ON MERTHIOLATE AND FUNGI ASSOCIATED WITH RINGWORM

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INTRODUCTION

During the latter part of the nineteenth century considerable confusion prevailed concerning the etiology of many kinds of human dermatitis. Insufficient descriptions of lesions and cases coupled with a confused terminology led to the merging of tinea, impetigo, and alopecia of various sorts into a mixed category. For example, "tinea" of the lips is probably rare at the present time and dermatitis of this area is usually of bacterial etiology, as has likely been true in the past.

By 1900 the mycotic skin conditions had begun to assume an entity separate from bacterial infections, in a large measure due to the work of Sabouraud, and an exact summary of the whole subject by this author[1] in 1910 further clarified the mycotic, as contrasted to bacterial, skin infections. Also it was recognized that fungi may infect any part of the human skin from the toes to the scalp. In any comprehensive bibliography of early fungus studies without doubt the names of Gruby, Bang, Whitfield, and Castellani would also occupy attention of the first order. We shall not attempt such a review, however.

In the last few years both clinical and laboratory studies[2 3 4 5 6] have dealt with the increasing prevalence of ringworm since the war, and the public health as well as medical problems presented by the increase and chronicity of this condition. The biology of the causative fungi has been dealt with in a comprehensive way by Weidman[3] and many recent studies have been made upon epidemiology, prevention, and treatment. Institutional attention to sanitation, involving the prevention of further spread of pathogenic fungi, and tests of many chemicals as agents of treatment have been the subjects of many reports.

Chemical agents suggested and used as agents of treatment have included salicylic acid, benzoic acid, phenol, menthol, thymol, volatile oils, iodine, silver nitrate, potassium permanganate, copper sulphate, zinc sulphate, zinc oxide, chrysarobin, calamine, sulphur, and thallium acetate. X-ray treatment has been used extensively. The British use of Whitfield's formula and the French use of tincture of iodine have been proverbial.

This laboratory in the last few years has examined the antiseptic action of the new mercurial, Merthiolate[7 8 9 10 11 12 13 14], from several points of view. This drug has been found to have, among other properties, high degrees of solubility in aqueous and protein media, a near absence of protein coagulation properties, very low toxicity for animals and humans, healing properties possibly due to sulphydryl, and adequate antibacterial properties. Buchsbaum and Bloom[15], on the basis of tests

against bacteria within living tissue cultures involving simultaneous antibacterial and anti-tissue effects, have reported a comparatively high value for Merthiolate. We have verified[16] this in tests of "white clot" or fibrin-containing media in which the physical conditions are somewhat similar to those found in infected tissues.

In view of the usefulness of Merthiolate as an antiseptic and its

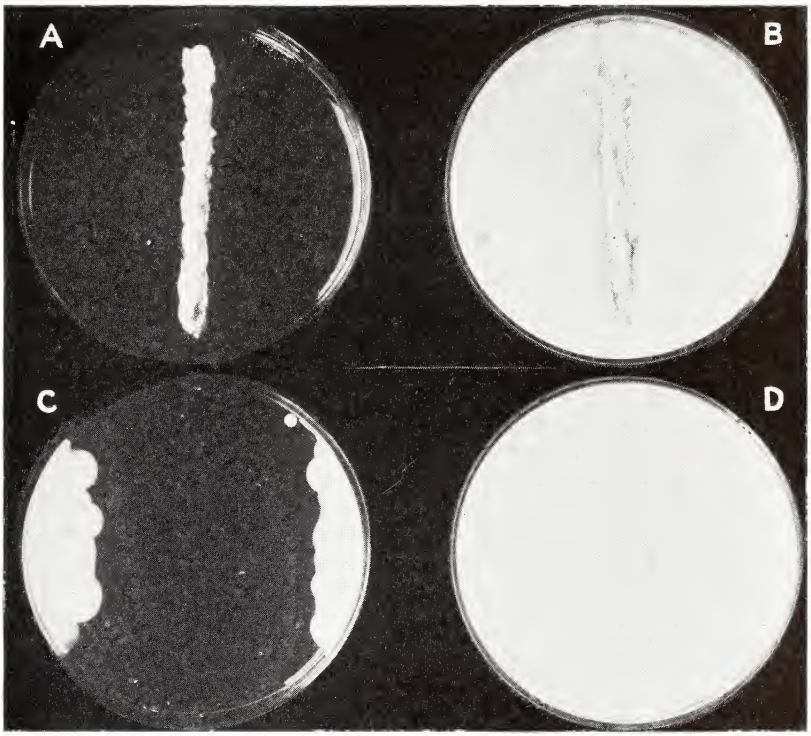

Fig. 1: *Trichophyton purpureum* No. 4183 Poured Agar Plates Prepared by Mixing 0.5 cc. Emulsion of Two Weeks Old Spore Containing Culture with 20 cc. Melted Agar, and, After Streaking with a Platinum Loop of the Indicated Materials, Incubating for Seven Days.

A. Merthiolate 1-1000 Cream, Containing Triethanolamine 1-80 and Carbitol 1-14.
B. Cream Base, Without Merthiolate, Containing Triethanolamine 1-80 and Carbitol 1-14.
C. Merthiolate Aqueous Solution 1-1000.
D. No Medication.

property of stimulating repair and healing it became of interest to examine the action of Merthiolate on pathogenic fungi.

Marshall[17] has reported that Merthiolate 1:10,000 kills *Trichophyton interdigitale* in five minutes but does not kill at a 1:100,000 dilution. Legge, Bonar, and Templeton[18] in an extensive comparative study showed that Merthiolate in all dilutions up to and including 1:10,000 killed all spores of *Trichophyton interdigitale* in five minute test exposures. In California they found this species more resistant to chemicals than *T. rosaceum.*

In this paper we shall present results of further tests of Merthiolate against pathogenic fungi, including the preparation of a cream vehicle which appears to bring about Merthiolate medication of the skin more adequately, and with more convenience, than may readily be done with liquids or solutions.

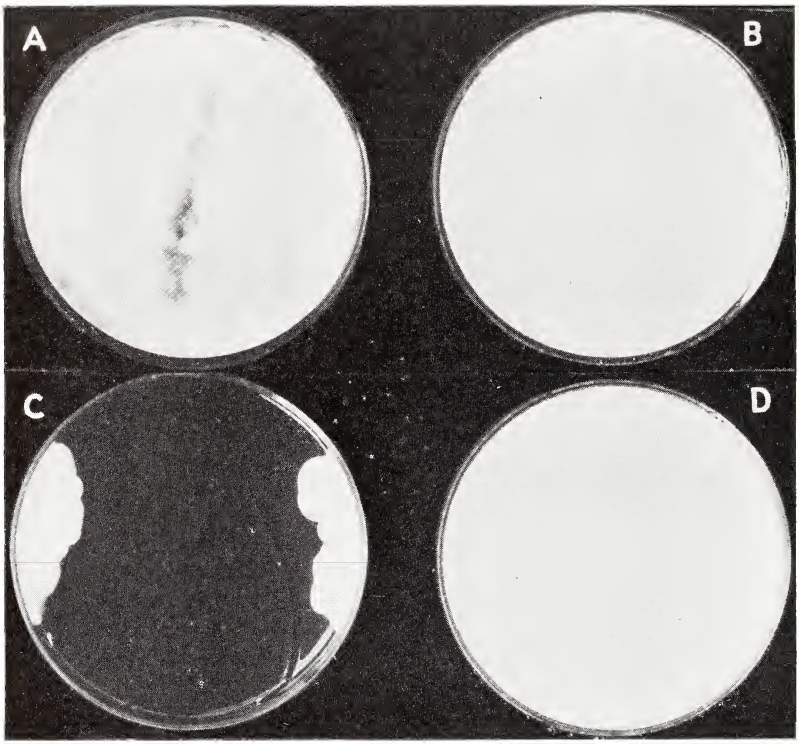

Fig. 2: *Trichophyton purpureum* No. 4183 Poured Agar Plates Prepared by Mixing 0.5 cc. Emulsion of Two Weeks Old Spore Containing Culture with 20 cc. Melted Agar, and, After Streaking with a Platinum Loop of the Indicated Materials, Incubating for Seven Days.

A. Triethanolamine Undiluted.

B. Carbitol Undiluted.

C. Merthiolate Aqueous Solution 1-1000 Containing Triethanolamine 1-80 and Carbitol 1-14.

D. No Medication.

EXPERIMENTAL

McCrea[19] has recently set forth a proposed standard method for the evaluation of fungicides. In preliminary test tubes tests of Merthiolate we have utilized these general test methods with certain modification as to volumes of test solutions. Instead of using 2 cc. volumes of chemical we prefer 5 cc. volumes which have been utilized in the F. D. A.[20] and other commonly used antibacterial laboratory procedures. Fungus test doses comprised 0.5 cc. taken from a 10 cc. water emulsion of a well spored agar slant of fungus culture. Medication of fungus

test doses with dilutions of chemical was conducted at 20° C. Ordinary platinum loop plantings of medicated spore culture were made after 5, 10, and 15 minute exposure intervals, to freshly melted deep-tube slants of glucose agar, which were incubated two weeks, then read. In McCrea's proposed standard method, the temperature of medication appears to have been omitted.

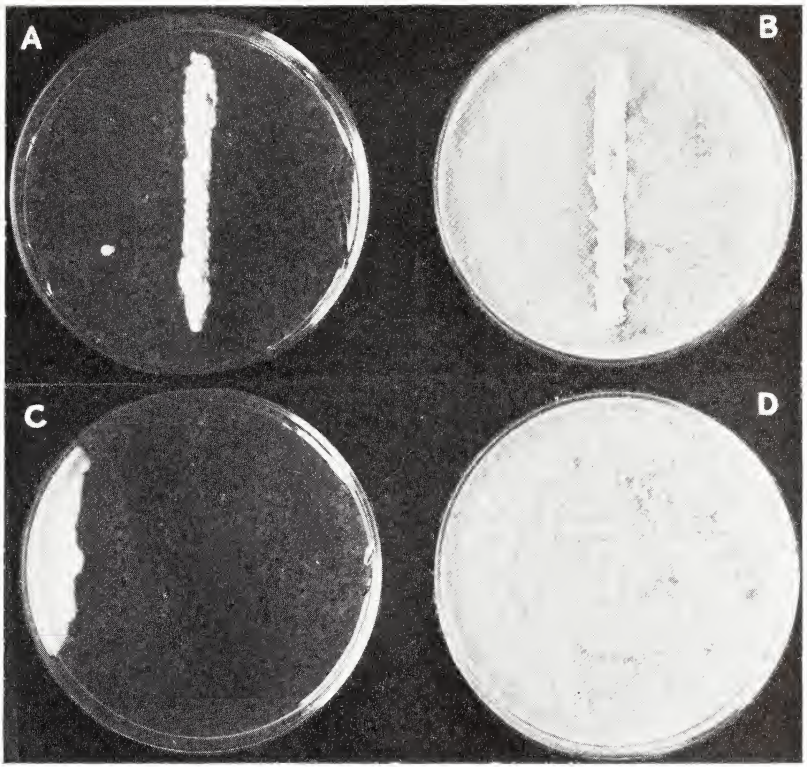

Fig. 3: *Epidermophyton rubrum* No. 655 Poured Agar Plates Prepared by Mixing 0.5 cc. Emulsion of Two Weeks Old Spore Containing Culture with 20 cc. Melted Agar, and, After Streaking with a Platinum Loop of the Indicated Materials, Incubating for Seven Days.

A. Merthiolate 1-1000 Cream, Containing Tricthanolamine 1-80 and Carbitol 1-14.
B. Cream Base, Without Merthiolate, Containing Tricthanolamine 1-80 and Carbitol 1-14.
C. Merthiolate Aqueous Solution 1-1000.
D. No Medication.

In order to make all preliminary experiments reproducible, three fungus test cultures were selected from the American Type Culture Collection. These were *Trichophyton purpureum* 4183, *Epidermophyton rubrum* 655. and *Microsporon lanosum* 650.

Fungicidal Action of Merthiolate in Aqueous Solution

Table I shows results of Merthiolate tests against the organisms just mentioned. It appears that in five minute exposures Merthiolate

in dilutions up to and including 1:10,000 is uniformly fungicidal for all three fungi. Merthiolate 1:20,000 is fungicidal for *Epidermophyton rubrum* and *Microsporon lanosum* only, while Merthiolate 1:40,000 is not effiective against any one of the three strains.

TABLE I

Test fungus	Trichophyton purpureum No. 4183			Epidermophyton rubrum No. 655			Microsporon lanosum No. 650		
Date tested	3-24-32			3-30-32			3-30-32		
Exposure of test culture to chemical (minutes)	5	10	15	5	10	15	5	10	15
Merthiolate 1:1000	—	—	—	—	—	—	—	—	—
5000	—	—	—	—	—	—	—	—	—
10,000	—	—	—	—	—	—	—	—	—
20,000	+	—	—	—	—	—	—	—	—
40,000	+	+	+	+	+	+	+	—	—
60,000	+	+	+	+	+	+	+	+	+
120,000	+	+	+	+	+	+	+	+	+

It may be mentioned that in these tests the plantings of medicated culture were made to deep tube slants of glucose agar, each containing about 25 cc. of culture medium, in order to eliminate growth inhibitory effects of any mercurial "carried over" with the inoculum. Some experimenters have occasionally resorted to the following alternative in order to distinguish lethal from inhibitory effects, namely replanting all negative medicated culture tubes with a second inoculum of non-medicated culture which in many instances might be many times the size of the medicated culture dose. It appears that the disadvantages of this procedure are obvious.

That the utilization of culture tubes containing a large amount of culture medium to dilute out the "carried over" chemical very highly is satisfactory is shown by the following experiment. A fungicidal test was conducted with three of the stronger dilutions of Merthiolate and *Trichophyton purpureum* 4183. The technique used was like that used

TABLE II

Test fungus	Trichophyton purpureum 4183					
Exposure of test culture to chemical (minutes)	5		10		15	
Culture Series	direct culture	sub-culture	direct culture	sub-culture	direct culture	sub-culture
Merthiolate 1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	—
5000	—	—	—	—	—	—
Saline	+		+		+	

in the first tests, as recorded in Table I, except that the culture tubes upon which medicated culture was planted were subcultured to a further series of tubes of fresh media, and readings of both series of tubes made after two weeks' incubation. The results of this test are shown in Table II. The primary series of culture tubes, which had received medicated culture plantings directly, were negative as was shown to

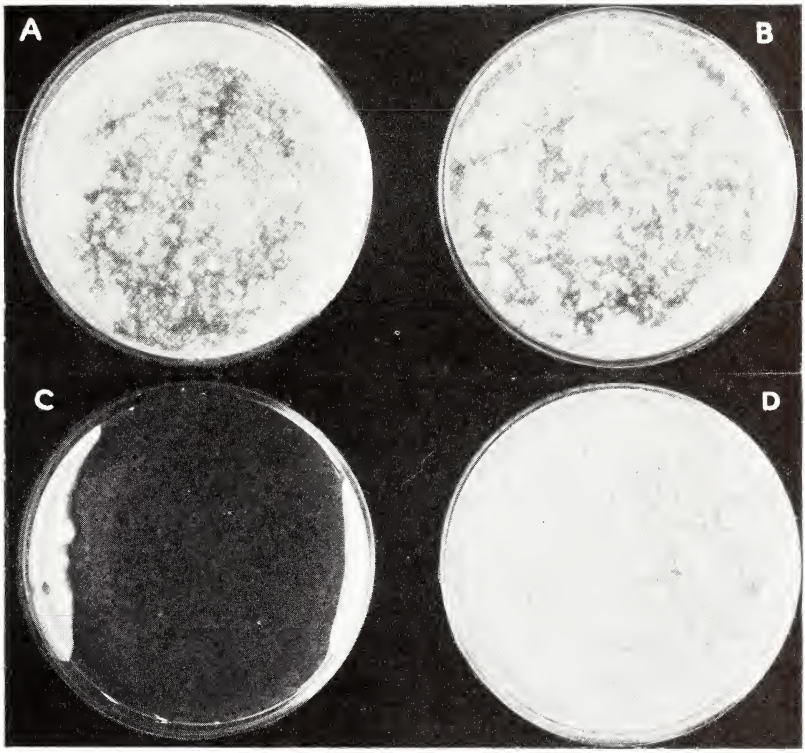

Fig. 4: *Epidermophyton rubrum* No. 655 Poured Agar Plates Prepared by Mixing 0.5 cc. Emulsion of Two Weeks Old Spore Containing Culture with 20 cc. Melted Agar, and, After Streaking with a Platinum Loop of the Indicated Materials, Incubating for Seven Days.

A. Triethanolamine Undiluted.

B. Carbitol Undiluted.

C. Merthiolate Aqueous Solution 1-1000 Containing Triethanolamine 1-80 and Carbitol 1-14.

D. No Medication.

be the case in Table I. Also the secondary series of culture tubes showed no growth, and this result appears to add certainty to primary culture readings.

The results set forth in Tables I and II verify those previously reported by Marshall,[17] and by Legge, Bonary, and Templeton[18] as referred to above, and show that Merthiolate in aqueous solution has ample "test-tube" fungicidal properties.

ACTION OF MERTHIOLATE IN A SPECIAL CREAM VCHICLE

In order to prepare Merthiolate in a form favoring better contact with infecting fungi in mycotic lesions of the skin for attempts at clinical use, due attention has been paid to the histology of the skin. The difficulties of adequate skin medication have been dealt with from the

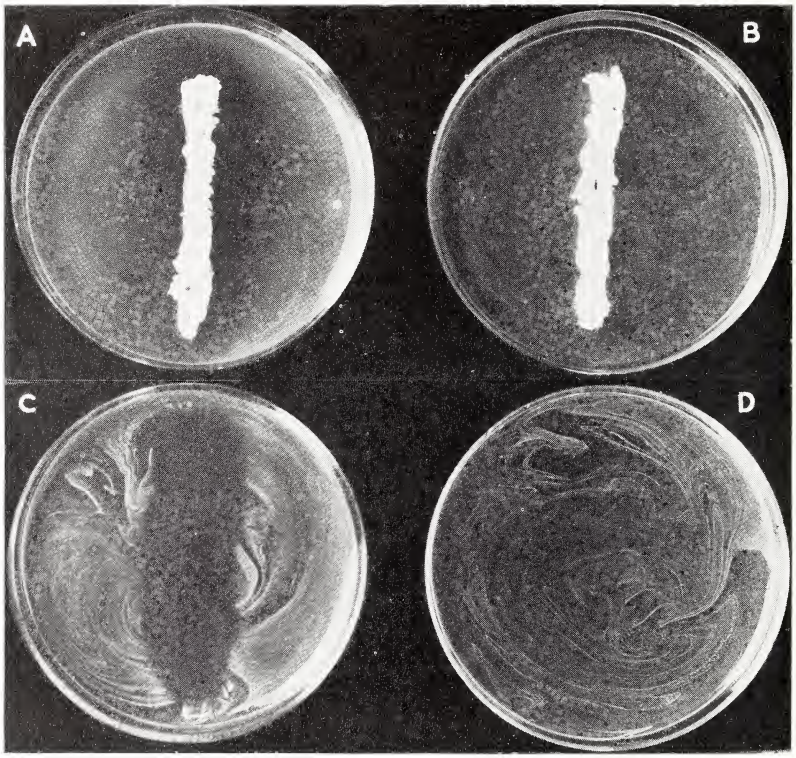

Fig. 5: *Staphylococcus aureus* Poured Agar Plates Prepared by Mixing 0.1 cc. Twenty-Four Hour Broth Culture with 20 cc. Melted Beef Extract Agar, and, After Streaking with a Platinum Loop of the Indicated Materials, Incubating at 37° C. for Twenty-Four Hours.

A. Merthiolate 1-1000 Cream, Containing Triethanolamine 1-80 and Carbitol 1-14.
B. Cream Base, Without Merthiolate, Containing Triethanolamine 1-80 and Carbitol 1-14.
C. Merthiolate Aqueous Solution 1-1000.
D. No Medication.

standpoint of overcoming the "insulating" effect of skin secretions and deposits through use of better oil-cutting accessory substances, and softening materials for horny layers.

Attention was directed[21] to certain unique properties of stearic acid cream vehicles or bases prepared with triethanolamine and carbitol, including excellent skin-softening and natural skin oil emulsification properties.

Fungicidal Tests of Solutions as Controls

Preliminary to the use of Merthiolate in a cream vehicle or base containing triethanolamine and carbitol, fungicidal tests were conducted to determine the individual and combined effect of these chemicals upon such organisms. The optimum concentrations of triethanolamine and carbitol in such a cream base appeared to be about 1:80 and 1:14 respec-

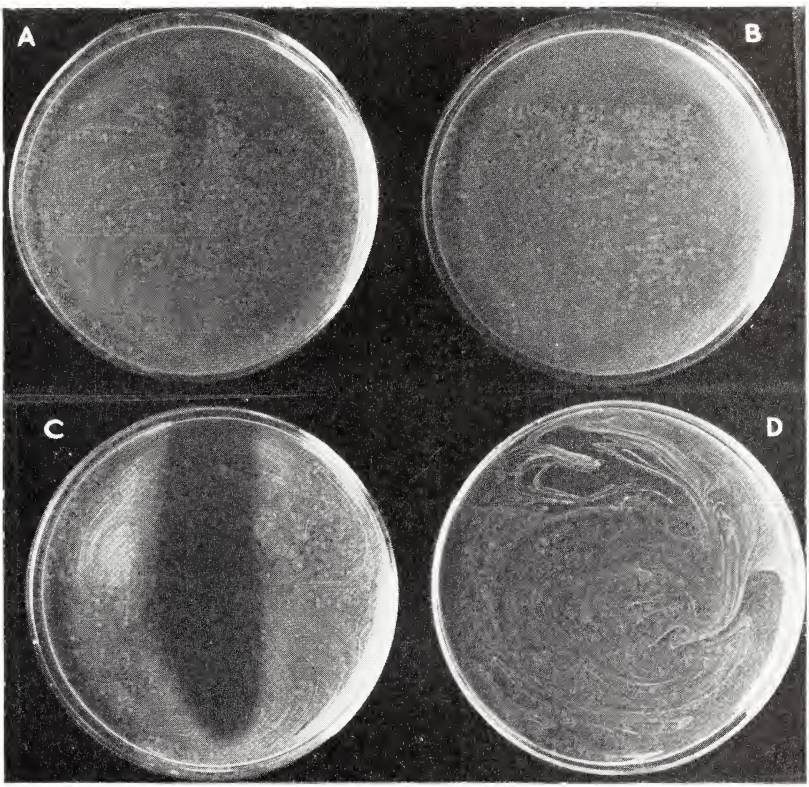

Fig. 6: *Staphylococcus aureus* Poured Agar Plates Prepared by Mixing 0.1 cc. Twenty-Four Hour Broth Culture with 20 cc. Melted Beef Extract Agar, and, After Streaking with a Platinum Loop of the Indicated Materials, Incubating at 37° C. for Twenty-Four Hours.

A. Triethanolamine Undiluted.

B. Carbitol Undiluted.

C. Merthiolate Aqueous Solution 1-1000 Containing Triethanolamine 1-80 and Carbitol 1-14.

D. No Medication.

tively, consequently these concentrations were tested in the combinations with Merthiolate. Table III shows the results of these tests. Both triethanolamine and carbitol when used undiluted proved fungicidal, but when used 1:5 and 1:3 respectively were not fungicidal. Concentrations of these chemicals of 1:80 and 1:14 respectively in a special cream base therefore would appear useful only indirectly as intended, and yet on the other hand not interfere with the fungicidal action of Merthiolate.

TABLE III

Test fungus	Trichophyton purpureum 4183			Epidermophyton rubrum 655		
Exposure of test culture to chemical (minutes)	5	10	15	5	10	15
Triethanolamine:						
Undiluted	—	—	—	—	—	—
1:5	+	+	+	+	+	+
1:10	+	+	+	+	+	+
1:15	+	+	+	+	+	+
1:20	+	+	+	+	+	+
Carbitol:						
Undiluted	—	—	—	—	—	—
1:2	+	+	—	+	—	—
1:3	+	+	+	+	+	+
1:4	+	+	+	+	+	+
1:5	+	+	+	+	+	+
Merthiolate:						
1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	—
3000	—	—	—	—	—	—
4000	—	—	—	—	—	—
5000	—	—	—	—	—	—
Merthiolate (with triethanolamine 1:80)						
1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	—
3000	—	—	—	—	—	—
4000	—	—	—	—	—	—
5000	—	—	—	—	—	—
Merthiolate (with carbitol 1:14)						
1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	—
3000	—	—	—	—	—	—
4000	—	—	—	—	—	—
5000	—	—	—	—	—	—
Merthiolate (with triethanolamine 1:80 and carbitol 1:14)						
1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	—
3000	—	—	—	—	—	—
4000	—	—	—	—	—	—
5000	—	—	—	—	—	—

TABLE IV

Test culture	Staphylococcus aureus 209			
Growth readings after incubation (hours)	24	48	72	168
Merthiolate:				
1:1,000,000	—	—	—	—
2,000,000	—	—	—	—
3,000,000	—	—	—	—
4,000,000	—	—	—	—
5,000,000	—	—	—	—
6,000,000	—	—	—	—
7,000,000	—	—	+	+
8,000,000	—	+	+	+
9,000,000	—	—	+	+
10,000,000	—	—	+	+
Merthiolate (from 1:1000 stock with triethanolamine 1:80)				
1:1,000,000	—	—	—	—
2,000,000	—	—	—	—
3,000,000	—	—	—	—
4,000,000	—	—	—	—
5,000,000	—	—	—	—
6,000,000	—	—	—	—
7,000,000	—	—	—	+
8,000,000	—	—	+	+
9,000,000	—	—	+	+
10,000,000	—	+	+	+
Merthiolate (from 1:1000 stock with carbitol 1:14)				
1:1,000,000	—	—	—	—
2,000,000	—	—	—	—
3,000,000	—	—	—	—
4,000,000	—	—	—	—
5,000,000	—	—	—	—
6,000,000	—	—	—	+
7,000,000	—	—	—	+
8,000,000	—	—	+	+
9,000,000	—	—	+	+
10,000,000	—	—	+	+
Merthiolate (from 1:1000 stock with triethanolamine 1:80 and carbitol 1:14)				
1:1,000,000	—	—	—	—
2,000,000	—	—	—	—
3,000,000	—	—	—	—
4,000,000	—	—	—	—
5,000,000	—	—	—	—
6,000,000	—	—	—	—
7,000,000	—	—	—	+
8,000,000	—	—	+	+
9,000,000	—	—	+	+
10,000,000	—	—	+	+

TABLE V

Test fungus	Staphylococcus aureus 209					
Exposure of test culture to chemical (minutes)	5		10		15	
Culture Series	direct culture	sub-culture	direct culture	sub-culture	direct culture	sub-culture
Phenol:						
1:60	—		—		—	
70	+		+		—	
Triethanolamine:						
Undiluted	+		+		+	
1:5	+		+		+	
10	+		+		+	
15	+		+		+	
20	+		+		+	
Carbitol:						
Undiluted	—	—	—	—	—	—
1:2	+		+		+	
3	+		+		+	
Merthiolate:						
1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	—
3000	—	+	—	—	—	—
4000	+	+	—	—	—	—
5000	+	+	+	+	—	+
Merthiolate (stock 1:1000 with triethanolamine 1:80)						
1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	+
3000	—	+	—	—	—	—
4000	+	+	—	—	—	—
5000	+	+	+	+	—	—
Merthiolate (stock 1:1000 with carbitol 1:14)						
1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	—
3000	—	—	—	—	—	—
4000	—	+	—	—	—	—
5000	+	+	—	+	+	+
Merthiolate (stock 1:1000 with triethanolamine 1:80 and carbitol 1:14)						
1:1000	—	—	—	—	—	—
2000	—	—	—	—	—	—
3000	—	—	—	—	—	—
4000	+	+	+	+	—	—
5000	+	+	+	+	—	+

Antibacterial Tests of Solutions as Controls

For further information as to the desirability of such modifications of Merthiolate *Staphylococcus aureus* inhibition tests were conducted with Merthiolate solution 1:1000 alone and as modified with triethanolamine and carbitol separately and together. The technique of such tests has been described previously[7]. The results of these tests are shown in Table

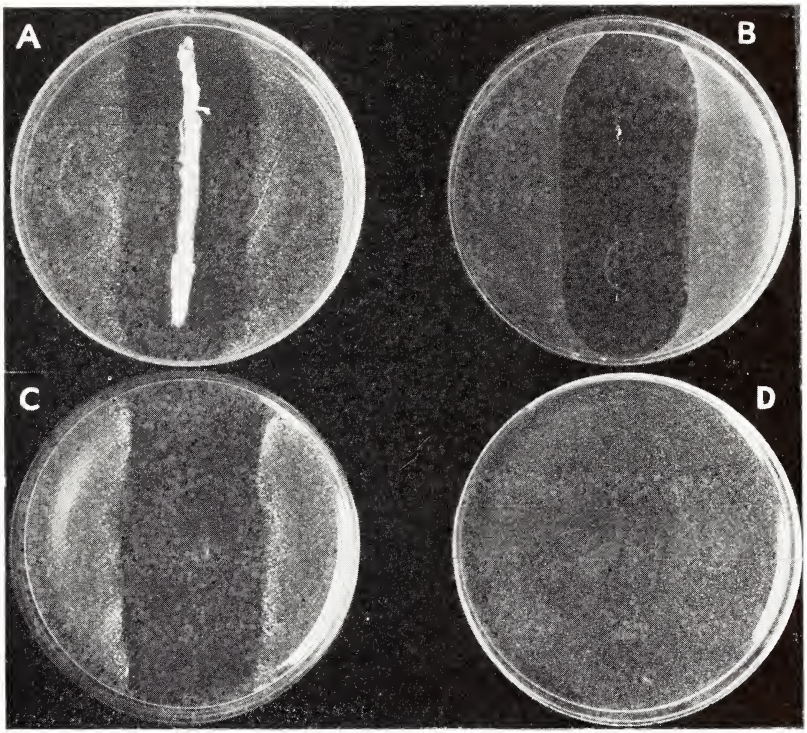

Fig. 7: *Staphylococcus aureus* Poured Agar Plates Prepared by Mixing 0.1 cc. Twenty-Four Hour Broth Culture with 20 cc. Melted Beef Extract Agar, and, After Streaking with a Platinum Loop of the Indicated Materials, Incubating at 37° C. for Twenty-Four Hours.

A. Merthiolate Cream 1-1000, Number 38586-1, Aged Twelve Months at Outside Weather Temperature in Collapsible Tubes.

B. Merthiolate Jelly 1-1000, Number P-25186, Aged Thirteen Months at Room Temperature in Collapsible Tubes.

C. Merthiolate Aqueous Solution 1-1000 as Control.

D. No Medication.

IV, and indicate that triethanolamine and carbitol do not add to or detract from the bacterial inhibitory properties of Merthiolate. The inhibitory properties are somewhat higher as shown here than we previously reported[7].

Germicidal tests were also conducted with Merthiolate, triethanolamine, and carbitol to determine further whether such combination is

desirable. The technique used is essentially that employed in the F. D. A. method[20]. Subcultures were made of culture tubes showing no growth following two days' incubation, and final results were read after seven days' incubation. These results are set forth in Table V, and indicate that carbitol, but not triethanolamine, has a week germicidal action, and that combinations of these with Merthiolate in concentrations mentioned above do not add to or detract from the well known germicidal action of Merthiolate.

FUNGUS PLATE TESTS OF MERTHIOLATE 1:1000 CREAM AND CONTROL MATERIALS

A series of fungus poured plate comparative tests of the general nature previously described[7,16] have been conducted with Merthiolate 1:1000 Cream. The fungi used were *Trichophyton purpureum* 4183 and *Epidermophyton rubrum* 655. The essential procedure utilized consisted in the preparation of glucose agar poured plates, containing fungi throughout the agar, and these were then streaked with a platinum loop of the test chemical. Following incubation of a week, large cleared areas appeared in the plates if the chemical tested possessed fungus inhibitory properties. Subcultures of the agar from such cleared areas have shown that under these conditions Merthiolate is also fungicidal as evidenced by uniform lack of growth in subcultures.

The fungus plates utilized in these tests have been photographed with suitable legends and are shown in Figures 1 to 4. An examination of these plates, including the proper controls, shows that Merthiolate has a readily demonstrable fungus inhibiting property, while triethanolamine has only a very slight action even when used undiluted. Neither carbitol nor the cream base has any demonstrable action on these fungi. It is also apparent that the utilization of triethanolamine and carbitol in the concentrations indicated does not affect either favorably or adversely the pronounced action of Merthiolate on fungi, and that these substances would serve only as indirectly acting materials, as planned, in a cream base with Merthiolate.

BACTERIAL PLATE TESTS OF MERTHIOLATE 1:1000 CREAM AND CONTROL MATERIALS

A further series of poured plates utilizing *Staphylococcus aureus* was prepared as has been described, and when the tests were completed these also were photographed as Figures 5 and 6. These tests show also that one may depend upon a strong antibacterial action of Merthiolate whether it be alone in aqueous solution or in a complex cream base, and also that the ingredients of the latter do not modify such antibacterial action in an appreciable way.

STABILITY OF MERTHIOLATE 1:1000 CREAM

Poured agar plate tests have been conducted as shown in Figure 7 which is nearly self explanatory in light of previous tests. A stability of action of one year is indicated, and deterioration has not been detected in this time.

Discussion

It appears that Merthiolate has fungicidal and fungus inhibiting action sufficient to justify its use in human mycotic skin conditions. We have attempted to improve upon a simple liquid solution for medicating the skin with Merthiolate through use of a semisolid stearate cream prepared by means of triethanolamine and carbitol which latter chemicals appear to have several desirable and unique properties. Both triethanolamine and carbitol in the concentrations suitable for preparation of a Merthiolate Cream base have been shown to have negligible direct action on fungi; however, these accessory materials expedite an emulsification of natural skin oils and thus assure good contact of Merthiolate and the skin.

Conclusions

1. Merthiolate has been shown to have a strong "test-tube" action on fungi associated with human mycotic skin conditions.
2. The action of Merthiolate in a special semisolid cream base on fungi is described from a laboratory point of view.

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INSECT GALLS ON SPECIES OF CUSCUTA

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In a study of the genus Cuscuta carried on now for nearly twenty years, I have had occasion to examine thousands of specimens either in the fresh condition or as herbarium specimens. Different species of these parasitic plants occur in nearly every part of the world and under a great variety of climatic conditions. They occur from Chile to Canada in the Americas, where they are most abundant, and from the Cape of Good Hope to about 60° north latitude in the Old World. Some species parasitize plants with woody stems seemingly as readily as they do those with succulent herbaceous stems, and occasionally they are to be found on the stems of Equisetum or of grasses. Hosts growing under the arid conditions of the desert or in salt marshes apparently serve as well for some Cuscuta species as do those occurring in more favorable growing regions.

One would expect to discover, in the examination of so large a number of specimens gathered from so wide a geographical range and growing under such variable ecological conditions, some specimens which were attacked by plant or animal parasites. In my experience I have seen no specimen which showed positive evidence of fungal parasitism. Saccardo records *Dendryphium macowanianum* as attacking *Cuscuta cassytoides*, and Peck described *Protomyces martindalii* as occurring on *Cuscuta Gronovii* which are the only references I have discovered giving