International Journal of Pharmaceutics, 34 (1986) 115–123
Elsevier

IJP 01145

The effects of antiseptics, disinfectants and preservatives on smooth, rough and deep rough strains of *Salmonella typhimurium*

A.D. Russell and J.R. Furr

Pharmaceutical Microbiology Research Laboratory, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff (U.K.)

(Received 2 June 1986)
(Accepted 3 July 1986)

Key words: Antiseptic; Disinfectant; LPS mutant; *Salmonella typhimurium*

Summary

The effects of some antiseptics, disinfectants and preservatives on a smooth strain and rough mutant strains (Ra, Rd_1 and Re) of *Salmonella typhimurium* are described. The inhibitors consisted of three phenolics, a homologous series (the parabens) of esters of *p*-hydroxybenzoic (4-hydroxybenzoic) acid, chlorhexidine diacetate and other cationic bactericides and two mercury compounds. The responses of the strains to these agents have been related: (a) to the nature of the bacterial outer membrane; and (b) to the physical properties of the compounds.

Introduction

Lipopolysaccharide (LPS)-deficient mutants of *Salmonella typhimurium* have been widely used as a means of assessing the permeability or impermeability of the outer cell membrane to hydrophobic antibiotics (Nikaido, 1976; Nikaido and Nakae, 1979; Nikaido and Vaara, 1985). These studies have demonstrated that re-organisation of the outer membrane rather than changes in the structure of LPS are responsible for the greater sensitivity of mutants with increasing degrees of roughness. Stickler and Thomas (1982) have pointed out that these *S. typhimurium* mutants have not been exploited further in studying the penetration of non-antibiotic inhibitors into the cells. Several such agents are used widely as antiseptics, disinfectants and pharmaceutical food or cosmetic preservatives (Hugo and Russell, 1982) and it therefore seemed to be of interest to investigate their effect upon these well-defined mutants.

The following were selected for study: the methyl (Me), ethyl (Et), propyl (Pr) and butyl (Bu) esters of *p*-hydroxybenzoic acid (4-hydroxybenzoic acid), dibromopropamidine isethionate (DBPI), phenylmercuric nitrate (PMN) and mercuric chloride, quaternary ammonium compounds (QACs), some phenolics and chlorhexidine. The strains examined were a smooth strain and various rough mutants (Ra, Rd_1 and Re) of *S. typhimurium*. In some experiments, the possible potentiating effect of the chelating agent, ethylenediamine tetraacetic acid, di-sodium salt (EDTA) was studied (Russell 1982).

Correspondence A.D. Russell, Pharmaceutical Microbiology Research Laboratory, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Post Box 13, Cardiff CF1 3XF, U.K.

Materials and Methods

Bacterial strains

Bacterial strains (in brackets, their LPS-type composition) consisted of TV 253 (smooth), SF 1591 (Ra), SF 1567 (Rd_1) and SF 1398 (Re). Cells were grown at 37°C in Nutrient broth (Oxoid, London) to a density of ca. 5×10^8 cfu/ml. Nutrient agar (Oxoid) was used as a solid medium.

Antibiotic sensitivity testing

Sensitivity to antibiotics was carried out by the conventional disc method, using Nutrient agar as medium. Molten agar at about 45°C was inoculated to give a final cell density of ca. $5–10 \times 10^5$ cfu/ml and discs placed on the surface of the set plates. After diffusion for 1 h at 20°C, plates were incubated at 37°C for 24 h and inhibition zone diameters measured. In other experiments, the cup-plate method was used, the cups being filled with antibiotic solutions (100 μg/ml).

Sensitivity to antiseptics, disinfectants and preservatives

Overnight 37°C broth cultures were diluted 1:100 in broth, and 1 μl volumes placed in a multipoint inoculator (Denley Instruments, Billingsburst) on to the surface of overdried (37°C, 2 h) agar plates containing the appropriate concentration of a drug. In some experiments, EDTA was also present (final concentration up to 10^{-3} M). Plates were incubated at 37°C for 24 h and the presence or absence of growth noted. The minimum inhibitory concentration (MIC) was the lowest concentration preventing growth.

MIC values of mutant strains were compared with the wild-type strain (TV 253). The MIC ratios obtained were considered in the context of the nature of the outer membrane and the physical properties of the test drug.

Results and Discussion

The characteristics of the smooth strain and rough mutants of *S. typhimurium* are presented in Table 1 and Fig. 1. Sensitivity testing with antibiotic discs was not entirely satisfactory because of the comparatively low content of an antibiotic per disc. Antibiotic solutions (100 μg/ml) were also examined by means of the cup-plate method. The results (Table 1) demonstrate that the wild-type strain is the most resistant to the hydrophobic antibiotics studied, with the deepest rough mutant the most sensitive. Table 2 presents the chemical, physical and microbiological properties of inhibi-

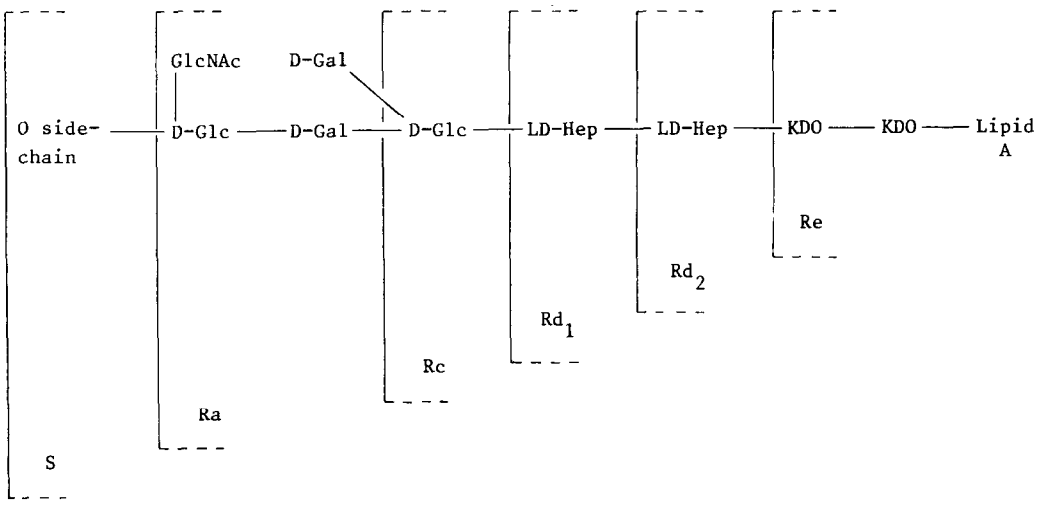

Fig. 1. Lipopolysaccharide (LPS) composition of smooth strain (TV 253) and rough mutants of *Salmonella typhimurium* used in this study.

TABLE 1

CHARACTERISTICS AND ANTIBIOTIC SUSCEPTIBILITY OF *SALMONELLA TYPHIMURIUM* STRAINS

Strain	Type of LPS Composition	Sensitivity (S) or resistance (R) to antibiotics *
TV 253	Smooth	R to Rif, NV, E, FD, Vanc
SF 1 591	Ra	R to Rif, NV, E, FD, Vanc
SF 1 567	Rd_1	Moderately S to Rif, NV, E; R to Vanc
SF 1 398	Re	S to Rif, NV, E, FD; R to Vanc

* Rif, rifampicin; NV, novobiocin; E, erythromycin; FD, fucidin; Vanc, vancomycin.

tors used in this study. Reference to certain aspects of Table 2 will be made where appropriate.

Tables 3–7 provide the MICs of various types of inhibitors against the *S. typhimurium* strains. Additionally, a ratio of MIC for smooth strain (TV 253) : MIC against mutant has been calculated which has been related to the degree of roughness of that mutant. In some instances, e.g. phenols (Table 4) and parabens (Table 6), a ratio of MIC of a phenolic : MIC of the test phenol, or of MIC of Me paraben : MIC of test paraben for each strain has been produced and related where possible to the partition coefficient of the particular inhibitor (Table 2).

Phenolics

The inhibitory concentrations of phenol, cresol and chlorocresol are described in Table 3. The results demonstrated, as would be expected (Hugo and Russell, 1982), that chlorocresol was the most active of the three agents against any one strain with phenol the least, and that the deep rough mutant (Re LPS) was the most sensitive to all of the inhibitors.

Additional data calculated from these results are presented in Table 4 and in Fig. 2. This information demonstrates that, for each strain, about half as much cresol and about one-twentieth as much chlorocresol are needed to bring about

TABLE 2

CHEMICAL, PHYSICAL AND MICROBIOLOGICAL PROPERTIES OF ANTISEPTICS, DISINFECTANTS AND PRESERVATIVES

Inhibitor [a]	M.W.	Solubility in water	log P [b]	MIC (μg/ml) [c]	
				Gram positive	Gram negative
Phenol	94.11	1 : 15	1.46–1.51	2000	2000
Cresol	108.1	1 : 50	1.95–2.01	600–1000	800
Chlorocresol	142.6	1 : 260	3.10	200–250	200
Me paraben	152.1	1 : 500	1.96	800	800–1000
Et paraben	166.2	1 : 1300	2.47	500	600–800
Pr paraben	180.2	1 : 2500	3.04	150	300–400
Bu paraben	194.2	1 : 6500	3.57	120	150–175
CHA	625.6	1 : 55	0.08 (not ion-corrected)	0.5–1	1–20
CPC	358.0	1 : 20	1.71	< 10	100 or more
DBPI	722.4	1 : 2		1–2	4–128
PMN	634.4	1 : 1500		0.1–0.2	1–2
Mercuric chloride	271.5	1 : 15		≤ 1	ca. 1

[a] CHA, chlorhexidine diacetate; CPC, cetylpyridinium chloride; DBPI, dibromopropamidine isethionate; PMN, phenylmercuric nitrate.

[b] P, partition coefficient (in octanol) from the following reference source: Hansch and Leo (1979).

[c] MIC data based on the following references: Cook (1954), Hugo (1971), Russell (1971), Wallhausser (1984). In our hands, *Staphylococcus aureus* strains are often more resistant to parabens than Gram-negative bacteria.

TABLE 3

EFFECT OF SOME PHENOLICS ON *SALMONELLA TYPHIMURIUM* STRAINS

Strain	LPS composition	Phenol		Cresol		Chlorocresol	
		MIC (μg/ml)	MIC * ratio	MIC (μg/ml)	MIC * ratio	MIC (μg/ml)	MIC * ratio
TV 253	Smooth	2000	1	1000	1	150	1
SF 1591	Ra	1500	1.33	750	1.33	100	1.5
SF 1567	Rd	1500	1.33	750	1.33	100	1.5
SF 1398	Re	750	2.67	500	2	50	3

* MIC vs smooth strain: MIC vs test strain

the same effect as phenol. Interestingly, the phenol : cresol and phenol : chlorocresol ratios were of the same order for each of the four strains.

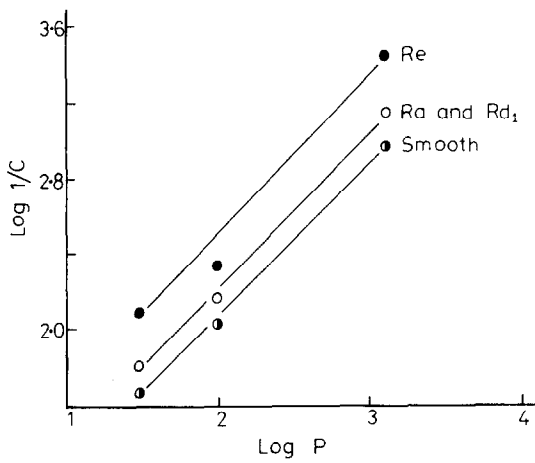

Fig. 2. Plot of the reciprocal of $\log_{10}$ molar inhibitory concentrations (log 1/C values) vs partition coefficients (log P) for phenol, cresol and chlorocresol.

Parabens

Four esters of *p*-hydroxybenzoic acid have been examined, and their activity is depicted in Table 5. As the homologous series is ascended, activity is increased (MIC values decrease). Of the four strains, the deep rough mutant (Re LPS) was the most sensitive to each compound. The Ra mutant showed an almost identical response to the smooth strain, the Rd_1 strain gradually became more sensitive, comparatively, as the homologous series was ascended, and the Re strain showed an accelerating rate of comparative sensitivity over the Me : Bu range of compounds.

A re-evaluation of the data is given in Table 6. The Me : Et ratios were very similar for the four test organisms. However, the Me : Pr ratios increased from 2.96 (smooth strain) to 4.74 (Re strain) and the Me : Bu ratios from 3.83 to 12.77. This is more clearly demonstrated in Fig. 3, where plots of $\log_{10}$ of 1/C (where C stands for the MIC value) against the octanol : water partition coefficients produce lines of markedly different slope.

TABLE 4

COMPARATIVE EFFECTS OF PHENOLICS ON *SALMONELLA TYPHIMURIUM* STRAINS

Strain	LPS composition	MIC (molar *) of phenolics			MIC ratios	
		Phenol	Cresol	Chlorocresol	Phenol : Cresol	Phenol : Chlorocresol
TV 253	Smooth	2.126×10^{-2}	9.251×10^{-3}	1.052×10^{-3}	2.3	20.21
SF 1591	Ra	1.595×10^{-2}	6.938×10^{-3}	7.013×10^{-4}	2.3	22.74
SF 1567	Rd_1	1.595×10^{-2}	6.938×10^{-3}	7.013×10^{-4}	2.3	22.74
SF 1398	Re	7.975×10^{-3}	4.626×10^{-3}	3.507×10^{-4}	1.72	22.74

* Molar values calculated from results presented in Table 3.

TABLE 5

EFFECT OF PARABENS ON *SALMONELLA TYPHIMURIUM* STRAINS

Strain	LPS composition	Me ester		Et ester		Pr ester		Bu ester	
		MIC (μg/ml)	MIC ratio *	MIC (μg/ml)	MIC ratio *	MIC (μg/ml)	MIC ratio *	MIC (μg/ml)	MIC ratio *
TV 253	Smooth	750	1	500	1	300	1	250	1
SF 1591	Ra	750	1	500	1	250	1.2	250	1
SF 1567	Rd_1	750	1	450	1.1	200	1.5	100	2.5
SF 1398	Re	500	1.5	300	1.67	125	2.4	50	5

* MIC vs smooth strain : MIC vs test strain.

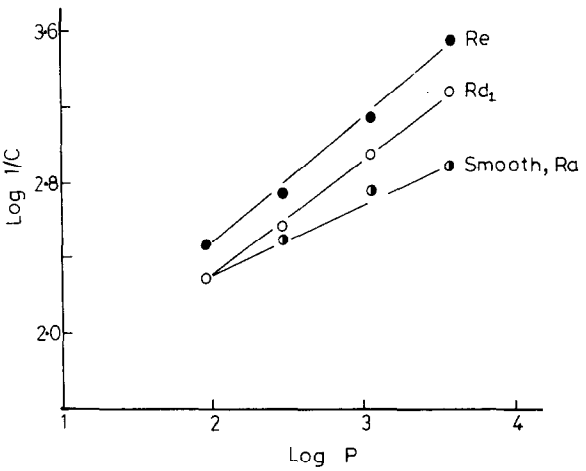

Fig. 3. Plot of the reciprocal of $\log_{10}$ molar inhibitory concentrations (log 1/C values) vs partition coefficients (log P) for Me, Et, Pr and Bu parabens.

Chlorhexidine

The smooth strain (TV 253) was more resistant (MIC 15 μg/ml) than the rough strains (Ra, Rd_1 : MICs 5 μg/ml) or the deep rough strain (Re-type LPS : MIC 1–2 μg/ml). This situation is rather different from that with *Escherichia coli*, in which it has been shown that wild-type, rough and deep rough strains are almost equally sensitive to chlorhexidine (El-Falaha et al., 1983; Russell and Furr, 1986). It was postulated that, with *E. coli*, the biguanide had little difficulty in penetrating the outer membrane to reach its primary target, the inner membrane (Hugo, 1982). *S. typhimurium* may be more resistant because of a different outer membrane, or because the inner membrane of the smooth strain is less susceptible to chlorhexidine action than that of the other strains. This is being examined further. It is, however, important to note that the latter reason has been put forward for the resistance of *Serratia marcescens* to chlorhexidine, although this was considered unimportant with envelope and mutant strains of *E. coli* and *Ps. aeruginosa* (El Falaha et al., 1985) and less important than the outer membrane with chlorhexidine-resistant *Providencia stuartii* (Ismaeel et al., 1986).

TABLE 6

COMPARATIVE EFFECTS OF PARABENS ON *SALMONELLA TYPHIMURIUM* STRAINS

Strain	LPS composition	MIC (molar *) of esters				MIC ratios **		
		Me	Et	Pr	Bu	Me : Et	Me : Pr	Me : Bu
TV 253	Smooth	4.935×10^{-3}	3.012×10^{-3}	1.667×10^{-3}	1.289×10^{-3}	1.64	2.96	3.83
SF 1591	Ra	4.935×10^{-3}	3.012×10^{-3}	1.389×10^{-3}	1.289×10^{-3}	1.64	3.55	3.83
SF 1567	Rd_1	4.935×10^{-3}	2.71×10^{-3}	1.111×10^{-3}	5.155×10^{-4}	1.82	4.4	9.57
SF 1398	Re	3.29×10^{-3}	1.807×10^{-3}	6.944×10^{-4}	2.577×10^{-4}	1.82	4.74	12.77

* Calculated from the results presented in Table 5: see also Fig. 3.

** Comparison of molar MIC values: the *higher* the values, the *more effective* is the test paraben in relation to the Me ester.

Cationic bactericides

Apart from chlorhexidine itself, two other types of cationic bactericides have been examined, viz. two QACs (cetrimide and CPC) and one diamidine (DBPI). Strain SF 1398 with Re-type LPS was the most sensitive to these three inhibitors, especially the diamidine (MIC 0.5 μg/ml) (Table 7).

A consideration of the LPS pattern in relation to sensitivity demonstrates that with the two QACs, the wild-type strain, the Ra mutant and the Rd_1 mutant showed high resistance and that significant sensitivity only became apparent with the Re mutant. In contrast, the Rd_1 mutant was considerably more sensitive than TV 253 or the Ra mutant to DBPI. Very little work has been carried out on the way in which diamidines enter bacterial cells, but it is known with the QACs that phospholipid molecules in the outer membrane may become exposed only in the deep rough mutants, and that these may play a role in permitting entry of these molecules.

Mercury compounds

Two mercury compounds were chosen for study, the inorganic agent mercuric chloride and the important preservative, phenylmercuric nitrate (PMN). The sensitivity of the various strains is described in Table 8. Since the concentration of PMN in pharmaceutical and cosmetic circles is expressed as a percentage, this value has also been included in Table 8.

The smooth strain of *S. typhimurium* was the most resistant to the organomercurial (M.W. 634.4), with the deep rough (Re) strain the most sensitive. The Rd_1 strain was slightly more sensitive than the smooth strain. When mercuric chloride (M.W. 271.5) was employed, the strains grew at 0.5 μg/ml, but were all inhibited at 1 μg/ml (Table 8). The values for the inorganic mercurial provide an interesting comparison with PMN for the Rd_1 and Re LPS strains. It seems likely that mercuric chloride can freely enter cells, whereas the larger organomercurial is prevented from doing so in the smooth and Ra-type LPS strains by the LPS which shields underlying structures. Elkhouly and Yousef (1974) list MICs of PMN and mercuric chloride against a strain (NCTC 7244) of *Pseudomonas aeruginose* as being 12 and 6 μg/ml, respectively, and Barr et al. (1970) quote a figure of 8.2 μg/ml as being the MIC of phenylmercuric acetate (PMA) against *Escherichia coli*. MICs of PMA of up to 25 μg/ml against pseudomonads and of up to 6.25 μg/ml against other Gram-negative bacteria are given by Croshaw (1977).

In his excellent review of inorganic and organic mercurials, Grier (1983) describes how variation of the R group in a series of organomercurial compounds of general structure R–Hg–X can affect activity as antifungal wheat seed protectants. It would be instructive to examine such a series on smooth and rough strains of *S. typhimurium* and *E. coli*.

Overall comments

Roantree et al. (1977) have demonstrated that *S. typhimurium* strains with LPS defects at the rfa level and deeper were more sensitive to the anti-

TABLE 7

EFFECT OF SOME CATIONIC BACTERICIDES * ON *SALMONELLA TYPHIMURIUM* STRAINS

Strain	LPS composition	CHA		CPC		Cet		DBPI	
		MIC (μg/ml)	MIC ratio **	MIC (μg/ml)	MIC ratio **	MIC (μg/ml)	MIC ratio **	MIC (μg/ml)	MIC ratio **
TV 253	Smooth	15	1	> 100	1	> 100	1	100	1
SF 1591	Ra	5	3	> 100	≥ 1	> 100	≥ 1	7.5	13.33
SF 1567	Rd_1	5	3	> 100	≥ 1	50	> 2	2.5	40
SF 1398	Re	2	7.5	7.5	> 13	10	> 10	0.5	200

* CHA, chlorhexidine diacetate; CPC, cetylpyridinium chloride; Cet, cetrimide; DBPI, dibromopropamidine isethionate.

** Ratio of MIC vs smooth strain : MIC vs test strain.

TABLE 8

COMPARATIVE RESPONSE OF *SALMONELLA TYPHIMURIUM* STRAINS TO PHENYLMERCURIC NITRATE (PMN) AND MERCURIC CHLORIDE

Strain	LPS deficiency	PMN			Mercuric chloride	
		(a) *	(b) **	Ratio ***	(a) *	Ratio ***
TV 253	Smooth	0.75	0.000075	1	1	1
SF 1591	Ra	1	0.0001	0.75	1	1
SF 1567	Rd_1	0.25	0.000025	3	1	1
SF 1398	Re	< 0.05	< 0.000005	> 15	1	1

* (a) MIC expressed as μg/ml.
** (b) MIC expressed as % w/v.
*** Ratio of MIC vs smooth strain : MIC vs test strain.

biotics bacitracin, novobiocin and polymyxin, whereas those with defects at the rfaG level (Rd_1 chemotype) were, in addition, more sensitive to vancomycin, erythromycin, nafcillin and cloxacillin. They interpreted these findings in the light of Nikaido's (1976) hypothesis that hydrophobic antibiotics gained access in deep rough strains where phospholipid patches appeared on the cell surface. Sanderson et al. (1974) had earlier noted that deep rough mutants of *S. typhimurium* were more sensi-

TABLE 9

EFFECT OF EDTA ON THE RESPONSE OF *SALMONELLA TYPHIMURIUM* STRAINS TO SOME INHIBITORS

Strain	LPS composition	Inhibitor *	MIC (μg/ml) in presence of EDTA **		MIC ratio in absence and presence of EDTA ***	
			5×10^{-4} M	10^{-3} M	5×10^{-4} M	10^{-3} M
TV 253	Smooth	Me Paraben	600	450	1.33	1.55
SF 1591	Ra		600	450	1.17	1.67
SF 1567	Rd_1		600	350	1.33	2.14
SF 1398	Re		300	250	2.0	2.0
TV 253	Smooth	Bu Paraben	200	100–125	> 1.25	1.75–2.0
SF 1591	Ra		150	75	1.5	3.0
SF 1567	Rd_1		125	75	1	1.33–2.0
SF 1398	Re		50	10	1	5.0
TV 253	Smooth	CPC	> 250	50	≥ 1	> 5
SF 1591	Ra		> 250	75	≥ 1	> 3
SF 1567	Rd_1		20	7.5	3.75	> 10
SF 1398	Re		< 5	< 5	ca. 1	> 1
TV 253	Smooth	CHA	10	5	1.25	2.5–3.0
SF 1591	Ra		2.5	2.5	2	2
SF 1567	Rd_1		2.5	2.5	2	2
SF 1398	Re		2.5	< 1	1	2.5

* CHA, chlorhexidine diacetate; CPC, cetylpyridinium chloride
** EDTA (10^{-3} M) alone did not inhibit colony formation of smooth, Ra and Rd_1 type strains, but Re-type was sometimes inhibited.
*** Experiments were carried out on two separate occasions in absence and presence of EDTA. MIC ratio = MIC in absence of EDTA/MIC in presence of stated EDTA concentration. The higher the ratio, the greater is the effect of EDTA.

tive to hydrophobic antibiotics and to vancomycin and deoxycholate, but less sensitive to tetracycline and ampicillin, than smooth strains. They made the further interesting points that actinomycin D inhibited RNA synthesis and lysozyme produced lysis in rough mutants in the absence of EDTA, whereas these agents were only effective against the smooth strains after EDTA treatment. When grown in the presence of Mg^{2+}, resistance of the Re strain of *S. typhimurium* was restored, although this was not due to the production of the normal set of outer membrane proteins (Stan-Lotter et al., 1979).

The effect of EDTA on the activity of Me and Pr parabens, CHA and CPC against the four strains demonstrated (Table 9) a moderate to marked increase in susceptibility of all the strains to the parabens, CPC and CHA. The deep rough mutant occasionally failed to grow on media containing 10^{-3} M EDTA. Increasing susceptibility of the parent strain to CHA differs from smooth *E. coli*, where inhibitory levels of the biguanide are virtually the same in the presence and absence of EDTA (Russell and Furr, manuscript in preparation).

Nikaido (1976) proposed that the removal of LPS by EDTA treatment resulted in reorganisation of the outer membrane, with production of exposed phospholipid bilayer regions, thereby allowing rapid penetration of hydrophobic molecules. Mackey (1983) has shown that EDTA will increase the cell surface hydrophobicity of *E. coli*. However Sukupolvi et al. (1984) have described three new classes of *S. typhimurium* mutants which appeared to contain 'normal' LPS but which were as sensitive to hydrophobic agents and to cationic detergents as heptoseless (deep rough) mutants. Overall, the following conclusions may be reached.

(i) Antibacterial activity of the inhibitors is related to their physical properties, in particular their hydrophobic nature, as well as to the hydrophobicity of the cell surface.

(ii) Unlike previous findings with *E. coli*, CHA apparently less readily enters smooth *S. typhimurium* cells than rough cells of this organism.

(iii) EDTA potentiates the activity of hydrophobic inhibitors, and of CHA, against not only the smooth but also against the deep rough strain.

References

Barr, F.S., Moore, G.W. and Gragg, B.J., Potentiating effects of organomercurials on bisphenols. *J. Pharm. Sci.*, 59 (1970) 262–263.

Cook, A.M., Comparative studies of methods of evaluating antibacterial substances. Part I. Evaluation of bacteriostatic action. *J. Pharm. Pharmacol.*, 6 (1954) 629–637.

Croshaw, B., Preservatives for cosmetics and toiletries. *J. Soc. Cosm. Chem.*, 28 (1977) 3–16.

El-Falaha, B.M.A., Russell, A.D. and Furr, J.R., Sensitivities of wild-type and envelope-defective strains of *Escherichia coli* and *Pseudomonas aeruginosa* to antibacterial agents. *Microbios*, 38 (1983) 99–105.

El-Falaha, B.M.A., Russell, A.D., Furr, J.R., and Rogers, D.T., Activity of benzalkonium chloride and chlorhexidine diacetate against wild-type and envelope mutants of *Escherichia coli* and *Pseudomonas aeruginosa*. *Int. J. Pharm.*, 25 (1985) 329–337.

Elkhouly, A.E. and Yousef, R.T., Antibacterial efficiency of mercurials. *J. Pharm. Sci.*, 63 (1974) 681–685.

Grier, N., Mercurials — inorganic and organic. In Block, S.S. (Ed.), *Disinfection, Sterilization and Preservation*, 3rd edn., Lea and Febiger, Philadelphia, 1983, pp. 346–374.

Hansch, C. and Leo, A.J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*, John Wiley and Sons, 1979, Chichester.

Hugo, W.B., Disinfection mechanisms. In Russell, A.D., Hugo, W.B. and Ayliffe, G.A.J. (Eds.), *Principles and Practice of Disinfection, Preservation and Sterilisation*, Blackwell Scientific, 1982, Oxford, pp. 158–185.

Hugo, W.B. and Russell, A.D., Types of antimicrobial agents. In Russell, A.D., Hugo, W.B. and Ayliffe, G.A.J. (Eds.), *Principles and Practice of Disinfection, Preservation and Sterilisation*, Blackwell Scientific, 1982, Oxford, pp. 8–106.

Hugo, W.B., Amidines. In Hugo, W.B. (Ed.), *Inhibition and Destruction of the Microbial Cell*, Academic Press, 1971, London, pp. 121–136.

Ismaeel, N., El-Moug, T., Furr, J.R. and Russell, A.D., Resistance of *Providencia stuartii* to chlorhexidine: a consideration of the role of the inner membrane. *J. Appl. Bacteriol.*, 60 (1986) 361–367.

Mackey, B.M., Changes in antibiotic sensitivity and cell surface hydrophobicity in *Escherichia coli* injured by heating, freezing or gamma radiation. *FEMS Microbiol. Lett.*, 20 (1983) 395–399.

Nikaido, H., Outer membrane of *Salmonella typhimurium*. Transmembrane diffusion of some hydrophobic substances. *Biochim. Biophys. Acta*, 433 (1976) 118–132.

Nikaido, H. and Nakae, T., The outer membrane of Gram-negative bacteria. *Adv. Microbial Physiol.*, 20 (1979) 163–250.

Nikaido, H. and Vaara, T., Molecular basis of bacterial outer membrane permeability. *Microbiol. Rev.*, 49 (1985) 1–32.

Roantree, R.J., Kuo, T.-T. and MacPhee, D.G., The effect of defined lipopolysaccharide core defects upon antibiotic resistances of *Salmonella typhimurium*. *J. Gen. Microbiol.*, 103 (1977) 223–234.

Russell, A.D., Modification of the bacterial cell envelope and enhancement of antibiotic susceptibility. In Stuart-Harris, C.H. and Harris, D.M. (Eds.), *The Control of Antibiotic-Resistant Bacteria: Beecham Colloquium No. 4*, Academic Press, 1982, London, pp. 119–168.

Russell, A.D., Destruction of bacterial spores. In Hugo, W.B. (Ed.) *Inhibition and Destruction of the Microbial Cell*, Academic Press, 1971, London. pp. 451–612.

Russell, A.D. and Furr, J.R., Susceptibility of porin- and lipopolysaccharide-deficient strains of *Escherichia coli* to some antiseptics and disinfectants. *J. Hosp. Infect.*, 6 (1986) 47–56.

Sanderson, K.E., MacAlister, T., Costerton, J.W. and Cheng, K.-J., Permeability of lipopolysaccharide-deficient (rough) mutants of *Salmonella typhimurium* to antibiotics, lysozyme and other agents. *Can. J. Microbiol.*, 20 (1974) 1135–1145.

Stan-Lotter, H., Gupta, M. and Sanderson, K.E., The influence of cations on the permeability of the outer membrane of *Salmonella typhimurium* and other Gram-negative bacteria. *Can. J. Microbiol.*, 25 (1979) 475–485.

Stickler, D.J. and Thomas, B.E., Intrinsic resistance to non-antibiotic antibacterial agents. In Russell, A.D., Hugo, W.B. and Ayliffe, G.A.J. (Eds.), *Principles and Practice of Disinfection, Preservation and Sterilisation*, Blackwell Scientific, 1982, Oxford, pp. 186–198.

Sukupolvi, S., Vaara, M., Helander, I.M., Viljanen, P. and Makela, P.H., New *Salmonella typhimurium* mutants with altered outer membrane permeability. *J. Bacteriol.*, 159 (1984) 704–712.

Wallhausser, K.H., Antimicrobial preservatives used by the cosmetic industry. In Kabara, J.J. (Ed.), *Cosmetic and Drug Preservation: Principles and Practice*, Marcel Dekker, New York, 1984, pp. 605–745.