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Decreased Permeation of Cephalosporins through the Outer Membrane of *Escherichia coli* Grown in Salicylates

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***Escherichia coli* K-12 cells grown in 1 to 5 mM sodium salicylate (SAL) or acetylsalicylate show increased phenotypic resistance to various antibiotics (J. L. Rosner, Proc. Natl. Acad. Sci. USA 82:8771–8774, 1985), including cephalosporins (this study). To determine whether these effects are caused by a decreased uptake of the antibiotics, the permeation of several cephalosporins through the outer membrane was measured. For *E. coli* K-12 grown in LB broth containing 5 mM SAL or acetylsalicylate, permeation of the outer membrane by the five cephalosporins tested decreased three- to fivefold compared with that in cells not grown in salicylates. Permeation of the outer membrane by cephaloridine decreased within 15 min of the addition of SAL to cells grown in broth and reached a minimum in 1 to 2 h. When cells were transferred from broth with SAL to broth without SAL, their permeability to cephaloridine increased slowly for the first 45 min and more rapidly over the next 1.5 h; the permeability then attained normal levels by 3 h. The permeability changes that occurred after media shifts, either to or from SAL, were prevented by concentrations of chloramphenicol that inhibited protein synthesis. These effects of SAL on outer membrane permeability are fully consistent with their effects on antibiotic resistance and with the report (T. Sawai, S. Hirano, and A. Yamaguchi, FEMS Microbiol. Lett. 40:233–237, 1987) that the outer membranes of SAL-treated cells are deficient in certain porins. Permeation of cephaloridine through the outer membrane also decreased when a virulent strain of *E. coli* K1 was grown in the presence of as little as 1 to 2 mM SAL. This raises the concern that high levels of salicylates in patients might interfere with cephalosporin or other antibiotic therapies.**

The effectiveness of beta-lactam antibiotics on gram-negative bacteria depends on their ability to cross the outer membrane barrier to a site where they can interact with enzymes involved in the synthesis of the peptidoglycan cell wall (for a review, see reference 8). Increased resistance of bacteria to beta-lactam antibiotics may occur by mechanisms that decrease the permeation of the outer membrane by these antibiotics or that increase beta-lactam hydrolysis by the β-lactamase(s) found in the periplasmic space (for a review, see reference 6).

Sodium salicylate (SAL), acetylsalicylate (AcSAL, or aspirin) and other membrane-permeable weak acids have been shown to induce phenotypic resistance in *Escherichia coli* K-12 to antibiotics (ampicillin, chloramphenicol, nalidixic acid, and tetracycline) that have dissimilar structures, targets, and modes of action (10). Sawai et al. (11) have shown that the outer membrane from *E. coli* grown in the presence of SAL have greatly reduced amounts of the outer membrane protein OmpF and slightly reduced amounts of OmpC. Since these proteins form channels that allow small hydrophilic molecules, such as the beta-lactam antibiotics, to penetrate the outer membrane of gram-negative bacteria, Sawai et al. (11) suggested that the increased antibiotic resistance of cells grown in SAL is caused by the decreased amounts of these porins.

We tested this hypothesis by measuring the permeation of the outer membrane by cephalosporins in intact bacteria. Substantially decreased rates of permeation by several cephalosporins were observed in cells that were grown in the presence of SAL or AcSAL. This effect of SAL on cephaloridine permeation was reversible and required protein synthesis, as would be expected from an agent that affects the outer membrane protein composition. Thus, the increased antibiotic resistance induced in *E. coli* by salicylates correlates well with the decreased outer membrane permeability to beta-lactam antibiotics and with the decreased levels of OmpF in the outer membrane.

MATERIALS AND METHODS

Bacteria. Strain JF568 is a derivative of *E. coli* K-12 (2) and is susceptible to beta-lactam antibiotics. Strain JF797 is a rifampin-resistant derivative of JF568, to which plasmid R471a was transferred by conjugation with the donor strain RS118 (12). The R471a plasmid carries the structural gene for the production of a TEM-1 β-lactamase (4) and renders JF797 resistant to beta-lactam antibiotics. A109 (O18ac:K1:H^-) is a pathogenic K1 strain of *E. coli* (1).

Media and chemicals. LB broth contained the following, per liter; 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract (Difco), and 5 g of NaCl (pH 7.4). LB bottom agar also contained 15 g of Bacto-Agar (Difco) per liter, and LB top agar contained 0.8% agar. For experiments with strain JF797, LB broth was supplemented with 200 μg of ampicillin per ml–5.0 mM $MgCl_2$. Stock solutions of SAL (aqueous) and AcSAL (aspirin) (in 85% ethanol) were diluted over 200 times with medium to the final concentrations (1 to 5 mM) indicated. Cephalexin and cepalothin were from Eli Lilly & Co. (Indianapolis, Ind.). Other chemicals, antibiotics, and salicylates were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Measurements of antibiotic-resistance. Agar double-diffusion tests were carried out by plating (in 2.5 ml of LB top agar), onto LB bottom agar, 0.1 ml of the indicated strain that was grown for 18 h on LB broth. Sterile paper disks were then placed onto the plate, and 25 μl of SAL (3.3 M) or 50 μl of AcSAL (0.94 M) was added to a central disk

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(diameter, 0.5 in. [1.27 cm]). A total of 10 μl of the indicated antibiotic solutions was then added to peripheral disks (diameter, 0.25 in. [0.64 cm]). The plates were allowed to dry for 30 to 60 min at room temperature and were then incubated at 37°C overnight. Asymetrical zones of inhibition surrounding the antibiotic disks, with more bacterial growth on the side of the disk facing the salicylate, indicated enhancement of resistance to the antibiotic (see Fig. 1). We then performed quantitative determinations of efficiency of plating (EOP) on LB plates containing antibiotic, with or without salicylates, as described previously (10).

Assay of cephalosporin hydrolysis. Bacteria were grown in LB broth (pH 7.4) with aeration at 37°C. The pH of the cultures varied by less than 0.2 units during growth and was not affected by the presence of salicylates or the other weak acids that we used. In preliminary experiments, the K_m and V_{max} values of the periplasmic β-lactamase for each of the cephalosporins used were determined. The rate of cephalosporin hydrolysis by intact cells, fluid from osmotically shocked cells, or cell lysates was determined as described previously (7). Briefly, intact cells or cell extracts were added to a solution containing 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and 5 mM $MgCl_2$ (pH 7.4; HEPES-Mg^{2+}) containing 1 mM of the cephalosporin in a total volume of 250 μl. The suspension was mixed and transferred to a cuvette with a 1-mm-wide light path, and the optical density changes at 260 nm was recorded at 37°C. The permeation coefficients for cephalosporins across the outer membrane barrier were calculated from the measured rates of hydrolysis (7). French press lysates were prepared from cells that were suspended in ice-cold HEPES-Mg^{2+} buffer and passed through a French pressure cell at 9,000 lb/in^2. The lysate was centrifuged (500 × *g*, 20 min) to remove debris, and the supernatant was assayed for activity. The fluids released by osmotic shock were prepared by the method of Neu and Heppel (5). Cells that were washed at room temperature in HEPES-Mg^{2+} buffer were suspended in the same buffer supplemented with 20% sucrose for 10 min and were then collected by centrifugation. The cells were dispersed rapidly into ice-cold deionized H_2O containing 0.5 mM $MgCl_2$ and kept at 0°C for 10 min to release the periplasmic materials. The cells were then removed by centrifugation, and the supernatant was assayed as described above.

RESULTS

Resistance to cephalosporins during growth in the presence of SAL and AcSAL. Salicylates render *E. coli* resistant to normally lethal levels of several antibiotics, including ampicillin (10). To test whether this was also true for cephalosporins, double-diffusion agar tests with strain JF568, ampicillin, and several cephalosporins were performed (Fig. 1A and B). The presence of SAL or AcSAL increased the resistance of this strain to ampicillin, cephalexin, cephaloridine, and cephalothin. This observation was verified by quantitative plating experiments with strain JF568 on LB agar in the presence or absence of the indicated antibiotics or salicylates (Table 1). The presence of either 2.5 mM SAL or 2.5 mM AcSAL increased the EOP on 16 μg of cephalexin per ml or 16 μg of cephalothin per ml by over 600-fold and increased the EOP on 4 μg of cephaloridine per ml by over 150-fold. Thus, SAL and AcSAL significantly increased the resistance of strain JF568 to cephalosporins.

Altered outer membrane permeability of cells grown in salicylates or other weak acids. The rate of cephalosporin uptake through the outer membrane can be estimated by measuring the rate of hydrolysis of the beta-lactam by a β-lactamase that was present in the periplasmic space (15). The rate of hydrolysis of cephaloridine in the periplasmic space of strain JF797 (the R471a derivative of strain JF568) was measured after growth in the presence or absence of 5 mM SAL (Fig. 2). The rate of hydrolysis of the beta-lactam was reduced in the presence of SAL. Control experiments (data not shown) demonstrated that the growth of *E. coli* in SAL or AcSAL did not significantly alter the total amount of cell-associated β-lactamase activity (as seen in French press lysates), the amount of β-lactamase released from the periplasm by osmotic shock (5), or the pH of the cultures.

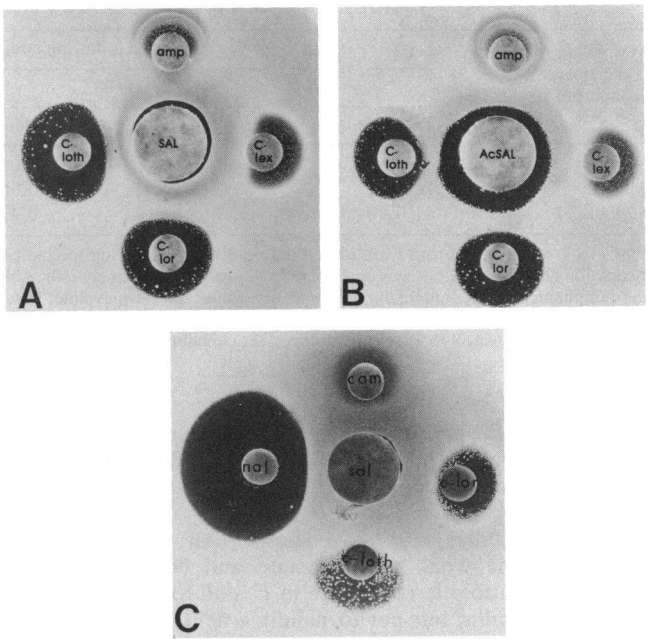

FIG. 1. Induction of resistance to several antibiotics by SAL and AcSAL. Plates for agar double-diffusion tests were prepared as described in the text. The indicated amounts of antibiotic were applied to the disks in 10-μl volumes. (A) Effects of SAL (25 μl of 3.3 M SAL) on the resistance of strain JF568 to ampicillin (amp) (5 μg), cephalexin (c-lex) (10 μg), cephaloridine (c-lor) (10 μg), and cephalothin (c-loth) (10 μg). (B) Same as described above for panel A, except that 50 μl of 0.94 M AcSAL (in 85% ethanol) was applied to the central disk. (C) Effects of SAL on resistance of strain A109 to chloramphenicol (cam) (10 μg), cephaloridine (c-lor) (10 μg), cephalothin (c-loth) (10 μg), and nalidixic acid (nal) (60 μg). Note in each case the reduced antibiotic susceptibility in the region of the antibiotic disk proximal to the salicylate-containing disk.

The effect of SAL on the hydrolysis of cephaloridine was independent of the growth phase of the cells. Untreated cells hydrolyzed cephaloridine at a rate of about 6.25 μM/min per 10^{10} cells, and this varied little over the entire growth phase (Fig. 3). The hydrolysis rate of cephaloridine in SAL-treated cells was fivefold lower than that of untreated cells and was independent of the growth phase. The slower growth in broth of SAL-treated cells (75 min doubling time versus 40 min doubling time for the control) may reflect reduced nutrient uptake through the outer membrane.

From results of these and similar experiments, the effects of salicylates on cephalosporin uptake rates into the periplasm were calculated (7). Table 2 shows the permeation coefficient calculated from the hydrolysis rates of six cephalosporins in strain JF797. The addition of either AcSAL or

TABLE 1. Effects of growth in salicylate on the EOP on LB agar containing antibiotics[a]

Salicylate (concn [mM])	EOP of the following strains grown on the indicated antibiotics and concn (μg/ml):						
	JF568				N117		
	Lexin (16)	Loridine (4)	Lothin (16)	Cam (6)	Amp (50)	Nal (5)	Tet (5)
None	<0.006	0.003	0.006	<0.001	<0.004	<0.004	<0.004
AcSaL (2.5)	0.52	0.45	0.56	0.20	1.09	0.46	0.95
SAL (2.5)	0.40	0.64	0.43	0.14	ND[b]	ND	ND

[a] Cells grown in LB broth were plated onto LB agar containing the indicated concentration of the antibiotic with or without the indicated salicylate. After incubation at 37°C, the EOP was determined as described previously (10). Abbreviations: Lexin, cephalexin; Loridine, cephaloridine; Lothin, cephalothin; Cam, chloramphenicol; Amp, ampicillin; Nal, naladixic acid; Tet, tetracycline. Data for ampicillin, nalidixic acid, and tetracyclin were obtained from a previous report (10). Typical peak concentrations (in micrograms per milliliter) of the following antibiotics in plasma, as commonly observed during human therapy (3), are as indicated: cephalexin, 9 to 18; cephaloridine, 35; cephalothin, 10 to 20; chloramphenicol, 10 to 13; ampicillin, 3 to 10; nalidixic acid, 0.6 to 3.5; tetracycline, 3.
[b] ND, Not done.

SAL lowered the uptake rates of all cephalosporins tested by three- to fivefold. Thus, these salicylates affect the permeability of the outer membrane to various beta-lactam antibiotics.

Previous studies (10) have shown that, in addition to salicylates, the weak acids acetate and benzoate induce phenotypic antibiotic resistance in *E. coli* to chloramphenicol and ampicillin but not to nalidixic acid or tetracycline. The effects of these weak acids on outer membrane permeability were compared with those of SAL (Table 3). The effect of benzoate (10 mM) was quite similar to that of SAL (5 mM). Acetate (50 mM) also reduced the outer membrane permeation of cephaloridine (to about 45% of that of the untreated control), but this was still threefold higher than the rate for cells treated with SAL. The weaker effects of acetate may reflect the fact that acetate is readily metabolized by *E. coli*. Nevertheless, these results indicate that weak acids other than salicylates reduce the permeability of the *E. coli* outer membrane to cephaloridine.

Changes in outer membrane permeability following transfer to or from SAL. The effects of salicylates on antibiotic resistance are reversible (10). Therefore, it was of interest to determine whether the permeability changes observed were also reversible and, if so, how rapidly they accurred. When SAL was added to cells that were growing in broth at 37°C, cephaloridine permeation decreased in less than 15 min and reached a minimal value (20% of the control) in 2 to 3 h (Fig. 4). Thus, maximal loss of permeability to cephaloridine was not instantaneous but required about two cell doublings.

In the reverse experiment, cells that were chronically grown in the presence of SAL were transferred to broth without SAL. Their permeability to cephaloridine increased slowly for about 45 min and more rapidly over the next 1.5 h, and then attained normal levels (those of nontreated cells) by 3 h (Fig. 4). Thus, the effect of SAL on outer membrane permeability was fully reversed after several generations of growth in its absence, consistent with the reversibility of SAL-induced antibiotic resistance (10).

Requirement for protein synthesis to effect permeability changes. Sawai et al. (11) have shown that SAL greatly reduces the amount of OmpF in the outer membrane and have suggested that this is caused by the inhibition of OmpF synthesis. If SAL decreases the permeability of growing outer membranes by reducing the amount of OmpF in the membranes, inhibition of protein synthesis in cells during their exposure to SAL should interfere with this process. The reverse should also be true: recovery of permeability

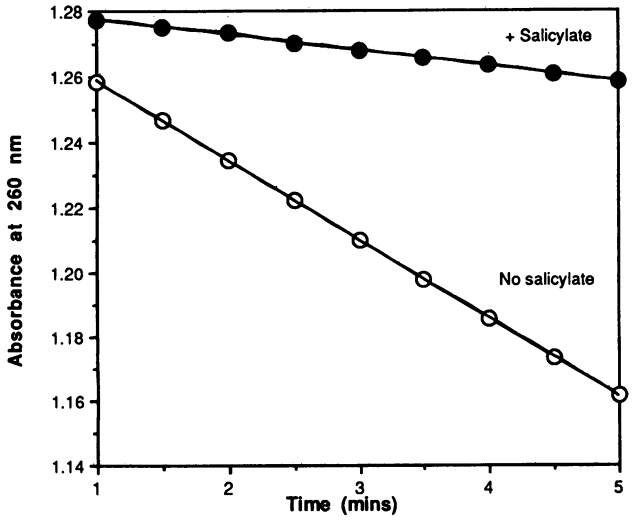

FIG. 2. Rates of hydrolysis of cephaloridine by JF797 grown in the presence (●) or absence (○) of 5 mM SAL. The experiment was done as described in the text.

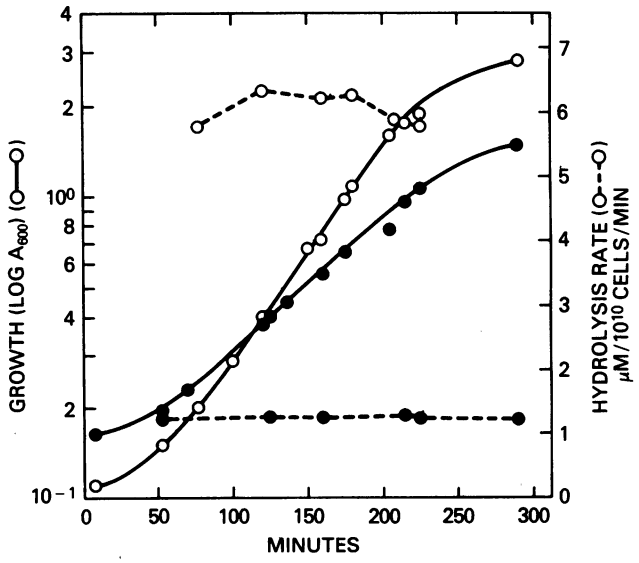

FIG. 3. Effects of SAL on the rate of hydrolysis of cephaloridine during different growth phases of strain JF797. Cells were grown in LB broth with 5 mM $MgCl_2$, with (●) or without (○) 5 mM SAL. At the indicated times, samples of each culture were removed and their A_{600} values were measured. The cells in the samples were collected by centrifugation, washed, suspended at an A_{600} of about 1.0, and assayed for the hydrolysis of cephaloridine.

TABLE 2. Effects of SAL and AcSAL on cephalosporin permeation of the outer membrane[a]

Cephalosporin	Growth medium[b]	Permeation (10^{-5} cm s^{-1}; mean ± SD)[c]	Relative permeation[d]
Cephaloridine	LB broth	43.4 ± 0.20	1.00
	+ SAL	11.6 ± 0.21	0.27
	+ AcSAL	12.0 ± 0.21	0.28
Cephalothin	LB broth	1.06 ± 0.02	1.00
	+ SAL	0.21 ± 0.01	0.20
	+ AcSAL	0.31 ± 0.03	0.29
Cefazolin	LB broth	3.44 ± 0.008	1.00
	+ SAL	0.84 ± 0.002	0.24
	+ AcSAL	1.27 ± 0.011	0.37
Cephalexin	LB broth	1.03 ± 0.006	1.00
	+ SAL	0.28 ± 0.002	0.27
	+ AcSAL	0.38 ± 0.007	0.37
Cephapirin	LB broth	12.2 ± 0.18	1.00
	+ SAL	2.30 ± 0.07	0.19
Cephalexin	LB broth	3.62 ± 0.03	1.00
	+ SAL	0.28 ± 0.002	0.08

[a] The hydrolysis rate of the indicated cephalosporins was determined and used to calculate the permeation as described previously (7).
[b] SAL or AcSAL was added to LB broth at 5 mM.
[c] Standard deviation of five determinations.
[d] Antibiotic permeation relative to that observed for cells grown in LB broth.

after removal of the cells from SAL should require protein synthesis. To test this, cells grown in the absence (or presence) of SAL were treated with 200 μg of chloramphenicol per ml (a concentration that inhibits protein synthesis in SAL-treated cells). The cells were then transferred to fresh broth with (or without) SAL. Table 4 (experiments 1 and 2) shows that if cells grown in the absence of SAL were treated with chloramphenicol during the incubation with SAL, decreased permeability to cephaloridine was not observed. Similarly, if cells were treated with chloramphenicol when they were removed from SAL, the recovery of permeability was prevented (Table 4, experiments 5 and 6). Chloramphenicol had no significant effect on outer membrane permeability in control cultures whose SAL concentrations were not changed during the experiment (compare experiments 3 and 4 and experiments 7 and 8 in Table 4). Thus, protein synthesis is required for both the loss of permeability caused by the addition of SAL and the recovery of permeability after the removal of SAL. This is fully consistent with an affect of SAL on outer membrane composition, as observed by Sawai et al. (11).

Reduced permeability in a pathogen grown in low levels of SAL. The effect of SAL on antibiotic resistance was tested in a pathogenic K1 strain of *E. coli* (A109). Agar double-diffusion tests showed that resistance of strain A109 to chloramphenicol, cephaloridine, cephalothin, and nalidixic acid was significantly increased in the presence of SAL (Fig. 1C) and AcSAL (data not shown). A109 produced colonies with 100% efficiency in the presence of 5 mM SAL on plates with 6 μg of chloramphenicol per ml, 10 μg of cephalothin per ml, or 5 μg of nalidixic acid per ml. In the absence of SAL, the EOP on the same plates was less than 0.005. Therefore, this pathogen is similar to the previously tested *E. coli* K-12 strains with regard to induction of antibiotic resistance by SAL and AcSAL. Similar results were obtained for *Salomonella typhimurium* LT2 (data not shown).

TABLE 3. Effect of growth in weak acids on cephaloridine permeation[a]

Expt	Weak acid supplement	Concn (mM)	Permeation (10^{-5} cm s^{-1}; mean ± SD)[b]	Relative permeation[c]
1	None		54.1 ± 0.07	1.00
2	Acetate	25	24.2 ± 0.81	0.45
3	Benzoate	10	9.4 ± 0.70	0.17
4	Salicylate	5	8.1 ± 0.01	0.15

[a] Strain JF797 was grown in LB broth with the indicated supplement.
[b] Standard deviation of five determinations.
[c] Antibiotic permeation relative to that observed for cells grown in unsupplemented LB broth.

Substantial resistance of *E. coli* K-12 to chloramphenicol has been achieved by growing it in as little as 1 mM SAL (10). The relation between the SAL concentration and cephaloridine hydrolysis for both the K1 strain (A109) and the K-12 strain (JF568) is shown in Fig. 5. Even at 1 mM SAL, significant reductions in cephaloridine permeation of the outer membrane were observed. Thus, SAL can increase the resistance to, and decrease the outer membrane permeation of, cephaloridine in a virulent pathogen.

DISCUSSION

Salicylates have previously been shown (10) to increase the phenotypic resistance of various *E. coli* K-12 strains to diverse antibiotics, including ampicillin, chloramphenicol, nalidixic acid, and tetracycline. Other weak acids, such as acetate and benzoate, have also been found to induce antibiotic resistance, but to a more limited extent (10). Since

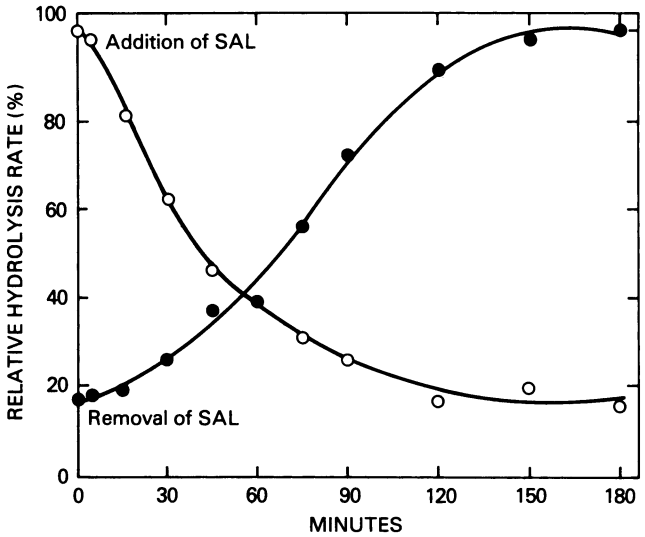

FIG. 4. Change in rates of hydrolysis of cephaloridine by strain JF797 after transfer to (○) or from (●) medium with SAL. JF797 was grown in LB broth, with or without 5 mM SAL, to a density of about 4×10^8 cells per ml. Cells from each culture were washed and suspended at the same density in LB broth without or with 5 mM SAL, respectively. Each culture was incubated at 37°C, and at the indicated times, samples of the intact cells were assayed for cephaloridine hydrolysis rates (in micromolar per 10^{10} cells per minute). These rates were then normalized to the rate found at either time zero (○) or 180 min (●).

TABLE 4. Effect of protein synthesis inhibition on changes in cephaloridine permeation of the outer membrane induced by growth in salicylate[a]

Expt	Effect of growth in the following on protein synthesis inhibition:			Permeation (10^{-5} cm s^{-1}; mean ± SD)[b]
	Medium 1: salicylate	Medium 2		
		Salicylate	Chloramphenicol	
1	−	+	−	16.2 ± 0.03
2	−	+	+	40.6 ± 0.07
3	−	−	−	46.7 ± 0.12
4	−	−	+	48.9 ± 0.07
5	+	−	−	47.1 ± 0.04
6	+	−	+	11.1 ± 0.11
7	+	+	−	11.3 ± 0.04
8	+	+	+	11.1 ± 0.10

[a] Overnight cultures of JF797 cells grown in LB broth at 37°C either without (−) or with (+) 5 mM SAL were diluted 1:40 into fresh medium (first medium) and grown to an A_{600} of 0.8. The cells were then centrifuged, washed, and suspended in an equal volume of LB broth with (+) or without (−) 5 mM salicylate and chloramphenicol (200 μg/ml) as indicated (second medium). The cultures were incubated at 37°C for 60 min. the cells were harvested, and the cephaloridine permeation was determined as described in the text. The concentration of chloramphenicol used (200 μg/ml) was shown in a separate experiment to completely inhibit the incorporation of ^{3}H-labeled leucine into acid-insoluble material by either culture.

[b] Standard deviation of five determinations.

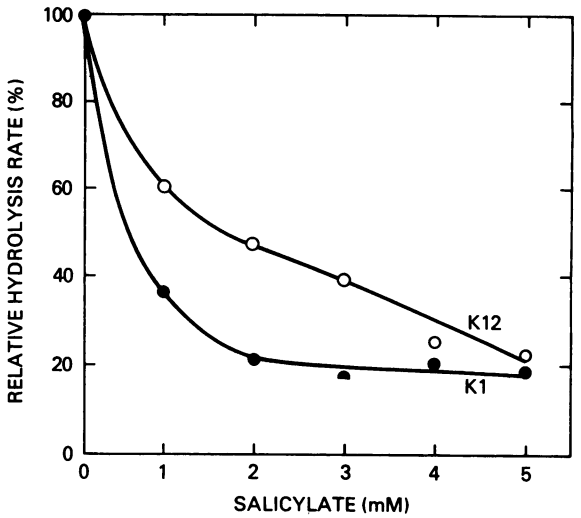

FIG. 5. Relative hydrolysis rates of cephaloridine as a function of SAL concentration for the K-12 strain JF797 (○) and the K1 strain A109 (●). Cells grown overnight in LB broth without (for the control with no SAL) or with 5 mM SAL were washed and diluted 1:40 into LB broth with the indicated concentrations of SAL. When the A_{600} of the cultures reached 0.8, the cells were harvested and assayed. Separate experiments showed that the presence or absence of SAL had no significant effect on the pH of the cultures (variation, <0.2 unit). The hydrolysis rate for each culture was measured and normalized to that of the control culture (grown without SAL).

these antibiotics differ both in their modes of action and in their chemical structures, it seemed plausible that the resistance was caused by an effect of the weak acids on antibiotic uptake. In gram-negative bacteria, these antibiotics must cross the outer membrane barrier to reach their sites of action. The measurements of cephalosporin permeation of the outer membrane reported here show that (i) SAL and AcSAL reduce the permeability of the outer membrane to a number of cephalosporins by three- to fivefold, (ii) benzoate reduces the permeation of cephaloridine by about fivefold, and (iii) acetate reduces this permeation by about twofold. The higher pK_a of acetate or its metabolism by *E. coli* could be responsible for the relatively weaker effects of acetate on permeability and drug resistance (10). Since SAL and AcSAL are effective at relatively low concentrations, are not metabolized by *E. coli*, and are medically significant drugs, they were studied more intensively.

The parallel effects of SAL (and AcSAL) on antibiotic resistance and outer membrane permeability suggest that the resistance to cephalosporins induced by salicylates can be attributed, at least in part, to the decreased uptake of beta-lactam antibiotics. (i) The induction of antibiotic resistance by weak acids was fully reversed when the cells were removed from the weak acids. The loss of permeability induced by the growth of cells in SAL was also reversed when the cells were transferred to medium without SAL (Fig. 4). (ii) The concentrations of SAL that increased drug resistance were similar to those that decreased permeability (Table 1 and Fig. 5). (iii) Different bacteria, such as the K1 and K-12 strains of *E. coli*, became more resistant to cephalosporins (Fig. 1) and less permeable to these antibiotics in the presence of SAL (Fig. 5). These data support the hypothesis that the reduction of outer membrane permeability induced by SAL is responsible for much of the observed increase in antibiotic resistance.

Decreased permeability to cephalosporins was detected 15 min after the addition of SAL to the cells (Fig. 4). However, since maximal loss of permeability took about 1 to 2 h of growth (two doublings) in the presence of SAL and required concomitant protein synthesis, a slow, cell-mediated process was implicated. All of these findings fit well with the observations of Sawai et al. (11) that SAL decreases the amount of OmpF in the outer membrane, that this is reversible by growth of cells in the absence of SAL, and that SAL reduces the amount of OmpF in the outer membrane when it is present at the same concentrations that increase antibiotic resistance and that decrese outer membrane permeability. A rapid decrease of OmpF in newly formed outer membranes could have caused the rapid initial decrease in permeability observed here. However, cell growth and division would be required for complete conversion of the outer membrane to the minimally permeable form that was observed. The recovery of permeability observed after the removal of SAL would then require the synthesis of outer membranes that contain OmpF to reverse this process.

The medical significance of these results has yet to be tested in animals, in which particular conditions could alter the interactions among bacterium, antibiotic, and salicylates. Nevertheless, it should be noted that both the concentrations of salicylates that were found to be effective in this study and the concentrations of antibiotics to which substantial resistance was engendered by the salicylates in vitro were not dissimilar from the concentrations encountered in certain therapeutic situations. The peak concentrations of salicylates in the plasma of patients with, e.g., rheumatoid arthritis treated with high doses of aspirin is expected to be in the range of 1 to 3 mM (9). These levels affect the permeation of the outer membrane by cephaloridine (Fig. 5). The peak levels of antibiotics commonly attained in plasma during therapies (3) are given in footnote *a* of Table 1. In many cases, these levels decline rapidly, and they may be higher or lower in specific tissues. Nonetheless, *E. coli* shows substantial resistance to these concentrations of antibiotics (except for cephaloridine and chloramphenicol) when 2.5 mM AcSAL or SAL is present in vitro (Table 1).

Thus, it is reasonable to suspect that high doses of salicylates could have untoward effects on certain antibiotic treatments.

The weak acids that induce antibiotic resistance and impermeability to cephalosporins are all chemotactic repellents (14). At least one promoter in *E. coli* appears to respond to the low internal pH engendered by weak acids such as SAL (13). Reduced permeability, negative chemotaxis, and other responses may be part of a "global" adaptation of various bacteria to weak acids in their environments.

ACKNOWLEDGMENT

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