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Mechanisms and Genetics of Resistance to Sodium Lauryl Sulfate in Strains of *Shigella* and *Escherichia coli*

L. M. CORWIN, SARA W. ROTHMAN,[1] ROSALIND KIM, AND L. A. TALEVI
Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts 02118

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The sensitivity to sodium lauryl sulfate (SLS) of *Shigella flexneri* and *Escherichia coli* is determined by at least three genes. One site is located near the lactose operon, and two loci are cotransducible with the arabinose operon. Calcium ions protect against SLS lysis. One gene is concerned with the relative ability of the bacterium to retain calcium against such chelating agents as ethylenediaminetetraacetic acid or phosphate buffer. This was first observed in a mutation from virulence to avirulence in *S. flexneri* with a concomitant loss of ability to penetrate the intestinal epithelium. The avirulent strain is far less sensitive to lysis by SLS in the presence of phosphate buffer than its virulent parent. The avirulent strain is also less sensitive to lysozyme and ethylenediaminetetraacetic acid. *E. coli* K-12 is much more sensitive to SLS than both of these *Shigella* strains. *An E. coli-S. flexneri* hybrid, which is unable to survive well in the gut and thus only produces an abortive infection, has inherited this extreme sensitivity to SLS.

It has been known for some time that one can obtain spontaneous, avirulent mutants of *Shigella flexneri* which are unable to penetrate into the intestinal epithelium (2). One such strain is a mutant of virulent *S. flexneri* 2a, strain M-42-43. This mutant (24570), obtained from S. Formal, is altered in several physical and biochemical characteristics from its virulent parent. It was isolated as a colonial variant, which compared to its parent was relatively opaque to oblique-light when grown on nutrient agar (2). The mutant is unable to utilize glycerol as a sole carbon source and is poorly able to oxidize succinate, fumarate, and malate. A major difference between the two strains is their relative resistance to the anionic detergent, sodium lauryl sulfate (SLS). The avirulent mutant is relatively insensitive to SLS. Nakamura demonstrated a gene governing sensitivity to SLS near the lactose (lac) operon in *Escherichia coli* (3). This was determined by the restoration of SLS resistance to a sensitive *lac*$^-$ mutant by mating with Hfr C, strain W-1895, and selecting for lactose utilization. W-1895 is itself sensitive to SLS. This implies that more than one gene may govern SLS sensitivity. This paper will present data concerning the chromosomal location of two genes governing sensitivity to SLS near the arabinose operon as well as a study of conditions which control this sensitivity.

[1] Present address: Department of Biochemistry, Tufts University School of Medicine, Boston, Mass.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains employed in this study and their characteristics are listed in Table 1.

Media. For reasons to be described later, Luria broth (LB) was used for most routine cultivation of bacteria to be tested for their sensitivity to SLS. Per liter of broth, it consists of 10 g of tryptone, 5 g of yeast extract, 2 g of glucose, and, depending on the experiment, $CaCl_2$ was added at a concentration of 2.5 mM. On occasion, Brain Heart Infusion (BHI) broth was used in place of LB. Minimal glucose media contained, per liter of distilled water: 10.5 g of K_2HPO_4, 4.5 g of KH_2PO_4, 0.05 g of $MgCl_2$, and 1 g of $(NH_4)_2SO_4$. Agar plates had, in addition, 15 g of agar per liter. The *S. flexneri* strains require in addition 20 μg of aspartic acid per ml and 5 μg of nicotinic acid per ml for growth in minimal medium.

Conjugation experiments. Crosses between donor *E. coli* Hfr C strain W-1895 and *S. flexneri* 2a strains M-42-43 and 24570 were performed in the following way. The male and female strains were grown to log phase in Penassay Broth to an optical density (OD) at 625 nm of 0.250 to 0.300. The male was then diluted 1:10 in broth, and 5 ml of the dilution was mixed with 5 ml of the undiluted recipient in a 125-ml flask. The mating mixture was incubated with very gentle shaking in a 37 C water bath. For interrupted mating, a 0.2-ml sample was diluted 1:20 in minimal salts, after which the culture was placed in a Vortex mixer at maximum

TABLE 1. *Genetic characteristics of bacterial strains*

Organism[a]	Auxotrophic characters			Utilization of					
	Met[b]	Nic	Asp	lac	ara	rha	xyl	mal	fuc
Shigella flexneri 2a									
M-42-43	+	−	−	−	−	−	−	−	−
24570	+	−	−	−	−	−	−	−	−
X-16 (hybrid)	+	−	−	−	+	+	+	+	+
No. 8 (hybrid)	+	−	−	+	+	ND	ND	ND	ND
Escherichia coli									
W-1895 (Hfr C)[c]	−	+	+	+	+	+	+	+	+

[a] Except for no. 8 hybrid, strains were obtained from S. Formal and L. Baron, Walter Reed Army Research Institute, Washington, D.C.

[b] Abbreviations used: Met = methionine; Nic = nicotinic acid; Asp = aspartic acid, lac = lactose; ara = arabinose; rha = rhamnose; xyl = xylose; mal = maltose; fuc = fucose; ND = not determined.

[c] The order of genes on Hfr C are ← purE (1) *lac* (5) *ara* (15) *rha* (30) *xyl* (35) *mal* (40) (minutes from origin).

speed for 60 sec followed by the addition of 0.1 ml of phage T6 (10^9 plaque forming units/ml), and the culture was then incubated for 10 min. Phage T6 was used when the recipient *Shigella* strains were T6-resistant. After suitable dilution, selection was made on enriched (5 ml of nutrient broth per 400 ml of selective medium) minimal agar plates supplemented with 0.5% of the selective sugar.

Transduction experiments. Transductions were carried out essentially by the methods of Gross and Englesberg (1) by using a 1:1 ratio of phage P1 vir to recipient bacteria. Recipient bacteria were grown to log phase in 10 ml of LB. A 0.5-ml amount of bacterial suspension, 0.5 ml of phage lysate prepared on donor bacteria by the confluent lysis technique, and 0.5 ml of a Mg-Ca solution (0.01 M $MgCl_2$ and 0.005 M $CaCl_2$) were mixed and incubated at 37 C for 20 min. Suitable dilutions were made, and samples were plated on selective media, which were minimally enriched. Proper bacterial and phage controls were always included.

Lysis by SLS. Bacteria were grown overnight in LB containing glucose and calcium. The cells were diluted 1:10 in LB, grown to log phase, and then centrifuged at 2,000 × *g* for 10 min. The pellet was resuspended in either 0.1 M phosphate buffer or 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer, both at *p*H 7.5, and adjusted to an OD at 625 nm to between 0.6 and 1.0. One volume of 2.5% SLS was added to nine volumes of suspended cells, and the decrease in OD was followed in a Gilford recording spectrophotometer at 37 C. For routine testing of many strains, it was found that studying lysis at 5 and 10 min in a Bausch & Lomb Spectronic 20 spectrophotometer was adequate to distinguish the different "lysis" types. Lysis was usually expressed as the percentage decrease in OD at 625 nm in 10 min except when more detailed kinetics were examined in the recording spectrophotometer. As will be seen in the next section, it was necessary to follow this protocol exactly to duplicate results.

For reasons not entirely clear, on occasion the rates of lysis of all strains seemed to increase when using the routine assay with the Bausch & Lomb spectrophotometer. This was ascertained by routinely including the M-42-43 and 24570 *Shigella* strains in the group to be assayed. If these two strains had elevated lysis rates, assays were repeated in the recording spectrophotometer. Very often this increase in lysis was seen to be due to a rapid initial drop in OD of 10 to 15% followed by the usual pattern of lysis characteristic for known strains. In testing the lysis characteristics of recombinants from matings or transductions, each organism was tested on three separate occasions. Any recombinants whose lysis characteristics could not be reproduced were not included. In experiments with strains whose lysis class could not be determined, it was found that often the type of lysis exhibited by the recipient parent strain was regained, indicating an instability of the recombinant.

Viability measurements. The effect of SLS on the survival of the virulent M-42-43 and avirulent 24570 *Shigella* strains was determined by incubation of the bacteria in the same manner as the SLS-lysis studies. The concentration of SLS was varied from 0.1 to 1.0%. After incubation for 20 min, the mixtures were suitably diluted for a plate count on BHI agar. The counts were compared to a control culture not treated with SLS but otherwise similarly treated.

Lysis by lysozyme and EDTA. Egg white lysozyme (0.1 ml) was added to 0.9 ml of a suspension of organisms which had been washed in 0.05 M Tris buffer (*p*H 7.5) and resuspended in the same buffer, yielding a final concentration of 25 μg of the enzyme per ml with an activity of 35 units/μg. Ethylenediaminetetraacetic acid (EDTA) was added next in a 0.1-ml volume, final concentration being 0.1 mM. The OD was measured at the start and after each addition, and lysis was measured as change in OD (△OD) 625 nm in the Gilford recording spectrophotometer at 37 C.

RESULTS

Conditions affecting susceptibility to SLS. There are several factors which affect lysis of bacterial cells by SLS (Table 2). The most obvious is the

TABLE 2. *Effect of growth and assay media on susceptibility[a] to sodium lauryl sulfate*

Growth medium	Assay buffer	Lysis rate of organism[b]		
		X-16	M-42-43	24570
BHI[c]	Tris[d]	3	2	1
LB + Ca^{2+}	Tris	8	2	1
LB − Ca^{2+}	Tris	10	2	1
BHI	0.1 M Phosphate	690	68	34
LB + Ca^{2+}	0.1 M Phosphate	50	28	7
LB − Ca^{2+}	0.1 M Phosphate	225	85	30
LB + Ca^{2+}	0.01 M Phosphate	5	0	0

[a] Lysis experiments performed as in the text. Rates determined from maximum slopes obtained from recording spectrophotometer.

[b] Values are expressed as 1,000 × ΔOD_{625nm} per min.

[c] BHI = Brain Heart Infusion broth; LB = Luria broth.

[d] Buffers are 0.05 M tris(hydroxymethyl) aminomethane (Tris), *p*H 7.5, and 0.1 M phosphate buffer, *p*H 7.5. Initial OD of samples varied from 0.956 to 1.105.

effect of the assay buffer. When SLS is added to cells suspended in Tris buffer, there is almost no lysis when compared to that seen in 0.1 M phosphate buffer. Lowering the phosphate buffer concentration to 0.01 M also considerably decreases the lytic effect of SLS. The second factor controlling lysis is the medium in which the bacteria are grown. In all strains, lysis in phosphate buffer is greater when the bacteria have been grown in media without added calcium. (LB-Ca and BHI). Calcium, apparently, has a marked inhibitory effect on lysis by SLS. Not shown in Table 2 is the effect of washing the cells several times in buffer, such as Tris buffer containing calcium ions. The washing of cells greatly increases their susceptibility to SLS as though some factor other than calcium ions is being removed which is required to prevent lysis. Finally, there is a genetic effect. An *E. coli-Shigella flexneri* 2a hybrid strain, X-16, is much more susceptible to SLS under the conditions studied here than the avirulent mutant and its virulent parent. It shall be established later that this is a general characteristic of the K-12 strain of *E. coli*. The avirulent *Shigella* mutant (24570) is quite stable to SLS lysis when compared to its virulent parent, M-42-43. This is most dramatically seen with cells grown in LB plus Ca. In many experiments, 24570 does not lyse at all for the first 10 to 20 min. In the rest of the experiments presented in this paper, lysis experiments were in LB plus Ca.

To follow the design of our experiments, it will be useful to interpret these data mechanistically within the framework of the following working hypothesis. Calcium ions prevent lysis by SLS presumably by being bound to a susceptible site and thus protecting it. Phosphate can apparently compete successfully for the calcium ions and thus make available the SLS-sensitive sites. Tris buffer, however, having no special affinity for calcium, cannot do this. One might assume that media without added calcium still contain enough divalent cations to bind to SLS-sensitive sites and protect against SLS when there is nothing in the assay medium to compete for the cations (i.e., in Tris buffer). Supporting evidence is available from studies of lysis with EDTA in Tris. Whereas neither SLS nor EDTA produces lysis of the virulent or avirulent *Shigella* strains in Tris buffer, the combination of the two results in rapid lysis of both strains. The difference between the virulent and avirulent bacteria might then be explained in terms of relative divalent cation-binding ability. If the avirulent mutant has increased cation-binding potential, possibly by a localized increased negative charge, it would be expected to retain the cations against the stress of the phosphate medium to a much greater degree than the virulent strain if the latter had a lesser negative charge. It has been found that with organisms grown under the conditions used to study SLS lysis, such a charge difference (as determined by microelectrophoresis) between virulent and avirulent strains does exist (Corwin and Talevi, *unpublished data*). Although the finding of the charge difference provides an explanation, it should not be necessary to demonstrate a charge difference in the whole cells to have localized calcium-binding sites. This point will have more relevance when we consider SLS lysis of *E. coli* K-12, which has a higher negative charge than any *Shigella* organism yet studied, possibly due to the piliation of *E. coli*.

Kinetics of SLS lysis and relation to survival. Kinetic studies of lysis by SLS in phosphate buffer are shown in Fig. 1. Three degrees of lysis are discernible from the graph derived from data obtained from a recording spectrophotometer and expressed as per cent lysis. Whereas avirulent strain 24570 shows an OD decrease of less than 10% over the 7-min time period, the virulent M-42-43 decreased almost three times as much. The strains W-1895 and X-16, which are an *E. coli* and *E. coli-Shigella* hybrid, respectively, exhibit a pattern of susceptibility to lysis by SLS which is an order of magnitude different from the *Shigella* strains.

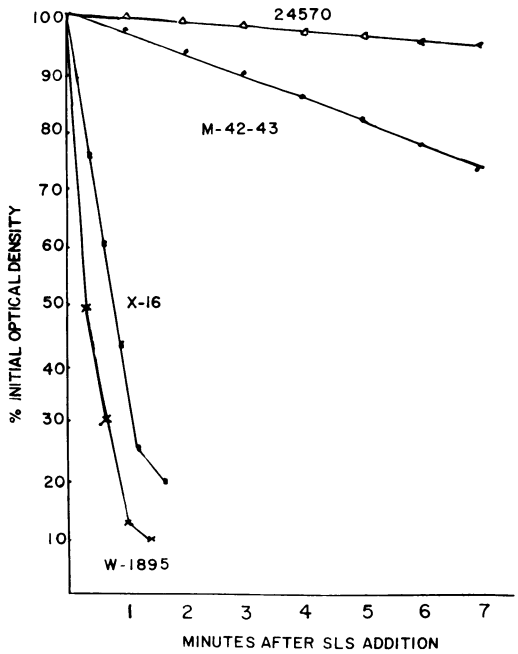

FIG. 1. *Rates of lysis of indicated strains by 0.25% sodium lauryl sulfate (SLS) as measured by the per cent loss of initial optical density at 625 nm. Initial optical density was between 0.9 and 1.0.*

The difference in lytic rates between the virulent and avirulent strains of *Shigella* can be correlated with their survival in SLS (Fig. 2). Up to a level of 0.25% SLS, there is at least a 10-fold increase in survival of the 24570 over its virulent parent M-42-43, although a certain percentage of both populations of bacteria are resistant to the lethal effect of SLS.

Lysis induced by lysozyme and EDTA. Gram-negative organisms are not lysed by lysozyme alone but must also be treated with EDTA for the enzyme to reach the glycopeptide layer and split the $\beta(1 \rightarrow 4)$ glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine (4). This requirement of EDTA to sensitize the cells probably involves the removal of stabilizing metal ions. Thus, some experiments were performed to see whether the resistance characteristics of the avirulent *S. flexneri* 24570 would be evident in this system (Table 3). Lysozyme and EDTA individually will not lyse these cells. However, when lysozyme and EDTA were added in that order, the 24570 was more resistant than its virulent parent, M-42-43, just as 24570 is also more resistant to SLS lysis. Another interesting result was obtained with the *E. coli* and *E. coli-Shigella* hybrid strains. The sensitivity to lysis by SLS was not seen with the lysozyme-EDTA system. Thus, *E. coli* sensitivity to SLS is probably not related to the relative calcium-binding since EDTA would have removed the calcium as effectively as the phosphate buffer.

Identification of genetic loci controlling SLS sensitivity. Nakamura has shown that there is a gene close to the lactose (*lac*) operon, which governs sensitivity to SLS (3). Specifically, Hfr C (strain W-1895) was mated with a *lac*-negative *E. coli* strain sensitive to SLS and yielded *lac*-positive recombinants, which were resistant to

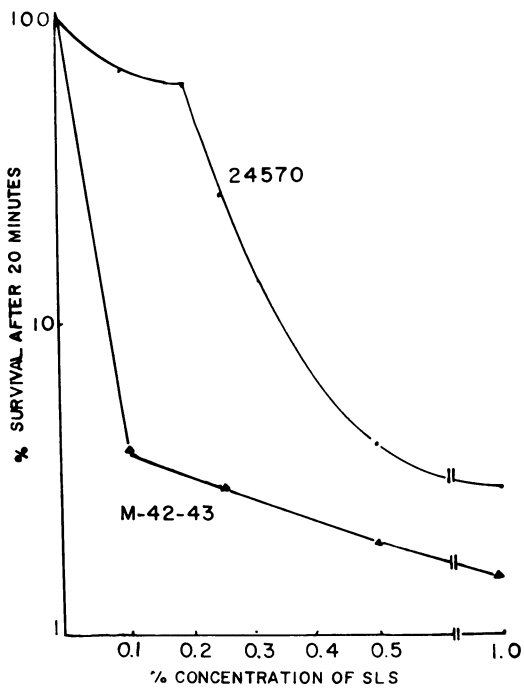

FIG. 2. *Relative survival of Shigella flexneri strains M-42-43 and 24570 versus increasing concentrations of sodium lauryl sulfate (SLS). The incubations with SLS were for a 20-min period.*

TABLE 3. *Lysis induced by lysozyme and EDTA*[a]

Organism	Lysis rate[b]
Shigella flexneri 24570	3
S. flexneri M-42-43	46
X-16 hybrid	32
Escherichia coli W-1895	32

[a] Experiment was performed in 0.05 M tris-(hydroxymethyl)aminomethane buffer, *p*H 7.5. The final concentration of lysozyme was 25 μg/ml and of ethylenediaminetetraacetic acid (EDTA) was 0.1 mM, which were added in that order.

[b] Values are expressed as 1,000 × ΔOD_{625nm} per min.

TABLE 4. *Sensitivity to sodium lauryl sulfate of selected recombinants and parents*

Strain	Per cent lysis/10 min	Lysis class[a]
W-1895, Hfr C *Escherichia coli*	50–75	S
M-42-43-*Shigella* (virulent)	20–40	I
24570-*Shigella* (avirulent)	0–19	R
Mating-W-1895 × M-42-43[b]		
lac selection 1	82	S
2	24	I
3	4	R
ara selection 1	75	S
2	21	I
3	0	R

[a] Lysis classes: S = sensitive; I = intermediate; R = resistant.

[b] Recombinants of the mating are typical examples from each lysis class.

TABLE 5. *Sodium lauryl sulfate sensitivity of recombinants of matings between W-1895 and Shigella strains*

Mating[a]	Selection	Lysis character		
		Sensitive	Resistant	Intermediate
W-1895 × M-42-43	lac (32)	34[b]	41	25
	ara (40)	58	17	25
W-1895 × 24570	lac (42)	36	50	14
	ara (59)	50	22	23
W-1895 × M-42-43 T6[r]	ara (118)	66	9	25

[a] Mating was carried out for 45 min with selection made either for lac or ara recombinants. Selected recombinants were then tested for sensitivity to sodium lauryl sulfate. Numbers in parentheses indicate number of recombinants tested in each cross.

[b] Per cent of recombinants.

SLS. A genetic analysis indicated close linkage between the two markers. When our attention was drawn to this work, we were surprised, since in our laboratory W-1895 was found to be very sensitive to SLS (Fig. 1 and Table 4). We performed a similar mating, this time with the virulent *Shigella* M-42-43 as the acceptor for W-1895 chromosomal material. When fermentation of either lactose or arabinose was used for selection, it was possible to find recombinants representative of all three lytic reaction classes as depicted in Table 4: sensitive, intermediate, and resistant. As will be seen later, the ratios of lytic classes obtained varied, depending on the sugar fermentation selected in the mating. Nevertheless, W-1895 is capable of converting the intermediate M-42-43 into either the sensitive or resistant type. Thus W-1895 contains a lac-linked resistance gene, which is recessive to one or more other genes conferring sensitivity. The remaining experiments indicate that there are two such genes close to the arabinose (*ara*) operon.

Recombination analysis of conjugation experiments involving the donor *E. coli* W-1895 and the two *Shigella* strains is presented in Table 5. When *lac* is the selected marker in a 45-min mating experiment, both crosses result in a slight preponderance of resistant recombinants. However, *ara* selection quite clearly indicates some linkage with a gene or genes conferring sensitivity from the *E. coli* donor. The large majority of the intermediate class M-42-43 was converted to sensitive recombinants. The avirulent 24570 was converted from resistant to both intermediate and sensitive strains by selection for arabinose fermentation. This conversion from the 24570 is the first evidence for two genes regulating SLS-resistance near the arabinose operon since recombinants from two different lysis classes were obtained.

Since the 45-min mating can easily allow for the inclusion of the *ara* region in the recombinants, the interpretation of the *lac* selection data may have been confused by various combinations of SLS sensitivity genes. Thus, an interrupted mating was performed by using a phage T6-resistant strain of M-42-43 as a recipient (Table 6). From this experiment, it is much clearer that the resistance gene from W-1895 enters the *Shigella* early, confirming Nakamura's finding that it is indeed near the *lac* operon (3). As the mating proceeds however, the number of resist-

TABLE 6. *Sodium lauryl sulfate sensitivity of lac-selected recombinants from interrupted mating[a] between W-1895 and M-42-43 T6[r]*

Time (min)	No. of recombinants	Lysis character		
		Sensitive	Resistant	Intermediate
5	20	25[b]	55	20
10	9	22	44	33
15	15	53	20	27
20	19	44	11	37
25	20	75	0	25
30	19	42	0	58

[a] Mating was interrupted by dilution and treatment with T6 phage as in the text.

[b] Per cent of recombinants.

TABLE 7. *Sodium lauryl sulfate (SLS) sensitivity of arabinose-positive recombinants from transduction into Shigella strains*

Donor	Recipient	No. of recombinants	Lysis character		
			Sensitive	Resistant	Intermediate
Hybrid (No. 8)[a]	M-42-43	49	61[b]	0	39
Hybrid (No. 8)	24570	65	43	12	45
W-1895	M-42-43	10	40	10	50

[a] Hybrid is an SLS-sensitive strain resulting from a cross between W-1895 and M-42-43. It is lac- and ara-positive and SLS-sensitive.

[b] Per cent of recombinants.

ant recombinants decreases rapidly to zero between 25 and 30 min after the onset of mating. It should be pointed out that the origin of W-1895 (Hfr C) is close to the *purE* locus and is within 5 min of the *lac* operon on the *E. coli* chromosome and about 14 min from the *ara* operon (4). There was an increase in sensitive recombinants at about 15 min and thereafter. The important conclusions that are qualitatively obtainable from these data are the confirmation of the approximate location of a resistance gene in W-1895 near *lac* and the presence of one or two *ara*-linked genes conferring sensitivity.

Since SLS-sensitivity information appears to be linked to the *ara* operon in *E. coli* W-1895, the possibility of contransducibility with *ara* was investigated. By using bacteriophage P1 vir to transduce the *ara* region from W-1895 to the virulent *Shigella* M-42-43, only 10 transductants were obtained (Table 7). However, four SLS-sensitive recombinants were obtained from the SLS-intermediate *Shigella*. Thus, at least one sensitivity gene is in fact very closely linked to *ara*. To get higher numbers of recombinants by transduction, an *E. coli-Shigella* hybrid strain (no. 8), which was *lac*- and *ara*-positive and SLS-sensitive, was obtained from a mating of W-1895 with M-42-43. This was used as a donor for transduction with the virulent and avirulent strains of *Shigella*. When the cross with M-42-43 was made with phage P1 vir grown on the no. 8 hybrid, there was a 61% conversion of the intermediate lysis class parent to the sensitive class. None of the 49 *ara*-positive recombinants was resistant to SLS, which is reasonable since the resistance locus in the strain is near *lac*, which is not cotransducible with *ara*. A similar transduction with SLS-resistant 24570 resulted in a conversion of 88% of the 65 recombinants to the sensitive and intermediate classes in approximately equal numbers. One might thus infer that the 24570 has two resistance genes near arabinose. When one is made sensitive from the hybrid, the intermediate form is produced. If both genes are converted to sensitivity, then the recombinant is sensitive to SLS. It might then follow that the virulent M-42-43 already has one gene for sensitivity at the *ara* site and the other gene, which is resistant, is converted to the sensitive allele in the transduction to sensitive recombinants. Other explanations are possible but for the present this hypothesis does explain the facts. It also serves to explain the sensitivity of the *E. coli* W-1895 parent, which although it has an SLS-resistance gene linked to the *lac* operon, is nevertheless sensitive because of the two dominant sensitivity genes at the *ara* locus.

DISCUSSION

Resistance to SLS seems to be a function of alleles of at least three genes: one linked to the *lac* operon and two others cotransducible with the *ara* region of the *E. coli* chromosome. This naturally gives rise to questions concerning the mechanisms by which resistance or sensitivity is achieved. In gram-negative organisms, it is known that the protein-lipopolysaccharide complexes can be disaggregated by SLS, leaving the glycopeptide layer if autolytic enzymes are not given a chance to function (6). There are several kinds of mutations that might make an organism resistant to SLS under the conditions of our assay. One such mutation would involve an increased ability to bind calcium and perhaps other divalent cations. The mutation of the virulent *S. flexneri* to the avirulent strain 24570 appears to be a mutation of this sort. It has been established that SLS does not lyse these organisms in Tris buffer but will if pretreated with EDTA, which does not itself cause lysis. When SLS lysis of various organisms grown in calcium-containing media is measured in phosphate buffer, differential patterns of lysis are obtained. Thus it would seem that, with calcium or other divalent cations protecting against lysis due to SLS, the relative ability of the organism to retain the cation against the competition of chelators such as EDTA and phosphate would be a factor determining its resistance to SLS.

TABLE 8. *Analysis of genotypes and phenotypes of Shigella and Escherichia coli strains*

Organism	SLS genotype[a]			Phenotype
	lac	ara 1	ara 2	
W-1895	R[b]	S	S	S
X-16 hybrid	R	S	S	S
M-42-43	S	R	S	I
24570	S	R	R	R

[a] lac, ara 1, and ara 2 represent the sodium lauryl sulfate (SLS)-sensitivity genes next to *lac* and *ara*, respectively. The ara 1 and ara 2 are formal representations of the *ara*- linked genes and do not indicate order.

[b] As before, R = resistant; I = intermediate; S = sensitive.

The lysis of *E. coli* K-12 and its *Shigella* hybrid (X-16) by SLS is much greater than either the virulent or avirulent *Shigella* strains tested, whether grown in calcium-containing media or not. This sensitivity is conferred upon the *E. coli* W-1895, presumably by the *ara*-linked genes, since the SLS gene near *lac* in W-1895 can produce resistance when transferred to certain more sensitive strains (3). The dominance relationships between the three SLS-sensitivity genes is not conclusively established, but one can deduce the following patterns from the strains studied. It is obvious that, since *E. coli* W-1895 is phenotypically sensitive to SLS and yet donates a resistant gene near the *lac* locus, one must conclude that if only the *lac*-linked gene is resistant then the organism is sensitive. Since *lac* selection after 5 min of mating between W-1895 and the virulent *S. flexneri* M-42-43 yielded many resistant recombinants, several other conclusions can be reached. The M-42-43 must have an SLS-sensitive allele at the *lac* region since it was converted to resistance too early for the *ara* region to be brought into the recipient. Since M-42-43 belongs to the intermediate lysis class, it is likely that one of its *ara*-linked SLS genes is resistant, especially since *ara* selection in the W-1895 × M-42-43 mating produced largely sensitive recombinants, of which the X-16 hybrid is an example. Finally it seems likely that the 24570 avirulent *Shigella* mutant must have two *ara*-linked resistant genes since transduction with a sensitive donor can convert this resistant mutant to both intermediate and sensitive recombinants, when selecting for arabinose fermentation. Its *lac*-linked SLS gene is probably sensitive since the 24570 is produced from the M-42-43 by a single mutation and the latter has an SLS-sensitive allele near *lac*. Table 8 illustrates the above analysis.

It is possible that one or more of these genes affects the susceptibility of the lipopolysaccharide to SLS, inasmuch as other strains of *E. coli* such as 0111:B4, 026:B6, and 055:B5 are quite resistant to SLS (Corwin and Talevi, *unpublished data*) and differ in their lipopolysaccharide structure from the *E. coli* K-12 strain used in these experiments. Second, when the combination of EDTA and lysozyme in Tris buffer is used to lyse the organisms, the difference between the virulent and avirulent shigellae is still seen, but the W-1895 is not lysed any more than the *Shigella* strains. This would indicate that *ara*-linked gene or genes affecting SLS sensitivity are not affecting calcium-binding but perhaps could be affecting the sensitivity of the lipopolysaccharide to SLS. Another possibility which we have considered is that one of these genes may affect the release by SLS of autolytic enzymes which can attack the glycopeptide layer. This could account for the rapid and almost complete lysis observed when the W-1895 is exposed to SLS. To support this, we have at times been able to use an SLS lysate of the hybrid X-16 strain to produce lysis of other strains in Tris buffer only when SLS is present. It should be remembered that SLS is ordinarily ineffective in Tris buffer by itself. However, since autolytic enzymes are thought to be destroyed by SLS (5), another possibility to be considered is that one of the genes conferring sensitivity to SLS may be one which makes the autolytic enzymes resistant to SLS. Therefore, in conjunction with SLS which can disaggregate the lipopolysaccharide, the SLS-resistant autolytic enzyme can complete the job of lysis by digesting the glycopeptide.

The impetus for this project was to study alterations in cellular properties which could prevent the penetration of *Shigella* organisms into the intestinal epithelial cells, a primary step in the pathogenesis of dysentery. The 24570 strain was a spontaneous mutant of the virulent M-42-43, which was avirulent because of its inability to penetrate intestinal epithelial cells (2). The resistance to SLS is one of the properties of the strain. Although it is tempting to rationalize its avirulence and lack of penetration in terms of SLS resistance, this would be premature for several reasons. Until the precise nature of each gene regulating SLS sensitivity is known, we are unable to determine whether the 24570 mutation is a point mutation or a deletion. Although the intermediate state of SLS sensitivity characteristic of the virulent M-42-43 can be crossed into the 24570 by mating or transduction, it has not been determined whether the SLS-sensitivity gene transferred is identical to that conferring virulence or whether it is some close, unrelated neighboring gene. One needs a reversion to both viru-

lence and the intermediate type of SLS lysis from the 24570 to prove the relationship, but no obvious means of selection is available to do this at the present time. Also it seems quite possible that the original mutation of M-42-43 to 24570 may involve a locus regulating SLS sensitivity which is different from any of the three loci we have studied. Thus, by proper combination of different alleles of these loci with the virulence locus, one might obtain a recombinant with the correct lysis phenotype of the virulent strain without regaining virulence. We are currently looking at other biochemical parameters which have been altered in the transition to the 24570, in the hope that an easily definable locus can be discovered which correlates with virulence.

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