On the Nature of the Recipient Ability of *Salmonella typhimurium* for Foreign Deoxyribonucleic Acids

By MOTOYUKI OKADA AND TSUTOMU WATANABE

*Department of Microbiology, Keio University School of Medicine,
Tokyo, Japan*

AND TADASHI MIYAKE

*Department of Molecular Biology, Keio University School of Medicine,
Tokyo, Japan*

(Accepted for publication 19 July 1967)

SUMMARY

A fertile mutant (*fer*) isolated from *Salmonella typhimurium* LT-7*mut* with a mutator gene *mut* was found to have a high recipient ability when used as a recipient of the *Escherichia coli* chromosome and F' and R factors, whereas LT-7*mut*⁺ and *S. typhimurium* LT-2*mut*⁺, both of which lack the *mut* gene, were poor recipients of these foreign deoxyribonucleic acids. LT-7*mut* exhibited an intermediate recipient ability. These episomal elements, however, were transferred from LT-7*mut* and LT-7*mut*⁺ to the *mut*, *mut*⁺ and *fer* substrains at frequencies comparable to an *E. coli* recipient. In contrast, the frequencies of transfer of these episomes from LT-7*fer* to the other substrains of *S. typhimurium* were considerably lower than those to LT-7*fer*. The efficiencies of plating (*e.o.p.*) of phage P-22 grown on LT-7*fer* were likewise lower on LT-7*mut*, LT-7*mut*⁺ and LT-2*mut*⁺ than on LT-7*fer*. The *e.o.p.* of phage P-22 on LT-7*mut*, LT-7*mut*⁺ and LT-2*mut*⁺ were increased to the value on LT-7*fer* by its growth on LT-7*mut*⁺ or LT-2*mut*⁺. The frequencies of transduction of an R factor to these substrains of *S. typhimurium* with phage P-22 grown on various substrains were parallel with the *e.o.p.* of this phage on these strains. It was further shown that the deoxyribonucleic acid of phage P-22, which had previously been propagated on LT-7*fer*, injected into LT-7*mut*⁺ and LT-2*mut*⁺ is rapidly degraded, whereas appreciable breakdown did not occur in LT-7*fer*. These results with P-22 indicate that LT-7*fer* is a mutant which is impaired in its capacity of restriction and modification of this phage. The growth of P-22 on LT-7*mut* resulted in partial increase of its *e.o.p.* on LT-7*mut*⁺ and LT-2*mut*⁺, indicating that the restriction and modification capacities of LT-7*mut* are partially affected. The above results, that LT-7*fer* acts as a good recipient in the conjugal transfer of *E. coli* chromosome and non-viral episomes and that the frequencies of transfer of the non-viral episomes among the substrains of *S. typhimurium* are comparable to those to LT-7*fer* and *E. coli*, are interpreted as due to the restriction- and modification-less nature of LT-7*fer*.

INTRODUCTION

Sexual recombination between *Escherichia coli* and *Salmonella* strains was first discovered by Baron, Carey & Spilman (1958) and intensively studied by several groups of investigators (Baron, Carey & Spilman, 1959; Baron, Spilman & Carey, 1959; Zinder, 1960; Miyake, 1962). A characteristic feature of the mating of *E. coli*

with salmonellas was the low recipient ability of the *Salmonella* strains. Baron, Carey & Spilman (1959) succeeded by chance in isolating a good genetic recipient in a salmonella, which is capable of producing recombinants at a much higher frequency than the original strain. They were led by this finding to propose that the low frequency of recombination initially observed represented the selection of rare, high-frequency recipients from an otherwise sterile culture (Baron, Spilman & Carey, 1959). Miyake (1962) reported that in the crosses of *E. coli* Hfr strains with *Salmonella typhimurium* LT-7*mut*, which carries a mutator gene *mut* (Miyake, 1959; Miyake, 1960), recombinants for a range of donor markers can be produced but only at very low frequencies. Fertile recipients with a high recipient ability (LT-7*fer*) were isolated from LT-7*mut* by indirect selection, without previous participation in recombination. Thus populations of LT-7*mut* were considered heterogeneous with respect to the ability to mate with *E. coli* Hfr donors (Miyake, 1962). Strains of *S. typhimurium* are usually poor recipients for the transfer of R factors (see Watanabe, 1963) and the sex factor F from *E. coli* (Watanabe, 1962). We have found that the frequencies of transfer of an R factor and an F' factor (Jacob & Adelberg, 1959; Hirota & Sneath, 1961) from *E. coli* K-12 to LT-7*fer* are considerably higher than those to LT-7*mut* and LT-7*mut*⁺, which does not carry the mutator gene *mut*, and to *S. typhimurium* LT-2, that is regarded as *mut*⁺ (Miyake, 1962). We have also found that the conjugal transfer of these episomal elements from LT-7*mut*, LT-7*mut*⁺ and LT-2*mut*⁺ to these strains occurs at frequencies almost equal to those to *E. coli* K-12 and LT-7*fer*. These results will be reported in the present paper and the nature of fertility of the *Salmonella* recipients will be discussed.

METHODS

Bacterial strains. *Salmonella typhimurium* LT-7*mut* carrying the mutator gene *mut* and a stable mutant derived therefrom, LT-7*mut*⁺ without the *mut* gene, were employed. *S. typhimurium* LT-2 (originally colicin I-resistant) and its cysteine-requiring mutant cysD-36 were also used. Strain cysD-36 was supplied by H. Ozeki. Cysteine-requiring mutants isolated in our laboratory from LT-7*mut*, LT-7*mut*⁺ and LT-7*fer*, respectively, were used as well. An Hfr strain of *E. coli* K-12 used was W2252/*col* I (methionine requiring and colicin I-resistant, it injects its chromosome from lactose genes in a counter-clockwise direction). CSH-2/*col* I (methionine-requiring and colicin I-resistant carrying an *F'*-*lac*⁺ (F₁₃ of Hirota & Sneath, 1961), W677/PTS (requiring threonine, leucine, proline, thiamine; not fermenting mannitol, xylose, maltose, galactose, lactose; resistant to phage T6 and high concentrations of streptomycin, F⁻), and W3102/*col* I (non-fermenting galactose and resistant to colicin I, F⁻), all of which are substrains of *E. coli* K-12, were also used.

Phage strains. Phage P-22 (identical to PLT-22) wild type and its clear plaque mutant were employed.

Culture media and conditions of cultivation. Liquid cultures were prepared in Penassay broth (Difco) with gentle shaking. Plating media were nutrient agar (Difco), a minimal medium agar described by Davis & Mingioli (1950) and this minimal medium agar without glucose and sodium citrate. When the utilization of lactose was the selected marker, this sugar was added in 0.2 % to the minimal medium without glucose and sodium citrate (minimal lactose agar medium). Semi-solid nutrient agar medium for phage titrations contained 0.7 % agar. Incubation was at 37°.

Drugs. The drugs used were sulfathiazole (Takeda), dihydro-streptomycin sulfate (Takeda), chloromycetin powder (Parke, Davis & Co.) and tetracycline hydrochloride (Lederle) and are abbreviated as Su, Sm, Cm and Tc, respectively. The concentrations of these drugs incorporated in the media were the same as those used by Watanabe & Fukasawa (1961*a*); 500 µg./ml. for Su and 25 µg./ml. for Sm, Cm and Tc.

Method for proving the presence of the *mut* gene. Whether or not the bacterial strains carry the mutator gene *mut* was studied by the procedure described by Treffers, Spinelli & Belser (1954) and Miyake (1960); each colony was suspended in a small amount of saline and streaked on nutrient agar containing Sm 100 µg./ml. After overnight incubation of the plates, the streaks of the *mut* clones gave rise to numerous Sm-resistant colonies, whereas those of the *mut*⁺ strains produced very few or no such colonies.

Procedure for isolating *fer* mutants of *Salmonella typhimurium* LT-7. Fertile mutants *fer* of *S. typhimurium* LT-7 were isolated by Miyake's printing method (1962); approximately 100 bacteria of LT-7 *mut* per plate were plated on nutrient agar. After overnight incubation the developed colonies were replica-plated on minimal lactose agar medium which had previously been inoculated with 0.1 ml. of an overnight broth culture of w2252/*col* I. After incubation of the replica plates for 48 hr some of the replica-plated colonies gave heavy growth due to the lactose-fermenting (*lac*⁺) recombinants produced. Corresponding colonies on master plates were picked and retested for their high recipient ability by using the standard procedure for conjugation, described below.

Method for transduction of an R factor. The method for transduction of an R factor 222 with phage P-22 in *Salmonella typhimurium* was the same as that described by Watanabe & Fukasawa (1961*b*).

Procedure for conjugation. *Escherichia coli* K-12 strain w2252/*col* I was used as a donor for sexual recombination. The donor and recipient strains were separately grown in Penassay broth to about 3×10^8 bacteria/ml. A 1 ml. portion of the donor culture was mixed with 9 ml. of a recipient culture in a 200 ml. Erlenmeyer flask and the flask incubated in a water bath for 2 hr without agitation. Dilutions in physiological saline of this mixed culture were plated on properly enriched minimal medium to select for the recombinants. When the recipients were the substrains of *Salmonella typhimurium*, the donor and recipient strains were mixed in a 1:1 or 1:9 ratio, and 0.1 ml. of the dilutions of the mixture was immediately plated on the selective medium (Zinder, 1960). In parallel with this procedure, the filter membrane method devised by Sanderson & Demerec (1965) was used in some experiments. The mixture of about 3×10^8 donor bacteria and about 3×10^8 recipient bacteria was placed on a Millipore filter (45 mm. diameter; HA type) by filtering the mixture of the Penassay broth cultures through the filter, and the filter with the bacteria on it was placed on the surface of a semisolid nutrient agar plate, which was then incubated for 5 min. to permit the formation of effective cell contacts. The Millipore filter was then transferred to 10 ml. Penassay broth in a 200 ml. Erlenmeyer flask and the bacteria were gently resuspended in the broth, and the mating was allowed to proceed for 90 min; Varying dilutions in physiological saline of this mixed culture were plated on minimal lactose agar medium. The resultant *lac*⁺ recombinants were scored after incubation for 48 hr.

Procedure for transfer of an R factor and an *F'*-*lac*⁺ factor. CSH-2/*col* I (222) was used as a donor for the transfer of the R factor 222 and w3747/*col* I (*F'*-*lac*⁺) as

a donor for the transfer of the $F'-lac^+$. The recipients were prototrophs of *Salmonella typhimurium*. The donor and recipient strains were separately grown in Penassay broth to about 3×10^8 bacteria/ml. A 5 ml. portion of the donor culture was mixed with 5 ml. of recipient culture in a 200 ml. Erlenmeyer flask, and the flask was incubated in a water bath without aeration for 16 hr (R) or 3 hr ($F'-lac^+$). Varying dilutions in physiological saline of the mixed culture with the R^+ donor were plated on minimal agar containing Cm 25 $\mu\text{g./ml.}$ In the case of the transfer of the $F'-lac^+$, varying dilutions in physiological saline of the mixed culture were plated on minimal lactose agar. After incubation for 48 hr the colonies of the clones which received the R factor or $F'-lac^+$ were scored.

Method for detection of spontaneous segregants of an R factor. Spontaneous segregation of an R factor 222 and its drug resistance determinants in various substrains of *Salmonella typhimurium* was studied by the method described by Watanabe & Lyang (1962); a Penassay broth culture of each substrain of *S. typhimurium* carrying 222 was diluted and plated on nutrient agars containing Sm, Cm, Tc 25 $\mu\text{g./ml.}$ + Su 500 $\mu\text{g./ml.}$ Two of the well-isolated colonies of each strain, which had thus been confirmed resistant to the four drugs, were used as starting materials. Five ml. of Penassay broth in a test-tube was inoculated with each colony and incubated for 24 hr without aeration. Each culture was then diluted with fresh Penassay broth by a factor of 10^5 and 0.1 ml. of this dilution transferred to 5 ml. fresh Penassay broth; subcultures were daily prepared thereafter in a similar fashion. After 10 subcultures, the cultures were diluted to 10^{-5} and 10^{-6} , and 0.1 ml. each of these dilutions was plated on drug-free nutrient agar. The patterns of drug resistance of the developed colonies were studied with a replica plating technique (Lederberg & Lederberg, 1952).

Method for detection of colicin I production. The production of colicin I by various substrains of *Salmonella typhimurium* was studied with the method described by Fredericq (1957).

General phage techniques. The general phage techniques followed were those described by Adams (1950).

Method for study of the breakdown of the injected phage nucleic acid. ^{32}P -labelled phage P-22 was prepared and purified by the procedure described by Watanabe *et al.* (1966). The method used to study the breakdown of the injected phage nucleic acid was also similar to that described by Watanabe *et al.* (1966).

RESULTS

Production of colicin I by various substrains of Salmonella typhimurium

Wild-type strain of LT-7 is known to produce colicin I (Ozeki, Stocker & Smith, 1962). We have shown that LT-7 mut^+ , LT-7 mut and LT-7 fer all produce the same colicin. This was shown by the finding that *Escherichia coli* CSH-2 but not CSH-2/*col* I is killed by the colicin produced by these strains. LT-2 mut^+ did not produce any detectable colicin. The production of colicin I by LT-7 necessitated the use of colicin I-resistant mutants of *E. coli* K-12 as recipients in the crosses with LT-7 substrains.

Sexual recombination between an Hfr strain W2252/col I of Escherichia coli and substrains of Salmonella typhimurium

As shown in Table 1, the frequencies of recombinants were highest in an *Escherichia*

coli recipient and second highest in LT-7*fer*. No recombinants were found with LT-7*mut*⁺ and LT-2*mut*⁺ as recipients. LT-7*mut* gave intermediate frequencies of recombinants. w2252/*col* I injects its chromosome with the lactose genes as the leading region, and the proline genes are so closely linked to the lactose genes that they are transferred to W677/PTS at almost equal frequencies.

Table 1. *Frequencies of recombinants in the crosses of Escherichia coli w2252/col I* with various substrains of Salmonella typhimurium*

The procedure for conjugation was that of Sanderson & Demerec (1965) which is described in the text.

Recombinants	Recipient				
	W677/PTS†	LT-7 <i>fer</i>	LT-7 <i>mut</i>	LT-7 <i>mut</i> ⁺	LT-2 <i>mut</i> ⁺
Selected marker	<i>pro</i> ⁺ ‡	<i>lac</i> ⁺ ‡	<i>lac</i> ⁺	<i>lac</i> ⁺	<i>lac</i> ⁺
Frequency per donor cell	2.0 × 10 ⁻²	3.3 × 10 ⁻³	1.4 × 10 ⁻⁵	< 10 ⁻⁸	< 10 ⁻⁸

* w2252/*col* I is a methionine-requiring, colicin I-resistant Hfr strain of *E. coli* K-12.

† W677/PTS is an F⁻ substrain of *E. coli* K-12, requiring threonine, leucine, proline and thiamin, non-fermenting mannitol, xylose, maltose, galactose and lactose, and resistant to phage T6 and high concentrations of streptomycin.

‡ Abbreviations: *pro*⁺ non-requirement for proline; *lac*⁺ fermentability of lactose.

Transfer of an R factor 222 to substrains of Salmonella typhimurium

The mixtures of the donor and recipient cultures in Penassay broth were incubated overnight without aeration. The frequencies of transfer of an R factor 222 from CSH-2/*col* I (222) to LT-7*mut*, LT-7*mut*⁺ and LT-2*mut*⁺ were very low even with overnight incubation and much lower than those to *Escherichia coli* W3102/*col* I and LT-7*fer* (Table 2). The filter membrane method (Sanderson & Demerec, 1965) did not improve the frequencies of R transfer. The frequencies of R transfer between the substrains of *Salmonella typhimurium* were rather high except with LT-7*fer* (222) as a donor and almost comparable to those between the substrains of *E. coli* K-12. Thus, the poor recipient ability of these strains was specific for the transfer of the R factor from *E. coli* K-12. LT-7*fer* (222) transferred its R factor at a high frequency to *E. coli* K-12, at a lower frequency to T-7*fer* and LT-2*mut*⁺ and at even lower frequencies to LT-7*mut* and LT-7*mut*⁺. When LT-7*fer* was a recipient, the frequencies of transfer of the R factor were always high regardless of whether the donor was a substrain of *E. coli* K-12 or a substrain of *S. typhimurium*. Similarly, when W3102/*col* I was a recipient, the frequencies of transfer of the R factor were always high regardless of whether the donor was a substrain of K-12 or a substrain of *S. typhimurium*. The frequencies of R transfer from LT-2*mut*⁺ (222) to LT-7*mut* and LT-7*mut*⁺ were slightly lower than those between the other strains of *S. typhimurium*. The frequencies shown in Table 2 cannot be taken to reflect the frequencies of transfer very accurately, because the frequencies varied to some extent from experiment to experiment.

Spontaneous segregation of an R factor in various substrains of Salmonella typhimurium

The Su, Sm, and Cm resistance markers of the R factor 222 segregated at high frequencies in all of the substrains of *Salmonella typhimurium*. In contrast, the

segregation of the Tc resistance marker occurred at much lower frequencies in all of these strains. These results are analogous to those reported previously by Watanabe & Lyang (1962) with *S. typhimurium* LT-2 and there seems to be no specificity in the frequencies and patterns of segregation of the drug resistance markers of the R factor 222 in these substrains of *S. typhimurium*.

Table 2. *Frequencies of transfer of an R factor 222* among various substrains of Escherichia coli K-12 and Salmonella typhimurium*

The procedure for the transfer of the R factor is described in the text. The duration of mixed cultivation was 16 hr. The values in this table represent the frequencies of R transfer in 1 ml. of the mixed cultures.

	Frequency of transfer of an R factor 222 by the following donor				
	CSH-2/col I† (222)	LT-7 <i>cys</i> ⁻ ‡ <i>fer</i> (222)	LT-7 <i>cys</i> ⁻ ‡ <i>mut</i> (222)	LT-7 <i>cys</i> ⁻ ‡ <i>mut</i> ⁺ (222)	LT-2 <i>cys</i> ⁻ ‡ <i>mut</i> ⁺ (222)
W3102/col I§	2.6×10^6	1.0×10^7	4.0×10^6	3.0×10^6	5.0×10^6
LT-7 <i>fer</i>	9.2×10^4	2.4×10^5	3.3×10^6	4.5×10^5	4.4×10^6
LT-7 <i>mut</i>	2.0×10^1	1.9×10^4	8.5×10^5	5.6×10^5	2.1×10^3
LT-7 <i>mut</i> ⁺	4.0×10^2	9.5×10^3	1.2×10^6	1.3×10^6	3.2×10^3
LT-2 <i>mut</i> ⁺	$< 10^1$	1.2×10^5	5.6×10^5	3.8×10^6	3.5×10^4

* R factor 222 carries the markers of resistance to sulphonamides, streptomycin, chloramphenicol and tetracycline.

† CSH-2/col I is an F⁻, methionine-requiring, colicin I-resistant substrain of *E. coli* K-12.

‡ *cys*⁻ stands for cysteine-requirement. LT-2 *cys*⁻ *mut*⁺ is identical to *cys*D-36.

§ W3102/col I is an F⁻, galactose-non-fermenting, colicin I-resistant substrain of *E. coli* K-12.

Table 3. *Frequencies of transduction of an R factor 222* to various substrains of Salmonella typhimurium with phage P-22*

The procedure for transduction of the R factor is the same as that described by Watanabe & Fukasawa (1961*b*)

Recipient	Frequency of chloramphenicol-resistant transductants per donor phage with the following donor			
	LT-7 <i>fer</i> (222)	LT-7 <i>mut</i> (222)	LT-7 <i>mut</i> ⁺ (222)	LT-2 <i>mut</i> ⁺ (222)
LT-7 <i>fer</i>	1.7×10^{-8}	2.2×10^{-8}	9.0×10^{-8}	1.2×10^{-7}
LT-7 <i>mut</i>	$< 4.8 \times 10^{-10}$	8.0×10^{-10}	1.6×10^{-8}	7.1×10^{-8}
LT-7 <i>mut</i> ⁺	$< 4.8 \times 10^{-10}$	8.0×10^{-10}	1.7×10^{-7}	1.8×10^{-7}
LT-2 <i>mut</i> ⁺	$< 4.8 \times 10^{-10}$	4.0×10^{-10}	4.0×10^{-7}	2.5×10^{-7}

* R factor 222 carries the markers of resistance to sulphonamides, streptomycin, chloramphenicol and tetracycline.

Transduction with phage P-22 of an R factor 222 in various substrains of Salmonella typhimurium

The frequencies of transduction of an R factor 222 with phage P-22 grown on LT-7 *mut*⁺ or LT-2 *mut*⁺ carrying the R factor 222 to various substrains of *Salmonella typhimurium* were about equal as seen in Table 3. In contrast, the frequencies of transduction of the R factor 222 with P-22 grown on LT-7 *fer* (222) were about 10^{-8} per infective phage in LT-7 *fer* but much lower in LT-7 *mut*, LT-7 *mut*⁺ and LT-2 *mut*⁺ (less than 4.8×10^{-10} ; Table 3). The phage grown on LT-7 *mut* (222) gave a similar but less pronounced tendency of transduction to the phage grown on LT-7 *fer*.

Frequencies of transfer of an F'-lac⁺ to various substrains of Salmonella typhimurium

The mixtures of the donor and recipient cultures in Penassay broth were incubated for 3 hr without aeration. The frequencies of transfer of the F'-lac⁺ to various substrains of *Salmonella typhimurium* from w3747/col I (F'-lac⁺) and various substrains of *S. typhimurium* carrying the F'-lac⁺ are shown in Table 4. The frequencies of lac⁻ bacteria were scored on each donor culture for the transfer experiments, because the frequencies of spontaneous segregation of the F'-lac⁺ were rather high in *Salmonella* strains. As seen in Table 4, the frequencies of transfer of the F'-lac⁺ to LT-7fer were higher than those to the other substrains of *S. typhimurium* when the donor was a substrain of K-12. The same tendency was observed also with LT-7fer (F'-lac⁺) as a donor. In contrast, the frequencies of transfer of the F'-lac⁺ from LT-7mut, LT-7mut⁺ and LT-2mut⁺ did not show marked differences among the various recipients (Table 4). These data gave some fluctuations in several independent experiments.

Table 4. *Frequencies of transfer of an F'-lac⁺* among Escherichia coli K-12 and various substrains of Salmonella typhimurium*

The procedure for studying the frequencies of transfer of the F'-lac⁺ is described in the text. The duration of mixed cultivation was 3 hr in this experiment. The spontaneous segregants without F'-lac⁺ were determined on each donor in the transfer experiments and were found to range between 0 and 60 %; LT-7cys⁻fer 0 %, LT-7cys⁻mut 50 %, LT-7cys⁻mut⁺ 60 %, and LT-2cys⁻mut⁺ 50 %. The values in this table show the frequencies of F' transfer in 1 ml. of the mixed cultures of the donor and the recipient.

Recipient	Frequency of transfer of an F'-lac ⁺ by the following donor				
	w3747/col I† (F'-lac ⁺)	LT-7cys ⁻ fer (F'-lac ⁺)	LT-7cys ⁻ mut (F'-lac ⁺)	LT-7cys ⁻ mut ⁺ (F'-lac ⁺)	LT-2cys ⁻ mut ⁺ ‡ (F'-lac ⁺)
LT-7fer	6.0 × 10 ⁶	2.0 × 10 ⁶	2.2 × 10 ⁴	4.0 × 10 ⁴	2.0 × 10 ⁵
LT-7mut	4.7 × 10 ⁴	2.8 × 10 ⁴	4.4 × 10 ³	7.8 × 10 ³	1.2 × 10 ⁵
LT-7mut ⁺	3.7 × 10 ⁴	9.5 × 10 ³	2.1 × 10 ³	5.0 × 10 ³	1.5 × 10 ⁵
LT-2mut ⁺	2.1 × 10 ³	1.8 × 10 ⁵	3.5 × 10 ³	3.5 × 10 ³	2.1 × 10 ⁵

* F'-lac⁺ is F₁₃ of Hirota & Sneath (1961).

† w3747/col I is an F⁻, methionine-requiring, colicin I-resistant mutant of *E. coli* K-12.

‡ LT-2cys⁻mut⁺ is identical to cysD-36.

Efficiencies of plating (e.o.p.) of phage P-22 on various substrains of Salmonella typhimurium

It was previously noted by Miyake (1962) that phage grown on LT-7fer gave a high e.o.p. on LT-7fer, a lower e.o.p. on LT-7mut and an even lower e.o.p. on LT-7mut⁺. The adsorption of this phage took place equally well to these strains. We have shown that a clear mutant of this phage gives an e.o.p. on LT-2mut⁺ as low as on LT-7mut⁺ (Table 5). The e.o.p. of this mutant phage on LT-7mut, LT-7mut⁺ and LT-2mut⁺ were found to be increased by its growth on any of these bacterial strains. The e.o.p. on LT-7mut, LT-7mut⁺ and LT-2mut⁺ of the phage grown on LT-7mut⁺ or LT-2mut⁺ were equal to those on LT-7fer, whereas the phage grown on LT-7mut gave slightly lower e.o.p. on LT-7mut, LT-7mut⁺ and LT-2mut⁺ than on LT-7fer.

We made single-plaque isolations from the plaques developed on LT-2mut⁺ plated

with phage P-22 which had previously grown on LT-7*fer*. A broth suspension of each purified plaque was prepared and LT-2*mut*⁺ was infected with it. All the lysates thus obtained gave equal *e.o.p.* on every substrain of *Salmonella typhimurium*. Conversely, the propagation on LT-7*fer* of phage P-22 which had previously grown on LT-2*mut*⁺ resulted in the reduction of *e.o.p.* on LT-7*mut*, LT-7*mut*⁺ and LT-2*mut*⁺.

Table 5. *Efficiencies of plating of a clear mutant of phage P-22 on various substrains of Salmonella typhimurium*

Indicator	Efficiency of plating of phage P-22 grown on:			
	LT-7 <i>fer</i>	LT-7 <i>mut</i>	LT-7 <i>mut</i> ⁺	LT-2 <i>mut</i> ⁺
LT-7 <i>fer</i>	1.0	1.0	1.0	1.0
LT-7 <i>mut</i>	1.0×10^{-4}	4.0×10^{-1}	8.6×10^{-1}	2.1
LT-7 <i>mut</i> ⁺	3.0×10^{-5}	1.3×10^{-2}	8.3×10^{-1}	1.4
LT-2 <i>mut</i> ⁺	6.0×10^{-5}	2.0×10^{-2}	1.2	1.4

Since LT-7*mut* cultures are heterogeneous, containing fertile and infertile cells as was pointed out above, the results of the *e.o.p.* of the phage propagated on LT-7*mut* on the other indicators might be due to the heterogeneous population of the phage, some phage particles being propagated on the fertile cells and other phage particles on the infertile cells. The following single burst experiments were carried out to test this possibility.

Single burst experiments with a clear mutant of phage P-22 in Salmonella typhimurium LT-7mut

A culture of LT-7*mut* containing 8.4×10^7 bacteria/ml. was infected with a clear mutant of phage P-22 propagated on LT-7*mut* at a multiplicity of input of 4. The infected culture was diluted with Penassay broth so that 1 ml. contained 0.3 infected bacterium and distributed in 50 small test-tubes in a volume of 1 ml. in each tube. The content of each tube, after incubation for 2 hr, was divided into two equal portions and was separately plated for phage titrations with LT-7*fer* and LT-7*mut*⁺, respectively, as indicators. All of the 13 samples which gave bursts yielded consistently higher titres on LT-7*fer* than on LT-7*mut*⁺, the ratio between them being about 3:1. Similar results were obtained in several other independent experiments. These results suggest that the low *e.o.p.* on LT-7*mut*⁺ of the phage grown on LT-7*mut* is probably not due to its growth on the heterogeneous population of LT-7*mut*.

Isotope studies on the fate of the injected phage nucleic acid

It was found that 30–40% of the deoxyribonucleic acid (DNA) of a clear-plaque mutant of phage P-22, which had previously been propagated on LT-7*fer*, was degraded to acid soluble fractions in 10 min. in LT-7*mut*⁺ and LT-2*mut*⁺, whereas less than 10% of the ³²P label of the phage DNA became acid-soluble in LT-7*fer* in 10 min. In contrast, less than 10% of the DNA of the clear mutant of P-22, which had previously grown on LT-2*mut*⁺, was degraded to acid-soluble fractions in 10 min. in any of the strains LT-7*fer*, LT-7*mut*⁺ and LT-2*mut*⁺.

DISCUSSION

As reported in the present paper, the conjugal transfer of F' and R factors occurred most frequently to LT-7fer among various substrains of *Salmonella typhimurium*. We have also confirmed with an Hfr strain a previous finding of Miyake (1962) that LT-7fer has a high recipient ability in the crosses with different Hfr strains of *Escherichia coli* K-12. Thus LT-7fer acts as an unusually good recipient among the substrains of *S. typhimurium* in the conjugal transfer of bacterial chromosomes and episomal elements.

We have shown that phage P-22 grown on LT-7fer gives a very much reduced *e.o.p.* on any other derivatives of *Salmonella typhimurium*, namely LT-7mut, LT-7mut⁺ and LT-2mut⁺. Phage P-22 grown on LT-7mut⁺ or LT-2mut⁺, however, gave almost equal *e.o.p.* on any of the substrains of *S. typhimurium*. In contrast, the phage propagated on LT-7mut gave slightly lower *e.o.p.* on LT-7mut, LT-7mut⁺ and LT-2mut⁺ than on LT-7fer. We have further shown that the DNA of a clear-plaque mutant of phage P-22, which had previously been propagated on LT-7fer, is rapidly degraded after its injection into LT-7mut⁺ and LT-2mut⁺, whereas appreciable breakdown did not occur in LT-7fer. On the contrary, the DNA of the clear mutant of P-22, which had previously grown on LT-2mut⁺, was not degraded appreciably in any of the substrains of *S. typhimurium*. These results indicate that the observed phenomena are due to host-controlled restriction and modification as defined by Arber & Dussoix (1962) and Arber (1965). They may be interpreted in the following two alternative ways: (1) that LT-7fer is a mutant which lacks the capacity of host-controlled restriction and modification; (2) that LT-7mut⁺ and LT-2mut⁺ lack the capacity of modification, whereas LT-7fer and LT-7mut are possessed of this capacity. In other words, it can be said that P-22 is modified by growth on LT-7fer or LT-7mut so that it will be restricted in LT-7mut⁺ and LT-2mut⁺. Thus phage P-22 acquires new host specificity (Arber & Dussoix, 1962) by growth on LT-7fer and will be restricted in LT-7mut, LT-7mut⁺ and LT-2mut⁺. The fact that P-22 grown on LT-7fer is still restricted on LT-7mut indicates that modifications of P-22 by LT-7fer and LT-7mut may be different. It should be pointed out that LT-7fer is regarded as restriction-less in either interpretation. The first interpretation seems more reasonable, since it is known that restriction and modification are usually impaired together (Arber & Morse, 1965; Boyer, 1964; Wood, 1966).

The results of transduction with phage P-22 of host chromosomal markers reported previously by Miyake (1962) and of an R factor reported in the present paper with various substrains of *Salmonella typhimurium* as recipients are also compatible with these interpretations, since the frequencies of transduction with P-22 grown on LT-7fer or LT-7mut were considerably higher to LT-7fer than to the other strains. In contrast, the phage grown on LT-7mut⁺ or LT-2mut⁺ transduced the R factor to various substrains at almost equal frequencies. Spontaneous segregation of an R factor or its drug-resistance markers occurred equally frequently in various substrains of *S. typhimurium*, indicating that the R factor replicates equally well in the various substrains, once it has started replication.

It has been found in several systems of host-controlled restriction and modification that a wide variety of foreign DNA are restricted and modified in common, including phage genomes, non-viral episomes and bacterial chromosomes (Arber, 1964; Arber,

1965; Arber & Morse, 1965; Boice & Luria, 1963; Pittard, 1964; Watanabe *et al.* 1964; Watanabe *et al.*, 1966). Our finding that LT-7*fer* acts as a good recipient for bacterial chromosomes and F' and R factors can be easily understood, if the restriction and modification are exerted in common upon these foreign DNA. In fact, as we will report in a following paper, we have been able to show that a number of mutants of LT-2 with high recipient abilities selected by the transfer of an R factor from *Escherichia coli* CSH-2 (222) behaved, after elimination of the R factor with acridine dyes, as good recipients of K-12 chromosomes. Furthermore, phage P-22 grown on any substrains of LT-2 and LT-7 gave highest *e.o.p.* on these mutants. These results seem to suggest that the aforementioned pleiotropic phenomena are caused by single genetic events.

If we adopt the first interpretation as to the modification of phage P-22, its modification is complete on LT-7*mut*⁺ and LT-2*mut*⁺ in view of the *e.o.p.* on the substrains of *Salmonella typhimurium* of the phage propagated on these strains. The modification of F' and R factors, however, did not give clear-cut results. As the results in Table 4 indicate, the frequencies of transfer of an F' from LT-7*mut*⁺ (F'-*lac*⁺) to LT-7*mut*, LT-7*mut*⁺ and LT-2*mut*⁺ were considerably lower than those to LT-7*fer*, whereas the frequencies of transfer of the F' from LT-2*mut*⁺ (F'-*lac*⁺) to those strains were almost equal. On the other hand, the frequencies of transfer of the R factor 222 from LT-2*mut*⁺ (222) to LT-7*mut*, LT-7*mut*⁺ were much lower than that to LT-7*fer*, whereas the frequencies of R transfer from LT-7*mut*⁺ (222) to various substrains of *S. typhimurium* were almost equal (Table 2). These results are not completely parallel with the results of the *e.o.p.* of phage P-22 on the substrains of *S. typhimurium*. This discrepancy may be at least partly due to the experimental conditions we used: we incubated the mixtures of the donor and recipient cultures overnight or for 3 hr, because the frequencies of transfer of R and F' factors were too low in some recipients to detect in the ordinary 1 hr incubation. The incubation for long durations may have caused some distortion of the frequencies of transfer of these episomes. Some other unknown factors might also be involved.

Eisenstark (1965) succeeded in isolating fertile mutants from LT-2*mut*⁺ through treatment with a mutagen, nitrosoguanidine; Miyake (1962) had presented some evidence that such mutations may occur in LT-2*mut*⁺ spontaneously. These results are compatible with our results and hypothesis that fertility is given by the restrictionless mutation. The fact that fertile mutants are easily isolated in LT-7*mut* is considered to be merely because this strain is a mutable one due to the presence of the mutator gene.

The hybrids between *Escherichia coli* and *Salmonella* exhibit a high recipient ability when used as recipients in the crosses with *E. coli* donors (Baron, Carey & Spilman, 1959; Miyake, 1962). This finding was interpreted as due to the selection of high-frequency recipients and this fits well our data and hypothesis. Johnson, Falkow & Baron (1964), however, later demonstrated that the increased recipient ability of the hybrids of *E. coli* and *S. typhi* is due to the presence of integrated *E. coli* genetic material. They further showed that the recipient ability of these hybrids was increased only when their substituted *E. coli* genetic section matched the leading region of the Hfr chromosome. They also mentioned that the *Salmonella* population is probably homogeneous with respect to its initial ability to mate with *E. coli* on the basis of the finding that the transfer of an F'-*lac*⁺ element occurred only

slightly less efficiently from an *E. coli* F' donor to *Salmonella* than it did to an *E. coli* F⁻ recipient. In contrast, in our results, the transfer of an F' from *E. coli* to the substrains of *S. typhimurium* except to LT-7fer occurred at much lower frequencies than to *E. coli* recipients. The discrepancies between the results of Johnson *et al.* (1964) and ours may probably be due to the difference in the strains employed; Johnson *et al.* (1964) employed *S. typhi*, whereas we used *S. typhimurium*.

This investigation was supported in part by U.S. Public Health Service research grant AI-04740 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

- ADAMS, M. H. (1950). Methods of study of bacterial viruses. *Meth. med. Res.* **2**, 1.
- ARBER, W. (1964). Host specificity of DNA produced by *Escherichia coli*. III. Effects on transduction mediated by λ dg. *Virology* **23**, 173.
- ARBER, W. (1965). Host-controlled modification of bacteriophage. *A. Rev. Microbiol.* **19**, 365.
- ARBER, W. & DUSSOIX, D. (1962). Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ . *J. molec. Biol.* **5**, 18.
- ARBER, W. & MORSE, M. L. (1965). Host specificity of DNA produced by *Escherichia coli*. VI. Effects on bacterial conjugation. *Genetics* **51**, 137.
- BARON, L. S., CAREY, W. F. & SPILMAN, W. M. (1958). Hybridization of *Salmonella* species by mating with *Escherichia coli*. *Int. Congr. Microbiol., Stockholm*, p. 50.
- BARON, L. S., CAREY, W. F. & SPILMAN, W. M. (1959). Genetic recombination between *Escherichia coli* and *Salmonella typhimurium*. *Proc. natn. Acad. Sci. U.S.A.* **45**, 976.
- BARON, L. S., SPILMAN, W. M. & CAREY, W. F. (1959). Hybridization of *Salmonella* species by mating with *Escherichia coli*. *Science, N.Y.* **130**, 566.
- BOICE, L. B. & LURIA, S. E. (1963). Behavior of prophage P1 in bacterial matings. I. Transfer of the defective prophage P1dl. *Virology* **20**, 147.
- BOYER, H. (1964). Genetic control of restriction and modification in *Escherichia coli*. *J. Bact.* **87**, 1652.
- DAVIS, B. D. & MINGIOLI, E. S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B₁₂. *J. Bact.* **60**, 17.
- EISENSTARK, A. (1965). Mutagen-induced hybridization of *Salmonella typhimurium* LT-2 $\times$ *Escherichia coli* K12 Hfr. *Proc. natn. Acad. Sci., U.S.A.* **54**, 117.
- FREDERICQ, P. (1957). Colicins. *A. Rev. Microbiol.* **11**, 7.
- HIROTA, Y. & SNEATH, P. H. A. (1961). F' and F mediated transduction in *Escherichia coli* K-12. *Japan. J. Genetics* **36**, 307.
- JACOB, F. & ADELBERG, E. A. (1959). Transfert de caractères génétique par incorporation au facteur sexuel d'*Escherichia coli*. *C. r. hebd. Séanc., Acad. Sci., Paris* **249**, 189.
- JOHNSON, E. M., FALKOW, S. & BARON, L. S. (1964). Recipient ability of *Salmonella typhosa* in genetic crosses with *Escherichia coli*. *J. Bact.* **87**, 54.
- LEDERBERG, J. & LEDERBERG, E. M. (1952). Replica plating and indirect selection of bacterial mutants. *J. Bact.* **63**, 399.
- MIYAKE, T. (1959). Fertility factor in *Salmonella typhimurium*. *Nature, Lond.* **184**, 657.
- MIYAKE, T. (1960). Mutator factor in *Salmonella typhimurium*. *Genetics* **45**, 11.
- MIYAKE, T. (1962). Exchange of genetic material between *Salmonella typhimurium* and *Escherichia coli* K-12. *Genetics* **47**, 1043.
- OZEKI, H., STOCKER, B. A. D. & SMITH, S. M. (1962). Transmission of colicinogeny between strains of *Salmonella typhimurium* grown together. *J. gen. Microbiol.* **28**, 671.
- PITTARD, J. (1964). Effect of phage controlled restriction on genetic linkage in bacterial crosses. *J. Bact.* **87**, 1256.
- SANDERSON, K. E. & DEMEREC, M. (1965). The linkage map of *Salmonella typhimurium*. *Genetics* **51**, 897.

- TREFFERS, H. P., SPINELLI, V. & BELSER, N. D. (1954). A factor (or mutator gene) influencing mutation rates in *Escherichia coli*. *Proc. natn. Acad. Sci., U.S.A.* **40**, 1064.
- WATANABE, T. (1962). Episomic resistance factors in *Enterobacteriaceae*. XVII. Further studies on the transduction of resistance factors in *Salmonella typhimurium*. *Med. Biol., Tokyo* **65**, 44.
- WATANABE, T. (1963). Infective heredity of multiple drug resistance in bacteria. *Bact. Rev.* **27**, 87.
- WATANABE, T. & FUKASAWA, T. (1961*a*). Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. I. Transfer of resistance factors by conjugation. *J. Bact.* **81**, 669.
- WATANABE, T. & FUKASAWA, T. (1961*b*). Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. III. Transduction of resistance factors. *J. Bact.* **82**, 202.
- WATANABE, T. & LYANG, K. W. (1962). Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. V. Spontaneous segregation and recombination of resistance factors in *Salmonella typhimurium*. *J. Bact.* **84**, 422.
- WATANABE, T., NISHIDA, H., OGATA, C., ARAI, T. & SATO, S. (1964). Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. VII. Two types of naturally occurring R factors. *J. Bact.* **88**, 716.
- WATANABE, T., TAKANO, T., ARAI, T., NISHIDA, H. & SATO, S. (1966). Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. X. Restriction and modification of phages by *fi*-R factors. *J. Bact.* **92**, 477.
- WOOD, W. B. (1966). Host specificity of DNA produced by *Escherichia coli*: Bacterial mutations affecting the restriction and modification of DNA. *J. molec. Biol.* **16**, 118.
- ZINDER, N. D. (1960). Hybrids of *Escherichia* and *Salmonella*. *Science, N.Y.* **131**, 813.