CHARACTERISTICS OF A HIGH FREQUENCY OF RECOMBINATION (HFR) STRAIN OF SALMONELLA TYPHOSA COMPATIBLE WITH SALMONELLA, SHIGELLA, AND ESCHERICHIA SPECIES

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Bacterial recombination, first demonstrated with the K-12 strains of *Escherichia coli* by Tatum and Lederberg,[1] has provided a versatile technique for the investigation of genetic organization in bacteria.[2-4] Recently, the sexual compatibility of bacteria from different genera has been extended to include hybridization between *E. coli* and Shigella,[5] and between *E. coli* and *Salmonella*.[6, 7] Only *E. coli* strains, however, have been shown to act as donors of genetic material (males) to the recipient (female) species of Escherichia, Shigella, and Salmonella. The *E. coli* male strains are of two types; the efficient donor or high frequency of recombination (Hfr) strain, and the F^+ strain, a much lower frequency mating strain which, however, has the ability to make female (F^-) strains of *E. coli* into males.[8, 9] Baron, Carey, and Spilman[7] have discussed the possibilities of developing donor strains of Salmonella previously.

It is the purpose of this communication to report on the characteristics of a *Salmonella typhosa* culture, strain ST-2, which behaves as an Hfr donor organism, compatible with many species and strains of Salmonella, Shigella, and Escherichia. Strain ST-2 is also able to confer Hfr ability on hybrids which have been selected for lactose utilization.

Materials and Methods.—Cultures: Strain ST-2 is a culture of *S. typhosa*, possessing the antigenic formula Vi 9,12:d- typical of typhoid strains. The fermentation pattern of ST-2 is like that of the well-known laboratory strain, *S. typhosa* Ty2 described by Felix and Pitt,[10] with the exception that ST-2 is able to ferment lactose and fucose. Other lactose fermenting (Lac^+) strains of *S. typhosa* obtained as a result of conjugation with *E. coli* K-12 have been reported by Baron, Spilman, and Carey.[11]

Species and strains of Shigella and Salmonella came from the culture collection of the Walter Reed Army Institute of Research. Strains of *E. coli* K-12 include W-1895 Hfr, W-1177 (F^-), and derivatives of these which will be mentioned below. These cultures, obtained from Dr. P. D. Skaar, were originally isolated by Dr. J. Lederberg and Dr. L. L. Cavalli. Other strains of K-12 were received from Dr. R. Weinberg; these were K-3 (F^-), K-152 (Hfr), and K-166 (Hfr).

Phages: The Vi typing phages described by Craigie and Felix[12] were obtained from Dr. Cora Gunther. Methods and procedures employed in propagating and studying these phages have been described by Baron, Formal, and Spilman.[13]

Media: The routine culture media as well as the methods and media used for selective purposes in recombination experiments have been described in detail.[7]

Experimental and Results.—The characteristics of strain ST-2 and its ability to recombine with Lac^- cultures are presented in Table 1. The results demonstrate that this strain of *S. typhosa* is compatible with diverse species of enteric bacteria. In some of these crosses, the frequency of recombination expressed as the ratio of

TABLE 1

CROSSES OF STRAINS WITH *Salmonella typhosa* ST-2 (HFR)
(Lac$^+$ or Lac$^+$S^r Selection)
ST-2 = Lac$^+$ Ara$^-$ Xyl$^+$ Rha$^-$ Fuc$^+$ Inos$^-$ Dul$^-$ Indol$^-$ S^s

Organism	Strain Designation	Lac$^+$ Recombinants
Salmonella typhimurium	LT-2	+
S. worthington	DC-158	+
S. paratyphi B	DC-3	+
S. typhimurium	TM-9S^r-2	+
S. norwich	DC-201	+
S. typhimurium	LT-7	+
S. champaign	WR-178	+
S. canoga	WR-244	+
S. kentucky	WR-98	+
S. illinois	WR-121	+
S. typhosa	0901	+
S. montevideo	WR-129	+
S. urbana	WR-117	+
S. typhosa	643	+
S. emek	WR-265	+
S. muenchen	WR-54	+
S. choleraesuis	DC-153	+
S. typhosa	Ty2	+
S. daytona	WR-205	+
S. florida	WR-162	+
S. lexington	WR-118	+
S. adelaide	WR-170	+
S. javiana	WR-160	
Shigella dysenteriae 2a	WR-15-1	+
Sh. flexneri 2a	WR-2457T	+
Sh. boydii 11	WR-27-2	+
Sh. flexneri 2b	WR-M58B	+
Sh. boydii 703	WR-38-1	+
Sh. flexneri 1a	WR-1-2	+
Sh. flexneri 5	WR-M90-A	+
Sh. sonnei phase II	WR-13-1	+
Sh. flexneri 4b	WR-G-2	+
Escherichia coli K-12	K-3(F$^-$Lac$-$)	+
E. coli K-12	CS-100(F$^-$Lac$-$)	+
E. coli K-12	K-166(Hfr Lac$-$)	−
E. coli K-12	K-152(Hfr Lac$-$)	−
E. coli B	B/r$_1$(F$^-$Lac$-$)	+
Paracolobactrum ballerup	WR-107	+

TABLE 2

FREQUENCY OF RECOMBINATION WITH HFR STRAINS

Recipient Strain	Donor Strains: E. coli W-1895	Donor Strains: S. typhosa ST-2
Salmonella typhimurium TM-9	4×10^{-8}	2×10^{-3}
S. typhimurium TM-9S^r-2	1×10^{-4}	3×10^{-3}
S. typhimurium TM-10	0	2×10^{-3}
S. typhimurium LT-2	0	6×10^{-4}
S. typhimurium LT-7	1×10^{-7}	4×10^{-4}
S. typhosa 643	$<10^{-8}$	5×10^{-4}
Shigella flexneri 2a	2×10^{-4}	4×10^{-4}
Escherichia coli B/r$_1$ (Lac$^-$)	..	2×10^{-3}

recombinants to the number of ST-2 cells (Table 2) was approximately 2×10^{-3}, which is somewhat higher than the results reported[7] in matings of *E. coli* Hfr $\times$ *S. typhimurium* F$^-$.

After purification from these crosses, Lac$^+$ hybrids were tested for acquisition of the ability to mate as males. This was accomplished by backcrossing the hybrids with Lac$^-$ strains on minimal lactose (ML) agar in the case of Lac$^+$ hybrids of *Shigella flexneri* which are unable to grow without nicotinic and aspartic acid

(nic$^-$ asp$^-$). Lac$^+$ hybrids of nutritionally independent strains (*S. typhimurium* and other Salmonella species) were crossed with Lac$^-$ S^r strains on Eosin-Methylene Blue (EMB) agar containing streptomycin (600 micrograms/ml). These efficient selective mechanisms were used to eliminate the male parent, since hybrids result from genetic transfer to the female. Therefore, hybrids will be referred to by the name of the female parent. The results in Table 3 show that these Lac$^+$ hybrids

TABLE 3

DEMONSTRATION OF HFR ABILITY BY HYBRIDS OF CROSSES WITH *S. typhosa* ST-2 (Selection for Lac$^+$ Hybrids)

Recipient Strain	% HFR Hybrids
Salmonella typhimurium TM-9	100
S. typhimurium LT-2	100
S. typhosa Ty2	100

Mating Procedure		HFR Hybrids (Lac$^+$)
S. typhosa ST-2 Hfr Lac$^+$	× *S. typhimurium* LT-2 Lac$^-$	+
S. typhimurium LT-2 Hfr Lac$^+$	× *S. typhimurium* TM-9 Lac$^-$	+
S. typhimurium TM-9 Hfr Lac$^+$	× *S. typhosa* Ty2 Lac$^-$	+
S. typhimurium LT-2 Hfr Lac$^+$	× *Sh. flexneri* 2a Lac$^-$	+
S. typhosa Ty2 Hfr Lac$^+$	× *Sh. flexneri* 2a Lac$^-$	+
Sh. flexneri 2a Hfr Lac$^+$	× *S. typhimurium* TM-9 Lac$^-$	+
Sh. flexneri 2a Hfr Lac$^+$	× *S. typhosa* Ty2 Lac$^-$	+
Sh. flexneri 2a Hfr Lac$^+$	× *S. flexneri* 2a Lac$^-$	−
S. typhosa ST-2 Hfr Lac$^+$	× *E. coli* B/r$_1$ Lac$^-$	+

have acquired the ability to mate as male strains as a consequence of their recombination with ST-2 and indicate the crosses which have been accomplished.

The following experiment was performed in order to determine whether ST-2 behaved in these crosses as a typical Hfr strain, as the recombination frequencies observed appeared to indicate, or as an F$^+$ strain exhibiting a frequency more usually ascribed to Hfr strains. This is particularly pertinent since the F$^+$ character can be transferred to an F$^-$ strain by contact alone with the F$^+$ strain without the occurrence of genetic recombination.[8, 9] A Lac$^+$ hybrid of *S. typhimurium* strain TM-9, was obtained as an F^0 (sterile) to F$^-$ mutant[14] from a cross with *E. coli* Hfr strain W1895. This hybrid, which was unable to act as a donor, was mixed in nutrient broth with ST-2. Both organisms were grown together at 37° C and at intervals the mixed culture was streaked on ML agar plates, as a selective device to recover the TM-9 Lac$^+$ organisms. Growth of ST-2 is not supported by this medium, since it has a nutritional requirement for cystine and tryptophan (cys$^-$ try$^-$). Attempts made in this manner to detect transfer of the F$^+$ character by contact were not successful, as judged by the lack of male sexual potency of these TM-9 Lac$^+$ isolates in subsequent crosses. Lac$^+$ TM-9 hybrids, which are fully competent as Hfr males, however, can be obtained following genetic recombination with TM-9 as the recipient strain and ST-2 as donor strain (100 per cent of these hybrids are Hfr after selection for Lac$^+$). Similar experiments using *E. coli* B also failed to show an acquisition of mating ability solely through contact with ST-2.

Strain ST-2 was next examined for its possible behavior as a female or recipient strain, as were the Lac$^+$ *S. typhosa* hybrids previously studied by Baron, Spilman, and Carey.[11] For this purpose, strain ST-2 was grown on nutrient agar plates, rather than in nutrient broth, harvested, and washed thoroughly; this method of cultivation results in a phenotypic change of donor cultures of *E. coli* from the male

to the female state.[15] Loss of donor ability was noted with ST-2 grown in this way. ST-2 was then mated with the *E. coli* Hfr strain, W-1895, on minimal media supplemented with cystine and tryptophan with either l-arabinose or l-rhamnose as the sole carbon source. Strain ST-2 was found to react as a recipient (phenotypically F^-) strain in these experiments with frequencies similar to those reported for other F^- *S. typhosa* previously studied.[11, 14] Thus, in this manner, it was possible to obtain hybrids of ST-2 which are able to utilize l-arabinose, and/or l-rhamnose, and which can produce indol. These hybrids still retained their ability to mate as males with the cultures listed in Table 1. A number of highly unstable forms, probably similar to the diploids described by Lederberg,[16] and a more stable hybrid which cross-reacted with antiserum to *E. coli*, identical in most aspects to a piliated hybrid of *S. typhosa*[17] previously studied, were observed and isolated in these experiments.

S. typhosa ST-2 was tested for lytic pattern with the Vi phage II typing phages. The results established this strain as a degraded Vi type, i.e. a strain lysed by all of the typing phages. All of the hybrids of ST-2 tested were still Vi^+ and susceptible to the Vi phages, including those hybrids which cross-reacted serologically with the *E. coli* donor strain.

The Lac^+ *Shigella flexneri* hybrids obtained from crosses with *S. typhosa* ST-2 were found to have retained their specific antigens in contrast to the majority of those isolated by Luria and Burrous[5] as a result of matings between *E. coli* and Shigella.

The selective marker employed throughout these mating experiments was the ability to utilize lactose (Lac^+). No instance of a spontaneous mutation to Lac^+ was observed with any of the Salmonella or Shigella cultures tested in these crosses; reversion of some Lac^- Escherichia strains to Lac^+ was observed on control plates, but recombination frequencies for Lac^+ hybrids of Lac^- *E. coli* were far in excess of the reversion frequencies. Since *S. typhosa* ST-2 is unable to utilize inositol, arabinose, or rhamnose, and cannot produce indol, loss of some of these abilities as unselected markers was tested for and detected in some Lac^+ hybrids. These results will be reported in a detailed communication at a later date.

The extension of compatibility with *E. coli* strains to Salmonella species was interpreted to be the result of F^- mutations in an otherwise F^0 (sterile) population of Salmonella cells.[14] The results now indicate clearly that Salmonella species which are F^0 in *E. coli* matings can, nevertheless, be fertile as recipients (F^-) at high frequency with *S. typhosa* ST-2. In addition, strains of *E. coli* K-12, Shigella, or Salmonella which behave as typical F^- cultures in crosses with *E. coli* show no fertility advantage over F^0 species of Salmonella, in crosses with ST-2. It was observed, however, that a Salmonella strain (*S. typhosa* Ty2) F^0 with regard to its mating ability with *E. coli* Hfr strain W1895, was able to mate with this strain of *E. coli*, after hybridization with ST-2. This experiment is depicted in Table 4, where it can be seen that the Lac^+ Hfr hybrid of *S. typhosa* Ty2 has acquired the ability to act as a recipient strain (female) in a cross with the *E. coli* Hfr.

Discussion.—The results of this preliminary survey of the mating capabilities of *S. typhosa* strain ST-2 indicate that this organism behaves as a male strain. Furthermore, it appears to act as a typical Hfr culture, as determined by the high frequency at which recombinants are detected as well as by its inability to transfer

TABLE 4

ACQUISITION OF FERTILITY WITH *E. coli* HFR BY A HYBRID FROM A CROSS WITH *S. typhosa* ST-2

Crosses	Frequency of Recombination Selection for LAC+	Selection for *l*-ARA+
1. *E. coli* Hfr × *S. typhosa* Ty2(F^0)	$< 10^{-8}$	0
2. *S. typhosa* ST-2 Hfr × *S. typhosa* Ty2	2×10^{-4}	0
3. *E. coli* Hfr × *S. typhosa* Ty2(Lac^+ Hfr)*	..	3×10^{-7}
4. *E. coli* Hfr × *S. typhosa* 643(F^0)	$< 10^{-8}$	0
5. *E. coli* Hfr × *S. typhosa* 643(Lac^+ F^-)†	..	2×10^{-6}

* Hybrid from cross 2 increase in frequency due presumably to mating with ST-2.
† Hybrid from cross 4 increase in frequency due to selection of F^0 to F^- mutant.

the fertility factor (F) by contact as would be expected of an F^+ culture. It is, nevertheless, able to transfer the Hfr character to recipient strains or species of Salmonella, Shigella, or Escherichia as a consequence of hybridization and these hybrids are now able to act as donor strains in subsequent matings, usually with the same high frequency of recombination as the original ST-2.

It is felt that this discovery may offer many unique opportunities and advantages for further genetic exploration. Heretofore, hybridization had been limited to certain strains of certain species with only *E. coli* strains capable of performing as Hfr or F^+ males. Now, Hfr strains of Salmonella, Shigella, and Escherichia can be derived easily, and in fact, occur in all hybrids selected for ability to utilize lactose (Lac^+) which have been tested. *S. typhosa* ST-2, and other Hfr strains of Salmonella, Shigella, and Escherichia which have been developed, still are able to act as F^- strains under the proper conditions. Thus, it is possible to introduce other markers into these strains by appropriate crosses without affecting their Hfr ability (in instances examined to date).

The observation that all the Salmonella, Shigella, and Escherichia cultures that have been tested with *S. typhosa* ST-2 were fertile, regardless of whether or not they were classified as F^- in matings with *E. coli*, is especially noteworthy. It now seems that all enteric strains studied can be fertile in certain crosses, though sterile in other matings, while the typical F^- strains previously described would be fertile in all crosses. This situation is pertinent to the present compatibility scheme, as preliminary findings demonstrate that an F^0 strain in an *E. coli* mating can be made fertile for this same *E. coli* donor, after hybridization with ST-2. Although the fertility here was at a lower frequency than that observable in the case of a typical F^- (one selected as an F^0 to F^- mutation by mating with *E. coli*[14]), this enhancement of fertility would be of obvious importance were it a general phenomenon.

The invariable transfer of efficient donor ability to Lac^+ hybrids indicates that this factor (Hfr) is linked to Lac in ST-2. Further studies will be necessary to determine the order of entry of markers in these crosses, although the Hfr linkage to Lac and other preliminary evidence demonstrate a marked difference in behavior of strain ST-2 as compared to the *E. coli* Hfr strains previously studied in this laboratory.

The alterations of the genomes of the enteric species which have been described may allow the creation of hybrids of novel biochemical and antigenic characteristics. Such changes may perhaps establish a rational basis for the difference in the pathogenicity of bacterial strains. This problem has been admirably presented by Burnet[18] in a discussion on speculations from a medical angle.

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The following is quoted from Dr. Burnet's article:

"Virulence is an inheritable character—from the point of view of medicine it is the most important character of all, and as such worthy of close genetic study. I am rather sorry that, for understandable reasons, very little refined genetic study has yet been made of the phenomena of bacterial virulence. It might be a happy thought if someone switched from *E. coli* B and K-12 to pathogenic strains of *S. pullorum* or *gallinarum* which can readily be tested for pathogenicity. . . ."

ON FINITE GROUPS OF EVEN ORDER WHOSE 2-SYLOW GROUP IS A QUATERNION GROUP

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We shall sketch a proof of the following theorem:[1]

THEOREM 1. *Let G be a group of finite even order. If the 2-Sylow group P of G is a quaternion group (ordinary or generalized), then G is not simple.*

Proof: If P has order 2^n, it can be generated by two elements α and β with

$$\alpha^{2^{n-1}} = 1, \qquad \beta^2 = \alpha^{2^{n-2}}, \qquad \beta^{-1}\alpha\beta = \alpha^{-1}.$$

The element $\mu = \alpha^{2^{n-2}}$ is the only element of order 2 in P and hence all elements of order 2 in G are conjugate to μ in G. Let $\chi_1 = 1, \chi_2, \ldots, \chi_k$ denote the irreducible characters of G and set $x_i = \chi_i(1)$. If σ is an element of G of even order, it can be shown without difficulty[2] that

$$\sum_{i=1}^{k} \chi_i(\mu)^2\chi_i(\sigma)/x_i = 0. \tag{1}$$

Let $\pi \neq 1$ be a fixed element of P and let ρ be an element of odd order of the