DRUG RESISTANCE OF ENTERIC BACTERIA

II. Transduction of Transmissible Drug-Resistance (R) Factors with Phage Epsilon[1]

KENJI HARADA, MITSUO KAMEDA, MITSUE SUZUKI, AND SUSUMU MITSUHASHI

Department of Microbiology, School of Medicine, Gunma University, Maebashi, Japan

Received for publication 3 September 1963

Abstract

Harada, Kenji (Gunma University, Maebashi, Japan), Mitsuo Kameda, Mitsue Suzuki, and Susumu Mitsuhashi. Drug resistance of enteric bacteria. II. Transduction of transmissible drug-resistance (R) factors with phage epsilon. J. Bacteriol. **86**:1332–1338. 1963.—Transmissible drug-resistance (R) factors, which transfer resistance to tetracycline (TC), chloramphenicol, streptomycin, and sulfonamide by cell-to-cell contact, were found to be transduced in the system of *Salmonella* E group with phage epsilon (ϵ_{15} and ϵ_{34}). The R^+ transductants of *S. newington* (S-84) and *S. chittagong* (S-224) were all found to be unable to transfer their R factors by conjugation, and their R factors were not eliminated by treatment with acridine dyes so far as tested. The R factors containing TC resistance were consistently segregated when transduced. At low multiplicities of infection, the R^+ transductants with ϵ_{15} were all nonlysogenic and unable to produce normal ϵ_{15} phage particles; among the R^+ transductants with ϵ_{34}, 34% were lysogenic and 66% were sensitive to ϵ_{34}.

It was found that multiple drug resistance was transferred in vitro from resistant *Escherichia coli* to shigellae, and also from resistant shigellae to *E. coli* (Ochiai et al., 1959; Akiba et al., 1960).

We confirmed this finding and demonstrated that this transmission is not mediated by transduction, transformation, or a filtrable agent, but by cell-to-cell contact (Mitsuhashi, Harada, and Hashimoto, 1960; Harada et al., 1961). This was also confirmed by blender treatment (Watanabe and Fukasawa, 1960*a*, *b*). It was proposed to designate as "R" (resistance) this transmissible drug-resistance factor (Mitsuhashi, 1960).

The transmissible drug-resistance factor can also be transferred by transduction in *E. coli* K-12 with phage P1kc (Nakaya, Nakamura, and Murata, 1960; Watanabe and Fukasawa, 1961*b*). In a previous paper (Mitsuhashi, Harada, and Hashimoto, 1961*a*; Mitsuhashi et al., 1962), it was reported that recombinant R factors were formed when two kinds of R factor were brought together in a host bacterium. In this paper, we report the transduction in *Salmonella* E group with phage ϵ_{15} (Iseki and Sakai, 1953) and ϵ_{34} (Harada, 1956*a*, *b*; Uetake, 1957).

Materials and Methods

Media. Liquid cultures were prepared in Brain Heart Infusion (BHI) broth (Difco). The nutrient agar was Heart Infusion (HI) Agar. The medium AGGa agar consisted of medium A (Davis and Mingioli, 1950) containing 0.5% glucose, 0.5% sodium glutamate, and 1.3% Difco agar. The medium ALGa agar consisted of medium A containing 0.5% lactose (instead of glucose), 0.5% sodium glutamate, and 1.3% Difco agar.

Drugs. Dihydrostreptomycin (SM) sulfate, chloramphenicol (CM), tetracycline (TC) hydrochloride, and sulfisoxazole (SA) were used. Drug resistance was determined according to the modified method described by Fukumi (1953), scoring the maximal concentration of drugs diluted by twofold steps that allowed the growth of tested organisms.

Strains of bacteria and phages. Salmonella newington C_2(S-84), *S. chittagong* (S-224), phage ϵ_{15} (Iseki and Sakai, 1953), and phage ϵ_{34} (Harada, 1956*a*, *b*; Uetake, 1957) were used as transduction systems. The *Salmonella* strains used and their O-antigenic structures are shown in Table 1. *E. coli* K-12 strain PA 200 was supplied by B. D.

[1] The outline of this paper was published in Med. Biol. (Tokyo) **62**:13–16, 1962 (in Japanese).

Davis. *E. coli* 58-161 is K-12 F^- and auxotrophic for methionine; *E. coli* PA 200 is K-12 F^- and auxotrophic for leucine, threonine, thiamine, histidine, arginine, and, in addition, has the following markers: lac^-, gal^-, mtl^-, xyl^-, $arab^-$, mal^-, T_1^r, and SM^r.

Preparation of bacteria and phage. Bacterial cultures were grown in BHI broth at 37 C for 18 hr. Phages ϵ_{15} and ϵ_{34} were grown on *S. newington* E_1 and E_2, respectively, in BHI broth at 37 C for 4 to 6 hr. After centrifugation at 8000 × *g* for 10 min, an equal volume of ether was added to the supernatant, which was allowed to stand at 37 C for 120 min to kill the remaining host bacteria. After evaporation of ether at 37 C by evacuation, the lysates were used as phage stocks for transduction. Phage stocks ϵ_{15} and ϵ_{34} were also obtained by ultraviolet induction of cells of lysogenic strains E_2 and E_3, respectively.

Factor sera. Rabbits were immunized with heat-killed *S. paratyphi* A 1015, *S. anatum* 293, *S. newington* C_2, *S. thomasville*, or *S. senftenberg.* Factor sera, anti O-1, anti O-10, anti O-15, and anti O-34, were prepared according to the method described by Edwards and Ewing (1955). Rabbits were immunized with phage ϵ_{15} or ϵ_{34} partially purified by methanol. The dilutions of anti-ϵ_{15} and anti-ϵ_{34} sera that neutralized completely 100 active phage particles at 37 C for 2 hr were 1:800 and 1:200, respectively.

Transmissible drug-resistance (R) factors. Six kinds of transmissible drug-resistance (R) factors, i.e., R_{10}(TC.CM.SM.SA), R_{12}(CM.SM.SA), R_{13}(TC.SM.SA), R_{14}(TC.CM), R_{16}(CM), and R_{26}(TC), were used. An R(TC.CM.SM.SA) factor is able to transfer TC, CM, SM, and SA resistance to a recipient cell by cell-to-cell contact. The different R factors originating from different strains isolated in field survey are distinguished by a numerical suffix according to Hirota's suggestion (Sugino and Hirota, 1962).

Transmission of R factors by mixed cultivation. The evening before an experiment, a single colony of each of the required strains (donor and recipient) was subcultured, from the stock agar plates maintained at 4 C, to BHI broth and incubated overnight at 37 C. Cultures for use in the experiment were prepared by inoculating 1 ml of the donor cell and 1 ml of the recipient cell culture into 9 ml of BHI broth and then shaking gently for 3 hr at 37 C. These cultures had a viable count of about 2×10^9 organisms per ml.

TABLE 1. *Somatic antigen and lysogenicity with epsilon phages of Salmonella strains*

Strain*	Somatic antigen	Lysogenicity
(S-84) E_1	3.10	ϵ_{15}^-, ϵ_{34}^-
(S-84) E_2	3.15	ϵ_{15}^+
(S-84) E_3	(3).(15).34	ϵ_{15}^+, ϵ_{34}^+
(S-224) E_2	3.15	ϵ_{15}^+
(S-244) E_3	(3).(15).34	ϵ_{15}^+, ϵ_{34}^+
(S-224) E_4	1.3.10.19	ϵ_{15}^-, ϵ_{34}^-

* (S-84), *S. newington* C_2; (S-224), *S. chittagong.*

The cultures were then mixed and reincubated with gentle shaking at 37 C. The ratio of donor cells to recipient cells was approximately 1:1.

After 4 hr of incubation, 0.1 ml of the approximately diluted mixed culture was spread on an appropriate selective medium. In the case of transmission of R factors from *E. coli* K-12 PA 200 F^- strain auxotroph to *Salmonella* strains, bromothymol blue ALGa agar containing CM (25 μg/ml), TC (25 μg/ml), SM (25 μg/ml), or SA (100 μg/ml) was used. In the case of transmission of R factors from *Salmonella* R^+ strains to *E. coli* strain K-12 58-161 F^-M^-, the mixed culture was centrifuged, washed twice with saline, and suspended in saline; the washed bacterial suspension was spread on medium ALGa agar containing methionine (10 μg/ml) and each drug.

Conditions of transduction. Transduction with phage ϵ_{15} was carried out with E_1 or E_4 cells of the strains *S. newington* C_2(S-84) and *S. chittagong* (S-224) as recipients which were sensitive to ϵ_{15} phage. Transduction with phage ϵ_{34} was carried out with cells of strains *S. newington* C_2(S-84) and *S. chittagong* (S-224) E_2 which were sensitive to ϵ_{34} phage.

Phage ϵ_{15} was grown on the R^+ donor strains of the E_1 or E_4 subgroup of *Salmonella* as described above, and phage ϵ_{34} was grown on R^+ cells of the E_2 subgroup of *Salmonella.* A 1.8-ml portion of phage (titer: 1.4×10^9 to 1.1×10^{10}/ml) and a 0.2-ml portion of recipient bacteria (ca. 1.4×10^9/ml) were mixed and incubated at 37 C. After 15 min of incubation, the culture was centrifuged at 8000 × *g* for 10 min, resuspended in saline solution, and spread on HI agar containing TC (25 μg/ml) or CM (25 μg/ml).

Two successive single-colony isolations were conducted on an appropriate selective plate containing each drug. The colonies which de-

veloped on the selective plate were picked and suspended in 1 ml of saline. A loopful of each bacterial suspension was streaked on HI agar containing CM (25 μg/ml), TC (25 μg/ml), or SM (25 μg/ml), or on AGGa agar containing SA (100 μg/ml), to check the multiple drug-resistance.

Transduction frequency. Phage grown on appropriate donor strains of R factor (titer: ca. 10^9 to 10^{10}/ml) and recipient bacteria (ca. 10^9/ml) were mixed. After 15 min at 37 C, the mixture was centrifuged at 8000 × *g* for 10 min. The number of free phage particles in the supernatant liquid was determined by the customary method (Lennox, 1955). The precipitated bacteria were suspended in an appropriate antiphage serum diluted 1:2 with saline and incubated at 37 C to neutralize free phage. After 10 min of incubation, bacteria were centrifuged, suspended in saline of the original volume, and spread on an appropriate selective medium containing each drug. In the case of transduction experiments with phages obtained by ultraviolet induction from the *Salmonella* strains lysogenized with ϵ_{15} or ϵ_{34} phage, 1 ml of phage (titer: ca. 10^4 to 10^7/ml) and 0.1 ml of recipient bacteria (ca. 10^9/ml) were mixed and incubated at 37 C. The transduction frequency was calculated by the following equation: number of transductants which received R factor per number of adsorbed phages.

Lysogeny test. Bacteria were grown to a concentration of ca. 10^9 cells per ml, the cultures were centrifuged, and the supernatant was treated with ether as described above. For ϵ_{15} phage, one loopful was spotted on an HI agar plate layered with E_1 or E_4 cells, and for ϵ_{34} phage on an HI agar plate layered with E_2 cells. The phage ϵ^+_{15} or ϵ^+_{34} cells were also detected according to the agglutination test by anti O-15 or O-34 serum because of the lysogenic conversion.

Immunity test. A loopful of culture was spread on HI agar to form a lawn of about 1 cm; a droplet of ϵ_{15} or ϵ_{34} phage was then deposited on the lawn.

Acriflavine treatment. Elimination of R factors by treatment with acriflavine was carried out according to the method described by Hirota and Iijima (1957) and was reported previously (Mitsuhashi, Harada, and Kameda, 1961*b*, *c*; Watanabe and Fukasawa, 1961*a*). The loss frequency of R factor was expressed as the ratio of number of R^- cells per total number of the cells examined.

RESULTS

Transduction of R factors to S. newington and S. chittagong with phages ϵ_{15} and ϵ_{34}. Phage ϵ_{15} was able to transduce R factors to E_1 or E_4 cells of *S. newington* C_2(S-84) and *S. chittagong* (S-224). Phage ϵ_{34} was also able to transduce R factors to (S-84) E_2 and (S-224) E_2. The R factors containing TC-resistance markers, i.e., R(TC.CM.SM.SA), R(TC.SM.SA), R(TC.CM), were segregated when transduced from the remaining resistance markers. The R factors without TC-resistance markers, i.e., R(CM.SM.SA) and R(SM.SA), were transduced together to recipient strains (Table 2).

Transferability of R factors in transductants. The transferability of R factors in transductants of *E. coli* K-12 58-161 F^- was tested according to the method described above. As seen in Tables 2 and 3, all R^+ transductants of *S. newington* (S-84) and *S. chittagong* (S-224) were so far unable to transfer their drug resistance by conjugation, whereas the R factors that were transferred by conjugation were all transferable by cell-to-cell contact.

The transferability and loss frequency of R factors in conjugants and transductants of *Salmonella* (S-84) and (S-224) are shown in Table 3. The R factors of *Salmonella* (S-84) R^+ or (S-224) R^+ that were transferred by conjugation were easily lost by treatment with acriflavine (experiments 2 and 4). However, the R factors of (S-84) or (S-224) R^+ transductants were all nontransferable by conjugation and were not eliminated by treatment with acriflavine (experiments 1 and 3).

Lysogenization with transducing epsilon phage. Relation between R^+ transductants and lysogenization with transducing epsilon phage was investigated. At lower multiplicities of infection, the R^+ transductants with ϵ_{15} phage were all stable R^+ and sensitive to ϵ_{15} phage, but, among the R^+ transductants with ϵ_{34} phage, 34% were lysogenic and 66% were sensitive to ϵ_{34} phage. The nontransferable R factors of R^+ transductants were transduced again with the homologous or heterologous epsilon phage to the one that had originally transduced the R factor. This will be described elsewhere.

TABLE 2. *Transduction of R factors with epsilon phages and transferability of R factors in transductants*

Phage	R factor	Donor	Recipient strain	Multiplicity of infection	Resistance patterns of transductants* when plated on		R factor†
					CM	TC	
ϵ_{15}	R_{10}(TC.CM.SM.SA)	(S-84)E_2.R^+	(S-84)E_1	1/10	CM.SM.SA (−6.8)	TC (−6.4)	R(CM.SM.SA) R(TC)
			(S-224)E_4				
	R_{12}(CM.SM.SA)		(S-84)E_1	1/1	CM.SM.SA (−7.3)		R(CM.SM.SA)
			(S-224)E_4		CM.SM.SA (−6.4)		R(CM.SM.SA)
	R_{26}(TC)		(S-84)E_1	1/1		TC (−7.6)	R(TC)
			(S-224)E_4			TC (−7.3)	R(TC)
ϵ_{34}	R_{10}(TC.CM.SM.SA)	(S-84)E_3.R^+	(S-84)E_2	1/1,000	CM.SM.SA (−3.9)	TC (−5.1)	R(CM.SM.SA) R(TC)
			(S-224)E_2		CM.SM.SA (−4.1)	TC (−5.4)	R(CM.SM.SA) R(TC)
	R_{12}(CM.SM.SA)		(S-84)E_2	1/100	CM.SM.SA (−4.2)		R(CM.SM.SA)
			(S-224)E_2		CM.SM.SA (−4.2)		R(CM.SM.SA)
	R_{26}(TC)		(S-84)E_2	1/1,000		TC (−5.0)	R(TC)
ϵ_{15}	R_{10}(TC.CM.SM.SA)	(S-224)E_2.R^+	(S-224)E_4	1/1,000	CM.SM.SA (−6.0)	TC (−5.7)	R(CM.SM.SA) R(TC)
	R_{12}(CM.SM.SA)			1/1,000	CM.SM.SA		R(CM.SM.SA)
	R_{13}(TC.SM.SA)			1/1,000		TC (−5.5)	R(TC) R(SM.SA)
	R_{14}(TC.CM)			1/1,000	CM (−5.7)	TC (−6.0)	R(CM) R(TC)
	R_{16}(CM)			1/1,000	CM (−5.4)		R(CM)
	R_{26}(TC)			1/1,000		TC (−6.0)	R(TC)
ϵ_{34}	R_{10}(TC.CM.SM.SA)	(S-224)E_3.R^+	(S-224)E_2	1/1,000	CM.SM.SA (−5.7)	TC (−5.5)	R(CM.SM.SA) R(TC)
	R_{12}(CM.SM.SA)			1/100	CM.SM.SA (−5.3)		R(CM.SM.SA)
	R_{13}(TC.SM.SA)			1/300		TC (−5.7)	R(TC)
	R_{14}(TC.CM)			1/100	CM (−5.7)	TC (−6.4)	R(CM) R(TC)
	R_{16}(CM)			1/500	CM (−5.7)		R(CM)
	R_{26}(TC)			1/100		TC (−6.4)	R(TC)

* Figures in each column indicate the logarithm of transduction frequency of R factors. Transductants were also plated on SA and not on SM; on SA, R_{13}(TC.SM.SA) resulted in SM.SA (−6.0).

† Transferability of R factors in transductants was tested by mixed cultivation with *E. coli* K-12 F^- auxotroph. In all cases, the transferability of R factors was less than 10^{-9}.

TABLE 3. *Comparison of R factors in conjugants and transductants of Salmonella E group*

Expt no.	Strain*		Transferability of R factor by conjugation†	Loss of R factor‡	
	Transductant	Conjugant		Spontaneous loss of R factor	Artificial elimination of R factor by acriflavine
1	(S-84) $E_2.R_{26}^+$(TC)		−	0/137	0/137
	(S-84) $E_3.R_{26}^+$(TC)		−	0/137	0/137
	(S-84) $E_2.R_{12}^+$(CM.SM.SA)		−	0/137	0/137
	(S-84) $E_3.R_{12}^+$(CM.SM.SA)		−	0/137	0/137
2		(S-84) $E_2.R_{26}^+$ (TC)	+ (−2.7)	1/110	41/100
		(S-84) $E_3.R_{26}^+$ (TC)	+ (−4.3)	1/110	36/100
		(S-84) $E_2.R_{12}^+$ (CM.SM.SA)	+ (−4.0)	31/199	413/500
		(S-84) $E_3.R_{12}^+$ (CM.SM.SA)	+ (−4.2)	29/200	166/200
3	(S-224) $E_2.R_{26}^+$(TC)		−	0/685	0/685
	(S-244) $E_3.R_{26}^+$(TC)		−	0/685	0/685
	(S-244) $E_2.R_{12}^+$(CM.SM.SA)		−	0/232	0/186
	(S-244) $E_3.R_{12}^+$(CM.SM.SA)		−	0/137	0/137
4		(S-224) $E_2.R_{26}^+$(TC)	+ (−3.4)	1/125	92/200
		(S-224) $E_3.R_{26}^+$(TC)	+ (−2.4)	1/125	206/500
		(S-224) $E_2.R_{12}^+$(CM.SM.SA)	+ (−3.2)	8/109	26/109
		(S-224) $E_3.R_{12}^+$(CM.SM.SA)	+ (−2.4)	37/500	157/300

* The R factors of R^+ conjugants were transferred by conjugation. The R factors of R^+ transductants subgroup E_2 or E_3 were transduced with ϵ_{15} or ϵ_{34} phage, respectively.

† Figures in parentheses indicate the logarithm of transmission frequency of R factor by mixed cultivation. Minus indicates that the transferability of R factors was less than 10^{-9}.

‡ Number of colonies in which the R factor was eliminated/total number of colonies tested.

DISCUSSION

With bacteriophage P1kc (Lennox, 1955) grown on *Shigella* with the resistance patterns (TC.CM.SM), (CM.SM), (TC.SM), or (SM), the antibiotic resistance was transduced into *E. coli* K-12 in a frequency of approximately 10^{-7} to 10^{-5} per phage particle adsorbed; this did not differ from the usual transduction frequency of ordinary characters (Nakaya et al., 1960). Watanabe and Fukasawa (1960*a*, 1961*b*) confirmed these findings and reported that R factors can be also transduced in *S. typhimurium* strain LT-2 with phage P-22.

Seven types of R factor are found from independently isolated bacteria: R(TC.CM.SM.-SA), R(CM.SM.SA), R(TC.CM.SA), R(SM.-SA), R(TC.CM), R(SM), and R(TC). A R(CM) factor is found in a segregant of a strain carrying R(CM.TC) factor (Mitsuhashi et al., 1961*d*). It was reported by this laboratory that eight types of R factor described above were transduced in *E. coli* K-12 with phage P1kc (Kondo, Harada, and Mitsuhashi, 1962). A majority of the transductants in *E. coli* K-12 had the same drug-resistance markers as the donor of R factors, and their R factors were transferable by conjugation (Kondo, Harada, and Mitsuhashi, 1962). About 10% of R factors containing (TC) marker, i.e. R(TC.CM.SM.-SA), R(TC.SM.SA), and R(TC.CM), were segregated when transduced in *E. coli* K-12 with P1kc, and segregation occurred consistently between (TC) and other resistance markers (Kondo et al., 1962).

In the systems of *Salmonella* E group epsilon phage, transductants of *S. newington* and *S. chittagong* were all unable to transfer their drug-resistance markers by conjugation. The R factors carrying (TC) marker were all segregated when transduced in the systems of *Salmonella* E group epsilon phages, and segregation was consistently found between (TC) and other remaining resistance markers.

The nontransmissible R(TC) factor of *S. newington* R^+(TC) transductant acquired transmissibility by cell-to-cell contact when infected with F factor (Harada et al., 1962). The details of these results will be described later.

At lower multiplicities of infection, the R^+ transductants with ϵ_{15} were all sensitive to ϵ_{15} phage and were unable to produce ϵ_{15} phage particles; among the R^+ transductants with ϵ_{34}, 34% were lysogenic and 66% were sensitive to ϵ_{34}.

The conversion of *S. anatum* (group E_1, somatic antigens 3,10) to group E_2 (antigens 3,15) by infection with phage ϵ_{15} and its virulent mutant ϵ_{15} *vir* was reported by Uetake, Luria, and Burrous (1958). The converted characters are controlled by vegetative phage as well as prophage. After infection with ϵ_{15} phage, the surviving cells segregate out for several generations a mixture of sensitive cells and phage-carrier cells. The pattern of segregation was confirmed by a single-clone experiment. All clones containing lysogenic cells also contain nonlysogenic, sensitive cells. The proportion of lysogenic cells in a clone containing lysogenic cells was 13%; the proportion of colonies containing lysogenic cells among all colonies was 8.5%. These values are in agreement with the final proportion of about 10% lysogenic cells found in the mass growth experiment (Uetake et al., 1958). In infection of *S. anatum* (group E_2) with phage ϵ_{34}, the proportions of phage-noncarrier cells in the progeny increased with the decrease of multiplicities of infection (Uetake and Hagiwara, 1959).

It is suggested that the existence of nonlysogenic colonies among R^+ transductants with epsilon phage may be accounted for by the transduction by recombinational incorporation of R factor into the bacterial chromosome and loss of the phage.

The existence of phage-noncarrier cells among R^+ transductants with epsilon phage might also be due to the existence of combined genetic elements, in which the resistance genes of R factor are associated with defective components of the epsilon phage genome such as λ *dg* (Arber, Kellenberger, and Weigle, 1958), PI *dl* (Luria, Adams, and Ting, 1960), and φ80 *dt* (Matsushiro, 1963). The details of the results which support this latter interpretation will be described later.

The R factors of R^+ transductants of *S. chittagong* and *S. newington* were nontransferable by conjugation and not eliminated by treatment with acriflavine. This suggests that the nontransferable R factors of R^+ transductants with epsilon phage are attached to the host chromosome, as indicated previously in the nontransferable R factor of R^+(CM.SM.SA) transductant of *E. coli* K-12 with phage P1kc (Kondo and Mitsuhashi, 1962).

Literature Cited

Akiba, T., T. Koyama, Y. Ishiki, S. Kimura, and T. Fukushima. 1960. Studies on the mechanism of development of multiply drug-resistant *Shigella* strains. Nihon Iji Shinpo No. 1866, p. 45–50.

Arber, W., G. Kellenberger, and J. Weigle. 1957. The defectiveness of lambda transduction phage. Schweiz. Z. Allgem. Pathol. Bakteriol. **20**:659–665.

Davis, B. D., and E. S. Mingioli. 1950. Mutants of Escherichia coli requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17–28.

Edwards, P. R., and W. H. Ewing. 1955. Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis.

Fukumi, H. 1953. On the methods for the determination of streptomycin and chloramphenicol sensitivity of *Shigella*. Nihon Iji Shinpo No.1598, p. 1600–1607.

Harada, K. 1956*a*. Studies on directed variation of *Salmonella* O-15 and O-34 by bacteriophage. I. Antigen transformation by two kinds of bacteriophage. Virus (Osaka) **6**:285–299.

Harada, K. 1956*b*. Studies on directed variation of *Salmonella* O-15 and O-34 by bacteriophage. II. Studies on antigen transformation by antiserum and antigen production governed by prophage. Virus (Osaka) **6**:300–307.

Harada, K., M. Kameda, M. Suzuki, and S. Mitsuhashi. 1961. On the drug-resistance of enteric bacteria. V. Mechanisms of the transmission of the drug-resistance between *Shigella* and *E. coli*. Japan. J. Bacteriol. **16**:6–16.

Harada, K., M. Kameda, M. Suzuki, and S. Mitsuhashi. 1962. Drug-resistance of enteric bacteria. Acquisition of transmissibility of noninfective R factor transduced by phage epsilon. Med. Biol. (Tokyo) **62**:21–24.

Hirota, Y., and T. Iijima. 1957. Acriflavine as an effective agent for eliminating F factor in *E. coli*. Nature **180**:655.

Iseki, S., and T. Sakai. 1953. Artificial transformation of O antigens in *Salmonella* E group. I. Transformation by antiserum and bacterial autolysate. Proc. Japan Acad. **20**:121–126.

KONDO, E., K. HARADA, AND S. MITSUHASHI. 1962. Drug-resistance of enteric bacteria. XII. Transduction of the transmissible drug-resistance factor by bacteriophage Plkc. Japan. J. Exptl. Med. **32**:139–147.

KONDO, E., AND S. MITSUHASHI. 1962. Drug-resistance of enteric bacteria. XV. Integration of R factor to the chromosomes of host bacteria. Gunma J. Med. Sci. **11**:59–62.

LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage PI. Virology **1**:190–206.

LURIA, S. E., J. N. ADAMS, AND R. C. TING. 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348–390.

MATSUSHIRO, A. 1963. Specialized transduction of tryptophan markers in *Escherichia coli* K-12 by bacteriophage ϕ80. Virology 19:475–482.

MITSUHASHI, S. 1960. On the genetics and drug-resistance of enteric bacteria. Kagaku (Tokyo) **30**:628–633.

MITSUHASI, S., K. HARADA, AND H. HASHIMOTO. 1960. Multiple resistance of enteric bacteria and transmission of drug-resistance to other strain by mixed cultivation. Japan. J. Exptl. Med. **30**:179–184.

MITSUHASHI, S., K. HARADA, AND H. HASHIMOTO. 1961*a*. Drug-resistance of enteric bacteria. VI. Combination of two types of R factor in a host bacterium. Med. Biol. (Tokyo) **61**:145–148.

MITSUHASHI, S., K. HARADA, AND M. KAMEDA. 1961*b*. Elimination of transmissible drug-resistance by treatment with acriflavine. Nature **189**:947.

MITSUHASHI, S., K. HARADA, AND M. KAMEDA. 1961*c*. Spontaneous and artificial elimination of transmissible drug-resistance factors. Japan. J. Exptl. Med. **31**:119–123.

MITSUHASHI, S., H. HASHIMOTO, K. HARADA, R. EGAWA, AND T. MATSUYAMA. 1961*d*. Studies on the drug-resistance of enteric bacteria. VII. Type of the transmissible drug-resistance factor. Gunma J. Med. Sci. **10**:59–66.

MITSUHASHI, S., K. HARADA, H. HASHIMOTO, M. KAMEDA, AND M. SUZUKI. 1962. Combination of two types of transmissible drug-resistance factors in a host bacterium. J. Bacteriol. **84**:9–16.

NAKAYA, R., A. NAKAMURA, AND Y. MURATA. 1960. Resistance transfer agents in *Shigella*. Biochem. Biophys. Res. Commun. **3**:654–659.

OCHIAI, K., T. YAMANAKA, K. KIMURA, AND O. SAWADA. 1959. Studies on inheritance of drug resistance between *Shigella* strains and *Escherichia coli* strains. Nihon Iji Shinpo No. 1861, p. 34–46.

SUGINO, Y., AND Y. HIROTA. 1962. Conjugal fertility associated with resistance factor R in *Escherichia coli*. J. Bacteriol. **84**:902–910.

UETAKE, H. 1957. Lysogenic conversion in bacterial antigens. Nisshin Igaku **44**:66–74.

UETAKE, H., AND S. HAGIWARA. 1959. Lysogenization and nuclear segregation. Virus (Osaka) **9**:574–577.

UETAKE, H., S. E. LURIA, AND J. W. BURROUS. 1958. Conversion of somatic antigens in *Salmonella* by phage infection leading to lysis or lysogeny. Virology **5**:68–91.

WATANABE, T., AND T. FUKASAWA. 1960*a*. "Resistance transfer factor" an episome in *Enterobacteriaceae*. Biochem. Biophys. Res. Commun. **3**:660–665.

WATANABE, T., AND T. FUKAWAWA. 1960*b*. Episomic resistance factors in *Enterobacteriaceae*. IV. Kinetics of transfer of resistance factors. Med. Biol. (Tokyo) **56**:98–100.

WATANABE, T., AND T. FUKASAWA. 1961*a*. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. II. Elimination of resistance factors with acridine dyes. J. Bacteriol. **81**:679–683.

WATANABE, T., AND T. FUKASAWA. 1961*b*. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. III. Transduction of resistance factors. J. Bacteriol. **82**:202–209.