GENETIC HOMOLOGY BETWEEN *ESCHERICHIA COLI* K-12 AND *SALMONELLA*

STANLEY FALKOW, R. ROWND, AND L. S. BARON

Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Washington, D.C., and the Department of Chemistry, Harvard University, Cambridge, Massachusetts

Received for publication July 30, 1962

ABSTRACT

FALKOW, STANLEY (Walter Reed Army Institute of Research, Washington, D.C.), ROBERT ROWND, AND L. S. BARON. Genetic homology between *Escherichia coli* K-12 and *Salmonella*. J. Bacteriol. **84**:1303-1312. 1962.—Recombinant analysis and interrupted mating procedures, in conjunction with molecular hybridization experiments, demonstrated that the genetic homology between *Escherichia* and *Salmonella* is incomplete. The most likely explanation of this incomplete homology is imperfect pairing between the deoxyribonucleic acid molecules of the two species. Despite the inhomologies, there is ample evidence that the order and distance of the genetic characters on the *Salmonella* chromosome are identical to those of *Escherichia*.

Hybridization between *Escherichia coli* and *Salmonella* was initially observed to occur at low frequency (Baron, Carey, and Spilman, 1958). Subsequently, a streptomycin-resistant mutant of *S. typhimurium* strain TM-9 S^r-2, isolated prior to mating experience, was found to behave as a high-frequency recipient (F^-) in matings with *E. coli*. The availability of this high-frequency recipient strain of *S. typhimurium* made further genetic analysis of hybrid progeny feasible (Baron, Carey, and Spilman, 1959*a*).

In addition to some strains of *S. typhimurium*, all strains of *S. typhosa* tested were fertile with *E. coli* at low frequency (Baron, Spilman, and Carey, 1959*b*). The concept that the initial recombinational event in *E. coli* × *Salmonella* mating was due to the presence of special recipient (F^-) cells in an otherwise sterile (F^0) *Salmonella* population was proposed as an explanation for the initial low level of fertility. According to this assumption, hybrids obtained from a mating between *E. coli* and *Salmonella* should recombine in further matings at a frequency typical of F^- cultures. This prediction has been confirmed by a number of workers (Baron et al., 1959*b*; Miyake and Demerec, 1959; Zinder, 1960*a*).

Further analysis of *E. coli* × *Salmonella* F^- matings indicated that the relative frequency of transmission of markers from *E. coli* into *Salmonella* was lac^+ ara^+ xyl^+. In addition, transfer of the *E. coli* determinants for the utilization of rhamnose (rha^+), fucose (fuc^+), and the production of indole (ind^+) as well as an *E. coli* antigenic component, pili (p^+), was demonstrated (Baron, Carey, and Spilman, 1960; Brinton and Baron, 1960).

The transfer of the *lac* to *ara* region of the chromosome of *E. coli* into *Salmonella* was studied by Zinder (1960*a*), with special reference to the macro- and microhomologies involved. It was pointed out that there was apparently considerable macrohomology, but the fine structure of the genetic material was diverse enough to restrict the chromosomal pairing necessary for transduction by phage PLT-22.

In the present study, the genetic homology between *Escherichia* and *Salmonella* has been examined over a large portion of the chromosome, as measured by recombinational analysis and interrupted mating procedures, in conjunction with molecular hybridization experiments.

MATERIALS AND METHODS

Bacterial strains. The characteristics of the strains employed in this study are shown in Table 1.

Media. Antibiotic Medium #3 (Penassay Broth) and meat-extract agar (both Difco) were used for routine cultivation of organisms.

Minimal medium consisted of a separately autoclaved Noble agar base (Difco) made up double strength (14 g/400 ml of water), to which were added 300 ml of physiological saline; 40 ml of a solution of KH_2PO_4 (60 g/liter) and K_2HPO_4 (140 g/liter); 40 ml of a solution of $(NH_4)_2SO_4$ (20 g/liter) and $MgSO_4 \cdot 7H_2O$ (2 g/liter); and 10

TABLE 1. *Bacterial strains used in studying genetic homology between Escherichia and Salmonella**

Strain	Source	Auxotrophic characters							Energy source utilization			p Antigen	Response to			Mating polarity
		TL	*try*	*M*	*cys*	*his*	*pro*	*arg/ura*	*lac*	*ara*	*xyl*		*S*	T_1	T_6	
Escherichia coli																
Hfr_3-W1895...	J. Lederberg	+	+	−	+	+	+	+	+	+	+	+	s	s	s	Hfr
Hfr-H.....	R. Weinberg	+	+	−	+	+	+	+	+	+	+	+	s	s	s	Hfr
AB-313.....	E. A. Adelberg	−	+	+	+	+	+	+	−	+	+	+	r	s	s	Hfr
1362.....	T. Matney	+	+	+	+	−	+	+	+	+	+	+	s	s	s	Hfr
58-161.....	J. Lederberg	+	+	−	+	+	+	+	+	+	+	+	s	s	s	F^+
Salmonella typhosa																
643 Lac^+3....	WRAIR	+	−	+	−	+	+	+	+	−	−	−	r	r	s	F^-
643 Lac^-3....	643 Lac^+	+	−	+	−	+	+	+	−	−	−	−	r	r	s	F^-
Salmonella typhimurium																
TM-9-1292....	WRAIR	−	+	+	+	+	−	+	−	−	−	−	r	r	r	F^-
TM-9-PAUL...	WRAIR	−	+	+	+	+	−	−	−	+	−	−	r	r	r	F^-

* Abbreviations used: *TL* = threonine + leucine; *try* = tryptophan; *M* = methionine; *cys* = cystine; *his* = histidine; *pro* = proline; *arg/ura* = arginine/uracil; *lac* = lactose; *ara* = arabinose; *xyl* = xylose; *S* = streptomycin; T_1 = phage T_1; T_6 = phage T_6; *p* = pili; Hfr = high frequency of recombination donor cell; F^- = high-frequency recipient; + = synthesized or utilized; − = not synthesized or utilized; r = resistant; s = sensitive.

ml of a 20% solution of the desired carbohydrate. Amino acids, when required, were added at a concentration of 20 μg/ml.

Eosin Methylene Blue Agar (EMB) without added carbohydrate (BBL), to which 20 ml of a 20% solution of the appropriate sugar were added per liter, was employed as a selective medium. Streptomycin sulfate (600 μg/ml) was added to the above media when required.

Isolation of deoxyribonucleic acid and molecular hybridization technic. Deoxyribonucleic acid (DNA) was prepared by the method of Marmur (1961) from the parental *Escherichia* and *Salmonella* strains, as well as from well-characterized hybrids. The DNA samples were heated and annealed with N^{15}-deuterated *E. coli* K-12 DNA (Schildkraut, Marmur, and Doty, 1961) and examined by cesium chloride density-gradient centrifugation (Meselson, Stahl, and Vinograd, 1957). Molecular hybrids consisting of one heavy-labeled and one unlabeled strand were recognized by their intermediate density.

Mating experiments. Cells in the exponential phase of growth were adjusted to a cell density of approximately 5×10^8 per ml and mixed in appropriate volume ratios. Generally, 1 ml of the donor culture was mixed with 4 ml of the recipient culture in a 125-ml flask. The mating mixture was agitated gently in a water bath at 37 C. Unless otherwise stated, mating suspensions were plated for selection of recombinants after 100 min of incubation.

Recombination studies. The blender technique of Wollman and Jacob (1955) was used to study the kinetics of chromosome transfer. Recombinant colonies were reisolated by streaking on a medium of the same composition as that used for initial selection. Replica plating (Lederberg and Lederberg, 1952) and inoculation into differential media were employed to determine unselected characters. The presence of piliation, p^+ (Brinton, 1959), was scored by a slide hemagglutination test (Brinton and Baron, 1960).

RESULTS

The results of a typical *E. coli* Hfr (Cavalli) × *Salmonella* F^- cross are presented in Table 2. The different classes of recombinants can be

ordered as *lac*$^+$-*ara*$^+$-*xyl*$^+$ on the basis of the frequency of their transmission. The order of the unselected marker *p*$^+$ between *ara*$^+$ and *xyl*$^+$ was established by its degree of association with the selected markers.

The chromosomal order *lac*$^+$-*ara*$^+$-*p*$^+$-*xyl*$^+$ was established also by interrupted mating experiments as illustrated in Fig. 1. The relative frequencies of transmission in time of penetration are as follows: *lac*$^+$ at 12 min, *ara*$^+$ at 22.5 min, *p*$^+$ at 25 min, and *xyl*$^+$ at 43 min. The recombinational analysis and interrupted mating data were essentially identical with use of strains of either *S. typhosa* or *S. typhimurium*. A comparison of these results with the data from interrupted matings obtained in *Escherichia* × *Escherichia* crosses (Fig. 2) indicates an apparent identity of gene order as well as distance (in time units) between markers.

Over 80% of the recombinants that had received the markers *lac*$^+$, *ara*$^+$, and *p*$^+$, in any combination, were found to be stable both biochemically and colonially. In contrast, all of the hybrids selected as *xyl*$^+$, as well as those hybrids selected on L-arabinose media that had acquired *xyl*$^+$, were highly unstable and could not be purified as stable positive cultures even upon continued subculturing on EMB media containing the selective sugar on which they had been isolated. In addition to their appearance on EMB medium, these unstable hybrids gave rise to two colonial forms on meat-extract agar. The two types consisted of a small dense colony which continually segregated translucent segments, and large stable translucent colonies (Fig. 3). These

TABLE 2. *Genetic transfer from Escherichia coli Hfr (Cavalli) to Salmonella F*$^-$*

Recombinants selected	Frequency per 100 ♂ cells	Frequency of unselected markers			
		lac$^+$	*ara*$^+$	*p*$^+$	*xyl*$^+$
		%	%	%	%
lac$^+$	0.63	—	24	14	0
ara$^+$	0.25	30	—	58	20
xyl$^+$	0.02	62	86	88	—

* Recombinant selections were made from standard 100-min matings. Streptomycin or methionine contraselections were employed. One hundred colonies of each class of selected recombinants were scored for the indicated unselected markers.

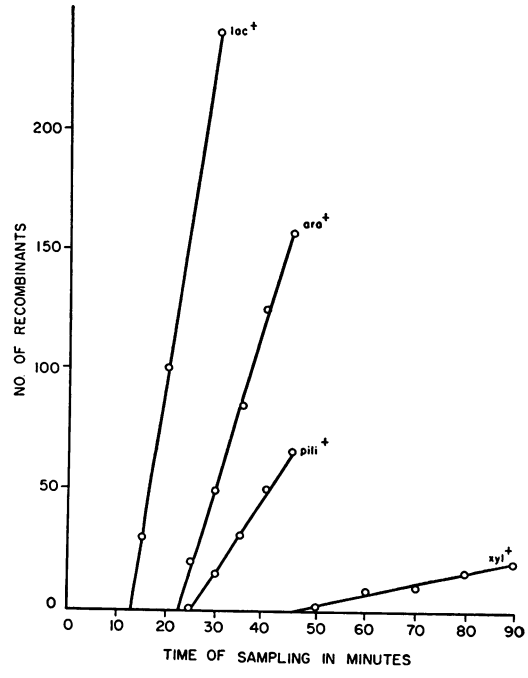

FIG. 1. *Kinetics of chromosome transfer from Escherichia coli Hfr (Cavalli) to Salmonella typhosa. Cells in the exponential phase of growth were mixed in the proportion, 1 Hfr to 10 F*$^-$ *in Penassay broth. At periodic intervals, samples were withdrawn, diluted in chilled saline, and agitated for 1 min in a microcup of a Servall omnimixer. Appropriate dilutions were plated on minimal media plus the specified carbon source, and recombinant selection was achieved by either methionine contraselection or streptomycin. Recombinant colonies were scored for pili by a slide hemagglutination test.*

unstable hybrids were considered to be comparable to the diploid heterozygotes described by Lederberg (1949) in *E. coli* K-12. The dense diploid form possessed the characteristics *lac*$^+$-*ara*$^+$-*p*$^+$-*xyl*$^+$, whereas the stable, haploid, translucent segregants had the characteristics of the unmated *Salmonella* parent.

Approximately 3% of the recombinants selected as *xyl*$^+$ were relatively stable diploids. Unlike the highly unstable cultures which could not be maintained for more than a few transfers, these clones (referred to as X30D type) could be maintained by careful selection and restreaking of typical colonies (Baron et al., 1960). Although clones of the X30D type were initially isolated on methionine-free media, after several transfers on a complete (EMB) medium, these cells

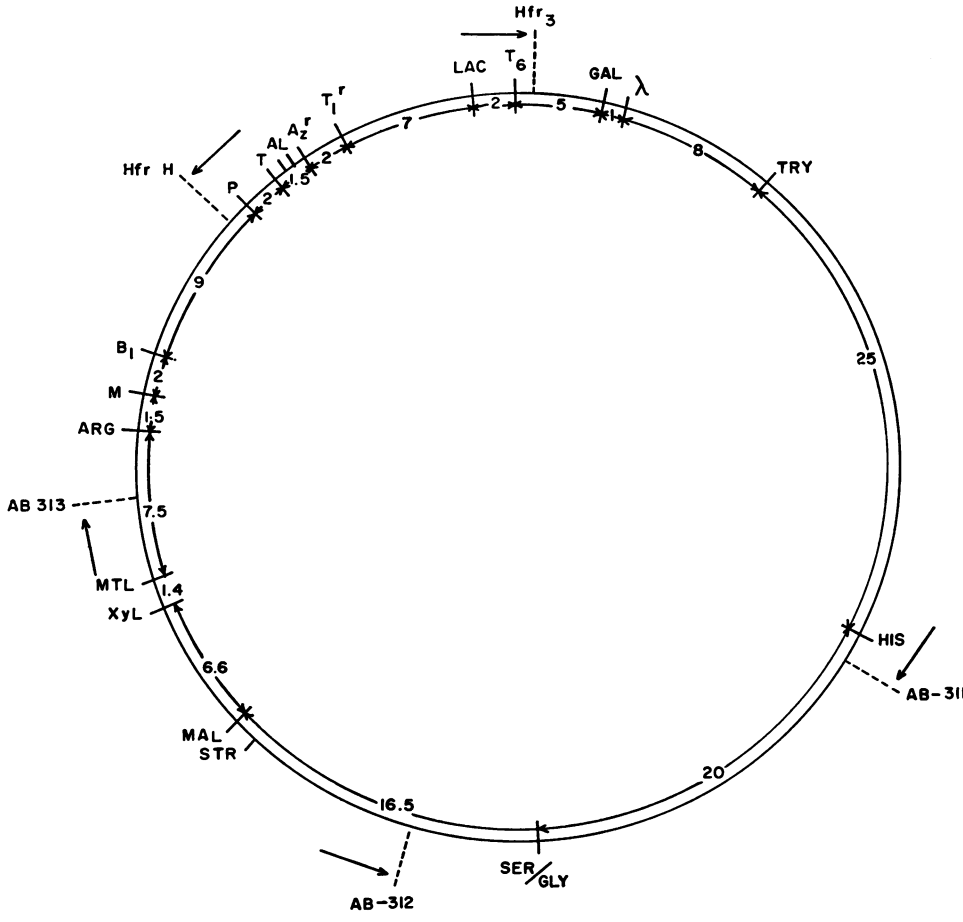

FIG. 2. *Chromosome of Escherichia coli K-12 as determined by interrupted mating experiments. This map was constructed from a series of interrupted mating experiments done in this laboratory and from the published reports of Jacob and Wollman (1961) and Taylor and Adelberg (1960). The experimental procedure was essentially the same as described for Fig. 1. Matings with the Vhf males* AB313, AB312 *and* AB311 *were interrupted with phage.*

would no longer grow on a medium devoid of methionine, but now required this amino acid for growth. Therefore, the X30D clone eventually employed for detailed study possessed the characteristics of *lac*$^+$-*ara*$^+$-*p*$^+$-*M*$^-$-*xyl*$^+$. This clone gave rise to two predominant haploid segregant types: X30P, which exhibited the same characters as X30D, appeared at a frequency of about 3×10^{-4}; and X30T, which was *lac*$^+$-*ara*$^-$-*p*$^-$-*M*$^-$-*xyl*$^+$, appeared at a frequency of about 1×10^{-4}. The X30D clone, therefore, was diploid only for the *ara*$^+$-*p*$^+$ region of the chromosome, but hemizygous for the other injected coli markers. A number of X30D-type clones have now been isolated from both *S. typhosa* and *S. typhimurium*. Whether isolated on a minimal, methionine-free medium or on a complete medium, they have all been initially *M*$^+$, rapidly becoming *M*$^-$, and diploid only for the *ara*$^+$-*p*$^+$ region.

To obtain a more satisfactory representation of the diploid region of X30D-type clones, a series of fermentation and nutritional mutants was obtained after ultraviolet irradiation of a *S. typhimurium* recipient clone. In this way, a *Salmonella* recipient was available which was *L*$^-$, *pro*$^-$, and *mal*$^-$ in addition to the characters mentioned previously.

This mutant stock was crossed with the *E. coli* Hfr strain and plated for selection of recom-

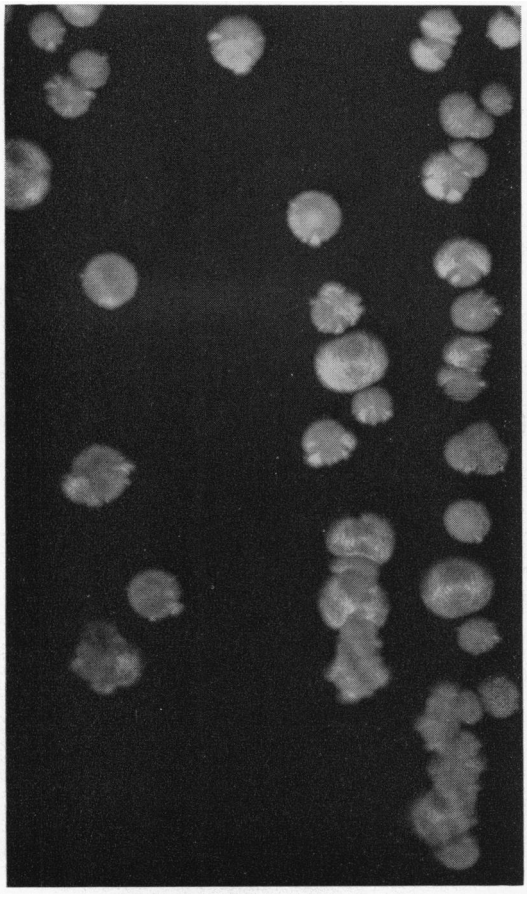

FIG. 3. *Appearance of unstable Salmonella typhosa hybrids on meat-extract agar. The small dense colonies contain diploid cells and continually segregate translucent (haploid) segments and large translucent colonies.*

binants on EMB-xylose-streptomycin medium. Diploids of the X30D type were isolated and analyzed. The X30D and X30P forms were *lac*$^+$-*pro*$^+$-*L*$^+$-*ara*$^+$-*p*$^+$-*M*$^-$-*xyl*$^+$-*mal*$^+$, whereas the X30T form exhibited the characteristics, *lac*$^+$-*pro*$^+$-*L*$^-$-*p*$^-$-*M*$^-$-*xyl*$^+$-*mal*$^+$.

Similar studies have been performed with a *pro*$^-$-*L*$^-$-*arg*$^-$/*ura*$^-$ *Salmonella* mutant. The *arg*$^+$/*ura*$^+$ marker which is normally injected at about 28 min (approximately 3 min after *p*$^+$) was lost in the transition, X30D → X30T, and is assumed to be part of the diploid region.

A study of the coliphage sensitivities of X30D and its haploid derivatives showed that X30D and X30P are sensitive to coliphages T_6 and T_1, whereas X30T segregants are T_6^s but T_1^r.

Apparently X30D is diploid for the region extending at least from T_1^s to *arg*$^+$/*ura*$^+$. X30P, presumably by crossing over, has integrated the entire *coli* linkage group extending from T_6^s-*xyl*$^+$. X30T has lost the diploid region and carries *Salmonella* genes in the region T_1^r-*arg*$^-$/*ura*$^-$ but has integrated the *coli* regions T_6^s-*pro*$^+$ and *M*$^-$ to *xyl*$^+$ (Fig. 4).

It should be emphasized that the mapping of the diploid region as described above and in Fig. 4 represents the reactions of the majority of X30D-type clones and its segregants. On several occasions, segregants have been isolated from X30D which are difficult to explain. For example, X30D-type clones have been isolated from the cross *E. coli* Hfr × *S. typhosa* T_6^s-*lac*$^+$. The vast majority of the X30T segregants isolated have typically lost only the T_1^s-*arg*$^+$/*ura*$^+$ region. An occasional X30T segregant, however, has also been T_6^r-*lac*$^-$, even though both parents which produced the initial recombinant were T_6^s-*lac*$^+$. The *Salmonella* and *Escherichia* parents employed in this cross have been maintained in this laboratory for over 3 years without any sign of instability for either T_6^s or *lac*$^+$. Unless during segregation there is a tendency to produce chromosomal deletions because of pairing or copying difficulties, we are, at present, unable to explain this phenomenon.

Donor strains of S. typhosa. Crosses between the *E. coli* strain 58-161F$^+$ and *S. typhosa* X30T have been especially interesting, since about 18% of the recombinants are now donors. The majority behave as typical F$^+$ donors; they transfer chromosomal determinants at a low frequency, but the sex factor at high frequency. These clones have made it possible to examine the transfer of *Escherichia* and *Salmonella* genes by the same organism. One clone F$^+$18 has been mated with suitable *E. coli* F$^-$ mutants with selection for recombinants receiving the *Escherichia* genes *TL*, *xyl*$^+$ and the Salmonella genes *mal*$^+$, *his*$^+$. The *coli* genes were transferred at a frequency of about $10^{-5}-10^{-6}$ per donor cell, and many of the recombinants showed linked substitutions, eg, 98% of *TL*$^+$ recombinants were *ara*$^+$; 38% of *xyl*$^+$ recombinants also received the *mtl*$^+$ (mannitol utilization) genetic determinant.

The transfer of the *Salmonella* genes *mal*$^+$ and *his*$^+$ to *E. coli* recipients occurred only at low

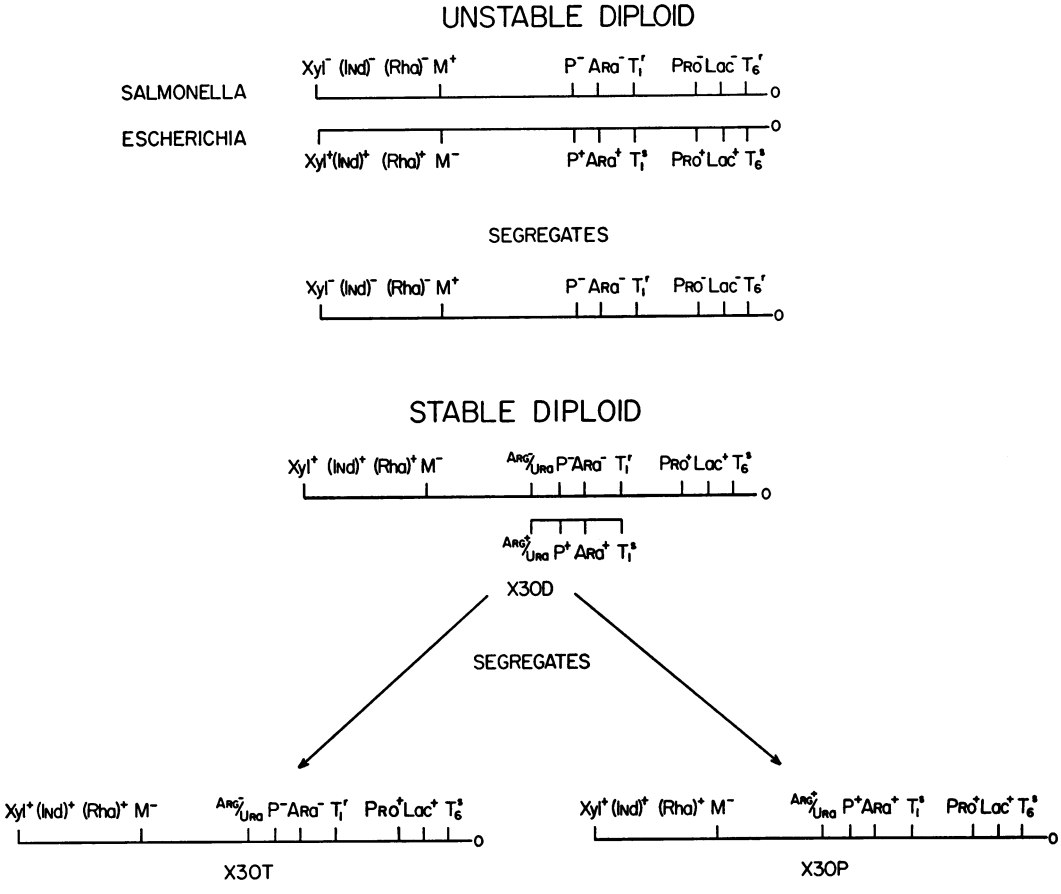

FIG. 4. *Summary of the diploid heterozygotes isolated from Escherichia coli Hfr (Cavalli) × Salmonella F⁻ after recombinant selection for xyl⁺. The order and location of the genes determining rhamnose utilization (Rha) and indol production (Ind) are approximations.*

frequency (10^{-7}–10^{-8} per donor cell), and linked substitutions were only rarely detected. F⁺18 donors have also been mated with *S. typhosa* recipients for both *coli* and *Salmonella* genes. Recombinants for *coli* genes were detected at reduced (10^{-8} per donor cell) frequencies. Unexpectedly, transfer of *Salmonella* genes to *Salmonella* recipients was also observed at a frequency of 10^{-8} per donor cell.

Our preliminary impression is that the sex factor of *E. coli* K-12 does not readily become incorporated by the *S. typhosa* chromosome, nor incorporate *typhosa* genes with the same facility as *Escherichia*. This is not to say that Hfr strains of *S. typhosa* cannot be isolated. Indeed, Hfr strains of two other *Salmonella* species, *S. typhimurium* and *S. abony* have already been reported (Zinder, 1960*b*; Makela, Lederberg, and Lederberg, *personal communication*). Our own experience, however, is that Hfr strains of *S. typhosa* are generally unstable. This, of course, may be a function of the strain we employed.

Several recombinants from a cross between 58-161F⁺ and an exceptional *lac⁻ ara⁻* × 30T segregant proved to be diploid heterozygotes (similar to X30D) for the regions *lac⁺-arg⁺/ura⁺* or T_1^s-*arg⁺/ura⁺*, as well as harboring the sex factor. Markers which lie outside of the diploid region are transferred at frequencies of 10^{-6}–10^{-8} per donor cell, depending upon the past history of the gene. These clones transfer the diploid region either partially or in its entirety at relatively high frequency (about 10^{-4} per donor cell) to *E. coli* F⁻ strains. The resulting recombinant

progeny are usually haploid and behave as typical F^+ donors. Thus, in these donor strains it appears that the diploid piece is transferred with the sex factor, although not necessarily attached to the sex factor. Recent studies indicate that the sex factor initiates conjugation, and the "fellow traveler" diploid piece acts as a short chromosome which is transferred in an oriented fashion.

In one instance, a donor clone F^+39D was isolated in which a diploid piece *lac*$^+$-*ara*$^+$-*p*$^+$ and the sex factor acted as a single unit of transmission and replication. This clone, however, was markedly unstable and no more than 4% of the cells could be maintained in this state even under the most rigid selective environment. The instability was primarily for the *ara*$^+$-*p*$^+$ genes, and relatively stable clones, F^+39T, were isolated in which the *lac*$^+$ genes and sex factor still act as a single unit of replication and transmission.

Mating between a variety of E. coli donor strains and Salmonella. In this investigation and previous studies from this laboratory (*see* Baron, 1960), the *E. coli* Hfr strain W1895 (Hfr 3 in Fig. 2) has been extensively employed because of the high frequency of transfer of the *lac*$^+$ region. Crosses between *Salmonella* and the *E. coli* Hfr strains, HfrH, AB313, and 1362 (similar to AB312 in Fig. 2) have now also been performed.

With each of these donor cultures, as was observed with the W1895 strain, the frequency of transmission of Hfr markers to *Salmonella* was decreased roughly 0.1 to 0.01 of that observed in *E. coli* × *E. coli* matings. While the relative gradient of transmission remained the same, there was, as shown in Table 3 for the 1362 strain, an apparent stretching of genetic linkages. Our interrupted mating data from *E. coli* to *Salmonella* as well as the preliminary studies of *Salmonella* Hfr, F^+ × *E. coli* matings and *Salmonella* × *Salmonella* crosses obtained in our laboratories and others (Zinder, 1960*a*, *b*; Smith and Stocker, 1962; Makela, Lederberg, and Lederberg, *personal communication*) show that

TABLE 3. *Summary of the characteristics of donor strains of Salmonella typhosa isolated from 58-161 F$^+$ × X30T crosses**

Donor strain	Recipient	Selection	Frequency	Remarks
S. typhosa F^+ 18	*E. coli* K-12	TL^{+coli}; xyl^{+coli}	10^{-5}–10^{-6}	Unselected markers *ara*$^+$; *mtl*$^+$ observed frequently
	E. coli K-12	His^{+Sal}; mal^{+Sal}	10^{-7}–10^{-8}	Unselected markers usually not observed
	S. typhosa F^-	Ara^{+coli}; xyl^{+coli}	10^{-7}–10^{-8}	Unselected markers usually not observed
	S. typhosa F^-	His^{+Sal}; mal^{+Sal}	10^{-7}–10^{-8}	Unselected markers usually not observed
S. typhosa diploid F^+	*E. coli* K-12	*Lac*$^+$; *ara*$^+$	10^{-4}	Usually recombinants receive all diploid markers but are haploid and typical F^+
	E. coli K-12	*Xyl*$^+$	10^{-6}	Markers outside of diploid region are transferred at usual F^+ frequency. However, many recombinants have also received diploid markers.
S. typhosa F^+ 39D	*E. coli* K-12 *S. typhosa*	*Lac*$^+$	10^{-2}	Recombinants receive all markers on diploid piece but are markedly unstable, segregating out *T* cells which carry only *lac*$^+$. The *lac*$^+$, however, is relatively stable and highly transmissible.

* Recombinant selections were made from a standard 100-min mating. Methionine contraselection was employed against the ♂ cell. The superscripts *coli* and *Sal* refer to the history of the gene, whether derived from *Escherichia* or *Salmonella*, respectively.

TABLE 4. *Frequency of occurrence of unselected markers in xyl⁺ Escherichia and Salmonella recombinants**

Cross	Recombinants selected	Frequency per 100 ♂ cells	Frequency of unselected markers				
			mal⁺	*xyl⁺*	*ara⁺*	*lac⁺*	*gal⁺*
			%	%	%	%	%
E. coli 1362 × *E. coli* F⁻.....	*xyl⁺*	12	87	—	25	10	5
E. coli 1362 × *S. typhimurium* F⁻.	*xyl⁺*	0.28	38	—	12	<1	0

* Recombinant selections were made from a standard 100-min mating. Histidine contraselection was employed against the ♂ cell. One hundred recombinants from each mating were scored for the indicated unselected markers.

order and distance between loci is essentially identical in the two organisms. We interpret the apparent stretching of linkages as showing that pairing interference occurs between the injected *Escherichia* material and the *Salmonella* chromosome. With all of the *E. coli* donor cultures tested, relatively short (ca. 12%) segments of the *E. coli* linkage group are rather easily integrated by *Salmonella*, whereas the integration of larger segments usually resulted in the formation of unstable diploids. Some markers (for example, *lac⁺*) appear to be more easily integrated than others (for example, *xyl⁺*), although it is not clear whether this is a property of the marker or adjacent regions. The significance of regions adjacent to the recombinational event is shown by the following experiments. The donor strain AB313 transfers its genes in the order, O-*mtl⁺*-*xyl⁺*-*mal⁺*, whereas the donor strain 1362 transfers with the orientation O-*Sr*-*mal⁺*-*xyl⁺*. Transfer from each of these male strains to a *xyl⁻ mal⁻ Salmonella* recipient with selection for *xyl⁺* or *mal⁺* was performed. The *Salmonella* recipient cells received *xyl⁺*-*mal⁺* from AB313 and 1362, 25 and 28 min, respectively, after the onset of conjugation. The majority of *xyl⁺*-*mal⁺* recombinants isolated after mating with AB313 were found to be relatively stable, whereas the majority of the *xyl⁺*-*mal⁺* hybrids selected after mating with strain 1362 were all highly unstable. Thus, there appears to be a region, presumably adjacent to the *mal⁺* region in *Salmonella*, which interferes with effective pairing and subsequent recombination for the *xyl⁺*-*mal⁺* genes of *Escherichia*.

Molecular hybridization between Escherichia and Salmonella. The results of the molecular hybridization experiments were presented in a preliminary report (Rownd, Falkow, and Baron, 1962) and will be reported in detail in a separate communication (Rownd et al., *in preparation*). For the purpose of this report, however, the results may be summarized as follows. (i) Heating and annealing of the *E. coli* parent W1895 with heavy isotope-labeled *E. coli* K-12 DNA (Schildkraut et al., 1961) resulted in the band profile expected for the random recombination of homologous single strands: 1 (light renatured):2 (hybrid):1 (heavy renatured). (ii) Heating and annealing of the *Salmonella* F⁰ and F⁻ DNA with labeled *E. coli* K-12 DNA did not disclose any trace of a band of intermediate buoyant density; therefore, molecular hybrids were not formed between the DNA preparations isolated from the parental strains. (iii) The DNA isolated from the X30D, X30P, and X30T strains formed molecular hybrids when heated and annealed with the heavy isotope-labeled *E. coli* DNA. The extent of molecular hybrid formation is in good agreement with the estimated extent of integrated *Escherichia* material carried by the genetic hybrid. Apparently, only the transferred genetic material meets the requirements for molecular hybrid formation with the heavy isotope-labeled DNA.

Thus, although *E. coli* mates well with *Salmonella*, it appears that major base sequence homologies on their DNA molecules are dispersed or are present on only a few molecules.

DISCUSSION

The results of this investigation strongly suggest that genetic homology between the *Escherichia* and *Salmonella* strains we employed is incomplete. This incomplete homology is evidenced by a number of observations: (i) the transfer of *coli* material to *Salmonella* is significantly less efficient than intraspecific *coli* matings; (ii) extensive transfer (> 12%) of *coli* material to *Salmonella* usually results in diploid heterozygotes; and (iii) the DNA of *coli* and *Salmonella* seem to possess only dispersed regions

of major homology in common. Also pertinent is the report of Zinder (1960) that there is apparently sufficient diversity between the fine structure of the genetic material of these two organisms to restrict transduction by phage PLT-22. These observations all indicate that the most likely explanation of the incomplete genetic homology between *Escherichia* and *Salmonella* is imperfect pairing between the DNA molecules of the two species, which results in a lowered frequency of recombinational events.

The low genetic homology which hinders integration of *coli* genes into the *Salmonella* chromosome (preliminary studies indicate that the reverse is also true) is often dependent upon segments of the chromosome adjacent to the character(s) under study. This has been demonstrated in two ways. One was by using the two donor strains AB313 and 1362 which transfer essentially the same amount of genetic material but differ in orientation of transfer. Recombinants that have received the *xyl*$^+$-*mal*$^+$ loci from these donors exhibit a marked difference in their integration capacity for these identical genes. The relatively stable diploid heterozygote, X30D, is another case in point. Usually, *Salmonella* integrates the *coli* genes *ara*$^+$-*p*$^+$ with comparative ease, yet integration of the *coli* region *M*$^-$-*xyl*$^+$ by *Salmonella* now seems to result in inefficient pairing in the *ara*$^+$-*p*$^+$ region. The explanation that low genetic homology is reflected in poor chromosomal pairing and the importance of regions adjacent to a recombinational event is certainly not unique to *coli* × *Salmonella*. This appears to be the favored interpretation for the observed low frequency of integration of *coli* genes by *Shigella* (Luria and Burrous, 1957; Franklin and Luria, 1961) and the lowered frequency of integration observed for heterospecific transformation (Schaeffer, 1958).

Despite the obvious inhomologies, there is also ample evidence from this investigation and others (Zinder, 1960*a*; Smith and Stocker, 1962) that the general features of the *Salmonella* chromosome are the same as *E. coli* K-12. Thus, the early linkage data of Baron et al. (1959*a*, *b*; 1960) and Zinder (1960*a*), and the present recombinational analysis and interrupted mating data over a large part of the chromosome, point out an essentially identical gene order for the two organisms.

E. coli K-12 has now been shown to be capable of transferring genetic material to both *Shigella* species (Luria and Burrous, 1957) and *Salmonella*. In both these instances, genetic homology has proved to be incomplete. It has been hypothesized (Ravin, 1960) that in the course of evolution the processes of mutation and perhaps recombination occurred in isolated, genotypically identical bacteria, which led to increasing divergence in the structure of their DNA. These differences of structure would be reflected in ineffective pairing during subsequent recombination. One might now ask the question whether the divergence between *Shigella* and *Salmonella* is greater or less than observed between these species and *E. coli*. *Shigella* probably shows a greater degree of homology with *Escherichia* than *Salmonella*, since it can be transduced by coliphage P1 (Lennox, 1955) and forms molecular hybrids with *Escherichia* DNA (Schildkraut et al., 1961). In preliminary experiments, we have demonstrated the transfer of genetic material from *Salmonella* Hfr to *Shigella flexneri*. The frequency of recombination observed was low (on the order of 10^{-6}) and the recombinants could be maintained only with great difficulty. Therefore, it seems, for the genetic segments we examined, that *Shigella* and *Salmonella* exhibit considerably more divergence with one another than might be expected from their individual relationship with *E. coli*. It should be interesting to examine the interrelationships observed from similar interspecific crosses. As has been pointed out previously (Luria and Burrous, 1957; Ravin, 1960), genetic examination of intermediate enteric organisms with *Escherichia*, *Salmonella*, and *Shigella* should yield important information as to the evolutionary phenomenon which have been and are occurring among the *Enterobacteriaceae*.

ACKNOWLEDGMENTS

The authors are indebted to J. A. Wohlheiter for his interest and suggestions, and to I. R. Ryman for his excellent technical assistance.

LITERATURE CITED

BARON, L. S. 1960. Genetic recombination between genera of the *Enterobacteriaceae*. Proc. 21st Ann. Biol. Colloq., p. 29–39. Oregon State College.

BARON, L. S., W. F. CAREY, AND W. M. SPILMAN. 1958. Hybridization of *Salmonella* species by

mating with *Escherichia coli*. Proc. Intern. Congr. Microbiol., 7th, Stockholm, p. 50.

BARON, L. S., W. F. CAREY, AND W. M. SPILMAN. 1959*a*. Genetic recombination between *Escherichia coli* and *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S. **45**:976–984.

BARON, L. S., W. M. SPILMAN, AND W. F. CAREY. 1959*b*. Hybridization of *Salmonella* species by mating with *Escherichia coli*. Science **130**: 566–567.

BARON, L. S., W. M. SPILMAN, AND W. F. CAREY. 1960. Diploid heterozygous hybrids from matings between *Escherichia coli* and *Salmonella typhosa*. J. Exptl. Med. **112**:361–372.

BRINTON, C. C., JR. 1959. Non-flagellar appendages of bacteria. Nature **183**:782–786.

BRINTON, C. C., JR., AND L. S. BARON. 1960. Transfer of piliation from *Escherichia coli* to *Salmonella typhosa* by genetic recombination. Biochim. Biophys. Acta **42**:298–311.

FRANKLIN, N. C., AND S. E. LURIA. 1961. Transduction by bacteriophage P1 and the properties of the *lac* genetic region in *E. coli* and *S. dysenteriae*. Virology **15**:299–311.

JACOB, F., AND E. L. WOLLMAN. 1961. Sexuality and the genetics of bacteria. Academic Press, Inc., New York.

LEDERBERG, J. 1949. Aberrant heterozygotes in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **35**:178–184.

LEDERBERG, J., AND E. M. LEDERBERG. 1952. Replica plating and indirect selection of bacterial mutants. J. Bacteriol. **63**:399–406.

LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **3**:22–41.

LURIA, S. E., AND J. W. BURROUS. 1957. Hybridization between *Escherichia coli* and Shigella. J. Bacteriol. **74**:461–476.

MARMUR, J. 1961. A procedure for the isolation of desoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.

MESELSON, M., F. W. STAHL, AND J. VINOGRAD. 1957. Equilibrium sedimentation of macromolecules in density gradients. Proc. Natl. Acad. Sci. U.S. **43**:581–588.

MIYAKE, T., AND M. DEMEREC. 1959. *Salmonella-Escherichia* hybrids. Nature **183**:1586.

RAVIN, A. W. 1960. The origin of bacterial species. Bacteriol. Rev. **24**:201–220.

ROWND, R., S. FALKOW, AND L. S. BARON. 1962. Genetic transfer and molecular hybrid formation between *E. coli* K-12 and *Salmonella typhosa*. Abstr. Biophys Soc. 6th Ann. Meeting, Washington, D.C.

SCHAEFFER, P. 1958. Interspecific reactions in bacterial transformation. Symp. Soc. Exptl. Biol. **12**:60–74.

SCHILDKRAUT, C. L., J. MARMUR, AND P. DOTY. 1961. The formation of hybrid DNA molecules and their use in studies of DNA homologies. J. Mol. Biol. **3**:595–617.

SMITH, S. M., AND B. A. D. STOCKER. 1962. Colicinogeny and recombination. Brit. Med. Bull. **18**:46–51.

TAYLOR, A. L., AND E. A. ADELBERG. 1960. Linkage analysis with very high frequency males of *Escherichia coli*. Genetics **45**:1233–1243.

WOLLMAN, E. L., AND F. JACOB. 1955. Sur le méchanisme du transfert matériel génétique a cours de la recombinaison chez *Escherichia coli* K 12. Compt. Rend. **240**:2449–2451.

ZINDER, N. D. 1960*a*. Hybrids of Escherichia and Salmonella. Science **131**:813–815.

ZINDER, N. D. 1960*b*. Sexuality and mating in Salmonella. Science **131**:924–926.