* LT-7 *mut* was prepared in the same way. After 90-minute incubation at 37°C with aeration, the two parental cultures were mixed in a 1:1 ratio, and 0.1 ml of the mixture was immediately plated onto appropriate selective agar plates. After 24–36 hours' incubation, the recombinants were scored, either directly or by first determining their genotypes through appropriate tests. The number of recombinants detected by this method was approximately one in 10^6–10^7 plated bacteria, although it varied according to the marker used. So far the markers *pro*, *thr*, *pan*, *leu*, *arg*, *asa*, *glt*, *tyr*, *try*, and *cys* have been tested and found to be recombinable.

Properties of recombinants: Ten colonies that appeared on minimal-lactose agar plates spread with 0.1 ml of a mixture of *E. coli* K-12 HfrCS-101 (*met*, *lac*$^+$, *mut*$^+$) and *S. typhimurium* LT-7 *mut* (*met*$^+$ *lac*$^-$ *mut*) were purified by single-colony isolation, and their properties were determined.

The recombinants were tested for ability to utilize lactose, by streaking saline suspensions on EMB medium; for prototrophy, by streaking on minimal medium; for presence of the *mut* gene, by streaking on nutrient medium containing 100 μg/ml streptomycin (MIYAKE 1960); for ability to utilize citrate, by streaking on medium containing citrate as the sole carbon source; and for agglutination, by mixing the recombinant cells with whole serum for *S. typhimurium*.

Results of these tests with ten recombinants (Table 2) showed that all carried the *lac*$^+$ marker of the *E. coli* parent, and that all behaved like *S. typhimurium* with regard to sensitivity to phage PLT-22, ability to utilize citrate, and aggluti-

nation with Salmonella serum. Only one carried the *mut*$^+$ gene of *E. coli*. In another experiment, two out of 30 recombinants were resistant to phage PLT-22 and sensitive to phage T6 of *E. coli*. The agglutination tests with the ten recombinants were positive, but the degree of agglutination seemed to be weaker in one case, suggesting that some recombinants are intermediate.

Fertility factor in Salmonella carrying a mutator gene: Our earlier hybridization experiments were made with *S. typhimurium tyr-7 mut* (*lac*$^-$) and *E. coli* HfrCS-101. The bacteria were plated on minimal-lactose plates supplemented with tyrosine, so that *lac*$^+$ of *E. coli* was the selected marker. Frequency of recombination was very low, and a large majority of the recombinants carried *tyr* in addition to *lac*$^+$, indicating that the two loci are not closely linked.

Several hybrid strains were established with recombinants from these early experiments, and two were used in a study to compare recombination between *E. coli* and Salmonella with that between *E. coli* and a hybrid. The results are shown in Table 3. In these experiments *tyr*$^+$, introduced by the *E. coli* parent, was the selective marker. The recombination frequencies were strikingly different, being much higher in experiments with the hybrids. Thus the hybrids were much more fertile than the Salmonella parent.

Two explanations were considered: (1) the higher fertility of the hybrids was due to the presence of a chromosomal or cytoplasmic material transferred from the *E. coli* parents; (2) it resulted from mutation of a fertility factor in the *S. typhimurium* parents, either chromosomal or cytoplasmic, stimulated by the *mut* gene. The second explanation was based on the assumption that, if the original

TABLE 2

Properties of parental strains and of ten hybrid recombinants

Parental strains	*met*	*lac*	*mut*	Phage sensitivity PLT-22	Phage sensitivity T6	Ability to utilize citrate	Agglutination (S. serum)
HfrCS-101	—	+	+	r	s	—	—
LT-7 *mut*	+	—	*mut*	s	r	+	+
Recombinants							
1–9	+	+	*mut*	s	r	+	+
10	+	+	+	s	r	+	+

TABLE 3

Frequencies of replacement of tyr-7 *by* tyr$^+$ *in crosses between* tyr-7 *Salmonella, or* tyr-7 *Salmonella–*E. coli *hybrids, and* tyr$^+$ *Hfr* E. coli *strains*

	HfrCS-101	HfrP4X$_6$	Control
Salmonella	79	19	5
Hybrid–1	>2000	>2000	4
Hybrid–2	>2000	>2000	6
Control	0	1	.

Number of *tyr*$^+$ colonies on two plates. Each plate received a bacterial suspension (ca. 2×10^8 cells), consisting of a 1:1 mixture of *S. typhimurium* (or hybrid) and *E. coli*. Minimal-glucose medium. Control figures represent numbers of spontaneous reversions.

Salmonella population was heterogeneous for fertility, we might have selected particularly fertile clones.

These explanations were tested in the following experiment. Approximately 100 cells per plate of *pro-214 mut* (*lac*$^-$) *S. typhimurium* were plated on nutrient agar. After overnight incubation at 37°C, the colonies were printed on minimal-lactose agar plates supplemented with proline, which had just been spread with 0.1 ml of an overnight broth culture of *E. coli* HfrCS-101. After 24–36 hours' incubation at 37°C, some of the printed colonies (0.1–1 percent) showed heavy growth, because of *lac*$^+$ recombinants produced on the plates, and the rest showed no growth. Corresponding colonies of both types were picked up from the original nutrient agar plates. The first type was designated *pro-214* fer and the second *pro-214* infer, and both were tested for fertility. Crosses between HfrCS-101 and six *pro-214* fer colonies produced more than one thousand hybrids per plate, whereas similar crosses with six *pro-214* infer colonies produced an average of 6.8 hybrids per plate. Thus, by this method, fertile clones of *mut* Salmonella were selected from a population that had never been in contact with *E. coli*.

These results, indicating that the presence of chromosomal or cytoplasmic material derived from the *E. coli* parent is not necessary for the increased fertility of hybrids, favor explanation (2). It is therefore deduced that a population of *S. typhimurium mut* is a mixture of fertile (fer) and infertile (infer) cells, and that only the former recombine with *E. coli* Hfr. Experiments with the fer Salmonella and an F+ strain (RT-18) of *E. coli* K-12 also revealed recombination, although the frequency was low (about 10^{-3} that of the Hfr cross). These findings suggested that fer in Salmonella is equivalent to F− in *E. coli* and that infer might be equivalent to F+. However, a cross between fer Salmonella and infer Salmonella produced negative results.

By the printing method, fertile clones were selected from the following *mut* Salmonella strains: *proA-214*, *proC-51*, *glt-2*, *arg-6*, *pan-5*, *leu-119*, and *thr-61*. All attempts to find fertile strains among *mut*$^+$ Salmonella were negative, a result supporting the previous assumption that changes (probably mutations) from the original state to the fertile state are increased by the presence of *mut*. Two hundred cells from a fertile clone were tested for fertility, and all were found to be fertile; therefore this character is assumed to be fairly stable.

In recent experiments, *lac*$^+$ recombinants were detected in a cross between *E. coli* HfrCS-101 and Salmonella *proB-9* (*lac*$^-$) *mut*$^+$. Overnight broth cultures of the two bacterial strains were mixed in a 1:1 ratio and bubbled very slowly for 60 minutes at 37°C. Ten ml of this mixture was added to 100 ml of synthetic medium containing lactose as the sole carbon source (0.2 percent), which was then incubated for five hours at 37°C with aeration, concentrated to 1/10 the volume of the original, and plated in 0.1 ml samples on minimal-lactose agar plates supplemented with proline. After 48-hour incubation, 50–80 colonies per plate were observed on the experimental plates and none on the control plates. This finding suggests two possibilities: (1) that even an infertile strain such as LT-2 (*mut*$^+$) has the ability to mate with Hfr, although very infrequently; (2)

that even in a *mut*$^+$ strain the change from infertility to fertility can occur, with very low frequency.

Transfer of the Hfr character from E. coli *to Salmonella*: It is known that the Hfr character, unlike the F+ character, is not transferable to F− cells by infection but can be transferred by conjugation (BERNSTEIN 1958); in other words, the factor responsible for Hfr is located on the bacterial chromosome. An attempt was therefore made to introduce the Hfr character into Salmonella by conjugation, and positive results were obtained. The *thr-61 mut* fer strain was used as the Salmonella parent because of the high stability of *thr-61* despite the presence of *mut*. Overnight broth cultures of this strain (*thr*, *mut*, *lac*$^-$) and of HfrP4X$_6$ (*thr*$^+$ *mut*$^+$ *lac*$^+$) were mixed in a 1:1 ratio, and 0.1 ml samples of the mixture (approximately 2×10^8 bacteria) were immediately plated on minimal-lactose agar supplemented with threonine. After 48-hour incubation, 20 to 30 colonies per plate were observed, whereas no colonies appeared on control plates containing either parent alone. Seventeen colonies were picked up at random, purified by single-colony isolation on minimal-lactose agar supplemented with threonine, and tested for *lac*, *thr*, and *mut*. All were *lac*$^+$ (*mut*), but only nine were *thr*$^+$. The other eight, carrying *lac*$^+$ *mut thr*, were grown overnight in broth and tested for ability to mate with *proC-51 mut* fer Salmonella by spot testing on minimal-glucose agar plates. After 48-hour incubation, six of the eight tests revealed *pro*$^+$ recombinants. Bacteria of all eight colonies were sensitive to Salmonella phage H5 and were agglutinated by Salmonella serum. Thus these hybrids, which received *thr*, *mut*, PLT-22 sensitivity, and serological reactivity from the Salmonella parent, and *lac*$^+$ from the *E. coli* parent, have the ability to mate with Salmonella. Since the rupture point of the HfrP4X$_6$ chromosome is between *pro* and *lac* and the order of injection is *pro–pan–leu–thr–*, selection for *lac*$^+$ recombinants was actually selection for the end marker. In another cross, between *tyr-7 mut* (*lac*$^-$) Salmonella and HfrCS-101, which injects *lac*$^+$ as the first marker, none of the *lac*$^+$ recombinants was capable of conjugating with Salmonella strains. From this finding it was deduced that the ability of a hybrid to mate with Salmonella is controlled by the *E. coli* Hfr factor, which enters last during transfer—a fact well established in studies with *E. coli* (JACOB and WOLLMAN 1961).

One of the eight tested hybrids (HfrSc-19) was crossed with several Salmonella strains carrying *mut*$^+$. In crosses with *proB-25*, *proAB-47*, *proAB-126*, *gua-2*, *leu-39*, *thi-1*, and *try-29*, about 10^3–10^4 recombinants were obtained in each case; in a cross with *adth-14*, 79; and in one with *thr-12*, 1. A cross with *pan-2* produced recombinants, although they could not be scored because of the heavy background. Thus the results were all positive, showing that even a Salmonella strain carrying *mut*$^+$ can mate with a hybrid carrying Hfr. The frequency of *pro*$^+$ recombinants resulting from a cross between *proC-51* fer and the hybrid HfrSc-19 was about one per 10^4–10^5 plated bacteria when the mixture was plated on minimal-glucose agar without preincubation.

Transfer of the pro-lac *region by recombination*: Recombination studies with

E. coli (JACOB and WOLLMAN 1961) have shown that the *pro* and *lac* loci, although located close together, recombine readily, and so the map distance between them is assumed to be approximately three percent of the total length of the genetic map. Since our strains of Salmonella, like a great majority of Salmonella strains, are *lac*$^-$, and no mutations to *lac*$^+$ have been observed, they are possibly deficient for the *lac* locus. We were therefore interested in determining the frequencies of recombination between *lac*$^+$ in *E. coli* and nearby loci in *S. typhimurium*. For these determinations we crossed three *pro* (*lac*$^-$) mutants of Salmonella (*proA-214*, *B-215*, and *C-51*) with three Hfr strains of *E. coli* (HfrCS-101, HfrH, and HfrP4X$_6$). In the experiments with HfrCS-101, *lac*$^+$ recombinants began to appear in ten to 15 minutes, and all of the approximately 10^4 recombinants scored were *lac*$^+$ *pro*$^+$, that is, none carried the *pro* marker of the Salmonella parent. Similar results were obtained in the experiments with strain HfrH, where recombinants began to appear after 20 to 30 minutes.

In the HfrP4X$_6$ experiments, however, all the *lac*$^+$ recombinants tested did carry the *pro* marker of the Salmonella. Thus it is evident that, in this system, recombination between *pro* and *lac* depends on the Hfr strain participating in the experiments. Recombination was not observed with strains having both markers on the same side of the break (Hfr attachment), but did occur with the strain in which the break is between the two markers. This finding supports the supposition that the *lac* locus is missing in strain LT-7 of Salmonella.

Transduction studies: After it had been found possible to introduce the *lac*$^+$ marker into the Salmonella genome by conjugation, an attempt was made to transfer *lac*$^+$ by transduction from a hybrid, *lac*$^+$*-1*, to *S. typhimurium* mutants. The results are shown in Table 4. They reveal three interesting points. (1) Phage grown on the hybrid had a high efficiency of transduction, both complete and

TABLE 4

Average number of prototrophs per plate (total three plates) obtained in transduction experiments with bacteria carrying certain markers and phage grown on a lac$^+$ *hybrid and on Salmonella LT-2 (phage multiplicity, 5×)*

Bacteria	Phage PLT-22 grown on Hybrid	LT-2	Control
proA-214 mut	2.3	399.0	3.7
proA-210 mut$^+$	1.7	67.7	1.0
leu-119 mut	12.7	463.0	3.0
leu-110 mut$^+$	1.3	518.7	0
thr-61 mut	15.3	220.3	0
thr-12 mut$^+$	2.0	408.3	0
try-29 mut	17.7	404.0	2.0
try-7 mut$^+$	0.3	136.0	0
tyr-7 mut	42.7	1240.0	1.0
tyr-5 mut$^+$	7.7	814.0	0
thr-61 mut fer	175.7	177.3	0
thr-61 mut infer	6.3	530.0	0
thr-61 in *lac*$^+$ hybrid (*mut*, fer)	131.3	134.7	0

abortive, with fer Salmonella and with a hybrid, lower efficiency with *mut* Salmonella, and lowest with *mut*$^+$. (2) Compared with phage grown on Salmonella, phage grown on the hybrid had a much lower efficiency of transduction, both complete and abortive, with all strains not carrying the fer factor. (3) There was no detectable transduction of the *pro* marker, even in *mut* Salmonella, whereas the other markers (*leu*, *thr*, *try*, and *tyr*) underwent positive transduction when they were carried in *mut* Salmonella and showed less definite results when they were carried in *mut*$^+$ Salmonella.

Order of loci: WOLLMAN and JACOB (1958) showed that the transfer of a chromosome from an Hfr to an F− bacterium can readily be interrupted by mechanical means. By timing the interruption, they were able to determine the sequence of entrance of genetic markers and thus to establish their order on the chromosome. They also demonstrated that in an experiment involving two or more markers there is a correlation between the time of entrance of a marker and the frequency of its appearance among the recombinants. Thus the order of markers can be ascertained either by timing their entrance or by determining their relative frequencies of appearance among the offspring.

Similar procedures were carried out to determine the order of several genes in the *S. typhimurium–E. coli* system. Since no fertile Salmonella strain carrying several markers was available, *lac*$^+$ in HfrCS-101 *E. coli* was used as a standard marker and the relative distances between the *lac* locus and other loci were measured. It is known that HfrCS-101 injects the *lac* locus early; and, as expected, *lac*$^+$ recombinants appeared in all the experiments several minutes earlier than did double recombinants involving *lac*$^+$ and the other markers included in the tests. Ratios of the numbers of double recombinants to total numbers of recombinants, determined 30–40 minutes after injection, were as follows: with *asa-5*, 217/318 (99.5 percent); *glt-2*, 145/147 (98.6); *arg-6*, 151/168 (89.9); *pan-5*, 227/300 (75.7); *leu-119*, 79/207 (38.2); and *thr-61*, 277/905 (30.6). These results indicate that the transfer of genetic markers from *E. coli* to Salmonella occurs in the same manner as transfer in the *E. coli* Hfr–F− system, that prototrophic alleles of the Salmonella auxotrophy genes exist in the *E. coli* chromosome, that *glu* and *asa* are very close to *lac*, and that the probable order of the other loci tested is *thr–leu–pan–arg–lac*. Previous experiments had shown that *pro* is located very close to *lac*.

DISCUSSION

We had made several unsuccessful attempts to cross Hfr strains of *E. coli* with various mutant strains of *S. typhimurium* before obtaining positive results in experiments with a Salmonella strain carrying a mutator gene (*mut*). The frequency of recombination was about one per 10^6–10^8. When hybrids isolated in these experiments were backcrossed to the same Hfr strain, the frequency of recombination was much higher, namely, one per 10^4–10^5. Analysis indicated that the presence of a fertility factor (fer) is necessary if mating is to occur. Apparently *mut*, in addition to increasing the frequency of mutation of all genes, also increases the frequency of changes from infertility to fertility, so that a

population carrying the *mut* gene is a mixture of fertile and infertile cells (about 1:100), and only the former recombine with *E. coli* Hfr. This hypothesis is supported by the finding that a fer mutant can be obtained from a population of *mut* Salmonella that has never been in contact with *E. coli*, and also by the fact that fer mutants are not found in a *mut*$^+$ strain. Since the fer mutant produces recombinants even with F+ of *E. coli*, it may be equivalent to *E. coli* F−.

The nature of the fer mutant—that is, whether it is due to a chromosomal mutation or a cytoplasmic change—has not been determined. The question is technically difficult to investigate because the fer character cannot be used as a selective marker.

That the Hfr factor is readily transferable from *E. coli* to Salmonella is evident from the crosses made with HfrP4X$_6$. In that strain the Hfr determinant is located between the *pro* and *lac* loci, and in the *E. coli* system *pro* is injected first and *lac* last. In hybridization experiments, therefore, if selection is made for *lac*$^+$, Hfr is usually present also. The same observation was made in crosses of Salmonella–Escherichia hybrids with strain HfrP4X$_6$: *lac*$^+$ recombinants carried the Hfr factor.

A considerable number of hybrids were analyzed. They were found to carry mainly the markers of the Salmonella parent, an indication of unidirectional transfer of markers from *E. coli* to *S. typhimurium*. In agglutination tests with Salmonella serum, the serological reactions of some hybrids were different from those of others. This fact suggests that several genes are involved in the control of serological characters, and that different hybrids carry different combinations of these genes.

The results of recombination experiments indicate an oriented transfer and incorporation of *E. coli* genetic material into the Salmonella genome, a high degree of homology between the chromosomes of these two bacteria, and a capacity on the part of *E. coli* genes to function in the Salmonella genome.

On the *E. coli* genetic map (JACOB and WOLLMAN 1961) the *pro* and *lac* loci are about three units apart, but in the hybrid crosses recombination involving these two loci was never observed. Furthermore, recombination between *lac* and *asa* or *glt*, which are in the same region as *pro*, was very rare among hybrids. These findings suggest that in hybrid crosses recombination in the *lac* region either does not occur or is greatly reduced. Since the Salmonella strains studied in these experiments are lactose-requiring and *lac*$^+$ mutants have not been found, it seems probable that the *lac* region is missing from their chromosomes.

A phenomenon comparable to host-induced modification was observed in phage PLT-22 grown on fer Salmonella; this phage showed a high efficiency of plaque formation on the fer Salmonella, lower efficiency on *mut* Salmonella, and lowest on *mut*$^+$. Since it is adsorbed equally well on these three types of bacteria, the differences are probably due either to different efficiencies of injection of the viral genetic material or to different efficiencies of virus multiplication in the three bacterial strains. The fact that the phage has the highest efficiency of transduction, both complete and abortive, for fer Salmonella, lower for *mut* Sal-

monella, and lowest for *mut*+ suggests the first-named possibility, that is, differences in efficiency of injection of the genetic material into the three strains.

SUMMARY

Recombination of genetic markers by conjugation was obtained in crosses between an *Escherichia coli* Hfr strain and *Salmonella typhimurium* carrying a mutator gene (*mut*). The properties of the hybrids were predominantly those of the Salmonella parent, indicating unidirectional transfer of genetic markers from Escherichia to Salmonella. Agglutination tests of the hybrids suggested segregation of serological factors.

Populations of *mut* Salmonella were found to be heterogeneous with respect to ability to mate with *E. coli;* cells having high fertility (and the strains derived from them) were designated "fer," those with low fertility "infer." Since fertile cells were not found in *mut*+ Salmonella, it is deduced that the frequency of changes (mutations?) from infer to fer is increased by the presence of the mutator gene, and that this explains why only strains carrying the *mut* gene can recombine with *E. coli.* Fertile cells had about 10^3 times higher frequency of recombination than infertile cells, and also demonstrated ability to mate with an *E. coli* K-12 F+ strain.

A *lac*+ hybrid, selected in a cross between *E. coli* HfrP4X$_6$ and *S. typhimurium thr-61* (*lac*$^-$) fer, behaves like Salmonella in most respects and is able to mate with Salmonella mutants, even when they do not carry *mut*. It has been tested with ten markers so far, always with positive results. Presumably this hybrid carries the Hfr factor, transferred from *E. coli* with the end marker *lac*+.

Results of a study of the mechanism of transfer of genetic material from *E. coli* to *S. typhimurium* indicate (1) that this transfer occurs in the same manner as in the *E. coli* Hfr–F− system, (2) that the *E. coli* Hfr chromosome carries prototrophic alleles of Salmonella auxotrophic markers, (3) that the probable order of the loci studied is *thr–leu–pan–arg–pro–lac*, and (4) that *glt* and *asa* are located close to *lac*.

One of the findings of this experiment suggests an abnormality of chromosomal structure (perhaps a deletion) in the *pro–lac* region in Salmonella.

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