A COMPARATIVE STUDY OF THE ANTISEPTIC PROPERTIES OF CERTAIN OINTMENTS EMPLOYING VARIOUS BASES.*,[1]

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Reddish and Wales (1) investigated twelve of the U. S. P. and fourteen of the N. F. ointments which are generally indicated in conditions demanding an antiseptic action, and found only five of the U. S. P. and six of the N. F. to show any antiseptic properties.

Antiseptic ointments are those ointments which, when in contact with an infected area for a long period of time, either kill the infective organisms, or prevent their further growth. If the antiseptic ointment does not kill but does prevent the growth of the micro-organisms in the infected area, it will not only prevent them from doing further damage but will render them easy prey to the leucocytes. Therefore, this investigation is not concerned with the bactericidal but rather with the bacteriostatic action of the antiseptic. It is pointed out by Reddish (2) that in the treatment of infected surfaces, it is necessary that the active ingredient of the ointment be released from the inert base in order that it may surround the microörganism, thereby inhibiting its activity. Should the antiseptic be so securely incorporated into the inert base that it cannot be released, the therapeutic value of such preparations so far as the antiseptic ingredients are concerned would be worthless. With this idea in mind, this investigation was undertaken in an attempt to determine whether or not such antiseptic medicaments are influenced by the substitution of different fatty and non-fatty bases, and, if so, to what degree.

A series of tests was made, utilizing the more commonly employed so-called "antiseptic ointments" official in the United States Pharmacopœia and in the National Formulary, substituting various fatty and non-fatty bases.

EXPERIMENTAL PROCEDURE.

Staphylococcus (pyrogenes) aureus (3) has been recommended as the test organism for antiseptics and disinfectants. Since this organism is found on the surface of the skin, as well as in abscesses, boils and carbuncles, it was selected as the test organism in this investigation.

In order to simulate as nearly as possible the conditions met in treating infected areas of the skin, plain nutrient agar recommended by Reddish (4) was utilized. A fresh strain of *Staphylococcus aureus* was isolated from a carbuncle of a patient in the Roper Hospital of Charleston. Before each series of tests was made, the organism was transferred into nutrient broth, incubated for twenty-four hours and its phenol coefficient determined by the Food and Drug Administration Method (5). To make reasonably sure of the organism conforming to the phenol resistance requirements (6), a fresh transfer was made from the stock culture each thirty days, and the test organism taken from the month-old stock culture.

Two methods were used throughout the investigation, the Agar Cup-Plate Method (7) with certain modifications and the Agar Plate Method (8). For the Agar Cup-Plate Method, twenty cubic centimeters of plain agar were melted and cooled to 40° C. To this was added 0.1 cc. of a 24-hour broth culture of *Staphylococcus aureus*. This was thoroughly mixed, poured into a Petri dish and allowed to harden at room temperature. By means of a sterile cork borer, a disc

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was cut out of the solidified agar, leaving a cup 1.5 cm. in diameter and of an approximate depth of 0.2 cm., which was large enough to hold the sample of ointment to be tested. By means of a sterile pipette, a few drops of melted agar were placed in the cup to seal the crevices so as to prevent the ointment from seeping under the agar. An accurately weighed sample (500 mg.) of ointment was molded in the cork borer and transferred to the cup with the aid of a plunger, made from a cork to fit the borer. This modification is unique and facilitates the handling of the ointment. Ointments of a soft consistency cannot, of course, be molded, but may be transferred directly to the cup from the weighing paper. The plates were then covered with porous clay tops which absorbed the water of condensation, incubated at 37° C. for 24 hours, and then examined for evidence of inhibition. If a clear zone appeared around the ointment, the ointment was declared to possess inhibitory properties. The width of the zones was measured with a millimeter rule under a hand lens. All tests were made in duplicate and were rechecked many times. The Agar Plate Method is similar in all respects to the Agar Cup-Plate Method, with the exception that the ointment is placed directly on the surface of the inoculated agar.

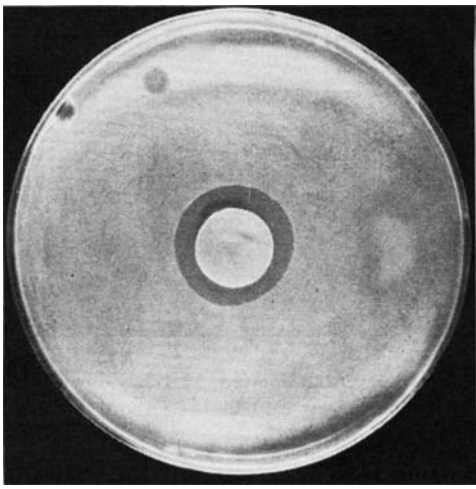

Fig. 1.—Yellow Mercuric Oxide Ointment, U. S. P. X. Agar Cup-Plate Method, 4-mm. zone.

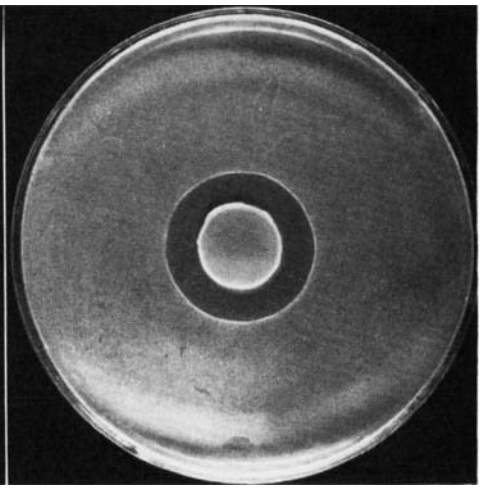

Fig. 2.—Ammoniated Mercury Ointment, U. S. P. XI. Agar Cup-Plate Method, 6-mm. zone.

YELLOW MERCURIC OXIDE OINTMENTS.

Bases.	Bacteriostatic Efficiency, Width of Zone of Inhibition Measured in Millimeters. Agar Cup-Plate Method.	Agar Plate Method.
U. S. P. XI	5 mm.	4 mm.
U. S. P. X (Fig. 1)	4 mm.	4 mm.
Wool Fat	5 mm.	4 mm.
Hydrous Wool Fat	5 mm.	5 mm.
Petrolatum	1 mm.	–
White Petrolatum	5 mm.	6 mm.
Rose Water Ointment	6 mm.	5 mm.
Protegin X*	5 mm.	5 mm.
Hydrogenated Fat[1]	7 mm.	6 mm.

IODINE OINTMENTS.

Bases.	Bacteriostatic Efficiency, Width of Zone of Inhibition Measured in Millimeters. Agar Cup-Plate Method.	Agar Plate Method.
U. S. P. XI (Fig. 5)	11 mm.	10 mm.
U. S. P. X	9 mm.	7 mm.
Wool Fat	6 mm.	5 mm.
Hydrous Wool Fat	5 mm.	6 mm.
Petrolatum	2 mm.	1 mm.
White Petrolatum	2 mm.	2 mm.
Rose Water Ointment	7 mm.	7 mm.
Protegin X*	9 mm.	9 mm.
Hydrogenated Fat[1]	12 mm.	15 mm.

– No zone.

* Product of the Goldschmidt Corporation, New York.

[1] Crisco, a product of Procter and Gamble, Cincinnati.

All conditions for carrying out the tests, including the manufacture of the ointments which were prepared to meet the pharmacopœial and National Formulary standards as to purity and strength, and the procedure for preparing the plates for incubation, were kept uniformly consistent throughout the entire investigation. Only chemically pure ingredients of known strength were used. The bases employed, minus the antiseptic, were plated as controls, and none were found to exhibit inhibitory properties.

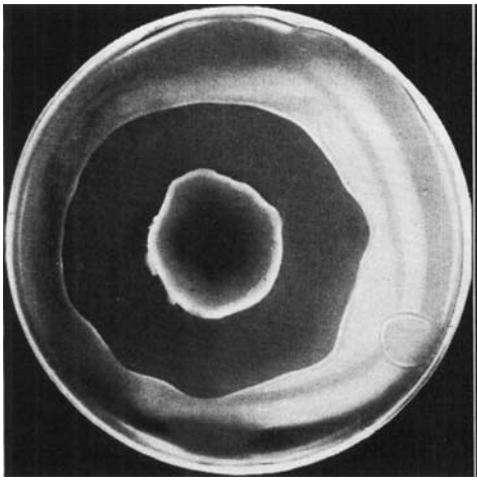

Fig. 3.—Ammoniated Mercury in Wool Fat Base. Agar Plate Method, 13-mm. zone.

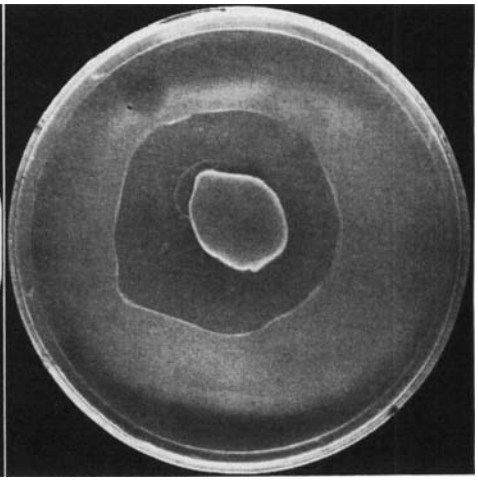

Fig. 4.—Ammoniated Mercury in Wool Fat Base. Agar Cup-Plate Method, 10-mm. zone.

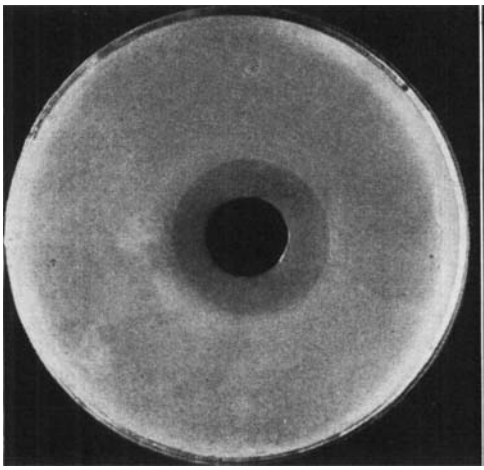

Fig. 5.—Iodine Ointment, U. S. P. XI. Agar Cup-Plate Method, 11-mm. zone.

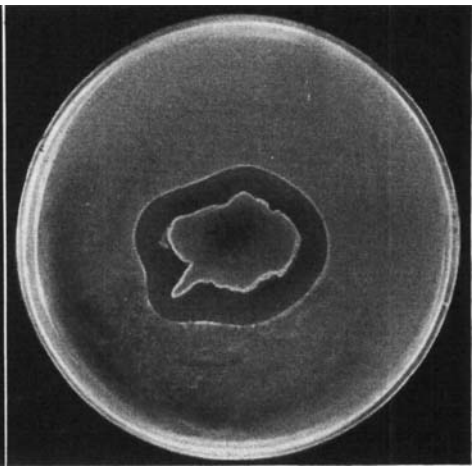

Fig. 6.—Ointment of Red Mercuric Oxide, N. F. VI. Agar Plate Method, 4-mm. zone.

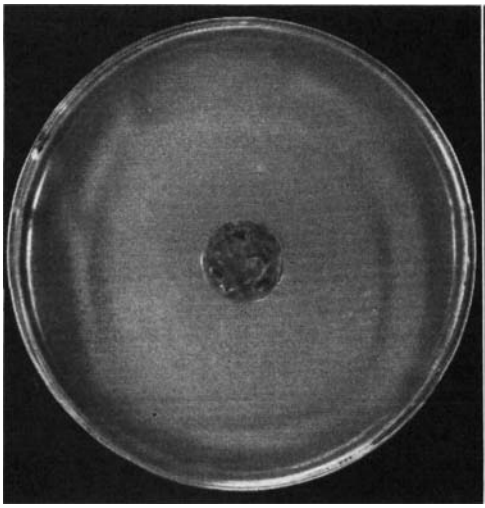

Fig. 7.—Phenol Ointment, U. S. P. XI. Agar Cup-Plate Method. No zone.

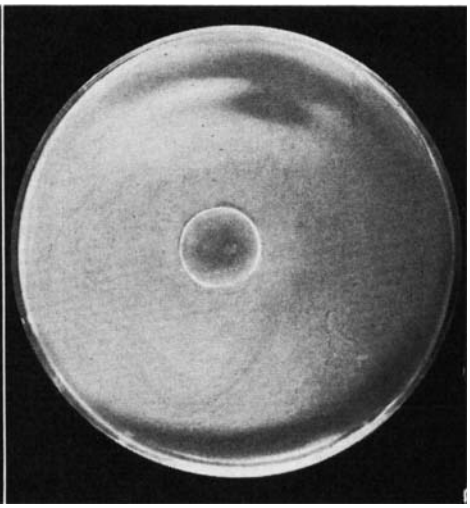

Fig. 8.—Sulfur Ointment, U. S. P. XI. Agar Cup-Plate Method. No zone.

AMMONIATED MERCURY OINTMENTS.

Bases.	Bacteriostatic Efficiency, Width of Zone of Inhibition Measured in Millimeters. Agar Cup-Plate Method.	Agar Plate Method.
U. S. P. XI (Fig. 2)	6 mm.	6 mm.
U. S. P. X	9 mm.	7 mm.
Wool Fat	10 mm.	13 mm.
Hydrous Wool Fat	10 mm.	10 mm.
Petrolatum	6 mm.	5 mm.
White Petrolatum	7 mm.	6 mm.
Rose Water Ointment	8 mm.	6 mm.
Protegin X*	8 mm.	8 mm.
Hydrogenated Fat[1]	7 mm.	6 mm.

OINTMENTS OF RED MERCURIC OXIDE.

Bases.	Bacteriostatic Efficiency, Width of Zone of Inhibition Measured in Millimeters. Agar Cup-Plate Method.	Agar Plate Method.
N. F. VI (Fig. 6)	4 mm.	4 mm.
N. F. V	5 mm.	4 mm.
Wool Fat	7 mm.	8 mm.
Hydrous Wool Fat	5 mm.	3 mm.
Petrolatum	5 mm.	4 mm.
White Petrolatum	6 mm.	4 mm.
Rose Water Ointment	11 mm.	8 mm.
Protegin X*	4 mm.	4 mm.
Hydrogenated Fat[1]	5 mm.	4 mm.

OINTMENTS OF MILD MERCUROUS CHLORIDE.

Bases.	Bacteriostatic Efficiency, Width of Zone of Inhibition Measured in Millimeters. Agar Cup-Plate Method.	Agar Plate Method.
N. F. VI	3 mm.	3 mm.
N. F. V	4 mm.	4 mm.
Wool Fat	3 mm.	4 mm.
Hydrous Wool Fat	2 mm.	2 mm.
Petrolatum	2 mm.	2 mm.
White Petrolatum	4 mm.	4 mm.
Rose Water Ointment	5 mm.	6 mm.
Protegin X*	5 mm.	3 mm.
Hydrogenated Fat[1]	5 mm.	4 mm.

PINE TAR OINTMENTS.

Bases.	Bacteriostatic Efficiency, Width of Zone of Inhibition Measured in Millimeters. Agar Cup-Plate Method.	Agar Plate Method.
U. S. P. XI	7 mm.	7 mm.
U. S. P. X	7 mm.	7 mm.
Wool Fat	3 mm.	2 mm.
Hydrous Wool Fat	5 mm.	4 mm.
Petrolatum	3 mm.	3 mm.
White Petrolatum	5 mm.	4 mm.
Rose Water Ointment	6 mm.	6 mm.
Protegin X*	4 mm.	3 mm.
Hydrogenated Fat[1]	2 mm.	1 mm.

* Product of the Goldschmidt Corporation, New York.

[1] Crisco, a product of Procter and Gamble, Cincinnati.

	Bases.	Bacteriostatic Efficiency, Width of Zone of Inhibition Measured in Millimeters. Agar Cup-Plate Method.	Agar Plate Method.
(1) Sulfur Ointments (2) Calamine Ointments (3) Phenol Ointments (4) Chrysarobin Ointments (5) Boric Acid Ointments (6) Zinc Oxide Ointments	U. S. P. XI	–	–
	U. S. P. X	–	–
	Wool Fat	–	–
	Hydrous Wool Fat	–	–
	Petrolatum	–	–
	White Petrolatum	–	–
	Rose Water Ointment	–	–
	Protegin X*	–	–
	Hydrogenated Fat[1]	–	–

– No zone.

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[1] Crisco, a product of Procter and Gamble, Cincinnati.

SUMMARY.

1. Six antiseptics (boric acid, calamine, chrysarobin, phenol, sulfur and zinc oxide) exhibited no bacteriostatic properties when combined with the bases employed in this investigation.

2. The tests, in most cases, proved conclusively that the antiseptics when combined with fatty bases, such as the wool fats, hydrogenated fats, etc., exhibit greater bacteriostatic properties than when combined with the non-fatty bases.

3. In the non-fatty group of bases, white petrolatum seems to permit the diffusion of the antiseptic agent into the agar to a much greater extent than petrolatum.

4. Contrary to Reddish's results (9), Calamine Ointment, U. S. P. X and calamine in combination with the bases used, produced no zones of inhibition.

5. Although it has been felt that not enough surface of the inoculated agar might be exposed to the ointment when using the Agar Cup-Plate method, the results showed in this work little or no variation when using either the Agar Cup-Plate or Agar Plate method.

6. It was found that a more accurate measurement of the zones could be made when using the Agar Cup-Plate method, as the margin of the zones produced was more regular.

REFERENCES.

(1) Reddish, Geo. F., and Wales, H. J., JOUR. A. PH. A., 18, 576 (1929).
(2) Reddish, Geo. F., *Ibid.*, 16, 655 (1927).
(3) *Circular 198*, U. S. Department of Agriculture, 9 (1931).
(4) *Ibid.*, 4 (1931).
(5) *Ibid.*, 4 (1931).
(6) *Ibid.*, 4 (1931).
(7) *Ibid.*, 14 (1931).
(8) *Ibid.*, 12 (1931).
(9) Reddish, Geo. F., and Wales, H. J., JOUR. A. PH. A., 18, 576 (1929).

According to James C. Munch, after reviewing various methods of measuring precision, a formula is developed to show how many animals are required for use in bioassaying products to give a desired degree of confidence in the results obtained.—Scientific Section, A. PH. A.