CHARACTERIZATION OF AN HFR STRAIN OF
SHIGELLA FLEXNERI

HERMAN SCHNEIDER AND STANLEY FALKOW

Division of Communicable Disease and Immunology, Walter Reed Army Institute
of Research, Washington, D.C.

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ABSTRACT

Schneider, Herman (Walter Reed Army Institute of Research, Washington, D.C.), and
1964.—An Hfr Shigella flexneri, strain 69, was obtained by terminal marker selection in a cross
between Hfr Escherichia coli and S. flexneri. The chromosome of this Hfr Shigella bears gross homol-
ogy to the E. coli chromosome: it can conjugate with both Shigella and E. coli; its order of gene
transmission is the same as E. coli; and interrupted matings show that distance between gene loci is
the same as for E. coli. The kinetics of transfer of the pro, thr, + leu, + and arg loci by Hfr S.
flexneri differ from Hfr E. coli, and may indicate that function of the sex factor, F, derived from
E. coli, is modified when integrated into the Shigella chromosome.

The taxonomic relationship within the family Enterobacteriaceae has been bolstered in recent
years by strong evidence of genetic homology among members of the Escherichia, Shigella, and
Salmonella groups (Ravin, 1960; Marmur, Falkow, and Mandel, 1963). Lennox (1955),
Luria and Burrous (1957) and Falkow et al. (1963), by transduction and conjugation, demon-
strated homology between the chromosomes of E. coli and various Shigella serotypes. Because of
decreases in frequencies of recombination and transduction, limited linked transfer, and the
presence of segregating diploid heterozygotes in many classes of recombinants, these workers con-
cluded that genetic homology between E. coli and Shigella is incomplete. On the molecular level,
Marmur, Schildkraut, and Doty (1961), and MacCarthy and Bolton (1963), demonstrated partial complementarity between DNA molecules of E. coli and Shigella, confirming existence of the

incomplete genetic homology between these species.

In the E. coli fertility system, chromosome transfer by genetic donors, or males, is mediated
by an episome, the sex factor or F. When integrated with the host chromosome, F confers the
Hfr state and promotes oriented chromosome transfer at high efficiency; when not integrated,
as in the F- state, F is transferred independently at high efficiency and, in addition, may transfer
the chromosome at low efficiency (Jacob and Wollman, 1961). The genus Shigella has been
firmly placed within the E. coli fertility system, but its role has been that of recipient or F-
parent. Luria and Burrous (1957) were able to convert F- Shigella to the F+ state by infection
with an E. coli episome. Although these F+ Shigella could transfer F with high efficiency,
transfer of Shigella chromosome was never observed. This report describes the isolation and
characterization of a donor Hfr Shigella strain capable of efficient, oriented chromosome trans-
fer.

MATERIALS AND METHODS

Cultures. A list of the bacterial strains and their genetic characteristics is presented in
Table 1.

Media. Antibiotic medium no. 3 (Penassay Broth, Difco) and meat extract agar (MEA;
Difco) were used for routine cultivation of organisms.

Minimal medium used to select and score recombinants was prepared according to Falkow,
Rownd, and Baron (1962). Carbohydrate utilization was determined on MEA containing 2% car-
bohydrate and 0.0012% bromothymol blue (DeWitt and Adelberg, 1962) and on Mac-
Conkey Agar (Difco).

Mating conditions. Standard mating conditions consisted of mixing log-phase Penassay cultures
**TABLE 1. Genetic characteristics of bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Auxotrophic characters</th>
<th>Utilization of</th>
<th>Response to streptomycin</th>
<th>Mating polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cys  pro  thr  leu  arg  his  nic  asp  ile  lys  met  rha  xyl  mol  lac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella flexneri 2a, strain 2475T</td>
<td>O. Felsenfeld</td>
<td>+  +  +  +  +  -  -  +  +  +  +  -  -  -  -  -</td>
<td>S</td>
<td>S</td>
<td>F⁻</td>
</tr>
<tr>
<td>S. flexneri 2a, strain 24570a</td>
<td>WRAIR</td>
<td>+  +  +  +  +  +  -  -  +  +  +  +  -  -  -  -  -</td>
<td></td>
<td>S</td>
<td>F⁻</td>
</tr>
<tr>
<td>S. flexneri 2a, strain 24570b</td>
<td>WRAIR</td>
<td>+  +  +  +  +  +  -  -  +  +  +  +  -  -  -  -  -</td>
<td></td>
<td>S</td>
<td>F⁻</td>
</tr>
<tr>
<td>S. flexneri 2a, strain 24570c</td>
<td>WRAIR</td>
<td>+  +  +  +  +  +  -  -  +  +  +  +  -  -  -  -  -</td>
<td></td>
<td>S</td>
<td>F⁻</td>
</tr>
<tr>
<td>S. flexneri 5, strain M90a</td>
<td>WRAIR</td>
<td>+  +  +  +  +  +  -  -  +  +  +  +  -  -  -  -  -</td>
<td></td>
<td>S</td>
<td>F⁻</td>
</tr>
<tr>
<td>S. flexneri, strain 69</td>
<td>WRAIR</td>
<td>+  +  +  +  +  +  -  -  +  +  +  +  -  -  -  -  -</td>
<td></td>
<td>S</td>
<td>F⁻</td>
</tr>
<tr>
<td>S. flexneri, strain 89c</td>
<td>WRAIR</td>
<td>+  +  +  +  +  +  -  -  +  +  +  +  -  -  -  -  -</td>
<td></td>
<td>S</td>
<td>Hfr</td>
</tr>
<tr>
<td>Escherichia coli K-12, strain P4X-6</td>
<td>F. Jacob</td>
<td>+  +  +  +  +  +  +  +  +  +  +  +  -  -  -  -  -</td>
<td></td>
<td>S</td>
<td>Hfr</td>
</tr>
<tr>
<td>E. coli K-12 strain AB1133</td>
<td>A. L. Taylor</td>
<td>+  +  +  +  +  +  +  +  +  +  +  +  -  -  -  -  -</td>
<td></td>
<td>R</td>
<td>F⁻</td>
</tr>
</tbody>
</table>

*Abbreviations: cys, cysteine; pro, proline; thr, threonine; leu, leucine; arg, arginine; his, histidine; nic, nicotinic acid; asp, aspartic acid; ile, isoleucine; lys, lysine; met, methionine; lac, lactose; + = synthesis or utilized; - = not synthesized or utilized; S = sensitive; R = resistant; F⁻ = recipient; Hfr = high frequency of recombination donor; WRAIR = Walter Reed Army Institute of Research.
in a volume ratio of 1 part donor culture to 4 parts recipient culture and incubation at 37°C with gentle agitation. Mating mixtures usually contained 10^8 to 2 x 10^9 recipients to 2 x 10^8 donors. Samples of the mating mixture were plated on selective media 110 min after mixing.

Interrupted matings were done according to a modification of the procedure described by de Haan and Gross (1962). Standard mating mixtures were gently diluted into appropriate volumes of liquid minimal glucose medium 5 min after the parental types were mixed, and were incubated at 37°C without agitation. Samples were removed at intervals, "blended" by violent agitation on a Vortex Jr. mixer for 100 sec, and then plated on appropriate selective media which had been prespread with 0.2 ml of Penassay Broth.

Scoring recombinants for unselected markers. Recombinants were transferred to master plates containing minimal agar of the same composition as the original selective media. Master plates containing 40 to 50 recombinants in the form of patches were then replicated onto appropriate selective media.

Antigenic analysis. Antigenic structure of Shigella recombinants was determined by slide agglutination with the use of type-specific Shigella antiserum and (f+) and (f−) antiserum kindly provided by F. Orskov (Orskov and Orskov, 1960).

RESULTS

Isolation and characterization of an Hfr Shigella. F+ recipients may be converted to the Hfr mating type by infection with wild-type F or F' episomes followed by selection for those recipients in which the episome is integrated with the recipient chromosome (Jacob and Wollman, 1961). Conversion to the Hfr state may also be accomplished by sexual conjugation if one selects for a terminal marker which is closely linked to the site of sex factor attachment. Such F− cells could then directly receive the sex factor in the integrated state (Jacob and Wollman, 1961; Miyake, 1962). Luria and Burrous (1957) were unable to detect chromosome transfer in Shigella made F+ with wild-type F, and we have observed only very limited chromosome transfer by Shigella carrying F' episomes. In view of these findings, the "terminal-marker selection method" appeared to offer the best possibility for detecting Hfr strains of Shigella. The terminal-marker selection employed consisted of crossing E. coli K-12, strain P4x-6, which transfers the lac+ locus terminally, with a genotypically lac−, F− S. flexneri 2a, strain 2457T.

Initial experiments with the use of conventional broth mating procedures usually yielded a few unstable lac+ Shigella recombinants, none of which received the F factor. However, a lac+ male Shigella, strain 69, was found as the result of a plate mating in which large numbers of E. coli and Shigella were used. Hfr recombinants were consistently obtained when conventional mating mixtures were allowed to incubate overnight before being plated on selective media. Under these conditions, the number of lac+ Shigella recovered varied from 0 to 15 per ml of mating mixture (≤ 1 x 10−7 per donor cell). Results of recombinant analysis from such an overnight mating experiment are presented in Table 2. Of a total of 58 lac+ recovered, 50 were unstable, segregating lac− Shigella. Stable lac+ Shigella were isolated from 48 of these recombinants after repeated transfer on MacConkey Agar, and all 50 proved to be serologically typical S. flexneri 2a. The remaining eight recombinants were stable lac+ on initial purification. These eight recombinants had lost their type-specific antigen, retaining only the group-factor antigen, and had in addition received the (f+) antigen, as evidenced by agglutination in (f+) antiserum (Orskov and Orskov, 1960). These findings, which indicate a gene order of lac−II antigen-F in these Shigella recombinants, also confirm the observation of close linkage between the II antigen and lac loci made by Luria and Burrous (1957).

Genetic analysis for other unselected markers indicates that only the lacF region of E. coli was inherited by the Shigella recombinants (Table 2).
This does not preclude the integration of other undetectable, small \textit{E. coli} chromosomal segments. However, in view of the limited degree of linked transfer from \textit{E. coli} to \textit{Shigella} reported by Luria and Burrou (1957) and Falkow et al. (1963), the possibility of the incorporation of segments of \textit{E. coli} chromosome other than the selected lac\(^+\)-F region to \textit{Shigella} appears unlikely.

Gradient of transmission of Hfr \textit{S. flexneri}, strain 69. One of the lac\(^+\), (f\(^+\)+) \textit{Shigella} recombinants, strain 69, was tested for its ability to conjugate with, and effect, genetic transfer to recipient strains in matings with various single auxotrophic F\(^-\) \textit{Shigella} strains and with an F\(^-\) \textit{E. coli} strain AB1133, possessing multiple auxotrophic markers. The results of these matings and of similar matings with \textit{E. coli} Hfr P4x-6 as the male are presented in Tables 3 and 4. These data clearly show that strain 69 exhibits efficient, oriented chromosome transfer, properties which are associated with the Hfr mating type. The recombination frequencies in crosses with F\(^-\) \textit{Shigella} strains indicate a common gene order of O(Origin)-leu-ile-lys-his for both Hfr strains, and in crosses with the F\(^-\) \textit{E. coli} strain the recombination frequencies are indicative of a common gene order of O-pro-thr+leu-arg-his for both strains. The recombination frequencies for most markers in the heterologous \textit{Shigella} Hfr X \textit{E. coli} F\(^-\) and \textit{E. coli} Hfr X \textit{Shigella} F\(^-\) crosses are tenfold less than the homologous \textit{E. coli} Hfr X \textit{E. coli} F\(^-\) and \textit{Shigella} Hfr X \textit{Shigella} F\(^-\) crosses, and simultaneous selection for the closely linked thr and leu markers is still less. Thus, although the common gene orders of the two Hfr strains indicate qualitatively similar gradients of transmission, the tenfold reduction in recombination frequencies seen in the heterologous crosses is indicative of quantitative differences in the gradients of transmission. These transmission data, therefore, indicate genetic homology between the two species, but the decreased recombination frequencies seen in the heterologous crosses also suggest that this homology is not complete.

Genetic analysis of these recombinants further demonstrates the incomplete genetic homology existing between \textit{Shigella} and \textit{E. coli} (Table 5). Although these linked transfer data indicate a similar gene order for both \textit{Shigella} and \textit{E. coli}, the data also show that the integration of unselected markers among recombinants from \textit{Shigella} X \textit{E. coli} crosses is limited to those markers closest to the selected marker. Also, the per cent linked transfer of unselected markers for each of the recombinant classes is much less than that seen in \textit{E. coli} X \textit{E. coli} recombinants.

**Kinetics of marker transfer.** Figure 1 shows the results of an interrupted mating between \textit{Shigella} Hfr 69 and \textit{E. coli} AB1133. The pro\(^+\) marker is transferred at 4 to 6 min, the thr\(^+\) leu\(^+\) markers at

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### Table 3. Comparative recombination frequencies for Hfr strains \textit{Shigella} flexneri 69c and \textit{Escherichia coli} P4x-6 in crosses with F\(^-\) \textit{Shigella*}

<table>
<thead>
<tr>
<th>Cross</th>
<th>Recombination frequency†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>leu(^+)</td>
</tr>
<tr>
<td>\textit{S. flexneri}</td>
<td>1.7 \times 10^{-2}</td>
</tr>
<tr>
<td>69 X \textit{S. flexneri}</td>
<td></td>
</tr>
<tr>
<td>\textit{E. coli} P4x-6</td>
<td>2.5 \times 10^{-3}</td>
</tr>
<tr>
<td>X \textit{S. flexneri}</td>
<td></td>
</tr>
</tbody>
</table>

* \textit{S. flexneri} 5, strain M90-1, was used to test leu\(^+\); \textit{S. flexneri} 2a, strain 24570a, was used for ile\(^+\), strain 24570b for lys\(^+\), and strain 24570c for his\(^+\).

† Expressed as number of recombinants per donor cell.

### Table 4. Comparative recombination frequencies between Hfr \textit{Shigella} strain 69 and \textit{Hfr Escherichia coli} strain P4x-6 when crossed with F\(^-\) \textit{E. coli} strain AB1133

<table>
<thead>
<tr>
<th>Cross</th>
<th>Recombination frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pro(^+)</td>
</tr>
<tr>
<td>\textit{S. flexneri} 69 X \textit{E. coli} AB1133</td>
<td>2.6 \times 10^{-2}</td>
</tr>
<tr>
<td>\textit{E. coli} P4x-6 X \textit{E. coli} AB1133</td>
<td>1.7 \times 10^{-1}</td>
</tr>
</tbody>
</table>

* Expressed as number of recombinants per donor cell.
TABLE 5. Transfer of unselected markers by Shigella flexneri Hfr 69 and Escherichia coli Hfr P4x-6 in crosses with E. coli F- AB1133

<table>
<thead>
<tr>
<th>Cross of E. coli AB1133 with</th>
<th>Selection</th>
<th>No. of clones tested</th>
<th>pro</th>
<th>thr</th>
<th>leu</th>
<th>arg</th>
<th>his</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli P4x-6</td>
<td>pro+</td>
<td>150</td>
<td>32</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. flexneri 69</td>
<td>pro+</td>
<td>150</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>E. coli P4x-6</td>
<td>thr+</td>
<td>149</td>
<td>46</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. flexneri 69</td>
<td>thr+</td>
<td>132</td>
<td>23</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>E. coli P4x-6</td>
<td>arg+</td>
<td>160</td>
<td>42</td>
<td>69</td>
<td>0.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. flexneri 69</td>
<td>arg+</td>
<td>121</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli P4x-6</td>
<td>his+</td>
<td>98</td>
<td>22</td>
<td>29</td>
<td>ND*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. flexneri 69</td>
<td>his+</td>
<td>116</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Not done.

14 min, and the arg+ marker at 24 min. Figure 2 presents the results of a comparable interrupted mating between E. coli Hfr P4x-6 and E. coli AB1133. These two figures show that the entry times for the pro+, thr+ + leu+, and arg+ markers are the same for both Hfr strains. Thus, the gene loci for the synthesis of proline, threonine, leucine, and arginine appear to be located at identical positions on both chromosomes.

The transfer curves of Shigella Hfr 69 for each of the markers do not rise linearly, but have an initial shallow slope followed by a sharp upward inflection 15 to 18 min after the marker is first detected. A similar break in slope is seen in the transfer curve for the arg+ locus by E. coli Hfr P4x-6 in Fig. 2, but not for the pro+ and thr+ + leu+ loci. Such changes in the slope of the E. coli Hfr transfer curves for these more proximal markers were observed in other experiments in which mating mixtures were not diluted by a large factor.

Heterogeneity of chromosome transfer was also detected by measuring the time required by a population of donor cells to complete transfer of a given marker (de Haan and Gross, 1962). The results of interrupted matings carried beyond the time when complete transfer has occurred are shown in Fig. 3. The time of entry of the leu+ marker into the recipient, E. coli AB1133, is the same for both E. coli P4x-6 and Shigella 69. However, E. coli P4x-6 accomplishes complete transfer of the leu+ marker in 20 min, whereas Shigella 69 requires 49 min for complete transfer, a ratio of 1:2.5. This same ratio also holds for transfer of the pro+ and arg+ markers. More than
HFR STRAIN OF SHIGELLA FLEXNERI

twice as many leu+ recombinants were recovered from mating mixtures diluted into minimal medium than when these same mating mixtures were diluted into an enriched medium, Penassay Broth (Fig. 3). In addition, the numbers of recombinants recovered from mixtures diluted into liquid minimal medium start to decrease soon after marker transfer is completed. These observations were first reported by de Haan and Gross (1962), who ascribe the increase in recombinants to greater stability of conjugating pairs in the presence of small amounts of available nutrient. Increase in pair stability permits one to detect what de Haan and Gross term chromosome withdrawal, exemplified in the descending portions of minimal medium curves after marker transfer has been completed.

DISCUSSION

We succeeded in isolating an Hfr Shigella by the rather circuitous means of terminal marker

FIG. 2. Kinetics of marker transfer in cross between Hfr Escherichia coli P4x-6 and F- E. coli AB1135. Standard broth mating was diluted 1:250 in minimal medium & min after mixing. Samples were blended and plated on selective media at 3-min intervals. Selection for pro+ and thr+ + leu+ recombinants was made after a further 1:4 dilution, and selection for arg+ recombinants was made without further dilution. Donors were counterselected by streptomycin (600 µg/ml). Note difference in scales of the right and left ordinates.

FIG. 3. Comparison of marker transfer kinetics of Shigella flexneri Hfr 69 and Escherichia coli Hfr P4x-6. Standard mating of S. flexneri Hfr 69 × E. coli AB1135 was diluted 1:100 in liquid minimal medium (●●●●) and 1:60 in Penassay Broth (O——O) 5 min after mixing with selection for leu+ recombinants. Standard mating of E. coli Hfr P4x-6 × E. coli AB1135 was diluted 1:600 in liquid minimal medium (●——●——●——●——●) and 1:50 in Penassay Broth (O——O——O——O——O) 5 min after mixing with selection for leu+ recombinants. Samples were blended by Vortex agitation before plating on selective media. Donors were counterselected by streptomycin (600 µg/ml). Note difference in the scales of the right and left ordinates.
selection. This Hfr *Shigella* can transfer its chromosome to F− *Shigella* at high frequency and to F− *E. coli* at lower frequencies. Our evidence indicates that the gene order from *O* to *his* on the *Shigella* chromosome is the same as on the *E. coli* chromosome. Moreover, the results of interrupted matings show that distances in time units (and presumably physical units as well) between the gene loci, *pro*+, *thr*+, *leu*+, and *arg*+, on the proximal 20% of the *Shigella* chromosome are the same as that of the *E. coli* chromosome.

A constant feature of the kinetic curves of Hfr *Shigella* strain 69 is their sharp upward break in slope 15 to 20 min after marker entry. A similar break in slope is also seen in the Hfr *E. coli* strain P4x-6 curve for the *arg*+ marker, a marker comparatively distal to the point of origin. This same type of curve can be seen for more proximal *E. coli* markers when dilutions are properly adjusted and if mating mixtures are sampled at shortly spaced intervals. Taylor and Thoman (personal communication) observed that the kinetics curves of a variety of Hfr *E. coli* strains also exhibit a similar upward break in slope soon after time of marker entry.

The asynchrony of chromosome transfer, reflected in the elapsed time between entry of a marker and complete transfer of this marker, has been ascribed primarily to variable delay in initiation of transfer after formation of effective pairs (de Haan and Gross, 1962). The nonlinear slope seen in the kinetics curves of both Hfr *Shigella* and Hfr *E. coli* suggests that there is also heterogeneity with respect to the rate of chromosome transfer in the two donor populations, the primary shallow slope representing a small population capable of a comparatively rapid rate of transfer and the secondary steep slope representing a large population which transfers at slower rates. The asynchrony of chromosome transfer in Hfr *E. coli* and Hfr *Shigella* is therefore probably due to both delay in initiation of transfer and variation in rate of transfer. However, the inordinate length of time required by the Hfr *Shigella* to complete transfer of a given marker, 2.5 times greater than Hfr *E. coli*, suggests that greater asynchrony, and therefore greater heterogeneity of chromosome transfer, is present in the Hfr *Shigella* population than in the Hfr *E. coli* population. It is possible that the greater heterogeneity of chromosome transfer seen in the Hfr *Shigella* population could be a function of the *E. coli* F− recipient, but preliminary studies of Hfr *Shigella* × F− *Shigella* crosses indicate that increased heterogeneity is present in this instance as well. Aberrant transfer kinetics, as compared with Hfr *E. coli*, were found by Johnson, Falkow, and Baron (1964) in *Salmonella* × *Salmonella* crosses. These workers reported that the rate of chromosome transfer of Hfr *Salmonella* is only 0.63 times that of Hfr *E. coli*.

The marked differences exhibited in the kinetics of chromosome transfer by *Shigella* and *Salmonella* harboring F may indicate that the function of the sex factor in heterologous but closely related organisms may be modified with respect to its ability to promote chromosome transfer. Other instances of modification of F function in hosts of the same species and in closely related species have been reported (Lederberg and Lederberg, 1955; Furness and Rowley, 1957; Bernstein, 1958; Luria and Burrous, 1957).

Recently, other Hfr *S. flexneri* have been recovered from matings between *E. coli* P4x-6 and *S. flexneri*. Preliminary experiments indicate that these Hfr *S. flexneri* do not differ from *S. flexneri* 69 in their recombination behavior and kinetics. Isolation of Hfr strains from other *S. flexneri* serotypes and from *S. dysenteriae*, *S. boydii*, and *S. sonnei* serotypes is possible, and should be of further interest in studying genetic homology and evolution in the family Enterobacteriaceae. Hfr strains of *Shigella* should also greatly aid in the study of virulence factors in *Shigella* and perhaps in the genetic transfer of the virulence factors of *Shigella* to *E. coli*.

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**Literature Cited**


