(8) G. Levy and R. H. Reuning, *J. Pharm. Sci.*, **53**, 1471(1964).
(9) M. Gibaldi, *Fed. Proc.*, **29**, 1343(1970).
(10) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.*, **13**, 976(1965).
(11) H. Matsumoto, *Yakugaku Zasshi*, **86**, 590(1966).
(12) W. Saski, *J. Pharm. Sci.*, **57**, 836(1968).

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Antibacterial Efficiency of Mercurials

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Abstract □ The minimum inhibitory concentrations (MIC) of five different mercurials against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were determined. Addition of sodium thioglycolate to the nutrient medium inactivated the mercurials, since higher MIC's were obtained. The extent of inactivation did not follow the stoichiometric relation with all tested mercurials and was different for each organism. The survivors-time relations of these compounds were measured using different concentrations of thioglycolate in the diluent. The sulfhydryl compound plays a role in the revival of damaged cells. The percentage of survivors was influenced by thioglycolate concentration; the highest survivors were obtained at an optimal concentration which was the same for all mercurials but changed with the test organism. *Ps. aeruginosa* was much more sensitive to the effect of the tested mercurials than was *Staph. aureus*. Mercuric chloride was the most efficient bactericide, followed by the phenylmercuric salts; thimerosal was the least. The possible mechanisms involved for such differences in efficiencies are discussed.

Keyphrases □ Mercurial compounds—antibacterial efficiency against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, effect of sodium thioglycolate in nutrient medium, role of sulfhydryl group □ Antibacterial efficiency—five mercurial compounds tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, effect of sodium thioglycolate in nutrient medium □ Thioglycolate (sodium)—effect on antibacterial efficiency of five mercurial compounds

The antibacterial action of mercurial compounds has been widely reported (1–7). Their mode of action was due to interference with essential metabolites and enzymes having sulfhydryl groups (8–11) or to reaction with the DNA (12) and RNA (13) of the cell. However, the antibacterial activity of these agents was found to be influenced by: *(a)* the chemical structure of the compound (14), *(b)* the type of test organism used [Gram-positive bacteria were reported to be more sensitive to mercurials than were Gram-negative ones (15)], and *(c)* the type of the quenching agent used to stop their effect, *e.g.*, sodium thioglycolate was a more efficient antagonizing agent than glutathione (11). Furthermore, previous work (16) with phenylmercuric nitrate showed that its antibacterial activity was greatly reduced by sodium thioglycolate when used in the diluent and at an optimum concentration. In this same study, thioglycolate was involved in the recovery of the damaged bacteria previously exposed to the effect of the mercurial.

Table I—Minimum Inhibitory Concentrations (MIC) of Different Mercurials against *Staph. aureus* and in the Presence of Sodium Thioglycolate

Mercurial Compound	Thioglycolate Concentration 0% MIC[a]	0.001% MIC	0.001% Hg to —SH[b]	0.003% MIC	0.003% Hg to —SH
Mercuric chloride	8.0	15	1:3	30	1:2.5
Thimerosal	1.0	4	1:12	7	1:14
Phenylmercuric acetate	0.5	4	1:8	7	1:13
Phenylmercuric borate	0.5	4	1:8	7	1:13
Phenylmercuric nitrate	0.5	4	1:8	7	1:13

[a] Micrograms per milliliter, average of six replicates. [b] Ratio of inactivated mercury molecules to thioglycolate molecules as given by: inactivated mercury molecules = (MIC of mercurial with thioglycolate − MIC without thioglycolate)/molecular weight of mercury compound.

In the present investigation, five commonly used mercurial compounds, one of which is inorganic, were tested for their antibacterial efficiencies on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, using sodium thioglycolate solution at different concentrations as the antagonizing agent. The purposes of this investigation were to study the effect of thioglycolate concentration on the antibacterial activity of mercurial compounds and to evaluate the antibacterial efficiency of these agents at the thioglycolate concentrations tested.

EXPERIMENTAL

Reagents—The following were used: mercuric chloride[1], phenylmercuric acetate[2], phenylmercuric nitrate[2], thimerosal[2], phenylmercuric borate[3], and thioglycollic acid[2] [neutralized aseptically with 1 *N* NaOH to prepare the stock sodium thioglycolate solution (16)].

Organisms—*Staphylococcus aureus* (NCTC 4163) and *Pseudomonas aeruginosa* (NCTC 7244) were used.

[1] Analar, British Drug Houses.
[2] Laboratory reagent grade, British Drug Houses.
[3] Laboratory reagent grade, Koch-Light Ltd.

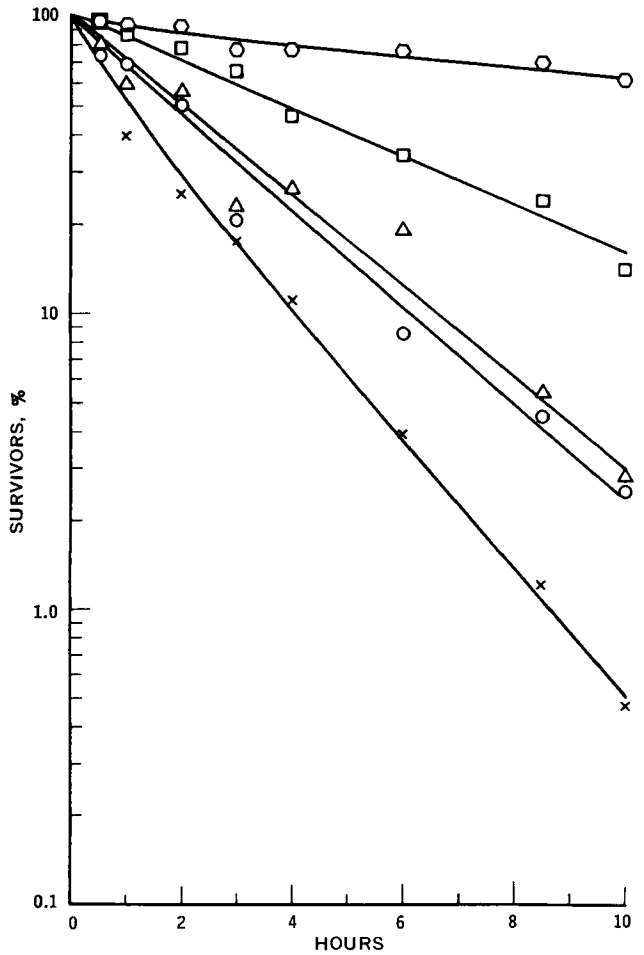

Figure 1—*Logarithm survivors–time curves of* Staph. aureus *in different solutions of mercurials when using sodium thioglycolate at 0.4%. Key:* ×, *mercuric chloride, 0.002%;* ○, *thimerosal, 0.5%;* ○, *phenylmercuric acetate, 0.02%;* □, *phenylmercuric borate, 0.02%; and* △, *phenylmercuric nitrate, 0.02%.*

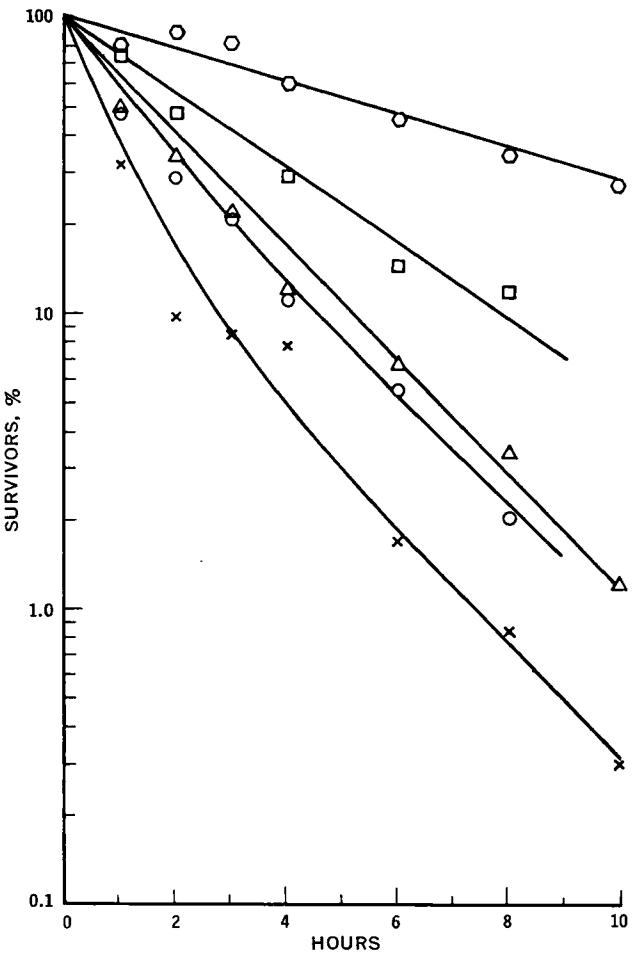

Figure 2—*Logarithm survivors–time curves of* Staph. aureus *in different solutions of mercurials when using sodium thioglycolate at 1.0%. Key:* ×, *mercuric chloride, 0.002%;* ○, *thimerosal, 0.5%;* ○, *phenylmercuric acetate, 0.02%;* □, *phenylmercuric borate, 0.02%; and* △, *phenylmercuric nitrate, 0.02%.*

Media—Nutrient broth[4] (16 g/liter), solidified when necessary with 15 g/liter of agar[5], was prepared.

Determination of Minimum Inhibitory Concentration (MIC)—The broth dilution method was adopted using as inoculum 0.1 ml (2×10^5 cells) of 24-hr broth culture. When sodium thioglycolate was included, 1 ml of thioglycolate solution at the required concentration was added aseptically to the broth before inoculation.

Determination of Bactericidal Activity—Bacterial activity was measured for each organism after treatment with the mercurial compound at appropriate concentrations for different time intervals, and the number of cells surviving treatments in each case was determined. The technique used for preparing bacterial suspensions and bactericidal reactions was the same as that used previously (16). Sodium thioglycolate solutions were used in all dilutions for the treated cells.

RESULTS AND DISCUSSION

Effect of Sodium Thioglycolate on MIC—It was previously shown that the bacteriostatic action of mercuric chloride could be eliminated by sulfhydryl-containing compounds in amounts close to the theoretical stoichiometric relation (17, 18). Therefore, the MIC's of these mercurials were determined alone and in the presence of different concentrations of sodium thioglycolate (Tables I and II) to see whether the same correlations apply. Sodium thioglycolate inactivates the mercurials since higher MIC's were obtained. However, the extent of inactivation varies with the mercurial agent and test organism, but it does not always follow the stoichiometric relation of mercury to sulfhydryl of 1:2. With *Staph. aureus*, the stoichiometric relation could be applied in the case of mercuric chloride. The ratio of inactivated mercury to sulfhydryl was 1:2.5. This result agreed with those reported on *Staph. au-*

Table II—Minimum Inhibitory Concentrations (MIC) of Different Mercurials against *Ps. aeruginosa* and in the Presence of Sodium Thioglycolate

Mercurial Compound	Thioglycolate Concentration 0% MIC[a]	0.001% MIC	0.001% Hg to —SH[b]	0.003% MIC	0.003% Hg to —SH
Mercuric chloride	6	8	1:12	15	1:8
Thimerosal	6	15	1:4	30	1:4
Phenylmercuric acetate	12	30	1:2	45	1:2.5
Phenylmercuric borate	12	30	1:2	45	1:2.5
Phenylmercuric nitrate	12	30	1:2	45	1:2.5

[a] Micrograms per milliliter, average of six replicates. [b] Ratio of inactivated mercury molecules to thioglycolate molecules as given by: inactivated mercury molecules = (MIC of mercurial with thioglycolate − MIC without thioglycolate)/molecular weight of mercury compound.

[4] Oxoid CM 15.
[5] New Zealand agar, Davis Gelatin Ltd., Warwick, England.

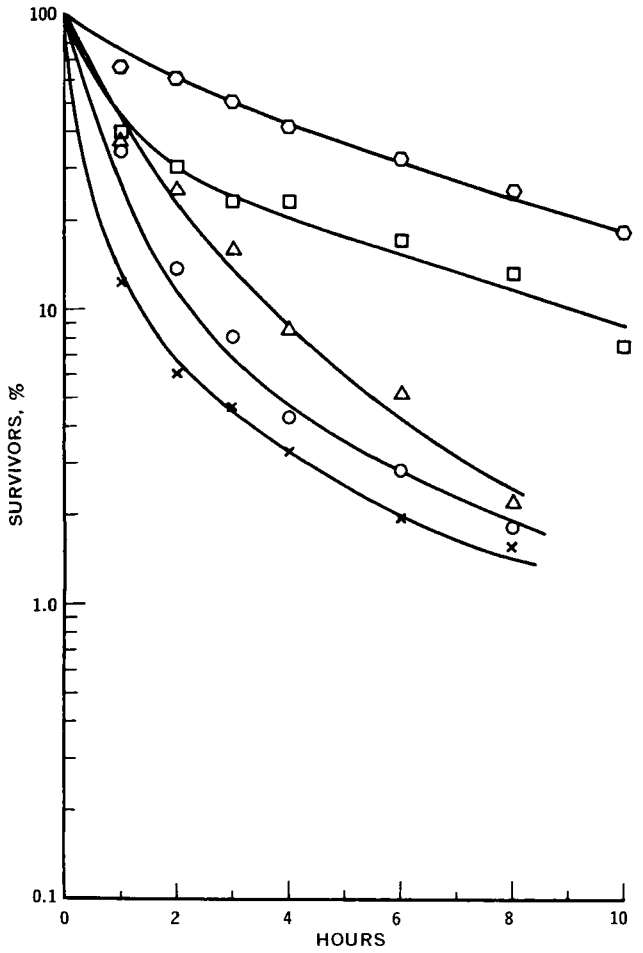

Figure 3—*Logarithm survivors–time curves of* Staph. aureus *in different solutions of mercurials when using sodium thioglycolate at 3.0%. Key: ×, mercuric chloride, 0.002%; ○, thimerosal, 0.5%; ○, phenylmercuric acetate, 0.02%; □, phenylmercuric borate, 0.02%; and △, phenylmercuric nitrate, 0.02%.*

reus (17) and *Escherichia coli* (18). However, with organic mercurials, ratios of 1:8 or higher were obtained. In the case of *Ps. aeruginosa*, the ratios were different. The mercury to sulfhydryl ratios were close to the stoichiometric relation with organic mercurials, while a higher ratio was obtained with mercuric chloride (Table II). The difference noticed in the mercury to sulfhydryl ratios of each mercurial was due to the type of organism used. This could indicate that, when the microorganism was present, the removal of the inhibitory action of the mercurial by the sulfhydryl compound was not solely due to simple chemical complexes between the free mercury molecules and sulfhydryl molecules but that there were other biological effects as well.

The organic mercurials (Table I) were found to be more inhibitory to *Staph. aureus* than to *Ps. aeruginosa*. These results agreed with similar, previously reported work (19, 20). Such findings are, however, different from those obtained with the bactericidal activity measurements; *Staph. aureus* was more resistant than *Ps. aeruginosa* to the effect of the tested mercurials. This might be attributed to a difference in the site of bacteriostatic and bactericidal action of the mercurial. As suggested previously (21, 22), when the action of the mercurial is bacteriostatic, it acts by physical adsorption on the cell surface; the bactericidal action is produced after the mercurial penetrates the cell and reacts with essential metabolites, causing structural changes in the cell.

Bactericidal Activity of Mercurials—*Staph. aureus*—The survivors-time curves of *Staph. aureus* bacteria treated with 0.002% $HgCl_2$, 0.5% thimerosal, and 0.02% phenylmercuric salts in 0.4, 1.0, and 3.0% sodium thioglycolate solutions are presented in Figs. 1–3, respectively. Even after 10 hr of treatment with such high concentrations of mercurials, viable cells were recovered; when 3% sodium thioglycolate was used, the level of mortality did not reach 99% (Fig. 3). The figures show the actual bactericidal efficiency of these agents when using high thioglycolate concentrations. The most active agent tested was mercuric chloride, although it was used at 0.002% concentration, and the least effective was thimerosal in spite of its high concentration (0.5%). The phenylmercuric salts occupied an intermediate position; however, the borate was slightly less active than the acetate and the nitrate.

The trend of reaction in Figs. 1 and 2 was logarithmic with all tested mercurials, which meant a first-order reaction. However, deviation from linearity occurred in Fig. 3, and the relation became more like a curve than a straight line. Although the bactericidal efficiencies of these compounds were different, it appeared from the shape of the survivors–time curves that their mechanism of action was the same. Difference in the bactericidal efficiency between mercurials was probably due to a difference in the rate by which the mercurial reached the vital molecules of the bacterial cell and became lethal. The results obtained are not in agreement with previous work (15) which showed that mercuric chloride was less active than organic mercurials. Such controversy is possibly due to the effect of sodium thioglycolate on the recovery of the damaged cells, particularly when the proper thioglycolate concentrations producing the highest revival were used. Sodium thioglycolate solution at 3% gave the highest number of survivors (Fig. 3).

Ps. aeruginosa—This organism was found to be much more

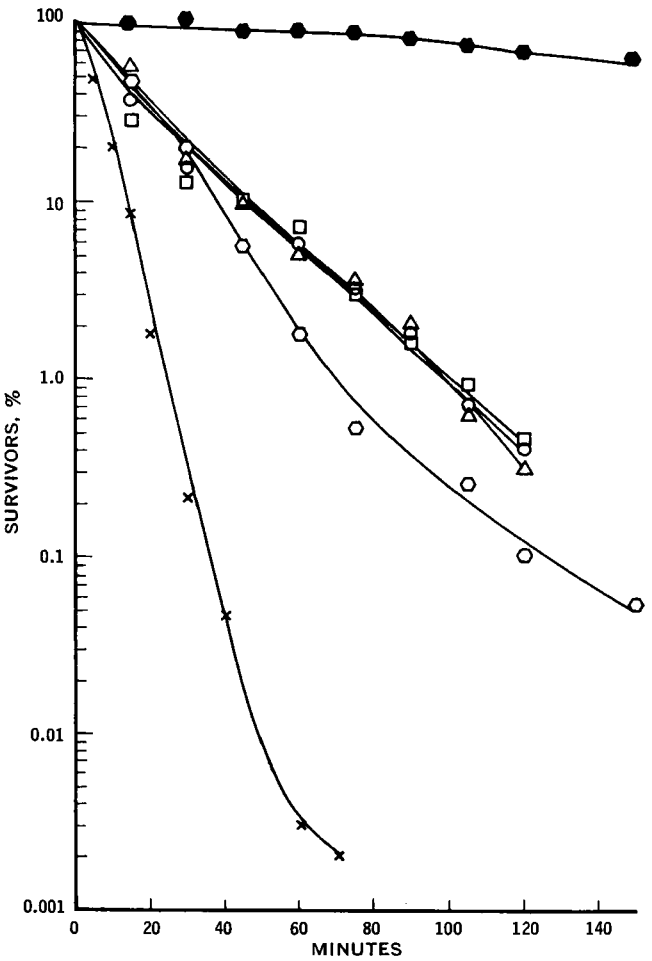

Figure 4—*Logarithm survivors–time curves of* Ps. aeruginosa *in different solutions of mercurials when using sodium thioglycolate at 0.04%. Key: ×, mercuric chloride, 0.002%; ○, thimerosal, 0.05%; ○, phenylmercuric acetate, 0.002%; □, phenylmercuric borate, 0.002%; △, phenylmercuric nitrate, 0.002%; and ●, thimerosal, 0.002%.*

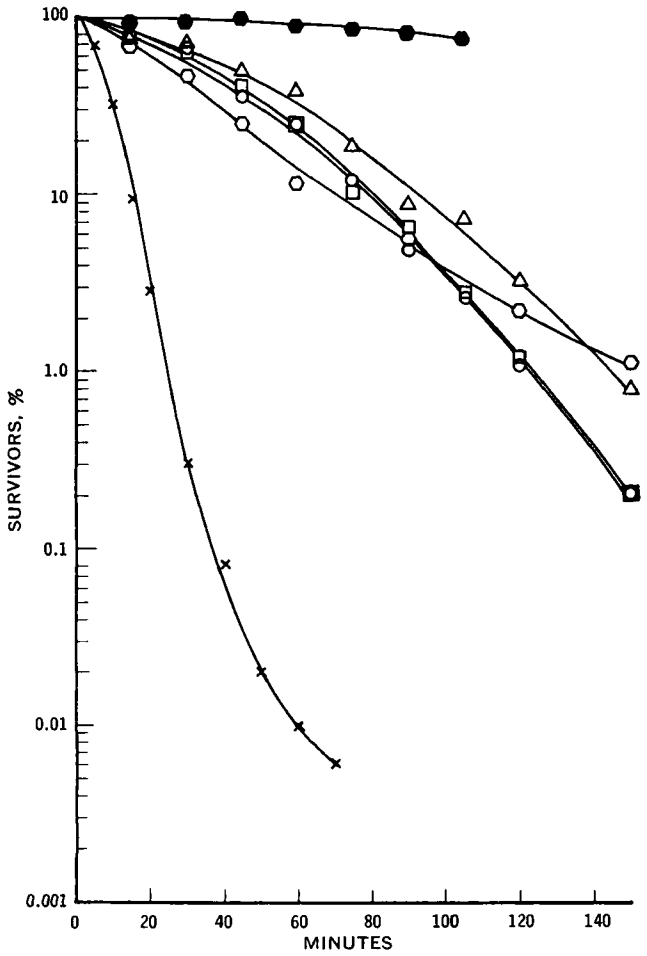

Figure 5—*Logarithm survivors–time curves of* Ps. aeruginosa *in different solutions of mercurials when using sodium thioglycolate at 0.2%. Key:* ×, *mercuric chloride, 0.002%;* ○, *thimerosal, 0.05%;* ○, *phenylmercuric acetate, 0.002%;* □, *phenylmercuric borate, 0.002%;* △, *phenylmercuric nitrate, 0.002%; and* ○, *thimerosal, 0.002%.*

sensitive to the effect of mercurials than was *Staph. aureus*. This finding is in agreement with those using phenylmercuric borate (21) and phenylmercuric nitrate (16). Consequently, to obtain reasonable survivors-time curves, the concentrations used were 0.05% for thimerosal and 0.002% for mercuric chloride and phenylmercuric salts (Figs. 4–6). The shape of the survivors curves did not follow a linear relation as it did with *Staph. aureus*. The results could indicate that the mode of action of mercurials on the two tested organisms was not the same. Furthermore, the order of bactericidal activities of these compounds differed from that obtained with *Staph. aureus*. Thimerosal at the 0.05% level appeared to be more active than the phenylmercuric salts. However, thimerosal at a concentration similar to that of the phenylmercuric salts (0.002%) proved to be less effective against *Ps. aeruginosa* (Figs. 4 and 5). The phenylmercuric salts behaved more or less similarly toward *Ps. aeruginosa* with respect to bactericidal activities. Mercuric chloride was still the most active bactericide among the tested mercurials.

With both microorganisms and under the test conditions, mercuric chloride was a remarkably efficient bactericide compared to the organic mercurials. The difference in bactericidal activity could be due to a difference in the nature of the interactions of these compounds with the cellular structures, such that the mercuric chloride interaction was not affected by the thioglycolate concentration to the same extent as the organic mercurial compounds. The sensitivity of *Ps. aeruginosa* toward the mercurials might be attributed to its relatively high lipid content of the cell wall (23, 24) as compared with *Staph. aureus* (25). The difference in the lipid content of each organism may play a role in the antibacterial activities of these agents as it affects the solubility of the mercurials in the cell membranes (22). In addition, it has been reported that the increased resistance of *Staph. aureus* to mercurials was due to a rise in its sulfhydryl content (26, 27).

Effect of Thioglycolate Concentration on Bactericidal Activity—The effect of sodium thioglycolate concentration on the number of cells surviving treatment with mercurials is shown quite clearly in Figs. 1–6. The number of survivors obtained with both tested organisms at any thioglycolate concentration used was remarkably higher than that obtained when no sodium thioglycolate was used or when the stoichiometric concentration was used. When sterile distilled water was used instead of thioglycolate, the 99% mortality of mercurial-treated bacteria was reached in <5 min with *Ps. aeruginosa* and in <10 min with *Staph. aureus* (Table III). With thimerosal-treated *Staph. aureus*, the 99% mortality was reached in 2 hr. Such significantly high survivors in the presence of thioglycolate could indicate that thioglycolate was an essential factor for the recovery and revival of the damaged bacteria. To obtain the highest level of survivors, sodium thioglycolate has to be used at an optimum concentration range which varied according to the test organism. With all tested mercurials, the optimal thioglycolate concentration for the recovery of *Ps. aeruginosa* was 0.2%; for *Staph. aureus*, it was 2–3%. Prolongation of bacterial treatments with mercurials affects the response of the damaged cells to thioglycolate concentration. The more severe the treatment the greater would be the response (16). These optimal thioglycolate concentrations are much higher than those customarily used in media for sterility testing. Since sterilization in the presence of mercurials results in severe damage to any ex-

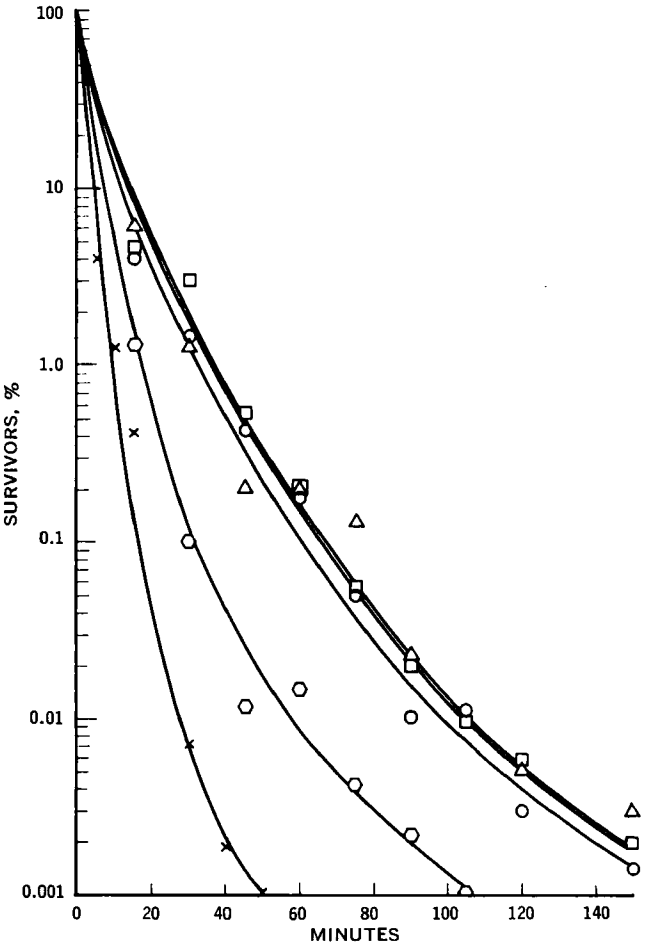

Figure 6—*Logarithm survivors–time curves of* Ps. aeruginosa *in different solutions of mercurials when using sodium thioglycolate at 1.0%. Key:* ×, *mercuric chloride, 0.002%;* ○, *thimerosal, 0.05%;* ○, *phenylmercuric acetate, 0.002%;* □, *phenylmercuric borate, 0.002%; and* △, *phenylmercuric nitrate, 0.002%.*

Table III—Effect of Sodium Thioglycolate Concentration on Bactericidal Efficiency of Mercurials against *Staph. aureus* and *Ps. aeruginosa*

Mercurial Compound		Thioglycolate Concentration			
		0%	0.4%	1.0%	3.0%
		Staph. aureus			
Mercuric chloride, 0.002%	A[a]	2 min	4 hr	3 hr	2.5 hr
	B[b]	10 min	8 hr	9 hr	10.0 hr
Thimerosal, 0.5%	A	90 min	>24 hr	>24 hr	>24.0 hr
	B	99 min	—	—	—
Phenylmercuric acetate, 0.02%	A	<2 min	6 hr	4 hr	2.5 hr
	B	2 min	11 hr	12 hr	14.0 hr
Phenylmercuric borate, 0.02%	A	5 min	11 hr	9 hr	9.0 hr
	B	10 min	21 hr	24 hr	>24.0 hr
Phenylmercuric nitrate, 0.02%	A	<2 min	7 hr	4 hr	3.5 hr
	B	2 min	11 hr	12 hr	13.0 hr
		Ps. aeruginosa			
		0%	0.04%	0.2%	1.0%
Mercuric chloride, 0.002%	A	<2 min	15 min	15 min	10 min
	B	5 min	25 min	25 min	15 min
Thimerosal, 0.05%	A	5 min	40 min	70 min	20 min
	B	10 min	70 min	155 min	30 min
Phenylmercuric acetate, 0.002%	A	<2 min	45 min	80 min	30 min
	B		100 min	125 min	60 min
Phenylmercuric borate, 0.002%	A		45 min	80 min	35 min
	B		100 min	125 min	65 min
Phenylmercuric nitrate, 0.002%	A		45 min	85 min	35 min
	B		100 min	125 min	60 min

[a] A = time to reach 90% mortality. [b] B = time to reach 99% mortality.

isting bacteria, the thioglycolate concentration becomes a critical factor for the revival of these damaged bacteria.

REFERENCES

(1) H. Engelhardt, *Disinfection*, **7**, 63(1922).

(2) J. H. Brewer, *Ann. N.Y. Acad. Sci.*, **53**, 211(1950).

(3) F. Monacelli, *Riv. Ital. Igune*, **10**, 111(1950).

(4) F. Bianchi and G. Malaguti, *Boll. Ist. Sieroter. Milan.*, **30**, 470(1951).

(5) J. E. Davison, *J. Pharm. Pharmacol.*, **3**, 734(1951).

(6) A. M. Cook and K. J. Steel, *ibid.*, **11**, 666(1959).

(7) M. R. W. Brown, *J. Pharm. Sci.*, **57**, 389(1968).

(8) P. Fildes, *Brit. J. Exp. Pathol.*, **21**, 67(1940).

(9) E. S. Cook and C. W. Ereke, *J. Biol. Chem.*, **162**, 43(1946).

(10) J. O. Harris, A. Eisenstpirk, and R. Deagsdore, *J. Bacteriol.*, **68**, 745(1954).

(11) A. M. Cook and K. J. Steel, *J. Pharm. Pharmacol.*, **12**, 219(1960).

(12) P. Pietsch and C. Corbett, *Nature*, **219**, 933(1968).

(13) Y. Kawade, *Biochem. Biophys. Res. Commun.*, **10**, 204(1963).

(14) E. C. McCulloch, "Disinfection and Sterilization," 2nd ed., Lea & Febiger, Philadelphia, Pa., 1945, pp. 266–286.

(15) K. E. Birkhaug, *J. Infect. Dis.*, **53**, 250(1933).

(16) J. P. Richards and A. E. Elkhouly, *J. Pharm. Pharmacol., Suppl.*, **19**, 209S(1967).

(17) P. Benigo and R. Santi, *Ric. Sci. Ricostr.*, **17**, 1117(1947).

(18) A. M. Cook and K. J. Steel, *J. Pharm. Pharmacol.*, **11**, 162T(1959).

(19) A. M. Cook, K. J. Steel, and B. A. Wills, *ibid.*, **8**, 721(1956).

(20) K. Toyoshi, K. Kato, K. Yamada, K. Komoda, and U. Kurimoto, *Yakugaku Zasshi*, **84**, 1149(1964).

(21) H. Hess and P. Speiser, *J. Pharm. Pharmacol.*, **11**, 694(1959).

(22) H. Meyer, *Pharm. Zentralh.*, **103**, 571(1964).

(23) K. Clarke, G. W. Gray, and D. A. Reaveley, *Biochem. J.*, **105**, 749(1967).

(24) R. A. Bobo and R. G. Eagon, *Can. J. Microbiol.*, **14**, 503(1968).

(25) A. V. Few, *Biochim. Biophys. Acta*, **16**, 137(1955).

(26) P. Benigo and R. Santi, *Ric. Sci. Ricostr.*, **17**, 184(1947).

(27) L. Vaczi, M. Fodor, H. Milch, and A. Rethy, *Acta Microbiol. Acad. Sci. Hung.*, **9**, 81(1961).

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