Journal of Industrial Microbiology, 13 (1994) 77–82

Published by The Macmillan Press Ltd

Enhancement of mycobactericidal activity of glutaraldehyde with α,β-unsaturated and aromatic aldehydes

M. D. Gordon, R. J. Ezzell, N. I. Bruckner and J. M. Ascenzi

Research Division, Johnson and Johnson Medical, Inc., PO Box 130, Arlington, TX 76004, USA

(Received 28 May 1993; revision received 2 October 1993; accepted 21 October 1993)

Key words: Glutaraldehyde; Mycobactericidal; Aldehydes

SUMMARY

Several α,β-unsaturated and aromatic aldehydes were evaluated for antimicrobial activity using *Mycobacterium bovis* as the test strain. Activity of most of the compounds was determined in the presence and absence of 2% glutaraldehyde. Several compounds highly active against this organism, e.g. 2-pentenal, benzaldehyde, and *o*-phthalaldehyde showed rapid kill of $>10^5$ CFU ml^{-1} in 5 min. Activity of α,β-unsaturated compounds substituted in the β_1 position showed increasing activity with increasing chain length. Of the aromatic aldehydes tested, benzaldehyde and *p*-dimethylamino benzaldehyde showed little activity alone, but when combined with 2% glutaraldehyde showed increased activity. Substituents added to the benzaldehyde ring (nitro, chloro, methyl, and methoxy) all detracted from the synergism, but still showed increased activity over the activity of 2% glutaraldehyde. The same affect was noted with disubstituted benzaldehyde compounds but not with substituted *o*-phthalaldehyde (2-formylformaldehyde).

INTRODUCTION

Aldehydes have biological activity which includes inhibition of metabolism in both eucaryotic [28,29] and procaryotic organisms [7,14,20,22,23], antitumor effects [30], cell division [12], and static and cidal activity against various bacteria [2,4,26], fungi [1,10,11,13], and viruses [3,18,27]. Several aldehydes occur naturally and have antimicrobial activities that provide protective mechanisms. For example; *trans*-2-hexenal, a fungicide, occurs naturally in the damaged leaves of *Gingko biloba* protecting the leaves from infection by fungi [19]. Citral and other terpene aldehydes found in grasses inhibit the growth of microbes in the stomachs of ruminants, thereby hindering complete utilization of ingested food [24].

Several factors influence the activity of aldehydes, such as carbon chain length, substituent groups, and degree of bond saturation. These factors affect the reactivity of the carbonyl carbon with sulfhydryl and amino groups, thereby affecting reaction with proteins and nucleic acids. Damage or rearrangement of these molecules can be the cause of the effects mentioned above. α,β-Unsaturated aldehydes react primarily with sulfydryl groups (primarily on proteins) forming a saturated aldehyde with a thioether linkage. The reactivity of the carbonyl group of the α,β-unsaturated aldehyde is affected by substituent groups on the carbon chain. The activity of aromatic aldehydes is also related to the reactivity of the carbonyl carbon(s), as demonstrated by the fact that while benzaldehyde is an active antimicrobial, benzyl alcohol and benzoic acid are not. The reactivity is also affected by substituent groups on the aromatic ring [28].

Glutaraldehyde (GA) is one of the most biologically active alkyl aldehydes in terms of its ability to kill bacteria, fungi, and viruses [5,16]; however, it does not possess the rapid mycobactericidal activity required by users of liquid disinfectants [9]. Extended exposure time above that effective for other vegetative cells is required for complete kill of $\geq 10^5$ CFU *Mycobacterium bovis* BCG. This is true for *Mycobacterium tuberculosis*, and most of the atypical mycobacteria. It has been demonstrated with *Mycobacterium cheloniae*, that a barrier to aqueous solutions exists [17] and lack of penetration of aqueous antimicrobials into the cell may account for their reduced activity.

In this paper we report the mycobactericidal activity of α,β-unsaturated aldehydes and aromatic aldehydes for their activity in the presence and absence of GA. Mycobactericidal activity is examined as a measure of activity since mycobacteria appear to be the most difficult of the vegetative organisms to inactivate with GA. Any increased activity provided by the added compounds can easily be discerned.

MATERIALS AND METHODS

Mycobacterial assays

Mycobacterium bovis ATCC 35743 was grown according to a previously published method [2]. Cells were stored in 2-ml aliquots at −70 °C until used. Frozen cultures were thawed at room temperature, diluted with saline containing 0.05% Tween 80 (Difco, Detroit, MI, USA) to a titer of approximately 10^5 CFU ml^{-1}. One milliliter of the cell suspension was added to 9 ml of the test solution at 20 °C

Correspondence to: J.M. Ascenzi, Research Division, Johnson and Johnson Medical, Inc., PO Box 130, Arlington, TX 76004, USA.

and aliquots removed at time intervals, diluted in an equal volume of neutralizer, serially diluted in saline and collected on 0.45-μm pore size membrane filters (Millipore Corp., Medford, MA, USA) which were placed onto Middlebrook 7H10 (Difco) with OADC (oleic acid-albumin-dextrose-catalase) enrichment (Difco) agar plates. Duplicate samples were plated and incubated for 21 days at 37 °C and counted using a binocular microscope. Data were plotted as survivors (S/S_0, where S is the number of organisms at any given time point and S_0 is the number of organisms at zero time).

Test solutions

GA solutions were prepared at a 2% concentration at a pH of 7.5. α,β-Unsaturated and aromatic aldehydes were added to the GA at the same molar concentration or to the limit of their solubility.

Neutralizer

Neutralization of aldehydes was accomplished with sodium bisulfite at a concentration 2.2 times the concentration of the total aldehyde concentration in the test solution. Antimicrobial activity of the neutralizer was assessed by the addition of equal volumes of neutralizer and test solution and spiking the mixture with 10^6 CFU ml^{-1} *M. bovis* and incubating it under the test conditions and plating for survivors up to an hour.

RESULTS

α,β-Unsaturated aldehydes

The structures of the compounds studied are shown in Table 1. Table 2 shows the relative activities of α,β-unsaturated monoaldehydes when combined with 2% GA solution. 2-Propenal had the greatest enhancement of activity against *M. bovis* BCG. This compound has no substituents on the carbon backbone. All other substituted compounds, except 2-hexenal, showed less activity, but still showed enhanced activity over 2% GA. Substitution in both β-positions (3-methyl-2-butenal) or in the α- and one of the β-positions (2-methyl-2-butenal), resulted in reduced activity relative to that of 2-propenal. 2-Hexenal and 3-phenyl-2-propenal are two compounds in this series that had low toxicity (as indicated by their widespread use in the food/flavor industry) and good activity enhancement. The most active compound, 2-propenal, is a highly toxic compound not likely to be used in products having the potential to come into contact with humans.

TABLE 1

Structure of α,β-unsaturated aldehydes studied

2-propenal	$H_2C{=}CH{-}CHO$
3-methyl-2-butenal	$H_3C{-}\underset{\displaystyle CH_3}{\underset{\vert}{C}}{=}C{-}CHO$
2-methyl-2-butenal	$H_3C\ \ CH{=}\underset{\displaystyle CH_3}{\underset{\vert}{C}}{-}CHO$
trans-2-hexenal	$H_3C{-}CH_2{-}CH_2{-}CH{=}CH{-}CHO$
2-pentenal	$H_3C{-}CH_2{-}CH{=}CH{-}CHO$
2-butenal	$H_3C{-}CH{=}CH{-}CHO$
3-phenyl-2-propenal	$\bigcirc{-}CH{=}CH{-}CHO$
2,4-hexadienal	$H_3C{-}CH{=}CH{-}CH{=}CH{-}CHO$

TABLE 2

Effect of α,β-unsaturated monoaldehydes on mycobactericidal activity of alkaline 2% glutaraldehyde solutions[a]

2% Glutaraldehyde + monoaldehyde (% w/w[b])	Time for total kill at 20 °C (min)
None (2.0% GA)	>90
2-propenal (0.19)	5–10
2-methyl-2-propenal (0.23)	30
2-butenal (0.23)	30
2-methyl-2-butenal (0.28)	60–70
3-methyl-2-butenal (0.28)	40–45
2-pentenal (0.28)	30
2-hexenal (0.33)	10
3-phenyl-2-propenal (0.10[c])	20
2,4-hexadienal (0.32)	20

[a]Test solutions were buffered with 0.6% dipotassium hydrogen phosphate. The pH of the test solutions was adjusted to 8.0 with 1*N*H_3PO_4.

[b]Equivalent to 3.33 mmol of monoaldehyde per 100 g of solution, except as noted.

[c]Solubility limit, equivalent to 0.77 mmol per 100 g of solution.

Of the series of 2-alkenal compounds in which an alkyl group is substituted in one β-position, the activity enhancement follows the order:

2-Hexenal > 2-Pentenal > 2-Butenal

β_1 = $CH_3CH_2CH_2$ CH_3CH_2 CH_3

The longer the alkyl chain, the greater is the activity (substituents greater than four carbons are not water soluble). The activity of several of the α,β-unsaturated monoaldehydes in the presence and absence of 2% GA is shown in Fig. 1. The data indicate enhanced activity of the combination over the activity of the 2% GA or monoaldehyde alone. 2-Butenal and *trans*-2-hexenal showed activity at low concentrations (<0.5%). 2-Propenal (0.19%) achieved complete kill of 10^5 CFU ml^{-1} *M. bovis* in ≤2 min. Neutralization controls indicate complete neutralization of the antimicrobial activity.

Aromatic aldehydes

A series of studies was done looking at the effect of mycobactericidal activity of aromatic aldehydes (structures shown in Table 3) in combination with GA. Table 4 shows increased activity with the addition of 0.3% benzaldehyde (BA) to solutions of 0.5 to 2.0% GA. Complete kill of $>10^5$ CFU ml^{-1} was achieved in <20 min even with the lowest level of glutaraldehyde tested, whereas 2% and 3% GA alone require >60 min for complete kill of the same test population.

Several monosubstituted BA compounds (Table 5) were evaluated in combination with 2% glutaraldehyde. Substitu-

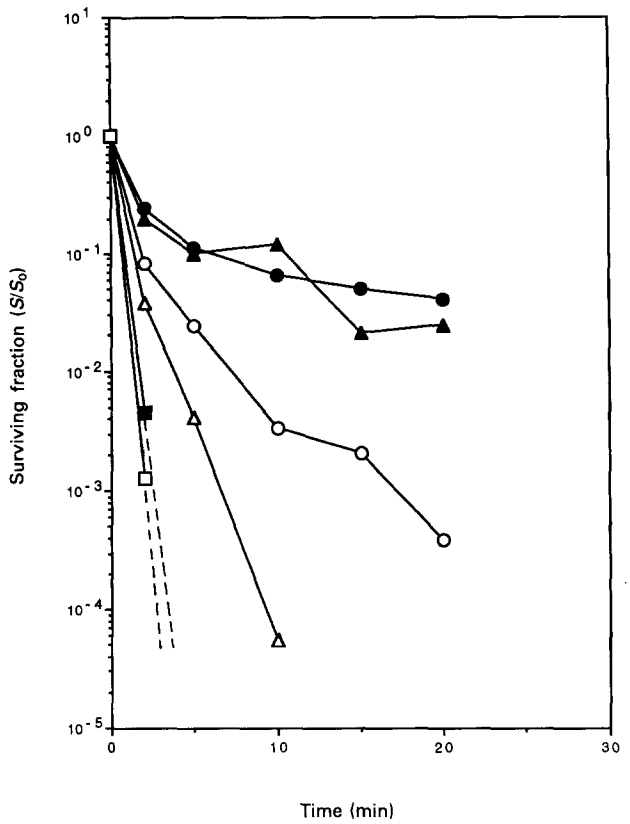


Fig. 1. Mycobactericidal activity of 2-butenal (—●—), *trans*-2-hexenal (—▲—), glutaraldehyde (—○—), GA + 2-butenal (—△—), GA + *trans*-2-hexenal (—□—), GA + 2-propenal (—■—). 2-propenal alone produced complete kill in <2 min.

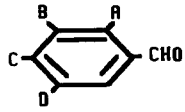
ents may also be present in the 2 (ortho-) and 3 (meta-) positions of BA with retention of good mycobactericidal activity enhancement. Although none had the enhancement effect of benzaldehyde, all provided enhancement that increased the activity over that of 2% glutaraldehyde. The activity of two representative aromatic aldehydes, benzaldehyde and *p*-dimethylamino benzaldehyde in the presence and absence of 2% GA are shown in Fig. 2. Neither showed activity alone, however, each increased the mycobactericidal activity of 2% GA.

The disubstituted BA compounds, 4-hydroxy-3-methoxy-, 3-hydroxy-4-methoxy-, 3,4-dioxymethylene-, 3,4,-dihydroxy-, and 3,4-dimethoxy-, were evaluated for activity when added to 2% GA at concentrations ≤0.55%. All resulted in enhancement activity and 3,4-dioxymethylene benzaldehyde had the greatest affect (Table 6). The aromatic dialdehyde, 2-formylbenzaldehyde (*o*-phthalaldehyde, OPA) was evaluated for activity when added to 2% GA. The activity of this combination was due largely to OPA as shown in Fig 3. These data indicate that OPA rapidly inactivates 10^5 CFU ml^{-1} *M. bovis* in less than 10 min at all three concentrations tested with complete kill in less than 5 min at 20 °C with 0.45% OPA.

Several monosubstituted OPA compounds (4-methoxy-, 4-hydroxy-, 4-chloro-, and 4-carboxy-OPA) were compared to OPA for mycobactericidal activity in the absence of 2%

TABLE 3

Structure of aromatic aldehydes evaluated



Compound	Substituent groups		
	A	*B*	*C*
Benzaldehyde	H	H	H
2-Hydroxybenzaldehyde	OH	H	H
3-Hydroxybenzaldehyde	H	OH	H
4-Hydroxybenzaldehyde	H	H	OH
4-Methoxybenzaldehyde	H	H	OCH_3
4-Methylbenzaldehyde	H	H	CH_3
4-Chlorobenzaldehyde	H	H	Cl
4-Nitrobenzaldehyde	H	H	NO_3
p-Dimethylaminobenzaldehyde	H	H	$N(CH_3)_2$
4-Hydroxy-3-Methoxybenzaldehyde	H	OCH_3	OH
3-Hydroxy-4-Methoxybenzaldehyde	H	OH	OCH_3
3,4-Dioxymethylenebenzaldehyde	H	OCH_3	OCH_3
3,4-Dihydroxybenzaldehyde	H	OH	OH
3,4-Dimethoxybenzaldehyde	H	OCH_3	OCH_3
2-Formylbenzaldehyde	CHO	H	H
4-Methoxy-2-Formylbenzaldehyde	CHO	H	OCH_3
4-Hydroxy-2-Formylbenzaldehyde	CHO	H	OH
4-Chloro-2-Formylbenzaldehyde	CHO	H	Cl
4-Carboxy-2-Formylbenzaldehyde	CHO	H	COOH
3-Carboxy-4-Methoxy-5-Methyl-2-Formylbenzaldehyde	CHO	COOH	OCH_3 (D-CH_3)

TABLE 4

Effect of glutaraldehyde concentration on mycobactericidal activity of benzaldehyde[a]

Concentration		Time for total kill at 20 °C (min)
Glutaraldehyde % (w/w)	Benzaldehyde % (w/w)	
2.0	—	>90
3.0	—	75
2.0	0.30	10
1.0	0.30	15
0.5	0.30	20

[a]See footnote a, Table 2.

GA (data not shown). The activity of 0.13% 4-methoxy-OPA was somewhat greater than that of an equimolar solution of OPA, while the 4-chloro compound had similar activity. However, the 4-hydroxy- derivative had less activity than OPA, and 4-carboxy-OPA had no substantial activity as did the trisubstituted OPA compound 3-carboxy-4-methoxy-5-methyl-OPA.

TABLE 5

Effect of monosubstituted benzaldehydes on mycobactericidal activity of 2% alkaline glutaraldehyde solution[a]

Monoaldehyde (%w/w[b])	Time for total kill at 20 °C (min)
Benzaldehyde (0.30[c])	10
2-Hydroxybenzaldehyde (0.41) (salicylaldehyde)	20
3-Hydroxybenzaldehyde (0.41)	20
4-Hydroxybenzaldehyde (0.41)	30
4-Methoxybenzaldehyde (0.45) (*p*-Anisaldehyde)	20
4-Methylbenzaldehyde (0.18[d]) (*p*-Tolualdehyde)	20
4-Chlorobenzaldehyde (0.06[e])	20
4-Nitrobenzaldehyde (0.06[f])	30

[a]See footnote a, Table 2.
[b]See footnote b, Table 2.
[c]Solubility limit equivalent to 2.83 mmol per 100 g of solution.
[d]Solubility limit equivalent to 1.50 mmol per 100 g of solution.
[e]Solubility limit equivalent to 0.43 mmol per 100 g of solution.
[f]Solubility limit equivalent to 0.40 mmol per 100 g of solution.

TABLE 6

Effect of disubstituted benzaldehydes on mycobactericidal activity of 2% alkaline glutaraldehyde[a]

Monoaldehyde (%w/w[b])	Time for total kill at 20 °C (min)
Benzaldehyde (0.30[c])	10
4-Hydroxy-3-Methoxybenzaldehyde (0.51) (Vanillin)	30–35
3-Hydroxy-4-Methoxybenzaldehyde (0.20) (Isovanillin)	35–40
3,4-Dioxymethylenebenzaldehyde (0.30[e]) (Piperonal)	20
3,4-Dihydroxybenzaldehyde (0.46)	70–90
3,4-Dimethoxybenzaldehyde (0.55) (Veratraldehyde)	35

[a]See footnote a, Table 2.
[b]See footnote b, Table 2.
[c]See footnote c, Table 5.
[d]Solubility limit equivalent to 1.31 mmol per 100 g of solution.
[e]Solubility limit equivalent to 2.00 mmol per 100 g of solution.

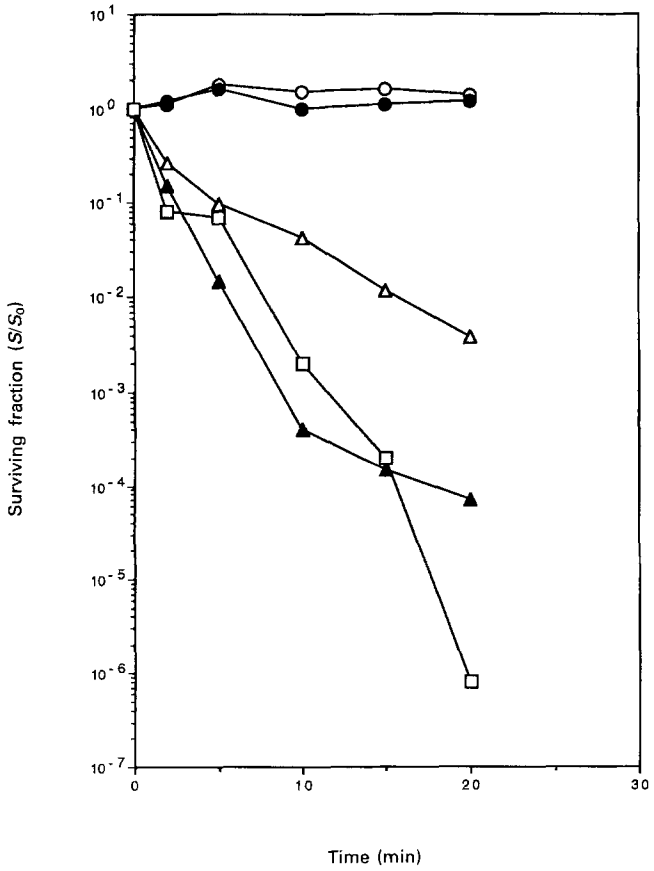


Fig. 2. Mycobactericidal activity of *p*-dimethyl amino benzaldehyde (—○—), benzaldehyde (—●—), GA (—△—), GA + *p*-DMB (—▲—), and GA + benzaldehyde (—□—).

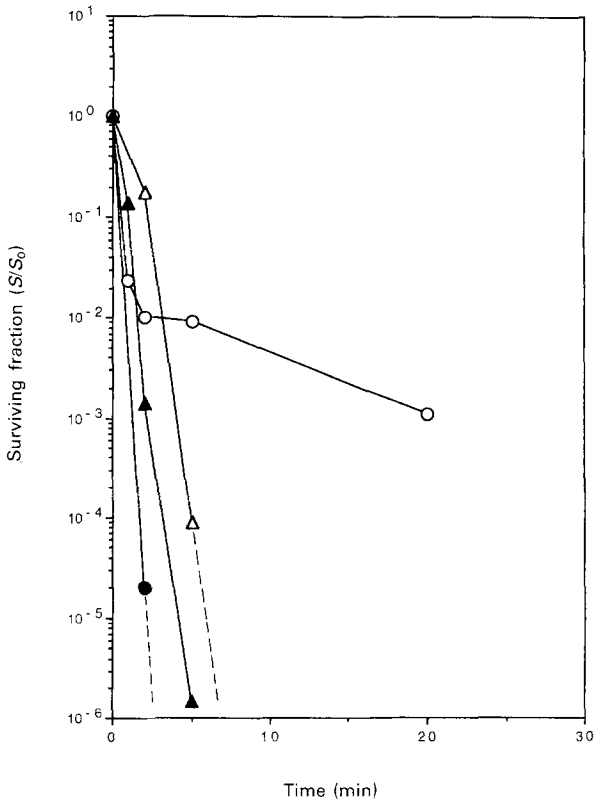


Fig. 3. Mycobactericidal activity of *o*-phthalaldehyde, 0.1% (—△—), 0.2% (—▲—), and 0.45% (—●—) compared to 2% glutaraldehyde (—○—).

DISCUSSION

Glutaraldehyde has been used in disinfectant formulations since the original description of the antimicrobial properties of GA by Pepper and Chandler [25]. The excellent biocidal properties have been documented regardless of the formulation although alkaline glutaraldehyde is a better sporicide than acid or neutral GA [15,21]. This pH dependence does not exist with other bacteria including mycobacteria.

The mycobactericidal properties of glutaraldehyde are less than its activity against other Gram-positive and Gram-negative bacteria. This is a concern for healthcare workers since GA has been the chemical of choice for disinfection of critical equipment. Cole et al. [8] showed that regardless of the claim on glutaraldehyde formulations, the reality of the situation requires that at least a 30-min soak of medical equipment is necessary for mycobactericidal activity whereas most vegetative organisms are eradicated in 10 min or less.

An alternative to GA as a disinfectant is desired because of the intermediate mycobactericidal activity, toxicity, and irritation potential. α,β-Unsaturated and aromatic aldehydes have been used in the flavor and food industry and therefore have been identified as safe for use in products that contact humans. An examination of their antimicrobial properties indicates that several may have excellent activity while others have none. In combination with GA, many of these additives can substantially increase the mycobactericidal activity of the formulation. Their activity appears to be related to the substituent groups on the molecule.

α,β-Unsaturated aldehydes show increased activity with increased chain length of the substituent group on the β_1 position, probably due to increased hydrophobicity and better penetrability of the molecule through the lipid matrix of the cell wall-membrane of mycobacteria. Disubstitution, whether in the α, β_1 or β_1, β_2 positions reduced the enhancement effect of the aldehyde significantly. This may be due to steric hindrance in the formation of the 1,4 addition product with amine or sulfhydryl groups, significantly reducing the antimicrobial activity of the aldehyde.

With the aromatic aldehydes, it is evident that either electron releasing (methoxy, methyl, chloro) or electron withdrawing (nitro) substituents in the 4 (para-) position of BA results in increased mycobactericidal activity. 4-Hydroxy BA showed the least amount of activity enhancement, which may reflect a pH dependency. Burton et al. [6] showed that the antimicrobial activity of substituted benzaldehydes was directly related to the partition coefficient of the aldehyde in hydrocarbon solvent. This indicated that activity may be related to its ability to penetrate the lipid cell wall-membrane complex. This is of particular relevance with the highly lipid cell wall-membrane complex of mycobacteria which possesses a barrier to aqueous solutes [17] lending support to the hypothesis that more hydrophobic chemicals are more likely to penetrate mycobacterial cells. Although penetration of the compound into the cell plays an important role in antimicrobial activity, the activity of the benzaldehyde compounds is directly related to the reactivity of the formyl group. While benzaldehyde is active at fairly low concentrations, benzoic acid and salicylic acid at concentrations as high as 10% in a 2% GA formulation show little or no activity enhancement over the activity of GA. This is supported by the work of Burton et al. [6] in which benzyl alcohol and benzoic acid were shown to be relatively inactive when compared to benzaldehyde.

REFERENCES

1 Aharoni, Y. and R. Barkai-Golan. 1973. Sensitivity to acetaldehyde vapours of *Alternaria tunuis* and *Stemphylium botryosum*. Phytopathol. Zeitschrift 78: 57–61.

2 Ascenzi, J.M., R.J. Ezzell and T.M. Wendt. 1987. A more accurate method for measurement of mycobactericidal activity of disinfectants. Appl. Eviron. Microbiol. 53: 2189–2192.

3 Bachrach, U., S. Don and H. Wiener. 1971. Antivirus action of acrolein, glutaraldehyde and oxidized spermine. J. Gen. Virol. 13: 415–422.

4 Beilfuss, W. 1976. Vergleichende untersuchungen der antimikrobiellen wirksamkeit α,β-ungesattigter aldehyde. Zbl. Bakt. Hyg., I. Abt. Orig. A 234: 271–280.

5 Boucher, R.M.G. 1975. On biocidal mechanisms in the aldehyde series. Can. J. Pharm. Sci. 10: 1–7.

6 Burton, D.E., K. Clarke and G.W. Gray. 1964. The mechanism of the antibacterial action of phenols and salicylaldehydes. Part III. Substituted benzaldehydes. J. Chem. Soc. July (1964): 2458–2460.

7 Chan, K. and T.M. Lau. 1979. Effect of formaldehyde on oxygen uptake and β-galactosidase activity in *Enterobacter aerogenes*. Microbios. Lett. 10: 69–74.

8 Cole, E.C., W.A. Rutala, L. Nessen, N.S. Wannamaker and D.J. Weber. 1990. Effect of methodology, dilution, and exposure time on the tuberculocidal activity of glutaraldehyde-based disinfectants. Appl. Environ. Microbiol. 56: 1813–1817.

9 Collins, F. 1986. Comparison of bactericidal activity of alkaline glutaraldehyde solution against a number of atypical mycobacterial species. J. Appl. Bacteriol. 61: 247–251.

10 Dabrowa, N., J.W. Landau and V.D. Newcomer. 1972. Antifungal activity of glutaraldehyde in vitro. Arch. Dermatol. 105: 555–557.

11 Dennis, C. and A. Gaunt. 1974. Effect of formaldehyde on fungi from broiler houses. J. Appl. Bacteriol. 37: 595–601.

12 Egyud, L.G. 1967. Studies on cell division: the effect of aldehydes, ketones and α-keto-aldehydes on the proliferation of *Escherichia coli*. Curr. Molec. Biol. 1: 14–20.

13 Gorman, S.P. and Scott, E.M. 1977a. A quantitative evaluation of the antifungal properties of glutaraldehyde. J. Appl. Bacteriol. 43: 83–89.

14 Gorman, S.P. and E.M. Scott. 1977b. Transport capacity, alkaline phosphatase activity and protein content of glutaraldehyde-treated cell forms of *Escherichia coli*. Microbios. 19: 205–212.

15 Gorman, S.P. and E.M. Scott. 1977c. Effect of alkalinization of the bacterial cell and glutaraldehyde molecule. Microbios. Lett. 6: 39–44.

16 Gorman, S.P., E.M. Scott and A.D. Russell. 1980. A review. Antimicrobial activity, uses and mechanism of action of glutaraldehyde. J. Appl. Bacteriol. 48: 161–190.

17 Jarlier, V. and H. Nikaido. 1990. Permeability barrier to hydrophilic solutes in *Mycobacterium chelonei*. J. Bacteriol. 172: 1418–1423.

18 Kremzner, T.L. and D.H. Harter. 1970. Antiviral activity of

oxidized polyamines and aldehydes. Biochem. Pharmacol. 19: 2531–2550.

19 Major, R.T., P. Marchini and A.J. Boulton. 1963. Observation on the production of α-hexenal by leaves of certain plants. J. Biol. Chem. 238: 1813.

20 McGucken, P.V. and W. Woodside. 1973. Studies on the mode of action of glutaraldehyde on *Escherichia coli*. J. Appl. Bacteriol. 36: 419–426.

21 Munton, T. J. and A.D. Russell. 1970. Aspects of the action of glutaraldehyde on *Escherichia coli*. J. Appl. Bacteriol. 33: 410–419.

22 Munton, T.J. and A.D. Russell. 1972. Effect of glutaraldehyde on the outer layers of *Escherichia coli*. J. Appl. Bacteriol. 35: 193–199.

23 Munton, T.J. and A.D. Russell. 1973. Effect of glutaraldehyde on cell viability, triphenyltetrazolium reduction, oxygen uptake, and β-galactosidase activity in *Escherichia coli*. Appl. Microbiol. 26: 508–511.

24 Oh, H.K., T. Sakai, M.B. Jones and W.M. Longhurst. 1967. Effect of various essential oils from Douglas fir needles upon sheep and deer rumen microbial activity. Appl. Microbiol. 15: 777–784.

25 Pepper, R.E. and V.L. Chandler. 1963. Sporicidal activity of alkaline alcoholic saturated dialdehyde solutions. Appl. Microbiol. 11: 384–388.

26 Rehn, D. and H. Nolte. 1979. Zur antimikrobiellen wirksamkeit substituierter aromatischer aldehyde und alkohole. Zbl. Bakt. Hyg., I. Abt. Orig. B 168: 507–516.

27 Sabel, F.L., A. Hellman and J.J. McDade. 1969. Glutaraldehyde inactivation of virus in tissue. Appl. Microbiol. 17: 645–646.

28 Scaife, J.F. 1970. Modification of the cytotoxic action of hydroxypentenal on cultured mammalian cells. Naturwissenschaften 57: 250–252.

29 Schauenstein, E. 1967. Autoxidation of polyunsaturated esters in water: chemical structure and biological activity of the products. J. Lipid Res. 8: 417–428.

30 Schauenstein, E., J. Zangger and M. Ratzenhofer. 1964. Uber die wirkung von hydroxyoctenal auf Ehrlich-acites-tumorzellen. Z. Naturforsch. 19b: 923.