Sulfo-Merthiolate as a Germicide

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Introduction

Twelve years ago we tested solutions of 'Sulfo-Merthiolate' (Sodium p-Ethyl Mercuri Thiophenylsulfonate, Lilly) for antibacterial action and toxicity. When tested by the Reddish method, it was found to be germicidal in dilutions of 1-1,000 and 1-2,000 but not 1-4,000 for *B. typhosus* and *Staphylococcus aureus*. It was tolerated in doses up to and including 20 mg. per kilogram intravenously by rabbits and in doses up to and including 40 mg. per kilogram intravenously by mice. Preparation of the drug at that time included difficulties of such order that a more complete laboratory study was not then attempted. In general, the drug is quite similar chemically to 'Merthiolate' (Sodium Ethyl Mercuri Thiosalicylate, Lilly) (1 to 11) and possesses the distinct advantage of being soluble over a wide acid, neutral, and alkaline pH range.

During the past two years more adequate amounts of 'Sulfo-Mer-

Table I.—Germicidal Test of 'Sulfo-Merthiolate'
Test culture: *B. typhosus*, Hopkins strain, in 0.5 cc. doses. "Medication" at 20°C

Germicide Dilutions (5 cc.)	Readings of culture growth in incubated broth tubes planted with test culture medicated for:					
	5 minutes		10 minutes		15 minutes	
	Direct culture	Sub-culture	Direct culture	Sub-culture	Direct culture	Sub-culture
Phenol 1-80	–		–		–	
1-90	+		+		–	
'Sulfo-Merthiolate' 1-1000	–	–	–	–	–	–
1-2000	–	–	–	–	–	–
1-3000	–	–	–	–	–	–
1-4000	–	+	–	+	–	–

thiolate' have been prepared and a wider laboratory study of the drug made. This report presents the results of these tests.

Experiments

Germicidal tests. 'Sulfo-Merthiolate' was subjected to standard germicidal tests against the Hopkins strain of *B. typhosus* and *Staphylococcus aureus* strain No. 209 at 20°C. Plantings were made in the usual way to tubes of the specified beef extract broth. After two days incubation at 37°C., all negative mercurial tubes were subcultured and reincubated, and both direct and subculture tubes were given final readings seven days after the test was done. The results of these tests are shown in Tables I and II.

These test-tube experiments show that 'Sulfo-Merthiolate' has a strong antibacterial action against the strains of bacteria commonly used in preliminary experiments on various germicides. Inasmuch as "phenol coefficients" of germicides tested in media nearly entirely aqueous in character can have very little importance, we shall not attempt to assay such figures.

The above tests in which 'Sulfo-Merthiolate' dilutions were made in water were repeated with 'Sulfo-Merthiolate' (but not phenol) dilutions made in sterile normal horse serum. One per cent 'Sulfo-Merthiolate'

TABLE II.—Germicidal Test of 'Sulfo-Merthiolate'
Test culture: *Staph. aureus* No. 209 in 0.5 cc. doses. "Medication" at 20°C

Germicide Dilutions (5 cc.)	Readings of culture growth in incubated broth tubes planted with test culture medicated for:					
	5 minutes		10 minutes		15 minutes	
	Direct culture	Sub-culture	Direct culture	Sub-culture	Direct culture	Sub-culture
Phenol 1-60	+					
1-70	+		+		+	
'Sulfo-Merthiolate' 1-1000	−	−	−	−	−	−
1-2000	−	−	−	−	−	−
1-3000	−	+	−	+	−	+
1-4000	−	+	−	+	−	+

TABLE III.—Germicidal Test of 'Sulfo-Merthiolate' in Normal Horse Serum Test culture: *B. typhosus*, Hopkins strain, 0.5 cc. doses. "Medication" at 20°C

Germicide Dilutions (5 cc.)	Readings of culture growth in incubated broth tubes planted with test culture medicated for:					
	5 minutes		10 minutes		15 minutes	
	Direct culture	Sub-culture	Direct culture	Sub-culture	Direct culture	Sub-culture
Phenol 1-80	−		−		−	
1-90	+		−		−	
'Sulfo-Merthiolate' 1-1000	−	−	−	−	−	−
1-2000	−	−	−	−	−	−
1-3000	−	−	−	−	−	−
1-4000	−	+	−	+	−	+

solution was diluted with whole normal horse serum to prepare the necessary series of germicide dilutions. By this technique the 1-1,000 'Sulfo-Merthiolate' dilution contained 90 per cent horse serum, the 1-2,000 dilution contained 95 per cent horse serum, et cetera; all mercurial dilutions therefore contained 90 per cent or more of whole normal horse serum. The results of these germicidal tests are shown in Tables III and IV. The results indicate that the antibacterial action of 'Sulfo-Merthiolate' persists well in strong serum concentrations. It was further observed that 'Sulfo-Merthiolate' was quite free of serum-precipitating properties.

Bacterial inhibition tests. 'Sulfo-Merthiolate' was diluted in sterile beef extract broth, and 5 cc. amounts of various dilutions were planted with a loop of twenty-four hour broth culture respectively of *B. typhosus*, Hopkins strain, and *S. aureus* No. 209. Both series of tubes were incubated at 37°C. for a week, and readings of growth noted as shown in Tables V and VI. The results indicate that 'Sulfo-Merthiolate' in dilutions of over one in one million to one in four million is inhibitory for ordinary bacteria. These values are somewhat higher than those originally reported of 'Merthiolate' (**1**) and equal those subsequently reported (**10**); however, at the time the present tests were conducted, 'Merthiolate' was found to be closely similar to 'Sulfo-Merthiolate' in

inhibition qualities as indicated by the results in footnotes at the bottom of Tables V and VI.

Fungicidal tests. 'Sulfo-Merthiolate' in aqueous solution has been tested against fungi associated with ringworm, using the technique heretofore reported (9). We have noted strong fungicidal action as indicated in Table VII. The 1-10,000 dilution of 'Sulfo-Merthiolate' (but not the 1-20,000 dilution) proved fungicidal for *Trichophyton purpureum* No. 4183, with final reading of all culture tubes being made after seven days incubation. Furthermore, poured plates of this organism showed "clearings" 7.5 cm. in width when streaked with a loop of 'Sulfo-Merthiolate' 1-1,000 and incubated seven days. These test results are quite similar to those reported for 'Merthiolate' (9) and compare favorably with results heretofore obtained with other drugs of this class.

Hemolytic, precipitation, and irritation tests. 'Sulfo-Merthiolate' in concentrations up to and including 1-200 (the strongest tested) in whole horse serum has caused no precipitation when heated at 37°C. for twenty-four hours. It is hemolytic for washed sheep cells (unit equivalent to erythrocytes contained in one-fortieth cc. of whole blood) in a concentration of 1-250 but not 1-500. These values are closely comparable to results obtained with 'Merthiolate' (1 to 11) and indicate considerable tolerance by body fluids and cells.

TABLE IV.—Germicidal Test of 'Sulfo-Merthiolate' in Normal Horse Serum
Test culture: *Staph. aureus* No. 209 in 0.5 cc. doses. "Medication" at 20°C

Germicide Dilutions (5 cc.)	Readings of culture growth in incubated broth tubes planted with test culture medicated for:					
	5 minutes		10 minutes		15 minutes	
	Direct culture	Sub-culture	Direct culture	Sub-culture	Direct culture	Sub-culture
Phenol 1-60	−		−		−	
1-70	+		+		+	
'Sulfo-Merthiolate' 1-1000	−	−	−	−	−	−
1-2000	−	−	−	−	−	−
1-3000	−	+	−	−	−	−
1-4000	−	+	−	+	−	−

TABLE V.—Bacterial Inhibition Tests of 'Sulfo-Merthiolate'
Test culture: *B. Typhosus*, Hopkins strain.

Germicide Dilutions in Broth (5 cc.)	Readings of culture growth in broth tubes containing varying dilutions of germicide and planted with 1 loop 24-hour broth culture and incubated for:				
	24 hours	96 hours	120 hours	144 hours	168 hours
'Sulfo-Merthiolate' 1-600,000	–	–	–	–	–
1-800,000	–	–	–	–	–
1-1,000,000	–	–	–	–	–
1-1,200,000	–	–	–	–	–
1-1,400,000	–	–	–	+	+
1-1,600,000	–	+	+	+	+

Note: On same day of this test 'Merthiolate' 1-1,200,000 proved inhibitory or same culture at 168 hours incubation.

We have subjected 'Sulfo-Merthiolate' to human skin tests on individuals quite susceptible to several irritating substances, including common adhesive plaster. In these tests different concentrations of 'Sulfo-Merthiolate' were applied to thoroughly cleansed skin of the upper arm by means of wet cotton pledgets held in place under rubber dam for eighteen hours. Dilutions of bactericidal activity (1-1000 to 1-4,000) showed no reaction whatever, either when aged up to a year or when heated at 60°C. for a month. To get a final end point of beginning irritation comprising slight redness, it was necessary to use a concentration of 1-25. Under the same condition 'Merthiolate' has given a slight reaction at a concentration of 1-100.

When tested under the same conditions, we have found 'Sulfo-Merthiolate' non-irritating to human skin when incorporated as a 1-1,000 concentration either in a dusting powder base, tragacanth jelly base, or tincture, or as a 1-2,000 concentration in an ointment base.

Toxicity tests. Six mice injected intravenously with an early preparation of 'Sulfo-Merthiolate' indicated the drug was tolerated in doses up to and including 40 mg. per kilogram. Twenty rabbits injected intravenously with four different preparations indicated that doses of 20 mg. per kilogram, but not 30 mg., were tolerated. We have recently treated forty-eight rats intravenously with 'Sulfo-Merthiolate' in doses of 20 mg. per kilogram up to 75 mg. per kilogram with increments of 5 mg.

doses, each of the twelve doses therefore being administered to four rats. At the end of seven days one rat out of four had died on the 30, 45, 55, 60, 65, and 70 mg. doses, and two rats out of four had died on the 75 mg. dose. We then treated sixteen rats intravenously with doses of 40, 60, 80, and 100 mg. per kilogram, using four rats on each dose. Within seven days none of the rats on 40 or 60 mg. doses had died. Two rats, quite ill on the 80 mg. doses, were sacrificed on the fifth day for study; the other two died by the seventh day. All four rats on the 100 mg. dose died by the seventh day, and one of these was found sufficiently early for microscopic study. Both of the rats treated intravenously with 80 mg. 'Sulfo-Merthiolate' per kilogram and sacrificed on the fifth day presented empty urinary bladders on autopsy and possibly were anuric. Only the kidneys showed noteworthy lesions. The first animal had many convoluted tubules with parenchymatous degeneration and a few with necrosis. Many tubules also showed evidence of regeneration and were lined by cells which had flattened, had basophilic cytoplasm, and showed numerous mitoses. The second animal's kidneys resembled those of the first. Many convoluted tubules were lined by regenerated cells and mitoses were frequently seen.

TABLE VI.—Bacterial Inhibition Tests of 'Sulfo-Merthiolate'
Test culture: *Staphylococcus aureus* No. 209.

Germicide Dilutions in Broth (5 cc.)	Readings of culture growth in broth tubes containing varying dilutions of germicide and planted with 1 loop 24-hour broth culture and incubated for:				
	24 hours	96 hours	120 hours	144 hours	168 hours
'Sulfo-Merthiolate' 1-1,000,000.....	–	–	–	–	–
1-2,000,000.....	–	–	–	–	–
1-3,000,000.....	–	–	–	–	–
1-4,000,000.....	–	–	–	–	–
1-5,000,000.....	–	+	+	+	+
1-6,000,000.....	–	+	+	+	+
1-7,000,000.....	+	+	+	+	+

Note: On same day of this test 'Merthiolate' 1-5,000,000 proved inhibitory for same culture at 168 hours incubation.

TABLE VII.—Fungicidal Test of 'Sulfo-Merthiolate'
Test culture: *Trichophyton purpureum* No. 4183 of the American Type Culture Collection in doses of 0.5 cc. of a 10 cc. water suspension of 7-day slant culture. "Medication" at 20°C

Germicide dilutions (5 cc.)	Readings of culture growth, after 7 days incubation, in agar tubes planted with test culture medicated for:		
	5 minutes	10 minutes	15 minutes
'Sulfo-Merthiolate' 1-1000.....	–	–	–
1-5000.....	–	–	–
1-10,000.....	–	–	–
1-20,000.....	+	–	–
1-40,000.....	+	+	+
1-60,000.....	+	+	+

The rat treated intravenously with 100 mg. 'Sulfo-Merthiolate' per kilogram and found dead on the sixth day was possibly anuric, the bladder being empty. Microscopically the kidneys were seen to be severely injured. The epithelium of many of the glomerular capsules and convoluted tubules showed severe parenchymatous degeneration. Some convoluted tubules showed fatty degeneration, and in many the cells were necrotic. In a few foci, there was evidence of slight regeneration.

Aside from the animals enumerated above, thirty guinea pigs have been injected hypodermically with 1 cc. of diphtheria toxoid preserved with 'Sulfo-Merthiolate' 1-10,000. No local lesions were observed. These guinea pigs will be referred to further.

'Sulfo-Merthiolate' as a biological preservative. Close similarity of 'Sulfo-Merthiolate' to 'Merthiolate' as noted thus far indicated the former might be used as a biological preservative as has 'Merthiolate' (11). A comparative test was, therefore, conducted, using a single lot of diphtheria toxoid subsequently divided into two portions. To one of these, 'Sulfo-Merthiolate' 1-10,000 was added, and, to the other, 'Merthiolate' 1-10,000 was added. Both antigens were then heated at 37°C. for four months. Immunization of guinea pigs was done with samples of toxoid removed after two months heating and after four months heating. In these tests a total of sixty guinea pigs of 300 grams weight were given single 1 cc. doses of the respective toxoids. For testing the degree of anti-diphtheria immunity which these toxoids incited, 10, 20, and 40

MLD of diphtheria toxin were administered to different subgroups of forty-five of these guinea pigs one month after they had received the single immunizing dose of toxoid. Fifteen guinea pigs had died of intercurrent pneumonias. Table VIII shows the results of these immunity tests. Totals of all survivals and deaths following the toxin doses used show that 48 per cent of the guinea pigs immunized with 'Sulfo-Merthiolate'-preserved toxoid survived and 40 per cent of the guinea pigs immunized with 'Merthiolate'-preserved toxoid survived. The actual number of animals used in each of the final groups was, however, too small to make exact comparisons. It appears that further experimental biological preservation with 'Sulfo-Merthiolate' is indicated, inasmuch as its showing in toxoid is a good one.

Discussion

The laboratory tests herein reported show that in biological action 'Sulfo-Merthiolate' (Sodium p-Ethyl Mercuri Thiophenylsulfonate, Lilly) is quite similar to 'Merthiolate' (Sodium Ethyl Mercuri Thiosalicylate, Lilly). Both compounds possess antibacterial action of about the same potency. Both have relatively low toxicity, in comparison with others of this class of drugs, when injected directly into the blood stream. There is almost complete freedom from protein precipitating qualities, and this germicide has a large margin of safety between undesirable toxic properties on the one hand and desirable antibacterial properties on the other hand.

The tests reported here, comprising evidence of both germicidal and strong bacteriostatic action as well as marked freedom from toxicity, are such as to indicate that 'Sulfo-Merthiolate' could exert a very pro-

TABLE VIII.—Comparative Immunizing Properties of Diphtheria Toxoid Preserved with 'Sulfo-Merthiolate' and Heated Two and Four Months at 37°C.

Diphtheria Toxoid Number	Number of Months Heated	Results of immunity tests conducted by injecting toxoid-treated guinea pigs with different amounts of diphtheria toxin					
		10 MLD		20 MLD		40 MLD	
		died	lived	died	lived	died	lived
B-4318A with 'Merthiolate' 1-10,000	2	2	2	0	4	1	3
	4	1	1	2	1	2	1
B-4318B with 'Sulfo-Merthiolate' 1-10,000	2	1	3	2	2	4	0
	4	1	3	2	3	2	2

longed antibacterial action when used clinically. On the basis of nearly all newer knowledge of immunology and chemotherapy, a moderately strong and persistent antibacterial action would be more likely to be effective than strong initial germicidal action without any mechanism (bacteriostasis) to make this persistent. 'Sulfo-Merthiolate', soluble in both acid and alkaline pH ranges and having both germicidal and bacteriostatic action, could, therefore, be recommended for further clinical trial.

Conclusions

1. A series of biological tests indicates 'Sulfo-Merthiolate' is a close counterpart of 'Merthiolate' in antiseptic action.

2. Concentrations of 1-2,000 to 1-3,000 of 'Sulfo-Merthiolate' are effective against staphylococci and typhoid bacilli when tested in blood serum.

3. Concentrations of 1-1,000,000 to 1-4,000,000 of 'Sulfo-Merthiolate' inhibit the growth of staphylococci and typhoid bacilli.

4. 'Sulfo-Merthiolate' in concentrations up to and including 1-200 does not precipitate serum. A 1-10,000 concentration is indicated as a biological preservative.

5. 'Sulfo-Merthiolate' in a concentration of 1-25 (i.e., forty times as strong as a stock 1-1,000 dilution) showed a beginning threshold of skin irritation.

6. White rats tolerate 40 to 50 mgm. 'Sulfo-Merthiolate' per kilo intravenously.

7. 'Sulfo-Merthiolate' is soluble over a wide acid and alkaline pH range.

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('Merthiolate," included in the titles of papers in the foregoing references, is the trade-mark of Eli Lilly and Company for its sodium ethyl mercuri thiosalicylate.)