

Maintenance of Antiseptic Action by "Merthiolate" as a Preservative

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Following its introduction (1, 2) as an antiseptic in 1931, "Merthiolate" (Sodium Ethyl Mercuri Thiosalicylate, Lilly) has been used in several laboratories for preserving vaccines, sera, and other biological products.

In 1933 we examined by staphylococcal plating methods already described (1) the approximate "Merthiolate" content of commercial typhoid mixed vaccine prepared in 1931. This vaccine was prepared in the usual way and then preserved with "Merthiolate" 1:10,000 and stored at 5° C. for two years. At that time, by the technique described, it produced "clearings" 9 to 11 mm. in width when streaked with a platinum loop on poured *Staphylococcus aureus* agar plates, as compared to "clearings" of 12 to 14 mm. in width produced by fresh "Merthiolate" 1:10,000 solution in similar quantities.

In their comparative studies dealing with vaccine and serum preservatives, Rosenstein and her associates (3) concluded that while both phenol and tricresol preservatives decreased in efficiency during ten months storage at 5° C., "Merthiolate" and mixed "Merthiolate" and phenol preservatives were apparently unimpaired during this time. Results of longer stability tests are believed to be desirable, and such tests have been conducted as follows. Recently we have examined, by staphylococcal test-tube inhibition methods already described (1), the approximate effective "Merthiolate" content of six lots of bacterial vaccine one to five years of age and eight lots of antitoxin one to seven years of age. All of these were regular commercial preparations preserved with "Merthiolate." Some were "house samples" and some had been outdated and returned and hence had been subjected to actual commercial handling for from one to three years.

In these tests the vaccines and antitoxins, along with fresh samples of "Merthiolate" 1:10,000 for control, were diluted with sterile broth so that the original "Merthiolate" content was reduced to a range from 1:50,000 to 1:4,000,000. Staphylococcal¹ inhibitory action of 5 cc. quantities of these various series of dilutions in tubes against one loop of twenty-four hour broth culture of *Staphylococcus aureus* No. 209 was then determined as has already been described. Readings of inhibitions for 24, 48, and 72 hours are shown in Tables I and II. These indicate that "Merthiolate"-preserved vaccines and antitoxins up to three years old exert an antiseptic action which is not substantially different from that of fresh "Merthiolate" 1:10,000 solution. All of the preparations up to three years of age, when diluted on the basis of original 'Merthiolate' content, inhibited *Staphylococcus* for 24, 48, and 72 hours incubation at dilutions of 1:3,000,000, 1:2,000,000 and 1:1,000,000, respectively. Both

¹Staphylococcus was selected on account of its intermediate vulnerability to "Merthiolate," diphtheroids being less resistant and pyocyanus more resistant. See Rosenstein, et al. (3).

vaccine and antitoxin decreased in antiseptic action after five and seven years. Some of the variation in the results tabulated may be due to the slight enrichment of the antitoxin series of tubes with the globulin carried over in accomplishing preservative dilution.

Similar staphylococcal inhibition tests have been applied to one- and two-year-old lots of pertussis vaccine preserved with 0.5% phenol. These vaccines, along with a phenol control solution, were diluted in broth using one part of vaccine to one, two, three, four, and five parts of broth and tested for staphylococcal inhibition. None of the test dilutions showed any inhibition, while the two strongest phenol control dilutions gave complete inhibition for three days. This indicates a rather rapid weakening of the phenol preservative which of course is never very highly inhibitory.

Similar inhibition tests have been applied also to six lots of tricresol-preserved vaccines and antitoxins which were available. The antitoxins contained originally 0.35% alcresta cresol and were seven, eight, and eight years old, respectively, while the vaccines contained 0.3% S. and G. tricresol and were seven, nine, and nine years old respectively. None of these, when diluted with one or more parts of broth, inhibited the staphylococcus, while control cresol solutions, when diluted with three parts of broth, caused inhibition. The cresols as well as phenol are never very highly inhibitory.

As further tests of the remaining vaccines and antitoxins which had shown losses in antiseptic action in the previous experiments, we conducted long-exposure germicidal tests in the ice box using one loop of twenty-four hour culture, diluted 1:100,000, planted into 1 cc. quantities of the undiluted vaccine or antitoxin. Sterility tests of these preparations were made after standing one day, two days, and longer. The results of these tests are shown in Table III. The five- and seven-year-old "Merthiolate"-preserved vaccines and antitoxins contained no viable Staphylococci after standing from two to ten days in the ice box. The one and two year old phenol-preserved vaccine was sterile after one day. The seven year old tricresol-preserved antitoxin did not become sterile in thirteen days.

Although we have not had all the samples of increasingly aged vaccines and antitoxins with different preservatives available for a thorough comparison up to seven or eight years, it appears that "Merthiolate" as a preservative alone maintains its antiseptic action almost fully for three years. At some point between three and five years its antiseptic action is considerably decreased. Seven-year-old "Merthiolate"-preserved antitoxin, however, is still able to sterilize itself of approximately 1,000 to 10,000 Staphylococci per cubic centimeter. Our results, together with those of Rosenstein et al, show that "Merthiolate" as a biological preservative can be depended upon, we believe, better than phenol and tricresol to exert prolonged antiseptic action together with less denaturation than any other antiseptic similarly studied and used.

Conclusions

1. When used as a preservative, "Merthiolate" maintains substantially full antiseptic action for three years.

TABLE I

Staphylococcal Inhibition Tests of "Merthiolate"-Preserved Vaccines to Measure Maintenance of Antiseptic Action

Dilution of Vaccine in Broth to Reduce "Merthiolate" Content to:	HOURS OF INCUBATION AT 37°C. AT WHICH BACTERIAL GROWTH WAS OBSERVED											
	24	48	72	24	48	72	24	48	72	24	48	72
	Staphylococcus Vaccine No. 913800 One year old			Staph-Strep Vaccine No. 915521 One year old			Streptococcus Vaccine No. 897174 Two years old			Coli Vaccine No. 896623 Two years old		
1—500,000	—	—	—	—	—	—	—	—	—	—	—	—
1—1,000,000	—	—	—	—	—	—	—	—	—	—	—	—
1—2,000,000	—	—	—	—	—	—	—	—	—	—	—	—
1—3,000,000	—	—	—	—	—	—	—	—	—	—	—	—
1—4,000,000	—	—	+	—	—	—	—	—	—	—	—	—
	Influenza-Pneumo Vaccine No. 887072 Three years old			Typhoid Mixed Vaccine No. 109294 Five years old			Fresh "Merthiolate" 1—10,000 Solution (Control)					
1—50,000				—	—	—						
1—100,000				—	—	—						
1—500,000	—	—	—	+	+	+	—	—	—			
1—1,000,000	—	—	—	+	+	+	—	—	—			
1—2,000,000	—	—	—	+	+	+	—	—	—			
1—3,000,000	—	—	—	+	+	+	—	—	—			
1—4,000,000	—	—	—	+	+	+	—	+	+			

TABLE III

Self-sterilizing power of aged vaccines and antitoxins, the preservatives of which made weak showings in previous tests

Preservative	Biological	Age in Years	Sterility of Staphylococcus Inoculated Biological After (days):					
			1	2	6	8	10	13
"Merthiolate"	Staphylococcus vaccine No. 35569	5	+	-	-			
	Typhoid vaccine No. 108256	5	+	+	+	-	-	
	Diphtheria antitoxin No. 32658	7	+	+	+	+	-	-
	Tetanus antitoxin No. 33328	7	+	-	-			
Phenol	Pertussis vaccine No. 915591	1	-	-				
	Pertussis vaccine No. 898469	2	-	-				
Tricresol	Streptococcus antitoxin No. 105286	7	+	+	+	+	+	+
	Streptococcus antitoxin No. 32656	7	+	+	+	+	+	+

In this test 1 c.c. quantities of the various biologicals were planted with a loop 1-100,000 dilution of 24-hour broth culture *Staph. aureus* No. 209, and then stored in the ice box. These preparations were then tested for sterility by planting a loop of each into the usual fermentation tubes of infusion broth, etc.

2. Although showing a decrease in antiseptic action after three years, "Merthiolate"-preserved antitoxin at the end of seven years still maintains a certain degree of self-sterilization.

References

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 2. (a) Jamieson, W. A. and Powell, H. M., 1931. Merthiolate as a preservative for biological products. *Amer. Journ. Hyg.* 14:218-224.
 - (b) Morgan, L. C., Jamieson, W. A., and Powell, H. M., 1933. Merthiolate as a preservative for biological products. II. The production and preservation of diphtheria toxoid. *Journ. Immunol.* 25:121-126.
 - (c) Powell, H. M., Jamieson, W. A., and Jones F. G., 1933. Merthiolate as a preservative for biological products. III. Action of Merthiolate on bacteriophage. *Journ. Immunol.* 24:185-192.
 3. (a) Rosenstein, C. and Levin, I., 1935. The bactericidal and antiseptic action of preservatives frequently used in biological products, and the effect of these preservatives on the potencies of the products. *Amer. Journ. Hyg.* 21:260-279.
 - (b) Falk, C. R. and Aplington, S., 1936. Studies on the bactericidal action of mixtures of phenol and Merthiolate. *Journ. Bact.* 31:8.
 - (c) Falk, C. R. and Aplington, S. P., 1936. Studies on the bactericidal action of phenol and Merthiolate used alone and in mixtures. *Amer. Journ. Hyg.* 24:285-308.
- The following, included in the titles of papers in the foregoing references, is a trade-mark which identifies a product of Eli Lilly and Company: "Merthiolate."