JOURNAL OF BACTERIOLOGY, Mar. 1968, p. 1078-1089
Vol. 95, No. 3
Copyright © 1968 American Society for Microbiology
Printed in U.S.A.

Effective Elimination of Drug Resistance and Sex Factors in *Escherichia coli* by Sodium Dodecyl Sulfate

MUNEMITSU TOMOEDA, MANABU INUZUKA, NORIKO KUBO, AND SACHIKO NAKAMURA

Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan

Received for publication 18 December 1967

A method for effective elimination of drug resistance (R) and sex (F) factors in *Escherichia coli* K-12 strains by treatment with sodium dodecyl sulfate (SDS) is presented. Growth of *E. coli* harboring R or F factors in Penassay Broth containing SDS led to the loss of all or part of these genetic elements. Appearance of drug-susceptible or F^- cells among survivors was observed after the culture reached the stationary phase. Drug-susceptible cells which had lost all of their resistance markers by SDS treatment could be efficiently infected with R or F factors. Among isolated segregants which came from resistant cells, tetracycline-susceptible cells were the major segregant class. Drug-susceptible cells gave no revertants to drug resistance. By treatment of F^+ cells with SDS, unusual F^+ cells which retained mating ability but showed resistance to M12 phage were also isolated, together with mutants of another type which lost mating ability but retained sensitivity to M12 phage. Since SDS is more toxic to R^+ cells than R^- cells, the isolation of drug-susceptible or F^- cells under these conditions may be partly attributable to selective growth of drug-susceptible or F^- cells in SDS-Penassay Broth.

The sex (F) factor in the autonomous state in *Escherichia coli* can be eliminated by treatment with cobalt or nickel ions (6) or with acridine dyes (7). F factors linked with a small fragment of bacterial chromosome, designated as F′, can also be eliminated by acridine dyes (1, 8) and by mutagenic agents (22). In contrast, F factors integrated into the chromosome are not eliminated by such agents (7). Loss of F factors and colicinogenic factors from thymineless strains of *E. coli* under conditions of thymine-limited growth has also been reported (2). Furthermore colicinogenic factor Col V has been eliminated by use of acridine dyes (11).

R factors of *E. coli* and *Shigella flexneri* are eliminated spontaneously on standing, and the frequency of loss is increased by treatment with acridine dyes (16, 19, 20), or with 4-nitroquinoline-1-oxide and its analogues (*unpublished data*). Mild penicillin treatment of R factors in *Salmonella paratyphi* also results in loss of R factors (12).

We found that an anionic surface-activating agent, sodium dodecyl sulfate (SDS), eliminates R and F factors in *Escherichia* with very high efficiency. The present paper deals with the action of SDS on *E. coli* K-12 derivatives carrying R and F factors (leading to their loss), the genetic identification and characterization of drug-susceptible and F^- strains obtained by the SDS treatment, and the possible mechanism of the action of SDS.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. The bacterial strains used in this work (Table 1) were *E. coli* K-12 derivatives. The principal strains were supplied by Y. Hirota, Osaka University.

The male-specific bacteriophage used was ribonucleic acid (RNA) phage M12 (9), supplied by Hirota. This phage lyses male strains of *E. coli* K-12 specifically. Phage P1kc was used for transduction.

Genetic markers. Nutritional requirements (− indicates dependence; +, independence) are designated by the following symbols: *thr*, threonine; *leu*, leucine; *pro*, proline; *pur*, purine; *met*, methionine; *thi*, thiamine.

Fermentation markers (− indicates nonfermentation; +, fermentation) are shown by the following symbols: *ara*, arabinose; *lac*, lactose; *gal*, galactose; *mal*, maltose; *xyl*, xylose; *mtl*, mannitol.

The symbols *str*, *chm*, *tet*, and *sul* denote the genetic

TABLE 1. *Strains of Escherichia coli K-12*[a]

F and R[b]	Strain	Genotype
$F^-R^+_{100\text{-}1}$	JE170	*lac-11D3 mal-5 str*^{r}
$F^-R^+_{100\text{-}1}$	JE177	*mal-5*
$F^-R^+_{100\text{-}1}$	JE2100	
$F^-R^+_{100\text{-}1}$	KE72[c]	*pur*$^-$
$F^-R^+_{100\text{-}1}$	KE75[d]	*lac-85*
F^+R^-	W6	*met*$^-$
F^+R^-	JE64	*thr*$^-$*leu*$^-$*lac*$^-$*thi*$^-$
F^+R^-	KE53[e]	*met*$^-$*str*^{r}
F^+_8 (F-*gal*)R^-	W4520	*met*$^-$chromosomal *gal*$^+$
F^+_{13} (F-*lac*)R^-	W3747	*met*$^-$chromosomal *lac*$^+$
F^-R^-	W3110	
F^-R^-	W3630	*mal-5*
F^-R^-	W4573	*ara-2 lac-85 gal-2 mal-1 mtl*$^-$*xyl-2 str*^{r}
F^-R^-	W4637	*lac-85 pur*$^-$
F^-R^-	KE70[f]	*pur*$^-$
F^-R^-	KE74[g]	*lac-85 pur*$^+$

[a] Only relevant markers are shown.
[b] Markers of $R_{100\text{-}1}$ factor are *i*$^-$*m*$^+$*str chm tet sul* (3, 17).
[c] Derived from KE70 by infection of the $R_{100\text{-}1}$ factor with JE170 as the donor.
[d] Derived from KE74 by infection of the $R_{100\text{-}1}$ factor using JE170 as the donor.
[e] Derived from W6 by spontaneous mutation to resistance to streptomycin.
[f] Derived from W4637F$^-$*lac-85 pur*$^-$ by mating with JE1031 (Hfr H)*met*$^-$$B_1^-$.
[g] Derived from W4637 by transduction with P1kc with W3110 as donor strain.

loci associated with drug resistance, and indicate resistance to: dihydrostreptomycin sulfate (Takeda Chemical Ind., Osaka, Japan), 20 μg/ml; chloramphenicol (Sankyo Co., Tokyo, Japan), 25 μg/ml; tetracycline hydrochloride [Lederle (Japan), Ltd.], 25 μg/ml; and sulfanilamide (Davis Co., Inc., Newark, N.J.), 200 μg/ml; + or − denotes presence or absence of R factors. $R_{100\text{-}1}$ (*i*$^+$*m*$^+$ *str chm tet sul*) is a mutant of R_{100} (3, 17); *i* is a genetic trait of the R factor concerning the inhibition of F mating; + or − indicates the inhibition or noninhibition of F mating, respectively; *m* is a genetic trait of R factors concerning the conjugal fertility associated with the R factor; + or − indicates the presence or absence of the conjugal fertility.

Materials and media. SDS (Tokyo Kasei Industries, Tokyo, Japan), was analyzed by emission spectroscopy. In addition to sodium, which was the major metallic component, copper, calcium, and magnesium were present as impurities, all in amounts less than 2.35 ppm.

Penassay Broth (Difco) was used for the SDS treatment and conjugation experiments.

L broth (15) was used for transduction experiments with P1kc and for phage M12 assay.

Davis glucose medium, a synthetic medium, was used for characterization of auxotrophic mutants.

A complete Eosin methylene blue (EMB) or synthetic Eosin methylene blue (EM)-sugar agar (13) with addition of one of the four drugs was used for scoring of drug-resistant colonies.

The synthetic EM-sugar agar was also used for scoring of sugar fermentation, and for selection of prototrophic recombinant colonies.

The *p*H of media was 7.6 unless otherwise stated.

SDS treatment. An overnight culture of R^+ cells in Penassay Broth, containing 25 μg of CM per ml, was diluted to 10^3 cells/ml in broth containing 10% (w/v) SDS and was shaken at 37 C. Cells were plated on EMB-glucose after appropriate dilutions in saline. All the colonies on the plate were tested for their drug-resistance characters by replica-plating on EMB-glucose containing chloramphenicol (25 μg/ml), tetracycline (25 μg/ml), or streptomycin (20 μg/ml), and on EM-glucose containing sulfanilamide (200 μg/ml).

SDS treatment of F^+ or F′ cells was carried out in a similar manner. The effect of the treatment on surviving colonies was determined by recombination techniques, and by tests for sensitivity to the male-specific phage M12, as described below.

Overnight cultures of the recipient strain, W4573, were streaked on EM-sugar medium, and they were spotted with one loopful of the culture being tested. After incubation at 37 C for 2 days, recombinant arose where the culture was spotted (14) if the test culture was a donor.

Test for sensitivity to male-specific phage. Phage M12 lysates in L-broth were streaked on EMB-glucose and were cross-streaked with the cultures (ca. 10^8 to 5×10^8 cells/ml) being tested. After incubation at 37 C overnight, the phage lysed the F^+ culture but not the F^- culture.

Infection with R and F factors. The drug resistance (R) factors were introduced into R^- (recipient) strains from appropriate R^+ (donor) strains by mixing equal volumes of exponential cultures (ca. 10^8 cells/ml) and incubating the mixed culture at 37 C overnight.

The F or F′ factor was introduced into F^- (recipient) strains from appropriate F^+ or F′ (donor) strains by a similar procedure, except that equal volumes of exponential cultures of F^- (ca. 2×10^7 cells/ml) and F^+ (ca. 4×10^8 cells/ml) strains were mixed.

RESULTS

Elimination of drug-resistance (R) factors by treatment with SDS. Strain JE2100, harboring the $R^+_{100\text{-}1}$ (*i*$^-$*m*$^+$*str chm tet sul*) factor, was treated with SDS at various concentrations by inoculating from 10^2 to 10^9 R^+ cells per ml. Survivors were analyzed at intervals for the presence or absence of drug resistance. Representative results are shown in Table 2. Loss of drug resistance of cells was observed at high frequency (up to 100%). Drug-susceptibility patterns characteristic of the susceptible cells thus obtained were stable after many generations. As the incubation time increased to 72 hr,

TABLE 2. *Elimination of drug-resistance (R) factors of Escherichia coli K-12 JE2100 R^+_{100-1} by treatment with various concentrations of sodium dodecyl sulfate (SDS)*

Expt no.	Inoculum size (cells/ml)	SDS concn (%)	Time of incubation (hr)	Viable count (cells/ml)	No. of colonies showing drug susceptibility/no. of colonies tested	Frequency of drug-susceptible colonies (%)
1	1.0×10^3	0	9	5.5×10^8	1/136	0.7
			18	4.1×10^9	0/335	0
			40	7.4×10^8	0/203	0
			72	—	0/258	0
		2	9	1.4×10^6	27/152	17.8
			18	8.5×10^8	8/76	10.5
			40	7.7×10^6	29/54	53.7
			72	1.4×10^3	14/14	100
		4	9	2.2×10^5	11/67	16.4
			18	9.3×10^8	51/150	34.0
			40	6.6×10^6	390/403	96.8
			72	2.6×10^3	23/25	92.0
		6	9	1.7×10^4	9/141	6.4
			18	5.9×10^8	11/94	11.7
			40	2.5×10^6	207/237	87.3
			72	10^2	—	—
		8	9	2×10^2	—	—
			18	1.1×10^9	132/205	64.3
			40	3.1×10^5	293/295	99.3
			72	1.8×10^3	18/18	100
2	3.1×10^3	0	24	4.8×10^8	0/90	0
		10	24	3.0×10^8	1/278	0.4
			48	4.4×10^6	512/662	77.3
			72	1.1×10^7	104/109	95.4
3	3.9×10^3	0	24	8.4×10^8	0/167	0
		10	24	1.8×10^7	116/525	22.1
	1.7×10^6	0	24	1.0×10^9	1/201	0.5
		10	24	1.5×10^7	19/361	5.3
	1.2×10^8	0	24	1.6×10^9	0/325	0
		10	24	2.8×10^7	54/527	10.2

the optical density (OD) and total viable count of the culture decreased, and the frequencies of drug-susceptible cells among the survivors increased. Formation of auxotrophic mutants was rarely observed.

The effect of inoculum size on the frequency of production of susceptible cells was insignificant. SDS at lower concentrations than 10% was also effective in producing susceptible cells. In most of the experiments reported below, broth containing 10% SDS was used.

Loss of drug resistance of R^+ cells caused by SDS treatment was also observed with JE177 R^+_{100-1}.

Kinetics of the elimination. W3110, as the F^-R^- reference strain, and JE2100, as the R^+ strain, were treated with 10% SDS in Penassay Broth at 37 C with shaking, and the survivors were analyzed at intervals. Representative results are shown in Fig. 1. Generation times for these strains in the exponential phase, when treated with SDS, were only slightly longer than without SDS. After the viable count reached a maximum, cells began to lyse rather slowly, and the net cell number of drug-susceptible cells increased.

Similar results were obtained with another R^+ strain, JE177.

Fluctuation experiment for the action of SDS on JE177 R^+ strain. JE177 was used as the R^+ strain in a fluctuation experiment. Penassay Broth containing about 10^3 R^+ cells/ml was diluted 10^{-1} with Penassay Broth, and the diluted culture was divided into 10 parts. A tube of the original culture (ca. 10^3 cells/ml) and 10 tubes containing the diluted cultures (ca. 10^2 cells/ml) were incubated at 37 C with shaking, and survivors were analyzed at intervals (Table 3). By inoculating 10^2 cells/ml, it was found that drug-susceptible cells were formed in every tube, though their frequency varied (0.7 to 74.3%).

Environmental effects on the elimination of R

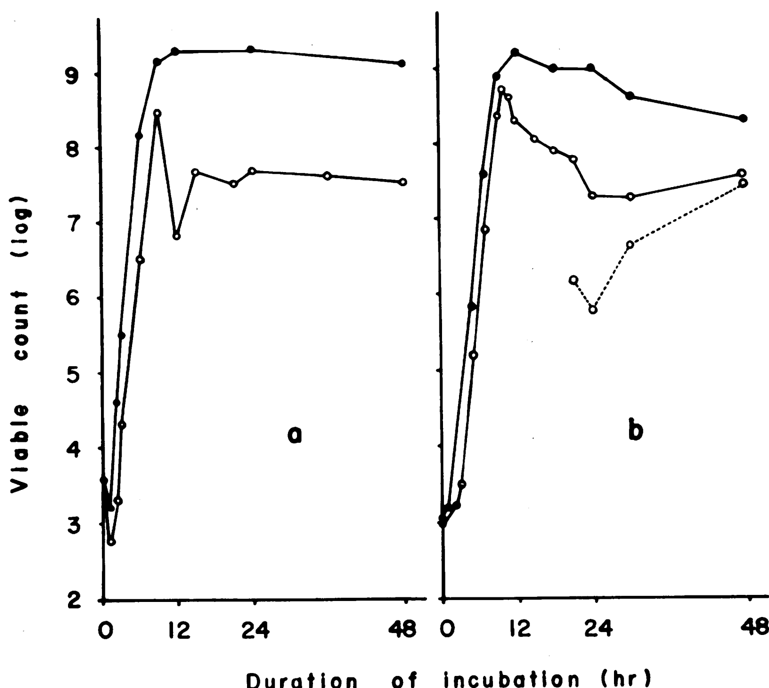

FIG. 1. *Kinetics of the SDS treatment of Escherichia coli W3110 (a) and JE2100 R^{+}_{100-1} (b). (a) W3110 cells (2.7 × 10^3 cells/ml) in Penassay Broth (10 ml) with or without 10% SDS were incubated at 37 C with shaking. Symbols: ●, viable count without SDS; ○, viable count with SDS. (b) JE2100 R^+ cells (1.1 × 10^3 cells/ml) in Penassay Broth (10 ml) with or without 10% SDS were incubated at 37 C with shaking. Symbols as in (a); ○ with dashed line represents viable count of drug-susceptible cells formed in the culture with SDS.*

factors by the SDS treatment. The effect of initial *p*H of the culture on the efficiency of elimination of R factors by SDS was determined by inoculating 10^3 JE2100 R^+ cells/ml into Penassay Broth at different *p*H values (Table 4). Higher frequencies of elimination were obtained at *p*H 7.0 and 7.6 than at *p*H 6.5 and 8.0.

The effect of medium on the efficiency of elimination of R factors by SDS was also examined with JE2100. Penassay Broth was replaced with a synthetic medium, Davis glucose medium, and a phosphate buffer medium without any carbon and nitrogen sources. These media were inoculated with ca. 10^8 to 10^9 cells/ml. In Davis glucose medium, drug-susceptible cells were formed in numbers not significantly different from those obtained in Penassay Broth (Table 5). However, no susceptible cells were formed in the phosphate buffer medium. Cells died more rapidly in both Davis medium and phosphate buffer medium than in Penassay Broth.

Similar results were obtained with strain JE177.

Effect of variation of JE2100 strain on the elimination frequency of R factors. It was realized at the beginning of the present investigation that higher frequencies of elimination of R factors by the SDS treatment were obtained when R^+ cells isolated from aged cultures of the R^+ strain JE2100 were used. Therefore, the possible effects of variation of the strain on the ease of elimination of R factors were investigated. JE2100 R^+ cells, obtained by infection of R_{100-1} factors from JE170, as donor, to W3110, as recipient, were inoculated into Penassay Broth containing chloramphenicol (25 μg/ml); and broth was incubated at 37 C overnight and then kept at room temperature. At intervals, a single R^+ colony was isolated, and the resistance markers of cells of the colony were confirmed by the replica-plating method. The cells from this colony were then used for the experiment.

It was found that, as the culture became older, the frequency of production of susceptible cells by SDS increased (Table 6). It was further observed that, when R^+ cells isolated from cultures more than 4 months old were used, spontaneous elimination of R factors took place at high frequencies.

Segregation patterns of R factors as the result of SDS treatment. Resistance and susceptibility to the four drugs of cells obtained by the SDS

TABLE 3. *Fluctuation experiment for action of SDS on the JE177 R+ strain*

Inoculum size (cells/ml)	SDS concn (%)	Time of incubation[a] (hr)	Frequency of drug-susceptible cells formed (%)	Tube no.
5.4×10^3	0	48	0	
	10	48	93.7	
5.4×10^2	0	72	0	0
	10	72	74.3	1
		72	51.6	2
		48	1.9	3
		72	14.8	4
		72	37.5	5
		72	21.5	6
		24	0.7	7
		72	19.2	8
		72	14.8	9
		72	61.4	10

[a] The time of incubation at which the highest frequency of drug-susceptible colony production was observed.

TABLE 4. *Effects of pH on the elimination of R factors of JE2100 by the SDS treatment*[a]

SDS concn (%)	*p*H	Viable count (cells/ml)	No. of colonies showing drug susceptibility/no. of colonies tested	Frequency of drug-susceptible colonies formed (%)
0	7.6	4.3×10^8	0/86	0
10	6.5	5.3×10^7	2/288	0.7
	7.0	1.5×10^7	42/132	31.8
	7.6	2.9×10^7	81/207	39.1
	8.0	5.2×10^6	24/212	11.3

[a] The inoculum was 3.1×10^3 cells/ml. Incubation was for 48 hr.

treatment were examined (Table 7). It was found that in JE2100 most drug-susceptible cells were segregants which had lost drug resistance only to tetracycline or to all four drugs; *str*^{s} *chm*^{s} *sul*^{s} cells were also found among the survivors. In JE177, the majority of sensitive cells were those which had lost drug resistance to all the drugs. Segregants of types other than *tet*^{s} or *str*^{s} *chm*^{s} *sul*^{s} (i.e., *chm*^{s}, *chm*^{s}*tet*^{s}, *chm*^{s}*tet*^{s}*sul*^{s}, *sul*^{s}, and *sul*^{s}*chm*^{s}*tet*^{s}) were also obtained with JE177. These patterns of drug resistance and susceptibility of susceptible cells were retained by the cells after many generations. The concentration of SDS, the inoculum size, and the duration of incubation had little effect on the segregation patterns of susceptible cells. It was noted that *tet*^{s} cells had lost the transfer locus (m+).

Segregation patterns of susceptible cells obtained in the variation experiment mentioned before gave similar results.

Reversion and infection with $R_{100\text{-}1}$ factors of drug-susceptible cells obtained by SDS treatment. If drug-susceptible cells obtained by the SDS treatment retained a locus with the susceptibility allele, they would give R+ revertants. Also, if drug-susceptible cells which lost all resistance markers retained a R-segment, they would not receive R factors. Reversion and infection experiments were thus carried out to test these predictions.

Reversion experiments with drug-susceptible cells obtained by SDS treatment were carried out with *tet* and *chm* as selected markers, and with W3110 and W3630 as R− reference strains. Segregants that were *tet*^{s} or *tet*^{s}*chm*^{s}, or cells susceptible to all the four drugs, were tested for reversion to resistance by inoculating 10^8 cells onto plates with tetracycline or chloramphenicol. No R+ revertant was obtained from these susceptible cells.

Infection experiments were carried out with the JE170 $R^+_{100\text{-}1}$ strain as the donor, and cells susceptible to all the four drugs obtained by SDS treatment as the recipient. These susceptible cells were found to be infected by $R_{100\text{-}1}$ factors, although the frequencies (14 to 57%) of infection were a little lower than those (48 to 58%) obtained with the W3630 R− strain as recipient.

Level of drug resistance and susceptibility expressed by drug-susceptible cells obtained by SDS treatment. The level of drug resistance and susceptibility of R+ and drug-susceptible cells isolated from the culture after the SDS treatment were compared with those of JE2100 or JE177 as R+ strains and W3110 as the R− strain. The levels of drug resistance were almost the same before and after the SDS treatment.

Reconstruction experiment between R+ and R− strains. To investigate the presence or absence of selective toxicity of SDS between R+ and R− cells, reconstruction experiments on the elimination of R factors from R+ cells to produce drug-susceptible cells by SDS were first carried out between KE72 (*pur*−*lac*+$R^+_{100\text{-}1}$) and KE74 (*pur*+*lac*$^-_{85}$), and between KE75 (*pur*+*lac*$^-_{85}$-$R^+_{100\text{-}1}$) and KE70 (*pur*−*lac*+). Overnight cultures (ca. 10^8 cells/ml) of R+ and R− strains were appropriately diluted in Penassay Broth with or without 10% SDS to give cell concentrations of ca. 10^3 cells/ml. The broth was supplemented with adenine (20 μg/ml) and thiamine (2 μg/ml). Equal volumes of R+ and R− cultures were mixed and incubated at 37 C with shaking, and survivors were analyzed at intervals, with *lac* as the distinguishing marker. Frequencies of production of drug-susceptible cells from R+ cells and also of R infection from R+ to susceptible cells during the SDS treatment were ex-

amined. Representative results with KE72 R^+ and KE74 R^- are shown in Fig. 2a. It was found that the maximal number of R^+ cells was lower than that of susceptible cells in SDS medium, and that after the SDS medium reached the stationary phase the viable count of R^+ cells decreased far more rapidly than that of R^- cells. This did not occur in the medium without SDS. It was also observed that, in the medium without SDS, the infection of R factors from *lac*$^+$*R*$^+$ to *lac*$^-$R$^-$ took place. The frequencies of *lac*$^-$R$^+$ in the whole *lac*$^-$ after 5, 12, 24, and 48 hr of incubation were found to be 0, 94, 98, and 100%, respectively. The infection of R factors was not observed during the SDS treatment. Similar results were obtained with KE75 R^+ and KE70 R^-.

Another reconstruction experiment was carried out with KE75 as R^+ strain and KE70 as R^- strain, as follows. R^+ (1.3×10^3 cells/ml) cells were inoculated in Penassay Broth supplemented with adenine (20 μg/ml) and thiamine

TABLE 5. *Effect of medium on the elimination of R factors by the SDS treatment*

Medium	Inoculum size (cells/ml)	SDS concn (%)	Time of incubation (hr)	Viable count (cells/ml)	No. of colonies showing drug susceptibility/ no. of colonies tested	Frequency of drug-susceptible colonies formed (%)
Penassay Broth	1.6×10^9	0	24	9.3×10^8	0/92	0
	1.1×10^9	10	48	4.1×10^7	53/100	53.0
Davis glucose	1.4×10^9	0	48	4.2×10^8	0/204	0
	1.1×10^9	10	48	1.5×10^4	—	—
			72	2.2×10^2	9/39	23.1
Phosphate buffer	1.2×10^9	0	48	2.0×10^8	0/404	0
	4.9×10^8	10	24	3.4×10^4	0/222	0
			48	2.6×10^3	0/521	0

TABLE 6. *Effect of variation of JE2100 on the ease of elimination of R factors by the SDS treatment*

Age of culture (months)	Inoculum size (cells/ml)	Time of incubation (hr)	No SDS			10% SDS		
			Viable count (cells/ml)	No. of colonies showing drug susceptibility/ no. of colonies tested	Frequency of drug-susceptible colonies formed (%)	Viable count (cells/ml)	No. of colonies showing drug susceptibility/ no. of colonies tested	Frequency of drug-susceptible colonies formed (%)
Freshly infected	1.0×10^3	24	7.9×10^8	0/146	0	5.0×10^7	4/124	3.2
		48	3.0×10^8	0/56	0	1.8×10^7	11/125	8.8
		72	1.2×10^8	0/24	0	1.6×10^6	99/315	31.4
2	2.2×10^3	0	2.2×10^3	0/100	0	—	—	—
		24	4.2×10^8	0/75	0	1.5×10^7	1/100	1.0
		48	2.0×10^8	0/19	0	2.2×10^7	50/187	26.7
		72	1.4×10^8	—	—	4.9×10^6	67/79	84.8
3	4.1×10^3	0	4.1×10^3	0/418	0	—	—	—
		24	9.4×10^8	0/185	0	1.1×10^7	6/207	2.9
		48	3.3×10^8	0/64	0	2.9×10^7	96/100	96.0
4	2.1×10^3	0	2.1×10^3	1/197	0.5	—	—	—
		24	6.9×10^8	1/114	0.9	1.7×10^8	188/271	69.4
		48	7.6×10^8	86/324	26.4	1.2×10^7	238/238	100
		72	3.6×10^8	44/55	88	—	—	—
5	2.2×10^3	0	2.2×10^3	1/174	0.6	—	—	—
		24	5.6×10^8	6/112	5.4	9.3×10^7	8/174	4.6
		48	4.6×10^8	172/181	95	3.9×10^5	59/74	79.7
		72	2.5×10^8	244/247	99	3.4×10^4	21/50	42.0

TABLE 7. *Segregation patterns of drug-susceptible colonies obtained by the SDS treatment*

Expt no.	Strain	Inoculum size (cells/ml)	SDS concn (%)	Time of incubation (hr)	Total frequency of drug-susceptible colonies among survivors (%)	Segregation patterns, resistant or susceptible to				No. of segregants per no. of drugs susceptible colonies tested	Frequency of segregants formed (%)
						str	*chm*	*tet*	*sul*		
8	JE2100	1.0×10^3	2	40	53.7	s	s	s	s	29/29	100
			4	40	97	s	s	s	s	50/50	100
			6	40	87.4	s	s	r	s	1/50	2.0
						s	s	s	s	49/50	98.0
			8	40	87.4	s	s	s	s	50/50	100
9[a]	JE2100	3.9×10^3	10	24	22.1	r	r	s	r	63/66	95.5
						s	s	s	s	3/66	4.5
		1.7×10^6	10	24	5.3	r	r	s	r	16/16	100
		1.0×10^9	10	24	37.6	r	r	s	r	91/92	98.9
						s	s	s	s	1/92	1.1
10	JE177	2.4×10^3	10	48	3.5	r	r	s	r	4/18	22.2
						r	r	r	s	1/18	5.6
						r	s	s	r	5/18	27.8
						r	s	r	s	1/18	5.6
						s	s	s	s	7/17	41.2
11	JE177	2.9×10^3	10	48	58.2	r	r	s	r	2/19	10.5
						r	r	r	s	2/19	10.5
						s	s	s	s	15/19	78.9
12	JE177	3.8×10^3	10	48	5.2	r	r	s	r	2/24	8.3
						s	s	r	s	3/24	12.5
						s	s	s	s	19/24	79.2

[a] Referred to previously as experiment no. 3 (Table 2).

(2 μg/ml), with or without 10% SDS, and the broths were incubated at 37 C with shaking. R$^-$ (2.0×10^3 cells/ml) cells were also inoculated in Penassay Broth with adenine and thiamine and with or without 10% SDS, and the broths were incubated at 37 C with shaking. After 10 hr, the R$^+$ broth without SDS was mixed with the R$^-$ broth without SDS in the ratio of $1:10^{-3}$, and the mixed broth was incubated at 37 C with shaking. The R$^+$ broth with SDS was also mixed with the R$^-$ broth with SDS in the same ratio, and the mixed broth was incubated at 37 C with shaking. Survivors were analyzed at intervals. As shown in Fig. 2b, R$^-$ cells, after being added to the R$^+$ culture containing 10% SDS, began to grow exponentially, whereas R$^+$ cells began to die. This did not occur when R$^-$ cells were added to the R$^+$ culture without SDS. Infection of R factors during the mixed cultivation was not checked.

Repeat of SDS treatment of R$^+$ strain. R$^+$ and drug-susceptible cells isolated during the SDS treatment of JE2100 were repeatedly treated with SDS, and survivors were analyzed. R$^+$ cells resistant to all four drugs and two kinds of susceptible cells (those susceptible only to tetracycline and those susceptible to all four drugs), isolated after 24 hr of SDS treatment, were treated again with 10% SDS-Penassay Broth at 37 C with shaking; the inoculum was ca. 10^3 cells/ml. R$^+$ cells were again isolated after 24 hr of treatment, and were treated again with 10% SDS.

It was found after each repeat of the SDS treatment that the frequencies of production of susceptible cells decreased, and that R$^+$ cells began to grow after a shorter lag and reached maximal growth a short time after susceptible cells did. These results were found to be reproducible.

Elimination of sex (F) factors by treatment with SDS. Strains W6 and KE53, harboring F factors, strain W3747, harboring F_{13} (F-*lac*), and strain W4520, harboring F_8 (F-*gal*) factors, were incubated with 10% SDS in Penassay Broth at 37 C. Survivors were analyzed at intervals for the presence or absence of fertility by examining, first, the mating ability toward certain F$^-$ strains chosen as recipients, and, second, sensitivity to the male-specific RNA

phage M12. As is shown in Table 8, SDS treatment of F^+ and F' cells gave colonies of four types with regard to their fertility. The first group was composed of colonies retaining mating ability (m^+) and showing sensitivity to M12 phage, which could be characterized as F^+ or F' cells unchanged during the SDS treatment. The second group was composed of colonies having lost mating ability (m^-) and showing resistance to M12 phage, which could be char-

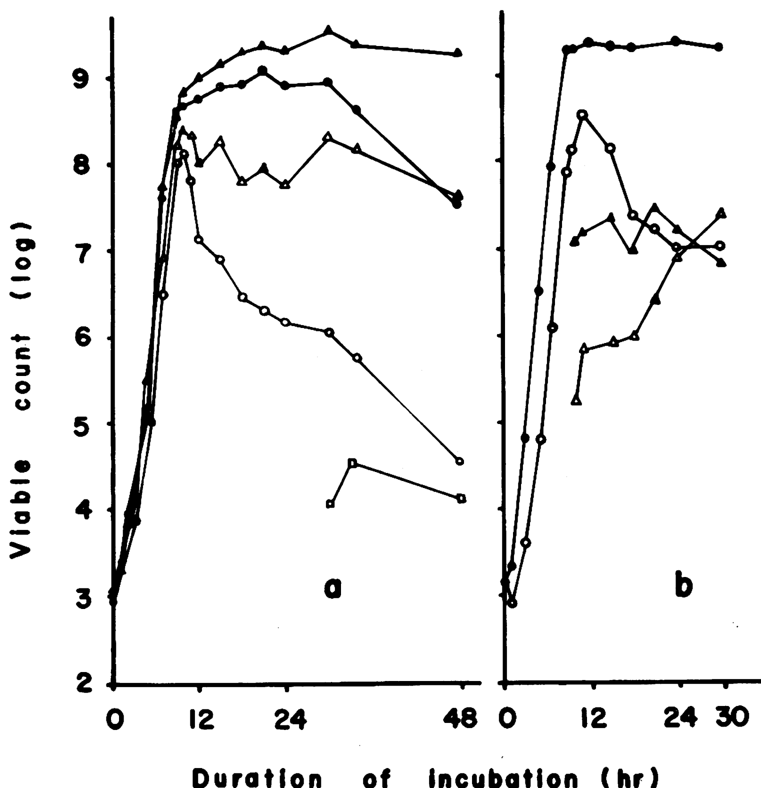

FIG. 2. *Reconstruction experiments between Escherichia coli KE72 (lac$^+$ pur$^-$$\overset{+}{R}_{100\text{-}1}$) and KE74 (lac$^-pur^+$) (a) and KE75 (lac$^-$ pur$^+$$\overset{+}{R}_{100\text{-}1}$) and KE70 (lac$^+pur^-$) (b). (a) Overnight cultures (ca. 10^8 cells/ml) of R^+ and R^- strains were diluted 10^{-5} in Penassay Broth supplemented with adenine (20 μg/ml) and thiamine (2 μg/ml) and with or without 10% SDS. Equal volumes of R^+ (8.6×10^2 cells/ml) and R^- (1.2×10^3 cells/ml) cultures were mixed and incubated at 37 C with shaking. Plating with appropriate dilutions at certain intervals was on EMB-lactose-agar. Fifty to one hundred lac$^+$ colonies were arbitrarily chosen to be streaked on EMB-lactose agar, which served as a master plate. Colonies grown on the master plate were replica-plated onto EM-glucose supplemented with adenine (20 μg/ml), thiamine (2 μg/ml), and certain amounts of streptomycin, chloramphenicol, tetracycline, and sulfanilamide, for examining the frequency of lac$^+$$R^-$ cells. This was the elimination frequency of lac$^+$$R^+$ to lac$^+$$R^-$. Fifty to one hundred lac$^-$ colonies grown on the original EMB-lactose-agar plate were arbitrarily chosen and were streaked on EMB-lactose-agar which served as a master plate. The colonies grown on the master plate were replica-plated onto EM-glucose supplemented with certain amounts of adenine, thiamine, and the four drugs, for examining the frequency of R infection from lac$^+$$R^+$ to lac$^-$$R^-$ during the SDS treatment. Symbols: ●, viable count of lac$^+$$R^+$ without SDS; ▲, viable count of lac$^-$$R^-$ without SDS; ○, viable count of lac$^+$$R^+$ plus lac$^+$$R^-$ with SDS; △, viable count of lac$^-$$R^-$ with SDS; □, viable count of lac$^+$$R^-$ formed by SDS. (b) Overnight cultures (ca. 10^8 cells/ml) of R^+ and R^- strains were diluted to 1.3×10^3 cells/ml and 2.0×10^3 cells/ml, respectively, in Penassay Broth supplemented with adenine (20 μg/ml) and thiamine (2 μg/ml) and with or without 10% SDS, and incubated at 37 C with shaking. After 10 hr, the R^- culture (1.0×10^8 cells/ml) was diluted 10^{-1} with Penassay Broth supplemented with adenine, thiamine, and with or without SDS, and 0.1 ml of the diluted culture was added to 10 ml of R^+ culture (1.2×10^9 cells/ml). The R^+ plus R^- culture was again incubated at 37 C with shaking, and survivors were analyzed by the procedure of (a). Symbols: ●, viable count of lac$^-$$R^+$ without SDS; ○, viable count of lac$^-$$R^+$ plus lac$^+$$R^-$ with SDS; ▲, viable count of lac$^+$$R^-$ added to the R^+ culture without SDS; △, viable count of lac$^+$$R^-$ added to the R^+ culture with SDS.*

TABLE 8. *Elimination of sex (F) factors of Escherichia coli K-12W6 F^+, W3747 F^+_{13} (F-lac), and W4520 F^+_8(F-gal) by treatment with SDS*

Expt no.	Strain	Inoculum size (cells/ml)	SDS (%)	Time of incubation (hr)	Viable count (cells/ml)	No. of colonies tested	No. of m^+M12^s colonies (F^+)	No. of m^-M12^r colonies (F^-)	No. of m^+M12^r colonies	No. of m^-M12^s colonies	Frequency of F^- colonies (%)
1	W6	1.7×10^3	0	48	2.3×10^9	50	50	0	0	0	0
			10	48	1.2×10^7	50	0	50	0	0	100
2	W3747	1.7×10^3	0	48	1.8×10^9	50	50	0	0	0	0
			10	48	3.3×10^7	50	6	41	3	0	82
3	W4520	2.0×10^3	0	48	1.4×10^9	100	100	0	0	0	0
			10	48	7.7×10^7	100	48	47	4	1	47
4	KE53	5.8×10^3	0	48	2.0×10^9	50	50	0	0	0	0
			10	48	8.2×10^7	50	1	49	0	0	98

acterized as F^- cells. The third group was composed of colonies retaining mating ability (m^+) and showing resistance to M12 phage, and, the fourth, of colonies losing mating ability (m^-) and showing sensitive to M12 phage. The colonies belonging to the third and fourth groups were characterized as being F^+ and F′ mutants.

The genetic trait of F^- isolates obtained, with respect to the absence of mating ability and resistance to M12 phage, was repeatedly examined and was found to be stable for many generations. It was found that the longer the incubation time with SDS, the higher the frequencies of F^- production were; this observation was also made for the experiments on R factors.

Kinetics of the elimination of F factors. It was proved by a kinetic study that the elimination of F factors by SDS can be most effectively attained in the stationary phase, as is the case for the elimination of R factors. The W3747 F^+-*lac* strain was treated with or without 10% SDS (Fig. 3). As the F^+-*lac* culture grew and reached stationary phase, F^- cells then increased as a function of time of treatment with SDS in stationary phase.

Infection experiments of F or F′ factors to F^- cells obtained by the SDS treatment. To prove the loss of F or F′ factors from F^+ or F′ cells by SDS, cells derived from F^+ or F′ cells and characterized as F^- were mixed at appropriate concentrations with W6 F^+ cells or W4520 F-*gal* cells as donors of F or F′ factors, and frequencies of infection by F or F′ factors into these F^- cells were compared to those with KE61 and W4354 as F^- reference strains. It was found that F^- cells obtained by the SDS treatment could receive F or F′ factors at as high frequencies (90%) as could KE61 or W4354 (88%).

DISCUSSION

SDS is a reagent routinely used in the laboratory for the killing and lysis of cells. It was shown (Fig. 1), however, that in Penassay Broth *E. coli* K-12 W3110 as R^-F^- reference strain can grow exponentially even with 10% SDS, and the generation time of the cells in the broth with 10% SDS was found to be almost the same as that in the broth without SDS. However, the maximal cell titers in SDS were found to be lower than those without SDS. After the culture reached the stationary phase, cells began to lyse rather slowly. This was found to be the case with the R^+ and F^+ or F′ strains and W4520 F^+_8 (F-*gal*) (Table 8, Fig. 3). Drug-susceptible and F^- cells were found to be present among survivors after the culture reached the stationary phase. It generally appeared that the population of drug-susceptible cells and F^- cells among survivors increased according to the duration of incubation time. Repeating the SDS treatment, however, resulted in a lowering of the elimination frequency of R factors.

Results of reversion experiments with drug-susceptible cells, and of infection experiments with R factors or F or F-*gal* factors, suggested that cells obtained by the SDS treatment have lost R or F factors. A part or all of these genetic elements might be deleted by the action of SDS.

It has been reported (16, 19, 20) that the frequency of elimination of R factors by acridine dyes varies between *Escherichia* and *Shigella*, and is very much lower (up to several per cent) in *Escherichia* than in *Shigella* (up to 100%). This suggests that R factors in *Escherichia* are more resistant to the elimination action of acridine dyes than R factors in *Shigella*. The action of SDS on the R factor proved to be effective enough to eliminate R factors present in *Escherichia* at frequencies of up to 100%. F factors were also eliminated by SDS with high efficiency and at an earlier time during the SDS treatment than R factors.

That R factors in R^+ cells isolated from aged cultures can be eliminated by SDS at much higher

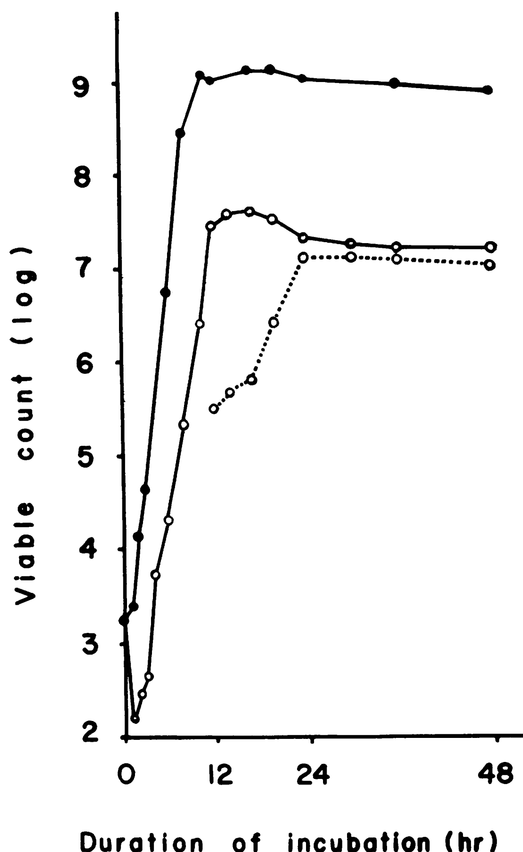

FIG. 3. *Kinetics of the SDS treatment of Escherichia coli W3747 F^{+}_{13} (F-lac). W3747 cells (1.7 × 10^3 cells/ml) in Penassay Broth (10 ml) with or without 10% SDS were incubated at 37 C with shaking. Symbols:* ●, *viable count without SDS;* ○, *viable count with SDS;* ○ *with dashed line, viable count of F^- cells formed during the SDS treatment.*

frequencies than R factors in R^+ cells isolated from fresh cultures, would be in accord with a similar earlier observation (18).

Action of acridine dyes on *E. coli* R^+ cells has been reported to lead to the complete loss of R factors (16, 19, 20). Action of SDS was now found to lead, besides producing drug-susceptible cells which lost all resistance markers, to the segregation of resistance markers (Table 7) in *E. coli*. The majority of segregants were those which showed sensitivity only to tetracycline; however, segregants of other types were also formed. When R factors were spontaneously eliminated from R^+ cells isolated from aged cultures, the major segregant cell type was *tet*^{s}. The segregation patterns obtained by the SDS treatment were found to be quite different from those observed with *Shigella* (19), in which the *str*^{s}*chm*^{s}*tet*^{s} segregants prevailed, and those observed with *Salmonella* (21), in which *str*^{s}-*chm*^{s}*sul*^{s} segregants were formed. In the meantime, *tet*^{s} cells obtained by the SDS treatment were found to have lost the transfer locus as well. This would support the earlier suggestion (4, 5, 15, 18) that TC and *t-m* regions are closely linked.

With respect to possible mechanisms of action of SDS toward the R and F factors, leading to their elimination, there is still no conclusive explanation. However, as shown in the reconstruction experiments (Fig. 2), in SDS-Penassay Broth the maximal growth of R^+ cells was lower than that of R^- cells. After the culture reached the stationary phase, the death rate of R^+ cells was found to be greater than that of R^- cells. The difference in death rates of R^+ and R^- cells was greater especially during the early stationary phase. It was also proved that when R^- cells, preincubated in SDS-Penassay Broth for 10 hr, were added to the R^+ culture incubated in SDS-Penassay Broth for the same period, R^- cells began to grow exponentially, whereas R^+ cells in the culture began to die. A similar result was obtained with SDS treatment of W3747 F^{+}_{13} (F-*lac*) (Fig. 3). Namely, F^- mutants which appeared in the survivors at the early stationary phase grew exponentially, whereas F^+ cells began to die gradually. These data would lead to the conclusion that the action of SDS toward R^+ or F^+ cells, yielding drug-susceptible or F^- cells, is a selection between R^+ or F^+ and R^- or F^-; i.e., SDS is more toxic for R^+ or F^+ than R^- or F^-. The experiment in which repetition of the SDS treatment of R^+ cells resulted in a more rapid growth of drug-susceptible cells than R^+ cells after treatment with SDS provides further support for the selection mechanism. This evidence might suggest that a locus of R^+ cells associated with sensitivity to SDS was eliminated, together with drug-resistance markers, by SDS, and that drug-susceptible cells thus formed became resistant to SDS.

The question of how and when the first drug-susceptible or F^- cell was formed during the SDS treatment has not been completely answered yet. There may of course be two ways, i.e., spontaneous elimination or elimination induced by SDS. As shown in Tables 2 and 3, elimination of R factors occurred consistently when 10^2 to 10^9 R^+ cells/ml were inoculated. These results could be accounted for by either or both of the two methods of elimination mentioned above, as the spontaneous elimination frequency of R factors was suggested to be rather high (10^{-5}; H. Hashimoto, *personal communications*). Meanwhile, experiments concerning the effect of SDS

on R^+ cells in a simple phosphate buffer without any carbon and nitrogen source failed to give any positive evidence for the elimination of R^+ induced by SDS; no drug-susceptible cell was formed. However, the failure to find susceptible cells among survivors in the phosphate buffer might be due to a successive death of susceptible cells, if once formed, in such a severe no-growth condition.

It has been revealed (10) that the bacterial chromosome and F factors as replicons are associated directly with a segment of bacterial membrane. An anionic detergent such as SDS may destroy the cell wall first and then the cell membrane, completely or partially, which would further result in the lysis of bacteria. R or F factors associated more closely with the cell membrane as smaller replicons than the chromosome would then be damaged more easily by SDS. The damage would be fatal, to the extent that they might be partially or wholly lost. Some sort of repair may then follow, and cells with R or F factors partially or completely eliminated might survive in the culture. That the *tet*^{s} cells were the major segregant obtained from the SDS treatment would suggest that the R factor may be associated with the cell membrane at a point around the *tet-m(t)* region. If a part of the chromosome was deleted by the action of SDS, it might be fatal.

It has been reported (23) that SDS treatment of R^+ cells led to temporary loss of ability of R transfer to R^- cells, and that the transfer ability was recovered when R^+ cells were transferred into a fresh culture without SDS. This might be due to loss or lysis by SDS of pili responsible for the R transfer. This information, coupled with our observations, might lead to speculation that R and F factors are located very close to the pili responsible for their conjugation, that SDS first causes lysis of the pili as the weakest structure on the cell wall, and that it leads to damage of R and F factors and to their partial or complete loss. Further experiments must be conducted before any conclusive explanation of the mechanism of action of SDS can be given.

ACKNOWLEDGMENTS

We are greatly indebted to J. Tomizawa, Y. Hirota, and Y. Sugino of the Faculty of Science, University of Osaka, for valuable discussions, and to T. Kiba of the Faculty of Science, Kanazawa University, for the spectroscopic analysis of sodium dodecyl sulfate. Thanks are also due to Drs. Hirota and Sugino for supplying bacterial strains and phage.

LITERATURE CITED

1. ADELBERG, E. A., AND S. N. BURNS. 1960. Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. **79**:321–330.
2. CLOWES, R. C., E. E. M. MOODY, AND R. H. PRITCHARD. 1965. The elimination of extrachromosomal elements in thymineless strains of *Escherichia coli* K-12. Genet. Res. **6**:147–152.
3. EGAWA, R., AND Y. HIROTA. 1962. Inhibition of fertility by resistance factor (R) in *Escherichia coli* K-12. Japan. J. Genetics **37**:66–69.
4. HASHIMOTO, H., AND Y. HIROTA. 1966. Gene recombination and segregation of resistance factor R in *Escherichia coli*. J. Bacteriol. **91**:51–56.
5. HASHIMOTO, H., AND S. MITSUHASHI. 1966. Drug resistance of enteric bacteria. VII. Recombination of R factors with tetracycline-sensitive mutants. J. Bacteriol. **92**:1351–1356.
6. HIROTA, Y. 1956. Artificial elimination of the F factor in *Bact. coli* K-12. Nature **178**:92.
7. HIROTA, Y. 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **46**:57–64.
8. HIROTA, Y., AND P. H. A. SNEATH. 1961. F′ and F mediated transduction in *Escherichia coli* K-12. Japan. J. Genet. **36**:307–318.
9. HOFSCHNEIDER, P. H. 1963. Untersuchungen über "kleine" *Escherichia coli* K-12 Bacteriophagen. Z. Naturforsch. **18b**:203–210.
10. JACOB, F., A. RYTER, AND F. CUZIN. 1965. On the association between DNA and membrane in bacteria. Proc. Roy. Soc. (London) Ser. B **164**:267–277.
11. KAHN, P., AND D. R. HELINSKI. 1964. Relationship between colicinogenic factors EI and V and an F factor in *Escherichia coli*. J. Bacteriol. **88**:1573–1579.
12. KAWAKAMI, M., AND O. E. LANDMAN. 1965. Experiments concerning the curing and intracellular site of episomes. Biochem. Biophys. Res. Commun. **18**:716–724.
13. LEDERBERG, J. 1950. Isolation and characterization of biochemical mutants of bacteria. Methods Med. Res. **3**:5–22.
14. LEDERBERG, J., L. L. CAVALLI-SFORZA, AND E. M. LEDERBERG. 1952. Sex compatibility in *Escherichia coli*. Genetics **37**:720–730.
15. LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
16. MITSUHASHI, S., K. HARADA, AND M. KAMEDA. 1961. Elimination of transmissible drug-resistance by treatment with acriflavine. Nature **189**:947.
17. SUGINO, Y., AND Y. HIROTA. 1962. Conjugal fertility associated with resistance factor R in *Escherichia coli*. J. Bacteriol. **84**:902–910.
18. WATANABE, T. 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol. Rev. **27**:87–115.

19. WATANABE, T., AND T. FUKASAWA. 1960. Episomic resistance factors in *Enterobacteriaceae*. III. Elimination of resistance factors by treatment with acridine dyes. Med. Biol. (Tokyo) **56**:71–74.
20. WATANABE, T., AND T. FUKASAWA. 1961. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. II. Elimination of resistance factors with acridine dyes. J. Bacteriol. **81**:679–683.
21. WATANABE, T., AND K. W. KYANG. 1962. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. V. Spontaneous segregation and recombination of resistance factors in *Salmonella typhimurium*. J. Bacteriol. **84**:422–430.
22. WILLETTS, N. S. 1967. The elimination of F*lac*$^+$ from *Escherichia coli* by mutagenic agents. Biochem. Biophys. Res. Commun. **27**:112–117.
23. YOKOTA, K., AND T. AKIBA. 1961. Studies on the mechanism of transfer of drug-resistance in bacteria. VII. Inhibition of transfer of the resistance-factor from *E. coli* to *Shig. flexneri* by some chemicals. Med. Biol. (Tokyo) **58**:188–191.