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Specific Action of Sodium Dodecyl Sulfate on the Sex Factor of *Escherichia coli* K-12 Hfr Strains

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A specific action of sodium dodecyl sulfate (SDS) on the sex (F) factor in the integrated state of *Escherichia coli* K-12 Hfr H strain is reported. Growth of Hfr cells in Penassay Broth containing SDS results in the elimination of part or all of the F factor, yielding low and nonfertile variants of defective Hfr type and F^+ cells and also F^- derivatives. Appearance of such variants was generally observed after the culture reached stationary phase. The frequencies of F^- cells then increased. F^- cells were usually isolated as the major population among survivors. Some defective variants of Hfr cells with an intermediate fertility between standard Hfr and F^+ cells had lost sensitivity toward the male-specific ribonucleic acid phage M12. Other defective Hfr variants with as much or less fertility than standard F^+ cells had also all lost sensitivity to phage M12. On single-colony isolation, they segregated nonfertile *female H* cells which, when infected with F, could restore high fertility with oriented transfer of the chromosome the same as that of the original Hfr H. Also, sensitivity to phage M12 was regained. *Female H* cells were characterized as those lacking fertility but still retaining a small segment of F or *sfa* locus at the original part of the chromosome, where newly infected F could attach. Similar results were obtained with two other Hfr strains. A possible mechanism of the specific action of SDS is discussed.

It is known that sex (F and F') factors in the autonomous state can be eliminated from host cells by treatment with cobalt or nickel ions (4), acridine dyes (1, 5, 6), or mutagenic agents (18). F and colicinogenic factors, harbored in thymineless strains, can be lost spontaneously under conditions of thymine-limited growth (2). Colicinogenic factor Col V can be eliminated by acridine dyes (9).

It is known also that multiple-drug-resistance (R) factors of *Escherichia coli* and *Shigella flexneri* can be eliminated spontaneously, an event accelerated by treatment with acridine dyes (14, 16, 17) or by 4-nitroquinoline 1-oxide and its analogues (*unpublished data*). R factors in *Salmonella paratyphi* can be eliminated by mild penicillin treatment (10).

We reported in a previous paper (15) that an anionic surface-activating agent, sodium dodecyl sulfate (SDS), eliminates with very high efficiency part or all of R and F factors existing in the autonomous state in *Escherichia*. A possible mechanism involved in the action of SDS was proposed as follows: SDS may first cause lysis of part of a surface structure of R^+ and F^+ cells, leading to damage of R and F factors located very close to the surface of bacteria (smaller replicons than the chromosome), and selective growth of drug-susceptible and F^- cells thus formed further favors the isolation of those cells.

This hypothesis prompted us to study the action of SDS on the F factor existing in the integrated chromosomal state of Hfr cells. Artificial elimination of the F factor in such an integrated state, for instance by acridine dyes, has not yet been successful (5).

In the present paper, we report on the specific action of SDS on the F factor of *E. coli* K-12 Hfr H, which results in the appearance of variants of several types which have lost part or all of the F factor. Similar results were obtained with *E. coli* Hfr 1 and Hfr 2 strains. The present paper deals with the action of SDS on three Hfr strains of *E. coli* K-12, genetic identification and characterization of survivors obtained by the SDS treatment, and the possible mechanism of the action of SDS.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. The bacterial strains used in this work were *E. coli* K-12 derivatives

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

F	Strain	Genotype[b]
Hfr H	JE1031	*thi⁻ met⁻*
Hfr H	KE120[c]	*lac⁻ thi⁻ met⁻*
Hfr 1	W1895	*met⁻*
Hfr 2	JE847	*met⁻*
F⁺	JE64	*thr⁻ leu⁻ lac⁻ thi⁻*
F⁻	W4573	*ara⁻₂ lac⁻₈₅ gal⁻₂ mal⁻₁ xyl⁻₂ mtl⁻ str^r*
F⁻	W2984	*pro⁻*
F⁻	W4354	*met⁻*
F⁻	JE346	*ara⁻ lac⁻₈₅ pur⁻ (ade, thi) gal⁻₂ try⁻ mal⁻ xyl⁻ mtl⁻ str^r T^r₆*
F⁻	JE2262	*pro⁻*
F⁻	KE118[d]	*thi⁻ met⁻*
F⁻	KE121[e]	*lac⁻ thi⁻ met⁻*

[a] Only relevant markers are shown.

[b] Genotype abbreviations for: nutritional markers (− dependence, + independence); threonine, *thr*; leucine, *leu*; proline, *pro*; purine, *pur*; tryptophan, *try*; thiamine, *thi*; methionine, *met*; fermentation markers (− nonfermentation, + fermentation); arabinose, *ara*; lactose, *lac*; galactose, *gal*; maltose, *mal*; xylose, *xyl*; mannitol, *mtl*. Drug-resistance marker (*r*, resistance): to streptomycin, *str^r*. Phage-resistance marker (*r*, resistance): to T_6, T^r_6.

[c] Derived from JE1031 Hfr H *lac⁺* by ultraviolet-induced mutation to nonfermentation to lactose.

[d] Isolated from the JE1031 Hfr H culture as M12 phage-resistant cells and characterized as F⁻.

[e] Isolated from the KE120 Hfr H *lac⁻* culture as M12 phage-resistant cells and characterized as F⁻.

mainly supplied by Y. Hirota, Osaka University. They are listed in Table 1.

The male-specific bacteriophage used was ribonucleic acid (RNA) phage M12 (7), and was supplied by Y. Hirota. This phage lyses male strains of *E. coli* K-12.

Materials and media. SDS was purchased from Tokyo Kasei Ind. (Tokyo). Penassay Broth (Difco) was used for SDS treatment and conjugation experiments. L broth (13) was used for the preparation of phage M12. Complete EMB-glucose-agar medium was used for scoring survivors after SDS treatment and for a cross-brush test to determine phage sensitivity. Synthetic Davis-glucose-agar medium and EM-glucose-agar medium (11) were used for characterization of auxotrophic mutants. Synthetic EM-sugar-agar media with necessary supplement were used for scoring of sugar fermentation and for the selection of prototrophic recombinant colonies.

The *p*H of SDS-Penassay Broth was adjusted to 7.6; the *p*H of other media was 7.0.

SDS treatment. An overnight culture of Hfr cells in Penassay Broth was diluted to about 10^3 cells/ml in broth containing 10% (w/v) SDS and shaken at 37 C. Survivors were plated on EMB-glucose-agar medium after appropriate dilution in saline. Colonies on the plate were then characterized for fertility by the recombination method and by a test for sensitivity to phage M12.

Recombination method. Exponential culture (about 5×10^8 cells/ml) of the recipient strain, W4573 F⁻, was streaked on EM-arabinose-agar medium and spotted with one loopful of the SDS-treated strain (about 5×10^8 cells/ml) being tested. After incubation at 37 C for 2 days, the presence of recombinants was scored to determine (12) whether the test culture was a donor.

For a quantitative analysis of fertility, overnight cultures (about 5×10^8 cells/ml) of strains to be tested as donor and of the recipient W4573 F⁻ were diluted 10^{-1}. The diluted cultures were incubated at 37 C with shaking to reach about 4×10^8 cells/ml. An 0.1-ml amount of the donor culture and 2 ml of the recipient culture were mixed and incubated at 37 C for 1 hr without shaking, diluted appropriately with saline, and plated on EM-agar supplemented with appropriate carbohydrates and dihydrostreptomycin sulfate (SM; 100 μg/ml). After incubation at 37 C for 2 days, *ara⁺ str^r*, *lac⁺ str^r*, and *gal⁺ str^r* recombinants grown on EM-arabinose, EM-lactose, and EM-galactose, respectively, were counted. Those recombinants were then randomly inoculated onto an EMB-glucose master plate. The colonies on the master plate were then replica-plated onto EMB-arabinose, EMB-lactose, EMB-galactose, and EMB-maltose supplemented with SM to determine the frequencies of inheritance of unselected *ara*, *lac*, *gal*, and *mal* markers, respectively.

When JE346 F⁻ was used as the recipient, *ara*, *lac*, and *try* loci were used as selected and unselected markers. Cultures to be tested, after mating and appropriate dilutions, were plated on EM-sugar-agar supplemented with tryptophan (20 μg/ml), adenine (20 μg/ml), thiamine hydrochloride (2 μg/ml), and SM (100 μg/ml). Recombinants *ara⁺ str^r*, *lac⁺ str^r*, and *try⁺ str^r*, which arose on EM-arabinose, EM-lactose, and EM-glucose, respectively, were counted. For the analysis of unselected markers, EMB-arabinose and EMB-lactose supplemented with SM, and EM-glucose supplemented with adenine, thiamine, and SM were used to score the *ara*, *lac*, and *try* markers, respectively.

Test for sensitivity to phage M12. Phage M12 lysates (about 10^9 phages/ml) in L broth were streaked on EMB-glucose and were cross-streaked with the culture (about 10^8 to 5×10^8 cells/ml) being tested. After incubation at 37 C overnight, the phage lysed the Hfr and F⁺ cultures but not the F⁻ culture.

Transfer of F factors. The F factors were transferred from appropriate F⁺ (donor) strains into F⁻ (recipient) strains by mixing their exponential culture (2×10^8 to 4×10^8 cells/ml) in a 20:1 ratio and incubating the mixed culture at 37 C overnight, unless otherwise stated.

Elimination of F factors by treatment with acridine orange. An overnight culture on Penassay Broth of the strain being tested was diluted to 10^3 cells/ml (in broth containing 20 to 30 μg of acridine orange per ml) and shaken at 37 C for 24 hr. Survivors were plated on EMB-glucose after appropriate dilutions

with saline. After incubation, the colonies grown on the plate were analyzed for fertility.

RESULTS

SDS treatment of Hfr strains: isolation of survivors affected by SDS. Strain JE1031 Hfr H *thi*$^-$ *met*$^-$ was incubated with 10% SDS-Penassay Broth at 37 C by inoculating 10^3 Hfr cells per ml. Survivors were analyzed at intervals for the presence or absence of fertility by the recombination method, and for sensitivity to the male-specific phage M12. Survivors were tentatively classified into five types by the analysis shown in Table 2. Fertility traits of survivors belonging to types I, II, III, and V were found to be stable after many generations. The fertility trait of survivors of type IV was, however, found to be unstable.

Representative results of SDS-treatment experiments are shown in Table 3. Viable counts, determined over a period of 72 hr, were generally found not to decrease below 10^7 cells/ml. Usually, as the time of incubation increased, frequencies of survivors of type I decreased down to 0%, whereas those of type V increased to 100%. Frequencies of survivors of types II, III, and IV varied according to the duration of incubation. The formation of auxotrophic or sugar fermentation mutants was not observed at a detectable frequency. Furthermore, mutation frequencies of T_1 and T_6 markers were not increased by treatment with SDS.

Similar results were obtained with strains W1895 Hfr 1 *met*$^-$ and JE847 Hfr 2 *met*$^-$.

Characterization of survivors. Characterization of survivors was carried out mainly with those derived from Hfr H.

Survivors of type V as F$^-$ cells. To determine whether nonfertile (m^-) survivors of this type showing resistance to phage M12 had lost the entire F factor, cells were subjected to F infection (incubation time, 1 hr) with donor strain JE64 F$^+$. The frequency of F infection of those cells was compared with that of reference strain W4354 F$^-$. Cells of this type could receive F factors at frequencies (~92%) similar to W4573 F$^-$ (94%; Table 4). The F infectants thus obtained were found to be F$^+$ and not Hfr males. Such strains, by conjugation with W2984 F$^-$, were found to carry an infectious F. Furthermore, they lost their fertility completely on treatment with acridine orange and, on mating with W4573 F$^-$, transferred their markers to the recipient at low frequencies typical of a standard F$^+$ strain, W6. It was therefore concluded that type V survivors behaved as F$^-$ strains.

Characterization of type V survivors derived from strains Hfr 1 and Hfr 2 indicated that they were also F$^-$.

Survivors of types II and IV as defective Hfr cells. Survivors of type II showing an intermediate fertility (m^+) between standard Hfr and F$^+$ cells were analyzed as follows. Cells of type IIa retaining sensitivity to phage M12 were crossed with JE346 F$^-$, and the frequencies of markers transferred from the donor to the recipient were determined. Observed frequency of recombinants selected for *ara*, *lac*, and *try* markers is shown in Table 5. The frequency of the *ara* marker (8.1×10^{-3}) was slightly lower than that of original Hfr H; however, those of *ara*, *lac*, and *try* markers showed a gradient of transfer similar to Hfr H. Examination of frequency of inheritance of unselected markers with each recombinant further supported the relative order of these markers. Cells of IIa type were also examined for infectious F factors. No transfer of F factors was observed to W2984 as F$^-$ recipient strain. Such evidence indicates that survivors of type II consist of Hfr cells, defective in that they are less fertile than standard Hfr cells and that in some instances they lost sensitivity to phage M12.

Survivors of type IV showed as much or less fertility (m^+) than standard F$^+$ cells, but had lost sensitivity to phage M12 and exhibited an unstable nature regarding fertility. On single-colony isolation, a large proportion (66%) of nonfertile (m^-) segregants was detected (recombination method). A clone retaining fertility after such isolation was again subjected to single-colony isolation. Of nonfertile clones, 46% were detected among the colonies. Upon further single-colony isolation in the same way, segregation of nonfertile clones was again observed, but less frequently (32%). The phenomenon of segregation

TABLE 2. *Classification of survivors isolated from the SDS-Penassay Broth of Hfr strains according to their fertility and sensitivity or resistance to the male-specific RNA phage M12*

Type of survivor	Fertility[a]	Sensitivity (*s*) or resistance (*r*) to phage M12
I	++++	*s*
IIa	+++	*s*
IIb	+++	*r*
III	++	*s*
IV	++ to +	*r*
V	−	*r*
Hfr H	++++	*s*
W6 F$^+$	++	*s*

[a] Fertility was classified as follows: ++++ and ++ represent as much fertility as Hfr and F$^+$ cells, respectively; +++ represents an intermediate between ++++ and ++; + represents less fertility than that of F$^+$ cells; − represents lack of fertility.

TABLE 3. *Elimination of F factors in Escherichia coli K-12 JE1031 Hfr H by treatment with sodium dodecyl sulfate: frequencies of survivors of various types*

Experiment no.	Inoculum size (cells/ml)	SDS concn (%)	Time of incubation (hr)	Viable count (cells/ml)	No. of colonies of various types formed/no. of colonies tested (frequency, %)[a]					
					Type I	Type IIa	Type IIb	Type III	Type IV	Type V
1	3.6×10^3	0	24	1.5×10^9	100/100 (100)					
			48	2.7×10^9	100/100 (100)					
			72	2.7×10^9	100/100 (100)					
		10	24	1.8×10^7	20/50 (40)		29/50 (58)			1/50 (2)
			48	4.8×10^7	0/50 (0)		6/50 (12)			44/50 (88)
			72	2.7×10^7	0/50 (0)		0/50 (0)			50/50 (100)
2	1.9×10^3	0	24	2.2×10^9	50/50 (100)					
			48	2.7×10^9	50/50 (100)					
			72	2.1×10^9	50/50 (100)					
		10	24	2.3×10^8	5/50 (10)			11/50 (22)	26/50 (52)	8/50 (16)
			48	7.2×10^7	4/50 (8)			7/50 (14)	38/50 (76)	1/50 (2)
			72	1.7×10^6	0/50 (0)			24/50 (48)	15/50 (30)	11/50 (22)
3	1.8×10^3	0	24	8.8×10^8	50/50 (100)					
			48	3.0×10^9	50/50 (100)					
			72	2.9×10^9	50/50 (100)					
		10	24	8.4×10^7	13/50 (26)	35/50 (70)	2/50 (4)			0/50 (0)
			48	2.0×10^8	0/50 (0)	45/50 (90)	1/50 (2)			4/50 (8)
			72	4.0×10^7	0/50 (0)	0/50 (0)	2/50 (4)			48/50 (96)

[a] See footnote *a*, Table 2, for fertility classification. Type I: ++++, M12-sensitive; type IIa: +++, M12-sensitive; type IIb: +++, M12-resistant; type III: ++, M12-sensitive; type IV: ++ to +, M12-resistant; type V: −, M12-resistant.

of nonfertile cells was observed repeatedly with other colonies of type IV. Those nonfertile cells were purified by repeated single-colony isolations and were tentatively named *female H* cells. Such strains were mated with the JE 64 F^+ donor, and infected recipients were analyzed. F infectants showing either an intermediate fertility between standard Hfr and F^+ cells or as much or less fertility than standard F^+ cells could be isolated at high frequencies (Table 6). F infectants with higher fertility had restored sensitivity to phage M12, whereas those with lower fertility still appeared resistant. The degree of fertility and sensitivity or resistance to the phage of F infectants were stable through many generations. Further characterization of F infectants was done as follows. Cells with higher fertility were crossed with W4573 F^-, and the frequencies of markers transferred from the donor to the recipient were determined. The frequency of the *ara* marker transferred ($\sim 6.6 \times 10^{-2}$) was almost as high as that of the Hfr H control experiment (Table 7). Furthermore, frequencies of *ara*, *lac*, and *gal* markers transferred showed a gradient, as was the case with Hfr H. The relative order of these markers was further supported by an examination of frequency of unselected markers for each recombinant. Furthermore, F infectants with either higher or lower fertility were found to lack an infectious F particle upon mating with JE2262 F^-, and did not lose their fertility on treatment with acridine orange. This evidence indicates that F infectants derived from *female H* cells can be characterized as defective Hfr H type with low fertility.

The conclusion that type IV survivors behave as defective Hfr cells was further supported by the fact that cells of this type, by conjugation with W2984 *pro*$^-$ F^-, were found to lack an infectious F particle, and that cells of this type, on mating with recipient strain W4573 F^-, transferred *ara*, *lac*, and *gal* markers at low frequencies similar to W6 F^+ (Table 5).

TABLE 4. *Frequencies of F infection of survivors (m^- met^-) of type V obtained by the SDS treatment with JE64 F^+ met^+ as the donor*[a]

Colony no. of type V tested	No. of m^+ colonies/ no. of met^- surviving colonies tested	Frequency of F infection (%)
1	30/50	60
2	39/50	78
3	40/50	80
4	35/50	70
5	46/50	92
6	33/50	66
W4354 F^-	47/50	94

[a] The original Hfr strain was Hfr H.

We also noticed that cells of type IV, when stored in the soft agar medium at room temperature for more than 3 months, lost the trait of segregating *female H* cells completely.

Survivors of type III as F^+ cells. Cells of this type showed as much fertility (m^+) as standard F^+ cells and sensitivity to phage M12. They were crossed with W4573 F^-, and frequencies of markers transferred from the donor to the recipient were examined. The frequencies of *ara*, *lac*, and *gal* transfer were as low as those with W6 F^+ in the control experiment (Table 5). No gradient of transfer was apparent. Cells of this type were also conjugated with a W2984 met^+ F^- recipient to determine whether they might have an infectious F. It was found that type III cells could transfer F factors to the recipient, though at frequencies ($\sim 70\%$) lower than JE64 F^+ (88%). A small proportion ($\sim 13\%$) of F infectants thus isolated was found to be resistant to phage M12. Further evidence for the autonomous state of F factors in cells of this type was gained from curing with acridine orange; all the survivors tested were

TABLE 5. *Frequencies of recombinants formed in cross between fertile (m^+) survivors of type I to IV, derived from Hfr H, and W4573 F^-*[a]

Type of m^+ survivors	Colony no.	Frequencies of transfer of genetic characters			
		ara$^+$	*lac*$^+$	*gal*$^+$	*try*$^+$
I	1	7.5×10^{-2}	2.0×10^{-2}	1.4×10^{-2}	5.7×10^{-4}
II	2	8.1×10^{-3}	2.0×10^{-3}		
III	3	1.4×10^{-5}	1.3×10^{-6}	6.8×10^{-6}	
IV	4	9.0×10^{-6}	7.0×10^{-7}	8.9×10^{-6}	
Hfr H		1.4×10^{-1}	3.3×10^{-2}	1.9×10^{-2}	
W6 F^+		2.8×10^{-4}	4.1×10^{-5}	2.5×10^{-5}	
W4573 F^-[b]		8.8×10^{-7}	$<4.0 \times 10^{-9}$	6.0×10^{-7}	

[a] The frequencies of recombinants were determined in samples taken after 1 hr of mating.
[b] Frequencies of revertants are shown.

TABLE 6. *Frequencies and characterization of F infectants formed in conjugation between female H cells as recipient and JE64 F+ as donor*

Colony no. of the recipient used	Frequency (%)[a] and characterization of F infectants isolated[b]			
	+++, s	++ to +, s	++ to +, r	(−)[c], (r)
1	2		84	14
2	96			4
3			54	46
4	2		64	34
5 [type V (F^-) cells]		98		2[d]

[a] Fifty colonies derived from the recipient were tested.

[b] See footnote *a*, Table 2, for fertility classification; M12-sensitive, s; M12-resistant, r.

[c] Nonfertile colonies showing resistance to phage M12 were characterized as *female H* cells unaffected by F infection.

[d] Type V (F^-) cells unaffected by F infection.

TABLE 7. *Frequencies of recombinants formed in cross between F infectants with intermediate fertility between those of standard Hfr and F+ cells, derived from female H cells by conjugation with F+, and W4573 F−*

Colony no. of F infectants tested	Frequencies of transfer of genetic characters[a]		
	ara+	*lac*+	*gal*+
1	4.3×10^{-2}	1.2×10^{-2}	8.4×10^{-3}
2	6.0×10^{-2}	1.1×10^{-2}	5.7×10^{-3}
3	6.6×10^{-2}	1.8×10^{-2}	7.4×10^{-3}
Hfr H	1.4×10^{-1}	3.3×10^{-2}	1.9×10^{-2}

[a] The frequencies of recombinants were determined in samples taken after 1 hr of mating.

found to be F^-. These results indicate that survivors of type III behave like F^+ males.

Survivors of type I as original Hfr H. Cells of this type, showing as much fertility (m^+) as original Hfr cells retaining sensitivity to phage M12, were crossed with a W4573 F^- recipient, and frequency of markers transferred from the donor to the recipient was determined. The frequency of transfer of the *ara* marker (7.5×10^{-2}) was almost as high as that of Hfr H in the control experiment (Table 5). Furthermore, the frequencies of *ara*, *lac*, and *gal* transfer showed a gradient. Examination of frequencies of unselected marker inheritance of each recombinant supported the relative order of those markers. Second, cells of this type were found to lack an infectious F in matings with a W2984 F^- recipient and were resistant to F elimination by acridine orange. Survivors of this type were therefore characterized as the original Hfr cells, being unaffected by the SDS treatment.

Kinetics of the SDS treatment. JE1031 Hfr H and its isogenic KE118 F^- were treated with 10% SDS in Penassay Broth at 37 C with shaking, and the survivors were analyzed at periodic intervals. Representative results are shown in Fig. 1. The generation time for these strains during the exponential phase and in the presence of SDS was only slightly longer than for control cultures devoid of SDS. However, the maximal cell density of SDS-treated cultures was lower than that of cultures without SDS. Cells began to lyse rather slowly, and the proportion of variants derived from Hfr cells increased. The frequency of survivors of type V or F^- was found to be 0 and 88% at 24 and 48 hr, respectively.

Reconstruction experiment between Hfr H and F− strains. To determine whether any selective toxicity of SDS on Hfr and F^- cells existed, reconstruction experiments dealing with the effect of SDS on the F factor of Hfr cells were carried out on JE1031 Hfr H (*lac*+) and KE121 F^- (*lac*−) strains. Overnight cultures (5×10^8 cells/ml) of Hfr and F^- strains were appropriately diluted in Penassay Broth with or without 10% SDS to give cell concentrations of about 10^3 cells/ml. Equal volumes of Hfr and F^- cultures were mixed and incubated at 37 C with shaking, and survivors were analyzed at intervals, with *lac* as the dis-

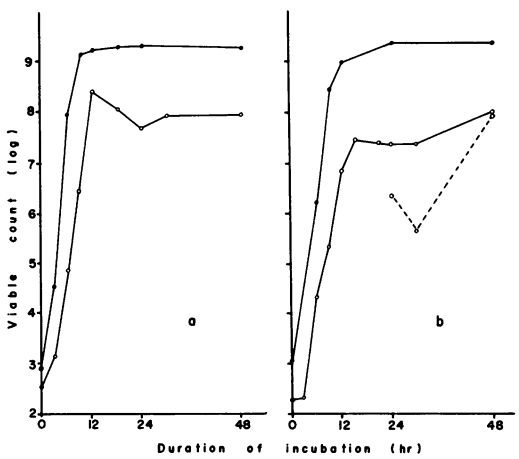

FIG. 1. *Kinetics of the SDS treatment of Escherichia coli KE118 F− (a) and JE1031 Hfr H (b). (a) KE118 cells (7.5×10^2 cells/ml) in Penassay Broth (10 ml) with or without 10% SDS were incubated at 37 C with shaking. Symbols: ●, viable count without SDS; ○, viable count with SDS. (b) JE1031 Hfr H cells (7.5×10^2 cells/ml) in Penassay Broth (10 ml) with or without 10% SDS were incubated at 37 C with shaking. Symbols as in (a); − −○− −, viable count of variants formed in the SDS medium.*

tinguishing marker. The frequency of production of variants from Hfr cells was also determined. Representative results are shown in Fig. 2. In SDS medium, the maximal number of Hfr cells was less than that of F^- cells and, after an SDS-treated culture reached the stationary phase of growth, the viable count of Hfr cells decreased far more rapidly than that of F^- cells. This did not occur in the medium without SDS. The proportion of variants (*lac*$^+$) of types II to V in the entire surviving population after 24, 30, and 48 hr was found to be 16, 34, and 83%, respectively. It was also observed that a small population (8%) of *lac*$^+$ cells isolated from the medium without SDS at both 24 and 48 hr behaved as F^- cells. They might be *lac*$^+$ F^- recombinants formed as the result of mating between Hfr *lac*$^+$ and F^- *lac*$^-$.

Another reconstruction experiment, carried out with KE120 Hfr H (*lac*$^-$) and KE118 F^- (*lac*$^+$), gave similar results.

DISCUSSION

It has been reported (15) that strains of *E. coli* K-12 can grow exponentially in 10% SDS-Penassay Broth and that the effect of SDS can lead to the loss of part or all of F, F′ and R factors in bacteria. We have now found by a kinetic study (Fig. 1) that JE1031 Hfr H cells can also grow exponentially in the SDS-broth, as did its isogenic F^-, KE118. After the culture reached a stationary growth phase, cells began to lyse slowly, the cultures retaining a viability of about 10^7 cells/ml as long as 72 hr. The appearance of variants of Hfr cells was observed after the culture reached the stationary phase, and their proportion among survivors increased rapidly, depending on the duration of incubation (*see* Table 3). Similar observations were found with two other Hfr strains, W1895 Hfr 1 and JE847 Hfr 2.

The relative proportion of survivors of type I to V varied as a function of time (Table 3). Usually, as incubation time went on, the proportion of survivors of type I (Hfr cells unaffected by SDS) decreased and that of type V (F^- cells which had lost F factors completely) increased. The proportion of survivors of intermediate types II to IV (low fertile variants) varied according to the Hfr strain used; Hfr H was found to give higher proportions of variants of these types than Hfr 1 and Hfr 2.

A genetic characterization of defective Hfr types classified as type II and IV seemed to be of interest and importance in connection with possible mechanisms involved in the action of SDS toward Hfr cells. They were found to lack infectious F particles and did not yield F^- clones on treatment with acridine orange. Variants of type

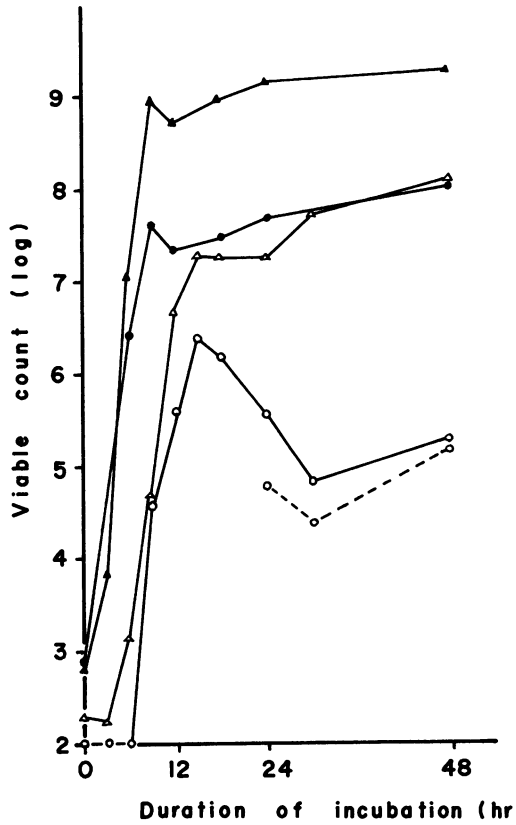

FIG. 2. *Reconstruction experiment between Escherichia coli JE1031 Hfr H (lac*$^+$*) and KE121 F*$^-$ *(lac*$^-$*). Overnight cultures (ca. 10*^{8} *cells/ml) of Hfr and F*$^-$ *strains were diluted 10*$^{-5}$ *in Penassay Broth with or without 10% SDS. Equal volumes of Hfr (7.5 × 10*^{2} *cells/ml) and F*$^-$ *(6.6 × 10*^{2} *cells/ml) cultures were mixed and incubated at 37 C with shaking. Plating with appropriate dilutions at certain intervals was on EMB-lactose-agar. One hundred lac*$^+$ *colonies were arbitrarily chosen and characterized by recombination method for presence or absence of fertility and by test for sensitivity to phage M12. Symbols: ●, viable count of lac*$^+$ *Hfr H plus lac*$^+$ *F*$^-$ *formed as the result of mating without SDS; ▲, viable count of lac*$^-$ *F*$^-$ *without SDS; ○, viable count of lac*$^+$ *Hfr H plus lac*$^+$ *F*$^-$ *and other variants; △, viable count of lac*$^-$ *F*$^-$ *with SDS; - -○- -, viable count of lac*$^+$ *F*$^-$ *and other types of variants formed in the SDS medium.*

II with an intermediate fertility between Hfr and F^+ cells still retained oriented transfer of chromosome the same as that of Hfr H. However, some of these variants had lost their sensitivity to the male-specific phage M12. Variants of type IV, with as much or less fertility than F^+ cells, had all lost sensitivity to phage M12. On repeated single-colony isolation, cells of type IV segregated nonfertile or *female H* cells retaining resistance to

phage M12 at high frequencies (~66%). When such *female H* cells were subjected to F infection, F infectants with as high a fertility as that of either type II or type IV were isolated. Those with high fertility showed oriented transfer of chromosome to F^- similar to that of the original Hfr H and had restored sensitivity to phage M12. F infectants with a lower fertility were resistant to male-specific phage and were found to lack free F particles. It appears, therefore, that F-infected *female H* cells behave as an Hfr type and not F^+ type of male.

These observations suggest that variants of type II and IV might have lost part of the F factor, still retaining defective F at the original site of the chromosome and showing sensitivity or resistance to phage M12 and immunity to F factors. These variants might, indeed, be similar to Hfr strains with a defective F isolated by Cuzin and Jacob (3). Variants of type IV also appear to be unstable, segregating *female H* cells which still retain a small segment of F, perhaps an *sfa* locus (1). After reinfection with F, *female H* cells had restored fertility and, in part, sensitivity to phage M12, presumably the result of recombination at the *sfa* locus between the F segment and newly infected F.

Hfr strains are known to be resistant to F elimination by acridine dyes (5). SDS may now be classified as an effective agent eliminating F from Hfr cells and producing other variants altered in their function of F.

With respect to possible mechanisms of the action of SDS on Hfr cells, leading to the formation of F^- as well as other defective variants, there is still no conclusive explanation. As shown in a reconstruction experiment (Fig. 2), however, maximal growth of Hfr cells was lower than that of F^- cells in SDS-Penassay Broth. After the culture reached stationary phase, the death rate of Hfr cells was found to be greater than that of F^- cells. The difference in death rates of Hfr and F^- cells was markedly greater during the early stationary phase. The proportion of F^- and other variants which appeared among the survivors during the early stationary phase increased as a function of time. The data lead to the conclusion that one action of SDS on Hfr cells is selective pressure; namely, SDS is more toxic for Hfr than F^-. The evidence suggests that a locus in Hfr cells associated with sensitivity to SDS is eliminated along with the fertility factor, and that F^- and other variants thus formed became resistant to SDS.

There still remains the question of how F^- and other variants were formed during the SDS treatment. Two mechanisms of elimination of F may be possible, namely, spontaneous elimination, perhaps via the intermediacy of F^+, or elimination induced by SDS. The fact that spontaneous F^- cells can be isolated from the Hfr H cultures as M12 phage-resistant colonies (*unpublished data*) would support the former possibility. On the other hand, the fact that SDS treatment of Hfr cells led to the isolation of variants of unusual Hfr type with a defective F supports the latter possibility, i.e., direct action of SDS on the F factor in Hfr cells. The F factor in bacterial chromosome of Hfr cells might be attached to the cytoplasmic membrane, in the vicinity of a surface antigen or F pili which it determines (8). Damage by SDS as an anionic detergent might be primarily lysis, if partial, of the surface structure of bacteria, and might have more effect on the F factor associated with the membrane than on the other site of the chromosome. The damage would be fatal to the extent that the F factor might be eliminated completely or in part. Some sort of repair mechanism might then follow, and cells with defective F or cells which had lost the entire F might be yielded. Further experiments on the mechanism in question are in progress.

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