THE BACTERIOSTATIC AND BACTERICIDAL ACTIONS OF SOME MERCURIAL COMPOUNDS ON HEMOLYTIC STREPTOCOCCI

In Vivo and in Vitro Studies

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Mercurial compounds have been employed as disinfectants since the beginning of bacteriology. Indeed, for a long period mercurial compounds, such as bichloride of mercury, headed the list of chemicals which were thought to be effective in the killing of micro-organisms. This perhaps may be attributed to the favorable publicity which Koch [1] in 1881 gave to bichloride of mercury during his work with the organism causing anthrax. In substance he stated that, without special preparation of the objects to be disinfected, bichloride kills by a single application of a very dilute solution, and in a few minutes even the most resistant forms of the organisms are killed.

Geppert [2] in 1889 first pointed out that false inferences were drawn that bacteria were killed by the action of bichloride of mercury when growth of the organisms was prevented by traces of the chemical in the culture medium. In support of his thesis Geppert claimed that the spores of Bacillus anthracis were capable of infecting animals even after the spores had been treated with bichloride of mercury and produced no growth on subculturing by the usual methods. To eliminate the bacteriostatic action of the small quantity of mercury adherent to the bacteria and of the small amount of the chemical carried into the subculturing medium in the inoculum, Geppert employed ammonium sulfide to inactivate the bichloride of mercury. By this technic Geppert showed that the spores of B. anthracis were not killed by 1:1,000 bichloride of mercury during a period of exposure of hours, in one case as long as twenty-four hours.

In 1891 Abbott [3] claimed that he had confirmed Geppert's work in part, but he did not do animal experiments. In his publication in 1891, Abbott concluded, ". . . it is plain that for use in surgical practice the solutions of corrosive sublimate do not possess all of the advantages hitherto attributed to them."

Do other compounds of mercury possess the same shortcomings as disinfectants [4] as does bichloride of mercury? Are non-spore forming pathogenic micro-organisms still infectious while in a state of bacteriostasis brought about by a mercurial compound? The answers to these important questions are provided by the experimental work to be described.

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1. Koch, R.: Ueber Desinfection, Mitt. a. d. k. Gesundheitsamte **1:** 234-282, 1881.
2. Geppert, J.: Zur Lehre von den Antiseptics: Eine Experimentaluntersuchung, Berl. klin. Wchnschr. **26:** 789-794, 819-821, 1889.
3. Abbott, A. C.: Corrosive Sublimate as a Disinfectant Against Staphylococcus Pyogenes Aureus, Bull. Johns Hopkins Hosp. **2:** 50-60, 1891.
4. Agents capable of destroying pathogenic micro-organisms and thus preventing infection.

REASONS FOR AND MEANS OF PREVENTING THE BACTERIOSTATIC ACTION OF MERCURIAL COMPOUNDS IN GERMICIDAL TESTS

It is generally known that it is exceedingly difficult to destroy bacterial spores. Our results, about to be described, indicate that virulent hemolytic streptococci (an example of non-spore forming organisms) are still infectious after being exposed for ten minutes to any one of three organic mercurial compounds obtained in the open market. The compounds studied were (1) the disodium salt of 2,7-dibrom-4-hydroxymercurifluorescein, merbromin, N. F., "mercurochrome," (2) sodium ethylmercurithiosalicylate, "merthiolate," (3) the anhydride of 4-nitro-3 hydroxymercuri-orthocresol, "metaphen." The fact that both vegetative cells and spores are still infectious while in a state of bacteriostasis is sufficient reason for taking precautions to eliminate the bacteriostatic effect of mercury when testing mercurial compounds in vitro for germicidal activity. In the in vitro test, micro-organisms are mixed with the chemical under test and at stated intervals portions, usually a standard loopful, are transferred to an appropriate subculturing medium. It is quite generally accepted that germicides in solution bring about their action by reacting chemically with the bacterial cell. When a portion of the germicide-culture mixture is transferred into the subculturing medium, in order to determine whether the organisms are still viable some of the germicide is introduced into the subculturing medium, both free in the solution and in combination with the bacterial cells. In testing strong solutions of germicides it was realized that in some cases it was possible to carry enough of the chemical in an inoculum the size of a standard loopful into the subculturing medium to give a concentration of the germicide in the subculturing medium great enough to exert a bacteriostatic action. Shippen [5] in 1928 recommended transferring four loopfuls from each subculture of the culture-germicide mixture to a second tube of broth. In this way the germicide would be diluted beyond the concentration at which it would be able to exert a bacteriostatic action. By this technic he found that bichloride of mercury in a dilution of 1:200 to 1:300 failed to kill Staphylococcus aureus in fifteen minutes' exposure. This concentration of bichloride of mercury is three to five times as great as usually recommended and is still incapable of destroying in fifteen minutes' exposure the vegetative cells of the common pus producing organism. Mere dilution of the inoculum does not eliminate the bacteriostatic action of the mercurial contained in it. Heinemann [6] pointed out that dilution of the inoculum as in the Shippen technic gave falsely high killing values in several instances. This was also demonstrated by Morton [7] with soaps containing mercurial compounds. It has been pointed out also that dilution does not neutralize the mercury which may be bound to the bacterial cell.

Bacterial cells which have been weakened by exposure to the action of a germicide are more susceptible to the bacteriostatic action of the germicide, so it is more difficult to eliminate the bacteriostatic action of the chemical. This was pointed out by Geppert [2] and was

5. Shippen, L. P.: A Fallacy in the Standard Methods of Examining Disinfectants, Am. J. Pub. Health **18:** 1231-1234, 1928.
6. Heinemann, B.: A Comparison of Methods Used for Evaluating the Bactericidal Properties of Mercurial Compounds, J. Am. Pharm. A. (Scient. Ed.) **32:** 298-301, 1943.
7. Morton, Harry E.: "Germicidal" Soaps: I. The Importance of a Clean Skin, the Action of Soaps in Freeing the Skin of Viable Micro-organisms, and Methods for Testing the Efficiency of Germicidal (Medicated) Soaps, J. A. M. A. **124:** 1195-1201 (April 22) 1944.

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emphasized in one of the early textbooks on bacteriology.[8] For a fuller discussion of the shortcomings of mercurial compounds as disinfectants reference should be made to the report of Brewer [9] in 1939.

A method more reliable than mere dilution for eliminating the bacteriostatic effect of a mercurial compound in the subculturing medium during the testing for germicidal action is chemical neutralization of the mercurial. Geppert employed ammonium sulfide to inactivate the mercury in the culture-germicide mixture which was subcultured and found that the dilutions of bichloride of mercury which were germicidal were much lower than when cultured by ordinary methods. Hunt [10] employed hydrogen sulfide for the same purpose. The thioglycollate medium described by Brewer [11] in 1940 for growing micro-organisms anaerobically was also found to be capable of neutralizing the bacteriostatic action of mercurial compounds.[12] The National Institute of Health made this the official medium for the sterility testing of biological products beginning July 1942, and it has been subsequently modified to a slight extent (Pittman,[13] N. I. H. circular.[14])

The objectives of our experiments were to determine (1) the effect of some of the organic mercurial compounds on an organism pathogenic for man and mouse and (2) whether the growth of the test organism in thioglycollate medium parallels infectivity for the animal body.

MATERIALS AND TECHNICS

Test Organism.—It is desirable to select for test organisms those which are pathogenic for man as well as for some laboratory animal. We selected for this work Streptococcus pyogenes, strain C203M. It gave a good reaction with group A serum (Lancefield). On extract agar containing 5 to 10 per cent normal horse blood the streptococcus colonies were surrounded by large zones of hemolysis of the red blood cells. The red blood cells in blood broth were readily hemolyzed. The culture was maintained in blood extract broth composed of Bacto-beef extract 0.3 per cent, Parke-Davis peptone 1 per cent, sodium chloride 0.5 per cent and distilled water. The p_H was approximately 7.2 after sterilization. Sterile defibrinated horse blood was added to the amount of 10 per cent. Approximately 10 cc. of medium was contained in the culture tubes. Fresh subcultures were made by transferring 0.5 cc. of a previous culture to a tube of fresh medium, incubating at 37 C. for twenty-four hours, then storing in the refrigerator at 4-11 C. The stock cultures were routinely subcultured every three months. Three out of three mice were killed within sixty-five hours following the intraperitoneal injection of 1 cc. of a 1 : 10,000,000 (1×10^{-7}) dilution of a twenty-four hour culture. The lethal dose constituted four colonies when grown in blood extract agar.

Experimental Animal.—White Swiss mice weighing 17-20 Gm. each were employed.

8. Sternberg, G. M.: A Manual of Bacteriology, Baltimore, William Wood & Co., 1892.
9. Brewer, John H.: The Antibacterial Effects of the Organic Mercurial Compounds, with Special Reference to Their Use as Germicides for the Sterilization of Surgical and Dental Instruments, J. A. M. A. **112**: 2009-2018 (May 20) 1939.
10. Hunt, G. A.: The Use of Cutaneous Staphylococcus Lesions in Mice for the Evaluation of the Germicidal Activity of Disinfectants, J. Infect. Dis. **60**: 232-237, 1937.
11. Brewer, John H.: Clear Liquid Mediums for the "Aerobic" Cultivation of Anaerobes, J. A. M. A. **115**: 598-600 (Aug. 24) 1940.
12. Marshall, M. S.; Gunnison, J. B., and Luxen, M. P.: Test for the Sterility of Biologic Products, Proc. Soc. Exper. Biol. & Med. **43**: 672-673, 1940. Brewer.[11]
13. Pittman, M.: A Study of Fluid Thioglycollate Medium for the Sterility Test, J. Bact. **51**: 19-32, 1946.
14. National Institute of Health Circular: Culture Media for the Sterility Test, first revision, Jan. 15, 1945.

Culture Mediums.—Bacto-Anaerobe medium with dextrose, experimental, contained in each liter Proteose-peptone No. 3, Difco, 20 Gm., Bacto-beef extract 3 Gm., Bacto-yeast extract 3 Gm., malt extract, Difco, 3 Gm., dextrose 5 Gm. and agar 1 Gm. Reaction of the medium after sterilization was approximately p_H 7.3.

Bacto-cooked meat medium No. 2 was a special lot for a contaminated wound project. Each liter of medium contained beef heart 454 Gm., proteose-peptone, Difco, 20 Gm., dextrose 2 Gm. and sodium chloride 5 Gm. Reaction of the medium after sterilization was approximately p_H 7.4.

Bacto-tryptose phosphate broth contained in each liter Bacto-tryptose 20 Gm., Bacto-dextrose 2 Gm., sodium chloride 5 Gm. and disodium phosphate 2.5 Gm. Reaction of the medium after sterilization was approximately p_H 7.3.

Hydrolyzed casein. This was received from Dr. C. M. Brewer, U. S. Department of Agriculture, who stated that it was "Trypticase-Pancreatic Digest of Casein" received from the Baltimore Biological Laboratory. A 3 per cent solution in distilled water containing 0.5 per cent sodium chloride was used. It had a reaction of p_H 6.8 after sterilization.

Bacto-fluid thioglycollate medium, Linden formula, was prepared in accordance with section 4 b of the National Institute of Health Bulletin "Fluid Thioglycollate Medium for the Sterility Test," dated Dec. 30, 1941. Each liter of the medium consisted of Proteose-peptone No. 4, Difco, 30 Gm., Bacto-dextrose 5 Gm., Bacto-yeast extract 2 Gm., sodium thioglycollate, Difco, 1 Gm., Bacto-agar 0.5 Gm., sodium chloride 5 Gm., dipotassium phosphate 2.5 Gm., Bacto-methylene blue (DA-6) 0.002 Gm. and distilled water. The reaction was approximately p_H 7.5 after autoclaving.

For a plating medium beef extract agar, made by adding 2 per cent agar to the beef extract broth, was used, to which was added 10 per cent sterile, defibrinated horse blood.

Disinfectants.—Samples of the three organic mercurial compounds "Mercurochrome," "Merthiolate" and "Metaphen" were purchased over the counter from various pharmacies and used undiluted from the original packages or diluted as for the standard phenol coefficient test.[15]

Technic.—In performing the disinfection tests, 1 cc. of a 24 hour old blood broth culture, ± 2 hours, was added to 10 cc. of the disinfectant in a test tube. After thorough mixing, 5 cc. of the culture-disinfectant mixture was transferred to an Esmarch dish to facilitate filling syringes. At intervals of five, ten and fifteen minutes 1 cc. of the culture-disinfectant mixture was removed from the test tube, 0.5 cc. inoculated into a tube containing about 11 cc. of blood broth, and the other 0.5 cc. portion inoculated into a tube containing about 11 cc. of fluid thioglycollate medium. At the ten minute interval 1 cc. portions or less, if the disinfectant was toxic for mice, of the culture-disinfectant mixture was injected intraperitoneally into white mice weighing 17-20 Gm. each. The temperature of medication was room temperature. After all subcultures had been made the tubes were thoroughly shaken and then incubated at 37 C. Subculture tubes which did not show growth were kept under observation for seven

15. Ruehle, G. L. A., and Brewer, C. M.: United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants, Circular No. 198, U. S. Dept. of Agriculture, Washington, D. C., December 1931.

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days before discarding. Growth in the subculture tubes was checked microscopically and in some cases by streaking onto blood agar plates. Streptococci were demonstrated in all critical tubes. The heart's blood from some of the mice which died following the injection of each culture-germicide mixture was streaked on blood agar plates and hemolytic streptococcus colonies were demonstrated. Representative cultures isolated from the heart's blood cultures were demonstrated to be group A by the technic of Brown.[16]

Controls.—Comparable amounts of the germicides which were contained in the dose of germicide-culture mixtures were injected intraperitoneally into mice to prove that the mice were not killed by the germicide. Virulence of the culture was demonstrated by adding 1 cc. of culture to 10 cc. of sterile distilled water, allowing to stand at room temperature for ten minutes, then injecting 1 cc. into each of four mice. Hemolytic streptococci were demonstrated in the heart's blood after death of the mice. Representative cultures isolated from the heart's blood of the dead mice were demonstrated to be group A by the technic of Brown.[16]

The transferring of a measured amount of culture-germicide mixture to the subculture tubes and to mice differed from the standard technic of transferring one loopful, but we feel that it is desirable and advantageous. The standard loop is estimated to hold about 0.02 cc. By transferring a larger volume one has a better chance of transferring viable organisms if they exist in the culture-germicide mixture. This is desirable, as our results indicate that a minimum of four streptococcus organisms of the strain used were required to produce a fatal infection in mice. Garrod [17] in 1935 pointed out that "an adequately large number of bacteria should be added to cultures in which bacteriostatic action is being studied." We found that a volume of 0.5 cc. could be employed conveniently in our in vitro tests. Tobie and Orr [18] transferred inoculums of 0.02 cc. with pipets because the loop was unsatisfactory, and Tice and Pressman [19] employed 0.05 cc. amounts. The toxicity of some of the compounds did not permit us to inject mice with more than 0.1 or 0.2 cc. The volume of culture-germicide mixture transferred by means of a pipet is arbitrary. By transferring larger volumes a more critical test is placed on the germicide, but this should not present an obstacle if the compound is an effective germicide.

EXPERIMENTAL

It would be very advantageous if cultures of virulent hemolytic streptococci could be grown in a blood-free culture medium with virulence of the culture maintained. The culture of hemolytic streptococcus strain C203M, recently passed through mice, was inoculated into the following five mediums: (1) extract broth containing 10 per cent horse blood, (2) Bacto-anaerobe medium, experimental, (3) Bacto-cooked meat medium, (4) Bacto-tryptose phosphate broth and (5) hydrolyzed casein medium. Serial transfers were carried through the mediums until the cultures had been grown in each medium for nineteen successive transfers. A sixth medium, Bacto-thioglycollate medium, Brewer's formula, was also included, but contamination of the culture caused us to drop that medium from the series.

Table 1.—*The Number of Hemolytic Streptococci Present in Twenty-Four Hour Cultures in Various Culture Mediums and the Virulence of the Streptococci for White Mice*

Dilution of culture	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Medium	Extract broth + 10 per cent horse blood				
Number of colonies	(47,000)*	(4,700)*	(470)*	47	4
Three mice injected with each dilution	Died 20 hrs.	Died 20 hrs.	Died 22 hrs.	Died 65 hrs.	Died 65 hrs.
	Died 20 hrs.	Died 22 hrs.	Died 22 hrs.	Died 65 hrs.	Died 65 hrs.
	Died 20 hrs.	Died 22 hrs.	Died 22 hrs.	Died 65 hrs.	Died 65 hrs.
	Bacto anaerobe medium with dextrose, experimental				
Number of colonies	(26,000)*	(2,600)*	Est. 200	26	7
Three mice injected with each dilution	Died 20 hrs.	Died 65 hrs.	Died 22 hrs.	Died 65 hrs.	Died 65 hrs.
	Died 22 hrs.	Died 65 hrs.	Died 65 hrs.	Died 65 hrs.	Survived
	Died 22 hrs.	Died 65 hrs.	Died 120 hrs.	Died 65 hrs.	Survived
	Bacto-cooked meat medium number 2, experimental				
Number of colonies	(57,000)*	(5,700)*	(5,0)*	57	4
Three mice injected with each dilution	Died 20 hrs.	Died 65 hrs.	Died 65 hrs.	Died 65 hrs.	Survived
	Died 22 hrs.	Died 65 hrs.	Died 72 hrs.	Survived	Survived
	Survived	Died 65 hrs.	survived	Survived	Survived
	Bacto-tryptose phosphate broth				
Number of colonies	(19,700)*	(1,970)*	197	20	3
Three mice injected with each dilution	Died 65 hrs.	Died 65 hrs.	Died 65 hrs.	Died 72 hrs.	Survived
	Died 65 hrs.	Survived	Died 7 days	Died 72 hrs.	Survived
	Died 65 hrs.	Survived	Survived	Survived	Survived
	Hydrolyzed casein medium				
Number of colonies	120	12	1	0	0
Three mice injected with each dilution	Died 65 hrs.	Died 65 hrs.	Survived	Survived	Survived
	Survived	Died 72 hrs.	Survived	Survived	Survived
	Survived	Died 8 days	Survived	Survived	Survived

* Colonies too numerous to count. Number estimated from colony count of plate with high dilution of inoculum. Survived = mice alive and healthy at end of eight days, when experiment was terminated.

Table 2.—*Comparison of Two Culture Mediums, with Modifications for Their Ability to Support Growth of Hemolytic Streptococci*

One medium capable of neutralizing the bacteriostatic action of mercurial compounds and one medium incapable of such action

Dilution of inoculum	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-0}	10^{-10}
Number of colonies developing from an inoculum of 0.5 cc.	..	..	11	2	0	..
Estimated number of organisms in inoculum	1,100	110	11	1 or 2	1 or 0	..
Beef extract broth	+	—	—	—	—	—
Beef extract broth + 10 per cent blood	+	+	+	+	+	—
Beef extract broth + 0.5 per cent glucose	+	+	—	—	—	—
Beef extract broth + 0.2 per cent cerulose	+	+	+	+	+	—
Beef extract broth + 10 per cent blood + 0.2 per cent cerulose	+	+	+	+	+	—
Bacto-thioglycollate medium, Linden's formula	+	+	+	+	+	—

16. Brown, J. Howard: A Simplified Method for Grouping Hemolytic Streptococci by the Precipitin Reaction, J. A. M. A. **111:** 310-311 (July 23) 1938.
17. Garrod, L. P.: The Effect of Bacterial Numbers on Minimum Bacteriostatic Concentrations, J. Infect. Dis. **57:** 247-251, 1935.
18. Tobie, W. C., and Orr, M. L.: Determination of Phenol Coefficients in Presence of Surface Tension Depressants, J. Lab. & Clin. Med. **29:** 767-768 (July) 1944.
19. Tice, L. F., and Pressman, R.: Antiseptics of the Quaternary Ammonium Type in the Presence of Positive and Negative Gelatin, J. Am. Pharm. A. (Sc. Ed.) **34:** 201-204 (Aug.) 1945.

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When the growths in the nineteenth serial transfers in each medium were 24 hours old, serial dilutions of each culture were made in extract broth. One cc. of each dilution was inoculated intraperitoneally into each of 3 mice, and 1 cc. was made into a blood agar poured plate. The results are summarized in table 1.

The results summarized in table 1 indicate that extract broth containing 10 per cent horse blood satisfactorily supported growth of the hemolytic streptococci and the organisms possessed the highest virulence for mice. Mediums not containing blood were not as satisfactory.

For the work to be described it is necessary to have two culture mediums which will support growth of hemolytic streptococci equally well. One of the culture mediums must be capable of neutralizing the bacteriostatic action of mercurial compounds, and for this medium we selected Bacto-thioglycollate medium, Linden's formula. The other medium must be incapable of neutralizing such bacteriostatic action, and beef extract broth with the addition of various substances was tried. The results are summarized in table 2.

From the results summarized in table 2, beef extract broth containing 10 per cent horse blood supported growth of hemolytic streptococci C203M as well as did Bacto-thioglycollate medium, Linden's formula. The addition of 10 per cent blood to the extract broth was preferred to 0.2 per cent cerulose because of the ease of reading growth in the medium containing blood and because it is the medium in which the organisms are maintained in culture.

Typical results obtained from numerous experiments wherein the cultures of hemolytic streptococci were exposed to the action of the various compounds and then subcultured and also injected into mice are given in table 3. It can be seen that, if subculturing of the mercurial germicide-culture mixture into thioglycollate medium resulted in growth of the culture, the organisms are still capable of producing a fatal infection in the animal body. Merthiolate 1 : 1,000, aqueous, when allowed to act on a culture of hemolytic streptococci for ten minutes was not a disinfectant because mice injected with such mixtures invariably died. In all 16 out of 17 mice injected with such mixtures died. Mercurochrome 2 per cent and metaphen 1 : 500 failed to kill streptococci within an exposure period of even fifteen minutes when the culture-germicide mixtures were subcultured into thioglycollate medium, and they failed to protect all of the mice from fatal infections. When the cultures of streptococci were treated with the marketed concentrations of these compounds diluted 1 : 2 and then injected into mice nearly all of the mice died. Perhaps the reason that growth of the streptococci in the thioglycollate subculturing medium was not accompanied with killing of all the animals is that fewer organisms are needed to initiate growth in the culture medium than are needed to kill a mouse. This is evident from the data in tables 1 and 2.

TABLE 3.—*Results* Obtained When Various Compounds Were Injected into Mice*

And when mixtures of streptococci and these compounds were subcultured into blood broth and thioglycollate medium after intervals of exposure of 5, 10, and 15 minutes and the culture germicide mixture injected into mice after an exposure of ten minutes

Material	Dilution	Blood Broth, Minutes			Thioglycollate Medium, Minutes			Number of Mice Injected	Amount Injected	Results
		5	10	15	5	10	15			
Mercurochrome 2 per cent	Undiluted	..	..	..	..	..	..	4	0.1 cc.	3 survived, 1 died 68 hours.
Mercurochrome 2 per cent	1:2	..	..	..	..	..	..	4	0.1 cc.	All survived.
Mercurochrome 2 per cent	Undiluted	—	—	—	+	+	+	4	0.1 cc.	1 died <15 hours, gram negative rod isolated from heart's blood. 1 died <15 hours, spreader isolated from heart's blood. 1 died 25 hours, no hemolytic streptococci isolated from heart's blood. 1 died 40 hours, hemolytic streptococci isolated from heart's blood.
Mercurochrome 2 per cent	1:2	—	—	—	+	+	+	4	0.1 cc.	All died <40 hours. Hemolytic streptococci isolated from heart's blood of all of the mice.
Metaphen 1:500..........	Undiluted	..	..	..	..	..	..	4	0.1 cc.	All survived.
Metaphen 1:500..........	1:2	..	..	..	..	..	..	4	0.2 cc.	All survived.
Metaphen 1:500..........	Undiluted	—	—	—	+	+	+	4	0.1 cc	2 survived, 2 died. Heart's blood of 1 mouse cultured and hemolytic streptococci found.
Metaphen 1:500..........	1:2	—	—	—	+	+	+	4	0.2 cc.	1 died 23 hours. 2 died <40 hours. Hemolytic streptococci in the heart's blood. 1 survived.
Merthiolate 1:1,000........	Undiluted	..	..	..	..	..	..	2	1 cc.	All survived.
Merthiolate 1:1,000........	1:2	..	..	..	..	..	..	2	1 cc.	All survived.
Merthiolate 1:1,000........	Undiluted	—	—	—	+	+	+	4	0.5 cc.	All died in 24 hours. Heart's blood of 2 mice cultured and hemolytic streptococci in both.
Merthiolate 1:1,000........	1:2	—	—	—	+	+	+	4	0.5 cc.	3 died in 24 hours. 1 died in 48 hours. Heart's blood of 1 mouse cultured and hemolytic streptococci found.
Phenol 1:80..............	....	..	..	..	..	..	..	4	0.5 cc.	All survived.
Phenol 1:80..............	....	—	—	—	—	—	—	4	0.5 cc.	3 survived, 1 died ½ hour from trauma during injection.
Phenol 1:100..............	....	—	—	—	+	—	—	4	0.5 cc.	All survived.
Phenol 1:120..............	....	—	—	—	+	+	+	4	0.5 cc.	All survived.
Phenol 1:140..............	....	—	—	—	+	+	+	4	0.5 cc.	1 died 24 hours; 2 died <41 hours. Hemolytic streptococci isolated from heart's blood of all 3 mice. 1 survived.

* These results are from one experiment but are typical of the results usually obtained in the various tests which were made.
— = no growth; + = growth of the test organism; .. = toxicity test on disinfectants.

COMMENT

In a preliminary report of this work [20] we made the statement that metaphen 1 : 500 was germicidal in an exposure of ten minutes but not of five minutes. These results were obtained when the culture-germicide mixture was subcultured to blood broth and at the end of the experiment transfers were made from each subculture blood broth tube to a second tube of blood broth and to a tube of thioglycollate medium. The subculturing from the primary subculture tubes, as recommended by Shippen,[5] does not give results as reliable as when the subcultures are made directly into a medium which will neutralize the bacteriostatic action of the mercurial compound as recommended by Brewer [11] and as required by the National Institute of Health [14] in the sterility testing of biologic products. In this particular experiment only 1 out of 4 mice injected with the mixture of culture and metaphen 1 : 500 died, but in another experiment 2 out of 4 animals died. The marketed solution of a germicide should be more effective in destroying pathogenic organisms.

20. Morton, H. E.; North, L. L., and Engley, F. B., Jr.: In Vitro and In Vivo Studies on the Bacteriostatic and Bactericidal Actions of Mercurial Disinfectants on Hemolytic Streptococci, J. Bact. **50:** 125-126 (July) 1945.

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Johnson and Meleney [21] reported that merthiolate was ineffective in preventing contamination of blood plasma, and Morgan, Simmons and Biggs [22] observed that organisms exposed to a concentration of 1:1,000 merthiolate for seven days at 5 C. proved viable when subcultured to thioglycollate medium. The longest period of exposure of hemolytic streptococci to merthiolate 1:1,000 was fifteen minutes at room temperature, and we always found the cultures viable when subcultured in thioglycollate medium. Although Graydon and Biggs [23] reported that the lag period may extend to weeks or even months when organisms are treated with sublethal doses of antiseptic, such as merthiolate, we observed no change in our subculture tubes between the second and sixth days of incubation, so terminated our observations on the sixth day. The results obtained within that period were obvious and satisfied the purpose of our experiment.

Finding that the marketed solutions of mercurochrome, metaphen and merthiolate failed to kill all the vegetative cells in a culture of hemolytic streptococci in vitro is not surprising in view of the results published by Hoyt, Fisk and Burde [24] and Nye.[25] It was to be expected that Green and Birkeland [26] would fail to find any therapeutic action when merthiolate (1:10,000 to 1:30,000) and metaphen (1:5,000 to 1:20,000) were applied to the chorioallantoic membranes inoculated with Staphylococcus aureus. In working with metaphen, mercurial M, merthiolate, mercurochrome and bichloride of mercury, Smith, Czarnetzky and Mudd [27] came to the conclusion that the activity of a mercurial antiseptic in serum is reduced to 0.33 to 0.007 per cent of its activity in saline solution. The marketed solutions of the three mercurials studied did not completely kill cultures of hemolytic streptococci in distilled water in an exposure of ten minutes, so any further reduction in antibacterial activity by the presence of serum would not leave much to be expected in the way of germicidal action. In spite of these facts the label on a bottle of "Solution Merthiolate, 1:1,000, Stainless" purchased as recent as June 1947 states that it is "a stable, stainless, organic mercury compound solution of high germicidal value, particularly in serum and other protein media." It is not highly germicidal and especially does not possess high germicidal value in the presence of serum and other protein mediums. The loss of antibacterial activity of mercurials in the presence of serum proves their incompatibility with serum. Not only is the antibacterial action of mercurial compounds much reduced in the presence of blood serum, but Waller [28] has found that a final concentration of 1:5,000 merthiolate destroyed the anti-Rh agglutinins in human serums.

The comparative in vitro studies of mercurochrome, metaphen and merthiolate on embryonic tissue cells and bacterial cells by Salle and Lazarus [29] cannot be ignored. These investigators found that metaphen, merthiolate and mercurochrome were 12, 35 and 262 times respectively more toxic for embryonic tissue cells than for Staphylococcus aureus. Nye [25] and Welch [30] also found the same three mercurial compounds more toxic for leukocytes than for bacterial cells. Not only is there the direct toxic action of the mercurial compounds on the cellular and humoral components of the animal body, but there is also the possibility of sensitization. Hollander [31] has recently reported a case of contact dermatitis produced by tincture of merthiolate.

We wished to learn whether organisms put in a state of bacteriostasis by a mercurial compound were harmless if introduced into the animal body. It is quite obvious that they are not harmless. There is a good correlation between the growth of hemolytic streptococci in thioglycollate medium and fatal septicemia in mice. In view of our recent knowledge of disinfectants, we wished to learn what might be expected of the three most commonly advertised mercurial compounds. The marketed aqueous solutions of the three compounds mercurochrome, merthiolate and metaphen are not effective disinfectants because when virulent organisms are treated with the substances for as long as ten minutes and introduced into the animal body either intraperitoneally or intradermally, the animals are not protected from infection. The substances are not effective germicides, as cultures treated with them are still viable. Neither are the substances effective antiseptics, as they are incapable of preventing sepsis when the pathogenic organisms are exposed as long as ten minutes to the substances. It can be stated that the substances have a bacteriostatic action. The efficacy of employing a bacteriostatic agent for topical application is beyond the scope of this paper. We only wish to point out that a pathogenic organism placed in a state of bacteriostasis by a mercurial compound is still capable of producing a fatal septicemia if introduced into the animal body and capable of producing a localized infection if introduced into the skin.[10]

SUMMARY

The organomercurial compounds "mercurochrome," "merthiolate" and "metaphen," as supplied in aqueous solutions on the market, possess many shortcomings as disinfectants. Aqueous solutions of these compounds may not completely kill cultures of virulent hemolytic streptococci, in that mice receiving an intraperitoneal injection of the culture-germicide mixture, after ten minutes' exposure of the organisms to the drugs, usually die, and hemolytic streptococci can be isolated from the heart's blood after death of the mice.

Hemolytic streptococci, an example of non-spore forming pathogenic organisms, while in a state of bacteriostasis produced by a mercurial compound, are still infectious for the animal body. Bactericidal rather than bacteriostatic action of the compounds is necessary to prevent a generalized infection.

Reports in the literature indicate that these three organomercurial compounds are more toxic for embryonic tissue cells and leukocytes than for bacterial cells.

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