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Efficacies of Selected Disinfectants against *Mycobacterium tuberculosis*

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The activities of 10 formulations as mycobactericidal agents in *Mycobacterium tuberculosis*-contaminated suspensions (suspension test) and stainless steel surfaces (carrier test) were investigated with sputum as the organic load. The quaternary ammonium compound, chlorhexidine gluconate, and an iodophor were ineffective in all tests. Ethanol (70%) was effective against *M. tuberculosis* only in suspension in the absence of sputum. Povidone-iodine was not as efficacious when the test organism was dried on a surface as it was in suspension, and its activity was further reduced in the presence of sputum. Sodium hypochlorite required a higher concentration of available chlorine to achieve an effective level of disinfection than did sodium dichloroisocyanurate. Phenol (5%) was effective under all test conditions, producing at least a 4-$\log_{10}$ reduction in CFU. The undiluted glutaraldehyde-phenate solution was effective against *M. tuberculosis* and a second test organism, *Mycobacterium smegmatis*, even in the presence of dried sputum, whereas the diluted solution (1:16) was only effective against *M. smegmatis* in the suspension test. A solution of 2% glutaraldehyde was effective against *M. tuberculosis*. This investigation presents tuberculocidal efficacy data generated by methods simulating actual practices of routine disinfection.

The resistance of mycobacteria to disinfectants has been considered intermediate between those of other vegetative bacteria and spores (5, 11, 14, 15, 17). This is attributed in part to their unusually high cell wall lipid content and the resultant hydrophobicity (5, 11, 14, 15). Therefore, it is generally believed that agents which can inactivate mycobacteria will also be effective against other types of vegetative bacteria. However, the lack of proper test protocols has rendered the data on the mycobactericidal efficacy of chemical disinfectants unreliable.

Of particular importance is the effectiveness of disinfectants against *Mycobacterium tuberculosis*. This organism is not usually included in such studies because of problems in the safe manipulation of this pathogen and its slow growth. *Mycobacterium smegmatis* or *Mycobacterium bovis* or both are frequently used to examine a disinfectant product for its tuberculocidal activity; *M. bovis* may be more closely related to *M. tuberculosis*, but the rapidly growing, less-pathogenic *M. smegmatis* is easier to work with. Although useful during protocol development and for the generation of efficacy data for *M. smegmatis* or *M. bovis*, the results of such studies cannot be safely extrapolated to *M. tuberculosis* since *M. tuberculosis* is generally considered the most resistant of these three organisms to disinfectants (5, 11, 17).

Further, the use of realistic organic loads to simulate actual practices of disinfection is often lacking in tuberculocidal efficacy testing. In sputum, the tubercle bacilli have greater protection from disinfectant action, since the disinfectant formulation must be able to penetrate the organic matter without becoming neutralized. Although it is understood that naturally contaminated sputum (bacteria may be present intracellularly) would ideally represent in-use conditions, the addition of sputum to test protocols is the next best alternative.

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In our previous investigation, reproducible, accurate mycobactericidal efficacy testing methods were developed (2). This study uses the same methods to determine the activities of a variety of disinfectants against *M. tuberculosis* and compares the results with those obtained previously with *M. smegmatis* (2). The germicidal efficacy of glutaraldehyde-phenate solutions, not included in the previous study, was also tested by using both *M. smegmatis* and *M. tuberculosis*.

MATERIALS AND METHODS

M. tuberculosis (H37Rv) and *M. smegmatis* (TMC 1515) were obtained from the Mycobacteriology Section of the National Laboratory for Special Pathogens, Laboratory Centre for Disease Control, Health and Welfare Canada, Ottawa, Ontario, Canada. Stock cultures were maintained on Lowenstein-Jensen medium. Sputum, used as the organic load, was a pool of mycobacterium-negative specimens. Test suspensions were prepared by suspending harvested bacterial cells in either normal saline containing 0.5% Tween 80 or sputum and homogenizing them for 1 min with sterile glass beads to obtain 10^9 and 10^8 CFU/ml for *M. smegmatis* and *M. tuberculosis*, respectively. These two test suspensions for each organism were used as the initial inocula for all tests.

Disinfectant tests were carried out in the wells of a 24-well plastic cell culture plate (Falcon; Becton Dickinson Labware, Lincoln Park, N.J.) as previously described (2). All disinfectants tested were diluted according to the instructions of the manufacturers, with tap water as the diluent; in-use concentrations of disinfectants are shown in Tables 1 and 2. The sodium hypochlorite and sodium dichloroisocyanurate solutions were diluted to the required concentration of available chlorine as measured by a colorimeter (model DR 100; Hatch, Loveland, Colo.). The 2% glutaraldehyde and the 2 or 2.4% glutaraldehyde-phenate solutions were freshly activated by using the activators provided with the products.

TABLE 1. Activities of disinfectants after 1 min of contact

Disinfectant (concn used)	Organic load	Reduction in CFU of *M. tuberculosis*		Rating[a]	
		Suspension test	Carrier test	Suspension test	Carrier test
Phenol (5% wt/vol)	Absent	$>(5.60 \pm 1.05) \times 10^5$ $>(1.63 \pm 0.12) \times 10^5$	$(7.67 \pm 0.58) \times 10^4$ $(9.67 \pm 0.58) \times 10^4$	Pass	Pass
	Sputum	$>(1.10 \pm 0.10) \times 10^5$ $>(1.60 \pm 0.26) \times 10^5$	$(1.40 \pm 0.06) \times 10^4$ $(2.30 \pm 0.10) \times 10^4$	Pass	Pass
Sodium hypochlorite (10,000 ppm of Av Cl/ml)	Absent	$(1.10 \pm 0.11) \times 10^3$ $(1.17 \pm 0.07) \times 10^3$	$(1.59 \pm 0.02) \times 10^3$ $(1.47 \pm 0.03) \times 10^3$	Pass	Pass
	Sputum	$(1.77 \pm 0.04) \times 10^3$ $(1.04 \pm 0.02) \times 10^3$	$(1.67 \pm 0.01) \times 10^3$ $(1.56 \pm 0.04) \times 10^3$	Pass	Pass
Sodium hypochlorite (6,000 ppm of Av Cl/ml)	Absent	$(2.54 \pm 0.15) \times 10^2$ $(5.48 \pm 0.09) \times 10^2$	$(1.20 \pm 0.10) \times 10^2$ $(1.30 \pm 0.05) \times 10^2$	Fail	Fail
	Sputum	$(1.26 \pm 0.02) \times 10^2$ $(2.03 \pm 0.50) \times 10^2$	$(2.33 \pm 1.53) \times 10^2$ $(1.90 \pm 0.44) \times 10^2$	Fail	Fail
Sodium dichloroisocyanurate (6,000 ppm of Av Cl/ml)	Absent	$(1.33 \pm 0.10) \times 10^4$ $(1.10 \pm 0.20) \times 10^4$	$(1.50 \pm 0.08) \times 10^3$ $(1.41 \pm 0.06) \times 10^3$	Pass	Pass
	Sputum	$(1.87 \pm 0.03) \times 10^4$ $(1.90 \pm 0.10) \times 10^4$	$(2.01 \pm 0.02) \times 10^2$ $(7.40 \pm 1.27) \times 10^2$	Pass	Fail
Ethanol (70% vol/vol)	Absent	$(3.63 \pm 0.29) \times 10^3$ $(3.00 \pm 0.10) \times 10^3$	$(9.96 \pm 0.40) \times 10^1$ $(8.65 \pm 0.73) \times 10^1$	Pass	Fail
	Sputum	$(1.30 \pm 0.23) \times 10^2$ $(2.67 \pm 0.47) \times 10^2$	7.00 ± 0.42 3.00 ± 0.15	Fail	Fail
Quaternary ammonium compound (0.04% dimethyl benzylammonium chloride)	Absent	1.93 ± 0.12 6.57 ± 0.06	ND[b] ND	Fail	
	Sputum	ND ND	ND ND		
Povidone iodine (1.0% titratable I_2)	Absent	$>(1.13 \pm 0.13) \times 10^5$ $>(1.27 \pm 0.23) \times 10^5$	$(5.27 \pm 2.41) \times 10^3$ $(2.30 \pm 1.42) \times 10^3$	Pass	Pass
	Sputum	$>(1.27 \pm 0.14) \times 10^5$ $>(1.27 \pm 0.12) \times 10^5$	$(7.67 \pm 0.51) \times 10^2$ $(8.70 \pm 0.30) \times 10^2$	Pass	Fail
Iodophor (0.008% titratable I_2)	Absent	$(6.97 \pm 0.19) \times 10^1$ $(6.13 \pm 0.58) \times 10^1$	3.70 ± 0.17 3.46 ± 0.06	Fail	Fail
	Sputum	3.70 ± 0.10 1.90 ± 0.25	1.30 ± 0.26 1.20 ± 0.13	Fail	Fail
Chlorhexidine gluconate (4%)	Absent	$(6.70 \pm 0.36) \times 10^2$ $(7.52 \pm 0.97) \times 10^2$	$(9.87 \pm 0.17) \times 10^1$ $(9.97 \pm 0.02) \times 10^1$	Fail	Fail
	Sputum	$(6.30 \pm 0.09) \times 10^2$ $(6.13 \pm 0.35) \times 10^2$	1.47 ± 0.23 1.73 ± 0.06	Fail	Fail

[a] Rating: Pass, $\geq$3-$\log_{10}$ reduction in CFU; fail, <3-$\log_{10}$ reduction in CFU.
[b] ND, Not done.

A minimal contact time of 1 min was selected for the testing of all disinfectants, except the glutaraldehyde solutions with which longer times (10 and 30 min) were used in accordance with manufacturer efficacy claims for disinfection. An ideal disinfectant is one that produces its effect after a short contact time, and for this reason, a contact time of 1 min was chosen in this study. In all tests, dilution of the reaction mixture (100-fold followed by further 10-fold dilutions) immediately at the end of the contact time was the method used to terminate disinfectant action. This method of neutralization was found to be effective and resulted in no residual disinfectant activity. Normal saline was used as both diluent and eluent for bacteria, and it contained 0.5% Tween 80 to prevent clumping of cells.

Disinfectant efficacy tests were carried out with *M. tuberculosis* in suspension (suspension test) and dried on stainless steel surfaces (carrier test). Previous studies using this methodology concluded that disinfectants showing low ac-

TABLE 2. Activities of glutaraldehyde-based disinfectants

Disinfectant (concn used)	Organic matter	Contact time (min)	Reduction in CFU of *M. tuberculosis*		Rating[a]	
			Suspension test	Carrier test	Suspension test	Carrier test
M. smegmatis						
2.0% Glutaraldehyde-phenate (1:16)	Absent	1	4.8 ± 0.50	ND[b]	Fail	
			4.5 ± 0.50	ND		
		10	(1.27 ± 0.10) × 10^5	(1.14 ± 0.04) × 10^3	Pass	Pass
			(1.35 ± 0.14) × 10^5	(1.36 ± 0.03) × 10^3		
	Sputum	1	ND	ND		
			ND	ND		
		10	(1.37 ± 0.04) × 10^5	4.30 ± 0.06	Pass	Fail
			(1.24 ± 0.06) × 10^5	4.86 ± 0.51		
2.0% Glutaraldehyde-phenate (undiluted)	Absent	1	>(1.56 ± 0.21) × 10^6	(8.80 ± 1.30) × 10^3	Pass	Pass
			>(1.70 ± 0.50) × 10^6	(6.31 ± 1.48) × 10^3		
		10	ND	(9.33 ± 0.55) × 10^4		Pass
			ND	(8.23 ± 0.45) × 10^4		
	Sputum	1	>(1.07 ± 0.21) × 10^6	(8.53 ± 1.03) × 10^2	Pass	Fail
			>(1.75 ± 0.56) × 10^6	(8.75 ± 0.25) × 10^2		
		10	ND	(9.10 ± 0.53) × 10^4		Pass
			ND	(9.43 ± 0.31) × 10^4		
M. tuberculosis						
2.0% Glutaraldehyde-phenate (1:16)[c]	Absent	1	8.86 ± 0.15	ND	Fail	
			7.98 ± 0.89	ND		
		10	9.00 ± 1.00	ND	Fail	
			7.30 ± 1.00	ND		
		30	9.37 ± 1.53	ND	Fail	
			8.56 ± 1.32	ND		
2.0% Glutaraldehyde-phenate (undiluted)	Absent	1	>(1.50 ± 0.36) × 10^5	(2.43 ± 0.02) × 10^3	Pass	Pass
			>(2.75 ± 0.44) × 10^5	(1.06 ± 0.03) × 10^3		
		10	ND	(1.90 ± 0.21) × 10^4		Pass
			ND	(2.07 ± 0.45) × 10^4		
	Sputum	1	>(3.23 ± 0.50) × 10^5	(2.73 ± 0.14) × 10^2	Pass	Fail
			>(2.07 ± 0.15) × 10^5	(2.34 ± 0.43) × 10^2		
		10	ND	(2.16 ± 1.10) × 10^4		Pass
			ND	(1.63 ± 0.12) × 10^4		
2.0% Glutaraldehyde (undiluted)	Absent	1	4.97 ± 1.52	ND	Fail	
			4.63 ± 0.11	ND		
		10	(5.30 ± 0.36) × 10^3	(2.70 ± 0.44) × 10^3	Pass	Pass
			(4.98 ± 0.67) × 10^3	(1.87 ± 0.10) × 10^3		
		30	>(2.67 ± 0.25) × 10^5	>(2.07 ± 0.15) × 10^5	Pass	Pass
			>(2.23 ± 0.12) × 10^5	>(1.77 ± 0.15) × 10^5		
	Sputum	1	6.03 ± 0.58	ND	Fail	
			6.50 ± 0.50	ND		
		10	(1.60 ± 0.02) × 10^3	(3.28 ± 0.02) × 10^3	Pass	Pass
			(1.08 ± 0.08) × 10^3	(2.98 ± 0.76) × 10^3		
		30	>(3.56 ± 0.67) × 10^5	>(3.90 ± 0.35) × 10^5	Pass	Pass
			>(3.76 ± 0.43) × 10^5	>(3.23 ± 0.45) × 10^5		

[a] Rating: Pass ≥3-$\log_{10}$ reduction in CFU; fail, <3-$\log_{10}$ reduction in CFU.
[b] ND, Not done.
[c] Glutaraldehyde-phenate (2.4%) also produced a <1-$\log_{10}$ reduction in CFU.

tivities in the suspension test also exhibited low activities in the carrier test (2). For this reason, carrier tests were not performed on those disinfectants that produced less than a 1-$\log_{10}$ reduction of *M. tuberculosis* in suspension test.

In the suspension test, 0.1 ml of the test inoculum was added to 0.9 ml of disinfectant. Controls for each suspension contained 0.9 ml of the diluent instead of the disinfectant. After the required contact time, 0.1 ml of the reaction mixture was removed and immediately diluted 100-fold in diluent. The sample was subjected to further 10-fold dilutions, up to 10^{-7}. Samples (1 ml) from the dilutions were spread on 7H11 agar (Difco Laboratories, Detroit, Mich.) in duplicate and incubated at 37°C for 4 days and 4 weeks for *M. smegmatis* and *M. tuberculosis*, respectively.

Carrier disks (1 cm in diameter) were cut from locally purchased stainless steel sheets (0.75 mm thick). For the carrier test, disks were placed in the wells of a 24-well cell culture plate as needed. Samples (20 μl) of each test suspen-

sion were placed on the disk surface and allowed to air dry for 2 h at room temperature (22 ± 2°C) in a class II biological safety cabinet. The contaminated area was then covered with 20 μl of disinfectant. Controls with each test suspension were covered with 20 μl of diluent instead of disinfectant. After the required contact time, 980 μl of diluent was added to each well to dilute the disinfectant and elute the bacteria from the steel carrier (simple mixing was sufficient to recover the bacteria from the carrier surface). Subsequently, the eluates were serially diluted up to 10^{-7} and spread on 7H11 agar and incubated as in the suspension test.

Tests were carried out in triplicate, with two batches for each disinfectant (six replicates). Disinfectant activity was determined by comparing growths on the control and disinfectant plates and is reported as the mean (± standard deviation) reduction in CFU for each disinfectant batch. Each disinfectant was tested for its capacity to cause up to a 5-$\log_{10}$ (99.999%) reduction in CFU of *M. tuberculosis* and up to a 6-$\log_{10}$ (99.9999%) reduction in CFU of *M. smegmatis* (maximum levels of detection).

RESULTS

Various minimum acceptable log reductions have been suggested; however, for the purpose of discussing the relative efficacy of the formulations tested, it is considered that at least a 3-$\log_{10}$ reduction in CFU of the organism by the test agent is required before a product is regarded as being effective and is given a pass rating (11, 12, 17). In all tests, control reactions containing no disinfectant resulted in complete recovery of the initial inoculum.

Table 1 outlines the results of the efficacies of eight disinfectants tested against *M. tuberculosis* after 1 min of contact. Phenol was found to be extremely effective in all the tests done. It produced at least a 5-$\log_{10}$ reduction (maximum level of detection) in suspension and a 4-$\log_{10}$ reduction of the test organism when dried on the surfaces of steel disks. The tuberculocidal activity was not affected by the presence of sputum.

Two disinfectants (quaternary ammonium compound and the low-concentration iodophor) were completely ineffective in all tests; they were unable to produce more than a 1-$\log_{10}$ reduction in CFU. Chlorhexidine gluconate, although slightly more efficacious in the suspension test, was also ineffective against *M. tuberculosis*, producing no more than a 2-$\log_{10}$ reduction in CFU.

In the suspension test, the povidone-iodine solution was more effective than the lower-concentration iodophore, producing at least a 5-$\log_{10}$ reduction in CFU (maximum level of detection). However, the test organism was resistant to its action in the carrier test in the presence of sputum.

Sodium hypochlorite required an available chlorine (Av Cl) concentration of 10,000 ppm (10,000 μg/ml) before an effective level of reduction could be obtained. One of the batches used for the suspension test was only marginally effective [$(1.10 \pm 0.11) \times 10^3$]. The sodium hypochlorite solution containing 6,000 ppm of Av Cl was not effective, producing a 2-$\log_{10}$ reduction in all tests. In contrast, the sodium dichloroisocyanurate solution containing 6,000 ppm of Av Cl was effective both in suspension tests and in the carrier test in the absence of sputum. This formulation was unable to effectively inactivate *M. tuberculosis* dried on stainless steel surfaces in the presence of sputum.

Ethanol was also less effective against *M. tuberculosis* dried on surfaces, compared with the results obtained in suspension. Even in suspension, this disinfectant was effective only in the absence of an organic load.

The results of tests with glutaraldehyde-based disinfectants (2% glutaraldehyde, undiluted glutaraldehyde-phenate, and a 1:16 dilution of glutaraldehyde-phenate) against mycobacteria are presented in Table 2. Undiluted glutaraldehyde-phenate was rapidly efficacious against both *M. smegmatis* and *M. tuberculosis* in suspension, resulting in at least a 5-$\log_{10}$ reduction in CFU after only 1 min of contact. However, 10 min of contact with the contaminated carriers was necessary to achieve more than a 3-$\log_{10}$ reduction of bacterial numbers with both of the mycobacteria tested, even in the presence of sputum.

The diluted solution of glutaraldehyde-phenate was effective against *M. smegmatis* only after 10 min of contact in the suspension test and in the carrier test in the absence of sputum. When *M. smegmatis* was dried in the presence of sputum, the diluted glutaraldehyde-phenate was ineffective. Both diluted solutions (2 and 2.4%) were ineffective against *M. tuberculosis* in all tests.

The 2% glutaraldehyde solution was not affected by the presence of sputum in the test suspension; however, a contact time of 30 min was required to cause a 5-$\log_{10}$ reduction in CFU of *M. tuberculosis*. Despite the superior activity of undiluted glutaraldehyde-phenate against *M. tuberculosis*, it was noted that the $\log_{10}$ reduction produced by this product in CFU of *M. smegmatis* was slightly lower than that we had found previously for 2% glutaraldehyde alone (2). Therefore, we were interested to know whether this could be due to inherent variations in the test protocol performed at different times or whether it was a real difference in susceptibility. Retesting of 2% glutaraldehyde alone and undiluted glutaraldehyde-phenate in parallel reproduced the slight difference in susceptibility observed.

DISCUSSION

The spread of tuberculosis through the use of improperly disinfected bronchoscopes has been documented (6, 10, 13). A total of 80% of laboratory-acquired tuberculosis cases have resulted from no obvious cause, and it has been suggested that the use of ineffective chemical disinfectants may be responsible for some of these cases of laboratory-acquired infections (4).

Quaternary ammonium compounds are good bactericidal agents, and they are widely used for the disinfection of environmental surfaces. However, precleaning of such surfaces is often necessary, because the effectiveness of quaternary ammonium compounds is reduced in the presence of soap and organic matter (8, 16). The formulation tested in this study proved to be ineffective against *M. tuberculosis*. Earlier studies have also demonstrated the poor mycobactericidal activities of quaternary ammonium compounds (2, 5, 15, 17).

The iodophore, containing 0.008% titratable I_2, was also ineffective against *M. tuberculosis*. In our earlier studies (2), this formulation proved to be ineffective against *M. smegmatis* as well. It should be noted here that the concentration of iodophore tested by us was lower than that used in previous studies; iodophore compounds, tested either undiluted (6) or at concentrations higher than 0.45% (10, 13), were able to inactivate *M. tuberculosis* on bronchoscopes.

The povidone-iodine solution was highly effective against *M. tuberculosis* in the suspension test but was unable to inactivate it in the carrier test in the presence of sputum. In contrast, *M. smegmatis* could be readily inactivated by povidone-iodine in both types of tests (2). The reports that alcoholic solutions of povidone-iodine have an enhanced mycobactericidal activity have been questioned (10, 13).

Chlorhexidine gluconate, a cationic biguanide, is a good bactericidal agent (8) and is commonly used in antiseptic formulations because it is mild and relatively nontoxic. However, it is known to be inhibitory to mycobacteria but is not lethal for them (14, 15). In this study, it proved to be ineffective against *M. tuberculosis*. This is in marked contrast to its high efficacy (>6-$\log_{10}$ reduction in CFU) against *M. smegmatis* (2).

In this study, sodium hypochlorite required a minimum of 10,000 ppm of Av Cl to be effective against *M. tuberculosis*, as opposed to 6,000 ppm needed for *M. smegmatis* in our earlier tests (2). Sodium dichloroisocyanurate, tested with 6,000 ppm of Av Cl, could not reduce the titer of the tubercle bacilli to the required level in the carrier test when sputum was present.

In previous studies with these two chlorine-based disinfectants, it was concluded that sputum has no noticeable effect on sodium dichloroisocyanurate but did cause a slight reduction in the efficacy of sodium hypochlorite (2). Other investigators (11, 17) have noted that the activities of chlorine donors, such as sodium dichloroisocyanurate, depend on the organic load and the test procedure.

Improper storage of stock or working solutions of sodium hypochlorite accelerates the escape of chlorine and can adversely affect their germicidal potential. Evaporation of chlorine or its neutralization by organic matter can also readily occur when sodium hypochlorite solutions are used in discard containers. Therefore, it is important that such solutions be changed frequently to ensure that the available chlorine in them does not reach a level ineffective for *M. tuberculosis* and other important pathogens.

Generally, 70% ethanol is considered a good tuberculocidal agent (5, 14–16). However, in our tests, ethanol (70%) proved to be effective against *M. tuberculosis* only in suspension in the absence of sputum. On the other hand, Lind et al. (11) reported a >3-$\log_{10}$ reduction in the titer of *M. tuberculosis* by 70% ethanol in a carrier test with a contact time of 15 min. The contact between a disinfectant and an environmental surface being treated is usually very brief; this is particularly true of alcohols or alcohol-based products because of their faster rate of evaporation.

Phenol (5%) could reduce the titer of *M. tuberculosis* by 4 $\log_{10}$ in the suspension test as well as in the carrier test, even in the presence of sputum. This finding is noteworthy since the same disinfectant proved ineffective against *M. smegmatis* in the carrier test (2). Phenol and phenol derivatives, known to be tuberculocidal even when organic matter is present (7, 10–12), are used in the disinfection of environmental surfaces. However, the strong odor and toxicity associated with phenolics make them unsuitable for use in food preparation areas and in places housing infants and children.

The diluted (1:16) glutaraldehyde-phenate remained ineffective against *M. tuberculosis* (without sputum) in the suspension test, even when the contact time was extended to 30 min. In view of this, the dilute form of this product was not tested against *M. tuberculosis* in the carrier test. Although it proved to be effective against *M. smegmatis* in the suspension test after 10 min of contact even in the presence of sputum, it failed to do so in the carrier test when sputum was added. A recent study also demonstrated that diluted (1:16) glutaraldehyde-phenate failed to inactivate *M. bovis* and a clinical strain of *M. tuberculosis* (3). These results are in agreement with those of Isenberg et al. (9), who found that this formulation could reduce the titer of *M. bovis* in organic soil by only <1 $\log_{10}$ after 10 min of contact.

The two mycobacteria also showed differences in their susceptibility to the undiluted aldehyde products tested. Undiluted glutaraldehyde-phenate was clearly superior against *M. tuberculosis* and achieved an effective level of disinfection after 10 min of contact. Glutaraldehyde (2%) required a longer contact time to cause an effective reduction. A 20-min exposure has been recommended as the minimum time needed to reliably kill *M. tuberculosis* with 2% alkaline glutaraldehyde (3). However, glutaraldehyde-phenate was slightly less efficient than glutaraldehyde alone in dealing with *M. smegmatis*. This suggests that *M. smegmatis*, when dried, may be less susceptible to the phenate component than is *M. tuberculosis*. This is supported by the results obtained for these two mycobacteria with 5% phenol. Whereas in suspension tests, 5% phenol could reduce the titer of *M. smegmatis* (in sputum) by >6 $\log_{10}$ after 1 min, in the carrier test, the drop in the titer was <2 $\log_{10}$ (2). When 5% phenol was tested against *M. tuberculosis* in sputum, it could reduce the titer by >4 $\log_{10}$ in 1 min in the suspension as well as the carrier test.

The mycobactericidal activities of glutaraldehyde-based disinfectants are critical, because such formulations are routinely used with instruments and heat-sensitive medical devices such as bronchoscopes. The dilution of such products either before or during use has been shown to render them ineffective against important pathogens, such as *M. tuberculosis* (1, 3, 18).

In general, *M. tuberculosis* appears to be more resistant to disinfection than the saprophytic *M. smegmatis*. This may be due to higher lipid levels in the cells of the tubercle bacillus (5). However, there are situations, as exemplified by phenol and glutaraldehyde-phenate, in which *M. smegmatis* proved to be more difficult to inactivate than *M. tuberculosis*. This suggests that the results of tests to determine the tuberculocidal efficacies of chemical disinfectants by using surrogate organisms must be interpreted with caution.

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