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The mycoplasmacidal properties of sodium hypochlorite

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SUMMARY

The effect of hypochlorite concentration on *Mycoplasma mycoides* ssp. *mycoides* viability was tested under a variety of conditions. The experimental variables employed included chlorine-cell contact time, chlorine concentration, carrier system and organic loading. Initial populations of 10^6 c.f.u./ml were killed (no survivors in 1 ml) by hypochlorite solution containing 25 p.p.m. available chlorine in 15 s in the absence of organic load and 50 p.p.m. available chlorine in 5 min in the presence of 1 % protein. Higher concentrations of hypochlorite were required to disinfect a porous carrier system in the absence or presence of protein. The results are in contrast to previous reports that *M. bovis* is killed only by high hypochlorite concentrations.

INTRODUCTION

Aqueous chlorine (hypochlorous acid [HOCl]), generally accepted as a universal water disinfectant, is a potent bactericide and virucide under optimal conditions (Dychdala, 1977) and is finding an ever increasing field of usefulness in the sterilization of milk and beverage bottles (Berger & Illingworth, 1971), dishes and utensils of various kinds and in the general disinfection of surfaces. At, or close to, pH 7 it is effective in micromolar concentrations (Tonney, Greer & Danforth, 1928; Tonney, Greer & Liebig, 1930; Kelly & Sanderson, 1958; Scarpino *et al.* 1972; Englebrecht *et al.* 1980; Harakeh & Butler, 1984), provided that the water is free from ammonia and organic compounds which contain nitrogen; in the presence of these compounds, however, the effectiveness of chlorine is diminished due to reactions yielding chloramines or other chloro-organic compounds, which are not potent biocides (Heicken, 1956; Dychdala, 1977).

Although there is a considerable literature on the effects of chlorine on viruses (e.g. Englebrecht *et al.* 1980; Scarpino *et al.* 1972) bacterial vegetative cells and spores (e.g. Tonney, Greer & Danforth, 1928; Tonney, Greer & Liebig, 1930; Foegeding, 1983), fungal vegetative cells and spores (Haas & Englebrecht, 1980; Rosenzweig, Minnigh & Pipes, 1983), protozoan cells and cysts (e.g. De Jonckheere

& Van de Voorde, 1976; Cursons, Brown & Keys, 1980) and algae (Kott, 1979), little is known about the conditions for mycoplasmacidal activity.

It is generally assumed that chlorine solutions are effective in killing mycoplasmas; however, we have found only two reports on this subject in the literature. These concern the cattle pathogen *Mycoplasma bovis*, which has been shown to be sensitive to high levels of hypochlorite: 100 p.p.m. available chlorine (av. Cl) for 1 min in the absence of protein and 10000 p.p.m. av. Cl for 1 min in the presence of milk (Jasper, Dellinger & Hakanson, 1976); 400 p.p.m. av. Cl for 1 min in the absence of protein and 3200 p.p.m. av. Cl for 60 or 20 min in the presence of milk or broth, respectively (Pfutzner, Scherwa & Trubner, 1983). These levels are much higher than would be expected for activity against an organism lacking a cell wall (q.v., 100% kill of *Escherichia coli*, 10 p.p.m. av. Cl for 15 s, Ferguson & Gibson, 1971), and are in contrast to the observations of Witzleb & Enghardt (1983), who found comparable antimicrobial activity of commonly used antiseptics (e.g. hexachlorophene, chlorhexidine gluconate) against human strains of mycoplasmas and *E. coli* and *Staphylococcus aureus*.

In man, mycoplasmas may cause pneumonia and genito-urinary disease (Taylor-Robinson & McCormack, 1979) and are possibly associated with arthritis (Cole & Ward, 1979) and other diseases of uncertain aetiology. Mycoplasmas also include many pathogens of importance in veterinary medicine (Van der Goot & Pijper, 1984), and cause major problems as contaminants of tissue cultures.

In this study we have investigated the mycoplasmacidal effectiveness of sodium hypochlorite using *Mycoplasma mycoides* ssp. *mycoides* as the test organism. This organism is the causative agent of bovine pleuropneumonia, a disease of economic significance in many African countries, India and elsewhere (Gourlay & Howard, 1979). 'Milton' solution was used as the source of sodium hypochlorite; this preparation has been reported to be stable, in a diluted form, for at least 1 year at room temperature (Morris, Kingsley & Chinwah, 1980). Its effectiveness against the test organism was investigated in the absence of organic matter and in the presence of various organic loads. Also, to simulate the use of hypochlorite in the disinfection of surfaces, we have further investigated its effectiveness against mycoplasmas absorbed into unglazed porcelain carriers. We considered it possible that, because of their small size, mycoplasmas might be absorbed deeper into surfaces, and therefore be better protected against disinfectants, than larger organisms.

MATERIALS AND METHODS

Organism

Mycoplasma mycoides ssp. *mycoides* strain T_1 was obtained from Dr G. R. Smith, Nuffield Laboratories of Comparative Medicine, Regent's Park, London, England.

Hypochlorite solutions

Hypochlorite solutions were dilutions of 'Milton'. The consumer product 'Milton' (Richardson-Vicks Ltd, Egham, Surrey, England) is sodium hypochlorite and 16·5% (w/v) sodium chloride. It is supplied as a 1 or 2% (w/v) solution of sodium hypochlorite; the latter was used here.

Media

Broth medium consisted of (g/l except where stated): tryptose (Oxoid), 20; yeast extract (Oxoid), 5; glucose, 5; glycerol, 5; benzylpenicillin, 0·125; Na_2HPO_4, 8·1; KH_2PO_4, 1·9; NaCl, 2·5; pig serum (Gibco) inactivated at 56 °C for 30 min, 100 ml/l. All components other than benzylpenicillin and serum were autoclaved together (121 °C for 15 min) after adjustment of the pH to 7·6. Benzylpenicillin was sterilized by membrane filtration (Millipore, pore size 0·22 μm). The complete medium was dispensed in 9 ml aliquots in disposable screw-top test tubes (Sterilin). Colony counts were made on agar containing (g/l except where stated): blood agar base (Oxoid), 30; glycerol, 10; benzylpenicillin, 0·125 and pig serum, 225 ml/l.

Preparation of inocula

A 48 h broth culture of *M. mycoides* with glycerol (1·5 M) as a cryoprotectant was dispensed in 2 ml aliquots in screw-top polypropylene ampoules (Sterilin), frozen in liquid nitrogen vapour to −80 °C at a cooling rate of approximately 17 °C/min, and plunged into liquid nitrogen. Stored ampoules were thawed in a water bath (37 °C for 4 min) and used to inoculate freshly prepared broth medium (200 μl per 9 ml of broth). After 48 h incubation at 37 °C cultures were diluted 1 in 20 in 1/4 strength Ringer solution to provide inocula for suspension and carrier tests. The viable count of inocula was in all cases $\geqslant 2 \times 10^7$ c.f.u./ml, and remained constant for a minimum of 4 h, whereas inoculations were always complete within 1 h.

Suspension tests

One ml of inoculum of *M. mycoides* suspension was added to acid-washed universal bottles containing 19 ml of 1/4 strength Ringer solution (pH 7·2) with various concentrations of hypochlorite and, where appropriate, 1·05% (w/v) filter-sterilized bovine serum albumin (BSA, Sigma A-8022). Bottles were shaken immediately and incubated at room temperature (19 °C). At suitable times, 1 ml aliquots were transferred to 9 ml of broth and mixed. Viable counts of broths and of tenfold serial dilutions in 1/4 strength Ringer solution were made in duplicate, using a surface drop-plate method (Postgate, 1969) and inoculating 80 μl (4 drops). Broths and plates were incubated for 7 days at 37 °C. Growth in broth cultures was determined visually and confirmed by optical density measurement (EEL colorimeter, OGR1 filter, 540 nm).

In all experiments 1 ml aliquots from broths which had received the shortest exposure time necessary to prevent visible growth at a particular hypochlorite concentration were subcultured to fresh broth. None of these subcultures showed visible growth after 7 days at 37 °C.

Carrier tests

The test employed was essentially similar to the Mallman Carrier Method as modified for Regulatory Sanitizer Evaluations (Shaffer, 1977). Porcelain carriers (16 mm, PBI porcelain insulators, Wellco Electric Ltd) were immersed in a suspension of *M. mycoides* prepared by adding 10 ml of inoculum to 190 ml of 1/4

Table 1. *The effect of hypochlorite on the survival of* M. mycoides (*c.f.u.*) *in the absence of organic load*

Hypochlorite conc. (p.p.m. av. Cl)	Time of exposure (min)								
	¼	½	1	2	5	10	15	30	90
0	$4{\cdot}2\times10^6$	ND	ND	ND	ND	ND	ND	ND	$4{\cdot}1\times10^6$
1	$3{\cdot}8\times10^6$	$3{\cdot}7\times10^6$	$4{\cdot}0\times10^6$	$3{\cdot}4\times10^6$	$3{\cdot}6\times10^6$	$3{\cdot}5\times10^6$	$3{\cdot}5\times10^6$	$3{\cdot}4\times10^6$	$3{\cdot}4\times10^6$
2	$3{\cdot}9\times10^6$	$4{\cdot}0\times10^6$	$4{\cdot}0\times10^6$	$3{\cdot}6\times10^6$	$3{\cdot}8\times10^6$	$3{\cdot}5\times10^6$	$3{\cdot}4\times10^6$	$3{\cdot}2\times10^6$	$3{\cdot}0\times10^6$
5	$2{\cdot}8\times10^6$	$2{\cdot}1\times10^6$	$1{\cdot}4\times10^6$	$1{\cdot}1\times10^6$	$5{\cdot}1\times10^5$	$5{\cdot}7\times10^4$	$3{\cdot}6\times10^3$	2×10^2	+
10	$1{\cdot}4\times10^6$	$1{\cdot}2\times10^6$	$1{\cdot}3\times10^6$	$8{\cdot}2\times10^5$	$3{\cdot}0\times10^5$	$2{\cdot}4\times10^3$	2×10^2	+	+
25	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0

M. mycoides was suspended in various concentrations of hypochlorite at an initial density of $4{\cdot}2\times10^6$ c.f.u./ml. One ml aliquots were transferred to broth and the number of survivors (c.f.u.) determined by plate counts. +, Growth in broth but no colonies observed on plates; 0, no growth in broth or on plates; +/0, conflicting results for growth in broth in replicate experiments in which plate counts were not determined.

ND, Not done.

strength Ringer solution, containing, where appropriate, 1·05% (w/v) BSA or 10·5% (v/v) inactivated pig serum or reconstituted 10% (w/v) dried skimmed milk. After 10 min at room temperature, carriers were removed, shaken, dried by placing on sterile filter paper for 30 min at 37 °C and transferred to 50 ml 1/4 strength Ringer solution containing various concentrations of hypochlorite. At suitable times carriers were removed to 9 ml of broth and vigorously shaken. Viable counts were made and broths were incubated as for the suspension tests.

Neutralization of mycoplasmacidal activity

Experiments were made to check satisfactory neutralization of hypochlorite carried over with test samples to nutrient media. One ml of hypochlorite solution (600 p.p.m. av. Cl) was added to 9 ml of nutrient medium; immediately after mixing, 1 ml of inoculum (10^6 c.f.u. in 1/4 strength Ringer solution) was added and at various time intervals (up to 2 h) samples were withdrawn for the determination of viable counts. There was no significant difference (90% level) between counts from time 0 to 2 h or between counts in the presence or absence of hypochlorite.

The concentration of hypochlorite used to test the neutralization capacity of the nutrient medium was the highest concentration used in suspension tests (600 p.p.m. av. Cl). The highest concentration in carrier tests was 1000 p.p.m. av. Cl; however, the volume of hypochlorite solution carried over, as determined by weight, was less than 0·1 ml. Sodium thiosulphate at 0·1% (w/v), commonly used for hypochlorite neutralization, was found to be inhibitory to the mycoplasma.

Table 2. *The effect of hypochlorite on the survival of* M. mycoides (c.f.u.) *in the presence of* 1 % (*w/v*) *BSA*

Hypochlorite conc. (p.p.m. av. Cl)	Time of exposure (min)								
	¼	½	1	2	5	10	15	30	90
0	$1{\cdot}0\times10^{6}$	ND	ND	ND	ND	ND	ND	ND	$9{\cdot}3\times10^{5}$
1	ND	ND	ND	ND	ND	ND	ND	ND	$7{\cdot}5\times10^{5}$
2	ND	ND	ND	ND	ND	ND	ND	ND	$7{\cdot}5\times10^{5}$
5	$1{\cdot}2\times10^{6}$	$1{\cdot}0\times10^{6}$	$7{\cdot}6\times10^{5}$	$8{\cdot}6\times10^{5}$	$5{\cdot}8\times10^{5}$	$6{\cdot}1\times10^{5}$	$3{\cdot}9\times10^{5}$	$4{\cdot}6\times10^{5}$	$1{\cdot}0\times10^{5}$
10	$8{\cdot}9\times10^{5}$	$1{\cdot}0\times10^{6}$	$8{\cdot}3\times10^{5}$	$9{\cdot}4\times10^{5}$	$8{\cdot}9\times10^{5}$	$7{\cdot}5\times10^{5}$	$6{\cdot}3\times10^{5}$	$2{\cdot}6\times10^{5}$	$2{\cdot}8\times10^{4}$
25	$4{\cdot}9\times10^{5}$	$3{\cdot}2\times10^{5}$	$2{\cdot}9\times10^{5}$	$1{\cdot}7\times10^{5}$	$1{\cdot}1\times10^{5}$	$6{\cdot}6\times10^{4}$	$7{\cdot}6\times10^{4}$	$4{\cdot}5\times10^{4}$	1×10^{2}
50	$8{\cdot}3\times10^{3}$	$4{\cdot}6\times10^{3}$	+	+/0	0	0	0	0	0
75	1×10^{2}	+	+/0	0	0	0	0	0	0
100	+	+	+/0	0	0	0	0	0	0
125	+	+	+	0	0	0	0	0	0
150	+	+	+/0	0	0	0	0	0	0
200	+	+/0	0	0	0	0	0	0	0
400	0	0	0	0	ND	ND	ND	ND	ND
600	0	0	0	0	ND	ND	ND	ND	ND

M. mycoides was suspended in various concentrations of hypochlorite in the presence of 1 % (w/v) BSA at an initial density of $1{\cdot}0\times10^{6}$ c.f.u./ml. One ml aliquots were transferred to broth and the number of survivors (c.f.u.) determined by plate counts. See Table 1 legend for key to symbols.

Analysis of hypochlorite solutions

Available chlorine and free available chlorine were determined by iodine/thiosulphate and diethyl-*p*-phenylenediamine titration methods respectively (Anon, 1980). Iodometric titrations may also measure chlorite and chlorate (decay products of hypochlorite); however, as fresh hypochlorite was used, the concentration of these products would have been low.

RESULTS

In the absence of an organic load, a contact time of 15 s with 25 p.p.m. av. Cl was sufficient to reduce the viable count of a suspension of *M. mycoides* from $>10^{6}$ c.f.u./ml to an undetectable level (i.e. no survivors in one ml). Lower concentrations of 10 and 5 p.p.m. av. Cl gave 99·9 % reduction in viability in 10 and 15 min respectively and 2 p.p.m. av. Cl gave only a marginal reduction in viable count after 90 min (Table 1). In the presence of an organic load (1 % BSA; see Table 2) a reduction in viable count from 10^{6} c.f.u./ml to an undetectable level was achieved within 15 s at 400 p.p.m. and within 5 min at 50 p.p.m. av. Cl; at 10 p.p.m. av. Cl there was relatively little reduction in viable count after 90 min.

In the absence of organic load the concentration of av. Cl ($\geqslant$75 p.p.m.) and the times required to kill *M. mycoides* absorbed into unglazed porous porcelain carriers (Tables 3 and 5) were increased compared to the values obtained for suspensions

Table 3. *The effect of hypochlorite on the survival of* M. mycoides (*c.f.u.*) *absorbed into porcelain carriers*

Hypochlorite conc. (p.p.m. av. Cl)	Time of exposure (min)					
	1	5	10	15	30	90
0	$2{\cdot}0\times10^{4}$	$1{\cdot}0\times10^{4}$	$1{\cdot}4\times10^{4}$	$3{\cdot}0\times10^{3}$	$2{\cdot}7\times10^{3}$	$2{\cdot}5\times10^{3}$
1	$8{\cdot}2\times10^{3}$	$6{\cdot}0\times10^{2}$	$3{\cdot}0\times10^{3}$	$1{\cdot}1\times10^{3}$	$2{\cdot}0\times10^{3}$	+
2	$1{\cdot}1\times10^{4}$	$5{\cdot}2\times10^{3}$	$3{\cdot}3\times10^{3}$	$2{\cdot}5\times10^{3}$	$1{\cdot}0\times10^{2}$	4×10^{2}
5	$2{\cdot}3\times10^{4}$	$8{\cdot}4\times10^{3}$	$3{\cdot}0\times10^{3}$	$8{\cdot}0\times10^{2}$	$2{\cdot}2\times10^{3}$	4×10^{2}
10	$1{\cdot}6\times10^{3}$	$9{\cdot}0\times10^{2}$	+	1×10^{2}	+	+
25	2×10^{2}	+	+	+	+/0	+/0
50	2×10^{2}	+	+/0	+/0	+/0	+/0
75	6×10^{1}	+	+	+/0	+/0	0
100	6×10^{1}	+	+/0	+/0	+/0	0
125	1×10^{2}	+/0	+/0	+/0	0	0
150	2×10^{2}	+/0	0	0	0	0
200	+	1×10^{2}/0	+/0	+/0	0	0

Porcelain carriers previously immersed in *M. mycoides* suspension ($2{\cdot}3\times10^{6}$ c.f.u./ml) were transferred to hypochlorite solutions. At various times carriers were transferred to broth, vigorously shaken, and the number of survivors (c.f.u.) determined by plate counts. Growth in the broth after incubation was also determined. See Table 1 legend for key to symbols.

Table 4. *The effect of hypochlorite on the survival of* M. mycoides (*c.f.u.*) *suspended in* 1 % (*w/v*) *BSA and absorbed into porcelain carriers*

Hypochlorite conc. (p.p.m. av. Cl)	Time of exposure (min)					
	1	5	10	15	30	90
0	$1{\cdot}2\times10^{5}$	$1{\cdot}2\times10^{5}$	$1{\cdot}2\times10^{5}$	$1{\cdot}2\times10^{5}$	$1{\cdot}2\times10^{5}$	$1{\cdot}1\times10^{5}$
1	$1{\cdot}8\times10^{5}$	$1{\cdot}6\times10^{5}$	$1{\cdot}4\times10^{5}$	$1{\cdot}4\times10^{5}$	$1{\cdot}2\times10^{5}$	$9{\cdot}2\times10^{4}$
2	$1{\cdot}9\times10^{5}$	$1{\cdot}5\times10^{5}$	$1{\cdot}3\times10^{5}$	$1{\cdot}2\times10^{5}$	$2{\cdot}0\times10^{4}$	$2{\cdot}3\times10^{4}$
5	$2{\cdot}1\times10^{5}$	$6{\cdot}5\times10^{4}$	$4{\cdot}9\times10^{4}$	$4{\cdot}4\times10^{4}$	$4{\cdot}2\times10^{4}$	$4{\cdot}4\times10^{3}$
10	$2{\cdot}4\times10^{4}$	$3{\cdot}2\times10^{4}$	$2{\cdot}9\times10^{4}$	$1{\cdot}8\times10^{4}$	1×10^{2}	4×10^{2}
25	$2{\cdot}4\times10^{4}$	$5{\cdot}0\times10^{4}$	$1{\cdot}8\times10^{3}$	$7{\cdot}7\times10^{3}$	3×10^{2}	2×10^{3}
50	$5{\cdot}3\times10^{3}$	$2{\cdot}7\times10^{3}$	2×10^{2}	+	+	+
75	9×10^{2}	3×10^{2}	1×10^{2}	3×10^{2}	$1{\cdot}0\times10^{3}$	5×10^{2}
100	4×10^{2}	+	+	+	+	+
125	$1{\cdot}9\times10^{3}$	3×10^{2}	4×10^{2}	+	+	+
150	$1{\cdot}0\times10^{3}$	$1{\cdot}5\times10^{3}$	+	+	+	+
200	$4{\cdot}0\times10^{2}$	+	+	+	+	0

Porcelain carriers immersed in *M. mycoides* suspension ($2{\cdot}0\times10^{7}$ c.f.u./ml) in the presence of 1 % (w/v) BSA were transferred to hypochlorite solutions. At various times carriers were transferred to broth, vigorously shaken, and the number of survivors (c.f.u.) determined by plate counts. Growth in the broth after incubation was also determined. See Table 1 legend for key to symbols.

of organisms (Table 1). In the presence of various organic loads (1 % (w/v) BSA, 10 % (w/v) milk and 10 % (v/v) serum) the concentration of hypochlorite required to kill was further increased (Tables 4 and 5). However, even in the presence of these loads absorbed mycoplasmas were killed by exposure to 250 p.p.m. av. Cl for 90 min or 1000 p.p.m. av. Cl for 30 min.

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Table 5. *The effect of hypochlorite on the survival of* M. mycoides *suspended in* 1% (*w/v*) *BSA*, 10% (*v/v*) *serum or reconstituted* 10% (*w/v*) *skimmed milk and absorbed into porcelain carriers*

Organic load	Hypochlorite conc. (p.p.m. av. Cl)	Time of exposure (min) 1	10	30	90
None	125	+	+	0	0
	250	+	+	0	0
	500	+	+	+	0
	1000	0	0	0	0
BSA	125	+	+	+	0
	250	+	+	+	0
	500	+	+	0	0
	1000	+	0	0	0
Serum	125	+	+	+	+
	250	+	+	0	0
	500	+	+	0	0
	1000	+	0	0	0
Milk	125	+	+	+	+
	250	+	+	+	0
	500	+	+	0	0
	1000	+	+	0	0

Porcelain carriers previously immersed in *M. mycoides* suspension (2.5×10^7 c.f.u./ml) in the presence of organic load were transferred to hypochlorite solutions. At various times carriers were transferred to broths and incubated. +, 0, Growth and no growth respectively.

In carrier tests the number of organisms transferred per carrier (based on an increase in weight of 0·05 g per carrier after immersion in cell suspensions) was between 10^5 and 10^6 c.f.u.

A portion of the cells, and possibly the majority, was washed off during exposure to hypochlorite and was not transferred to broth. However, in control experiments the number of organisms recovered in broth after thorough shaking of a carrier and broth was 10^4 and 10^5 c.f.u. The results show that after only 1 min exposure to 25 p.p.m. av. Cl, in the absence of organic load, 99% of these organisms were killed (Table 3). However, at much higher concentrations of hypochlorite and after exposure times of up to 90 min in some cases (25 and 50 p.p.m. av. Cl) there were still survivors; these organisms were presumably absorbed relatively deep into the carrier and protected from hypochlorite.

Available chlorine was reduced in the presence of 1% BSA (Fig. 1). One and 90 min after the addition of chlorine (free available chlorine, i.e. hypochlorous acid and hypochlorite ion) there was a linear relationship (correlation coefficients 0·992 and 0·997 respectively) between added chlorine and residual available chlorine; residual available chlorine may include both free available chlorine and combined available chlorine (in this case, organic chloramines formed by reaction with BSA). Within 10 s, 1% BSA reduced free available chlorine to <1 and 10 p.p.m., with 500 and 1000 p.p.m. added chlorine respectively.

There were no survivors at 15 s in the presence of 25 p.p.m. available chlorine without BSA, whereas there were survivors with 200 p.p.m. added chlorine

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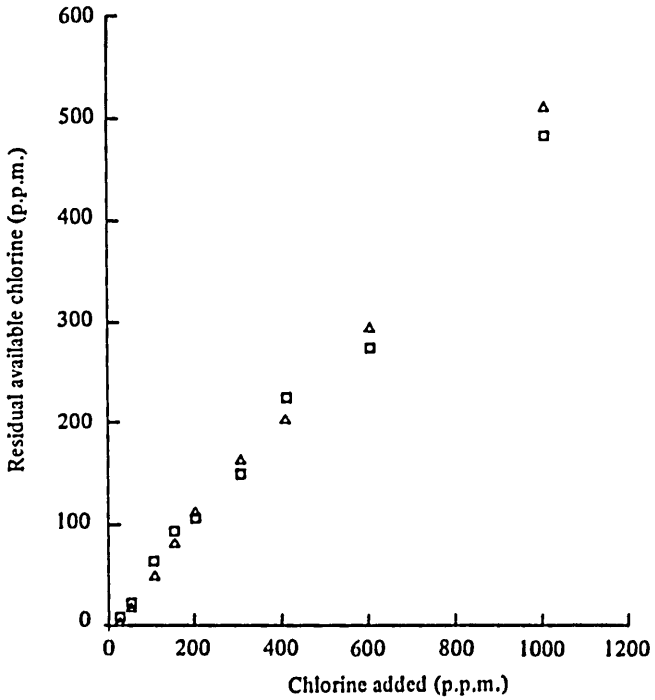

Fig. 1. Available chlorine at 1 (□) and 90 (△) min after the addition of 1 % BSA to various concentrations of hypochlorite.

(equivalent to 100 p.p.m. residual available chlorine) in the presence of 1 % BSA (Tables 1 and 2). This may indicate that the mycoplasmacidal activity of combined available chlorine is less than that of free available chlorine. However, the protection afforded by BSA in carrier tests (Tables 3 and 4) was greater than could be accounted for from reduction in free available chlorine due to the small amount of BSA transferred with carriers to hypochlorite solutions. In these tests, total BSA transferred was approximately 5 mg per 50 ml of hypochlorite; with 200 p.p.m. added chlorine this concentration of BSA reduced free available chlorine by less than 10 p.p.m.

DISCUSSION

The importance of mycoplasma infections in man is becoming increasingly recognized (Mårdh, 1983) and mycoplasmas include many animal pathogens of commercial significance (Tully & Whitcomb, 1979). The use of hypochlorite as a teat dip and sanitizer for the control of mycoplasma mastitis in cattle has been investigated (Jasper, Dellinger & Hakanson, 1976; Pfutzner, Scherwa & Trubner, 1983); however, the causative agent, *M. bovis*, was reported to be relatively insensitive to the action of hypochlorite. Our results demonstrate that *M. mycoides* ssp. *mycoides* is sensitive to chlorine and at concentrations markedly lower than those previously found for *M. bovis*. However, despite the absence of a cell wall in mycoplasmas, 10 p.p.m. available chlorine, which is sufficient to kill a range of bacterial and yeast suspensions within 15 min (Ferguson & Gibson, 1971), did not kill equivalent suspensions of *M. mycoides* in 90 min (Table 1). Thus, *M. mycoides* appears slightly more resistant to hypochlorite than most organisms.

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M. mycoides was protected from hypochlorite disinfection to a significant extent by absorption into porcelain carriers. We considered it possible that, because of the small size and plasticity of *M. mycoides*, the extent of absorption and protection might be greater than that of other bacteria. However, in parallel experiments, porcelain carriers previously immersed in cell suspensions (10^7 c.f.u./ml) of *Escherichia coli* and *Staphylococcus aureus* required 10 min exposure to 125 p.p.m. av. Cl to become sterile. Also, using a carrier test, Stedman, Kravitz & Bell (1954*a*) have reported that concentrations of up to 100 p.p.m. av. Cl for 10 min were required to kill 99·99% of bacterial populations. Thus, the extent of protection of *M. mycoides* in carrier tests is similar to that of other bacteria (see Table 3).

Differences in the concentration of hypochlorite required to disinfect organisms in suspension and absorbed into porous surfaces are to be expected, as these processes are subject to the consequences of differing physical forces. Stedman, Kravitz & Bell (1954*b*) have shown a wide variation in antimicrobial activity occurring with the same disinfectant on surfaces of differing porosities; their study serves to illustrate the inadequacies of test methods which do not attempt to duplicate the variety of 'in use' conditions in some reasonable manner.

Disinfection failures due to misuse of hypochlorite formulations could conceivably occur in many ways, although probably the most important is failure to remove protein residues by thorough cleaning of utensils or other surfaces before disinfection (Phillips, 1966). All types of organic material interact strongly with hypochlorite causing loss of activity. Using a bactericidal capacity test, Bloomfield (1973) showed that sodium hypochlorite activity was adversely affected by 1% milk. In addition, Ferguson & Gibson (1971) have shown that pre-inactivation of sodium hypochlorite with 5% milk considerably increases the exposure time required to achieve disinfection at 125 p.p.m. available chlorine. Similarly, Raunio & Wasz-Höckert (1971) have reported that in suspension tests the presence of 5% milk required a fourfold increase in hypochlorite concentration in order to achieve kill in the same time as in the absence of protein.

In this study, an organic load of 1% BSA increased the level of av. Cl required to kill mycoplasmas from 25 p.p.m. for 15 s to 50 p.p.m. for 5 min in suspension tests and from 125 p.p.m. for 30 min to 200 p.p.m. for 90 min in carrier tests. The levels of hypochlorite required in suspension tests to kill *M. mycoides* in the presence of 1% BSA are much lower than the values reported for *M. bovis* in broth (3200 p.p.m. av. Cl for 20 min, Pfutzner, Scherwa & Trubner, 1983; 10000 p.p.m. av. Cl for 1 min, Jasper, Dellinger & Hakanson, 1976). Organic loads of 10% serum or 10% milk gave essentially similar results in carrier tests to 1% BSA (Table 5).

The concentrations of organic load used here are much higher than those generally assumed to occur in practice. For example, levels of BSA as low as 0·1% have been suggested for testing the efficacy of chemical disinfection procedures in the presence of organic load (Beck *et al.* 1977), and a maximum organic load of only 0·1% milk would be expected in improperly cleaned bottles (Tan & Schnagl, 1983). However, if milk is present as congealed solids containing microbes it may present a greater barrier to disinfection. In carrier tests the extent of protection shown by BSA was greater than could be accounted for by a reduction in free available chlorine; this may indicate that BSA prevented direct chlorine cell interactions.

In conclusion, the concentration of hypochlorite required to kill *M. mycoides* was

significantly less than that reported for *M. bovis* in suspension tests and only marginally greater than values for other bacteria and yeasts in both suspension and carrier tests.

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