A BACTERIOLOGICAL STUDY OF THE ANTISEPTIC PROPERTIES OF VARIOUS PHENOL OINTMENTS

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INTRODUCTION

Phenol, because of its great affinity for petrolatum and other oils, loses its antiseptic properties, when incorporated in these substances as vehicles and after aging for a short period of time. This is due to the fact that phenol is so completely bound by the petrolatum that it is extremely difficult for it to be released to produce the antiseptic action. U.S.P. XII Phenol Ointment is such a preparation. The purpose of this study is to find a base which will give phenol its greatest antiseptic strength when used in 2% concentrations.

Phenol ointment has long been used as an antiseptic application. It has been official in the U.S.P. since 1873. At that time it contained 12.5% phenol. The amount was gradually reduced and at the present time the official ointment contains from 1.8 to 2.2% phenol. Originally it was called Ointment of Carbolic Acid then changed to Ointment of Phenol and now it is known as Phenol Ointment. Yellow wax with lard was originally used as the base. Now yellow wax with petrolatum is used.

In 1881 Koch advanced the theory that when phenol is dissolved in alcohol or oil, it no longer shows antiseptic properties. Since that time many different workers have proved that theory.

In 1933 Gershenfeld and Miller however showed that 2% phenol in a water-miscible base was much more effective than in a greasy base.

Gershenfeld and Brillhart in 1939 prepared the following bases:

a. Synthetic wax bases
b. Oxycholestrin bases
c. U.S.P. XI bases
d. Benzoinated lard bases
e. Petrolatum base
f. 2% cholestrin added to the d and e base

The synthetic wax group was found to be the most effective in tests in which the Agar and Agar Cup Plate methods were used. The following synthetic wax formula proved to be the best amongst the synthetic wax formulas.

Tegin	12%
Mineral Oil	2%
Glycerin	5%
Spermaceti	5%
Water	76%

This produced a zone of inhibition of 5 millimeters. The oxycholestrin base group was second in effectiveness. In this case a base which contained 74% water was the best. The other type bases showed little or no zones of inhibition. From these results we may conclude that water has a direct relationship on the antiseptic properties of a phenol ointment.

Clark, in the same year made a study on the antiseptic value of certain phenolic ointments. Ointments were prepared from the following:

2% sodium laurylsulfonate with 5% glycerin

2% sodium lauryl sulfonate

3% sodium benzoate

3% triethanolamine

Wool fat hydrous and anhydrous

Coconut oil

Crisco

Spry

The ointments were prepared and tested under the following conditions. Ointments were made using prolonged fusion and aged for 2 months or more. F.D.A. Serum Agar plate method was used. Plates were incubated for 24 hours at 37° C. using Staphlococcus aureus A 209 as the test organism. Tests were run with 2, 5, and 10% phenol in each ointment base. Clark concluded that:

1. Ointments of phenolic germicides are likely to have little, if any, antiseptic value in ordinary fatty bases unless very high in germicidal content.

2. The base composed of petrolatum, sodium lauryl sulfonate, glycerin, and water promised to be of value when used with phenolic germicides.

3. The addition of soap and glycerin or the inclusion of a small amount of alcohol in petrolatum appeared likely to be of value as a base for phenolic ointments.

4. "Before any phenolic ointment is claimed to be antiseptic it should be aged sufficiently to indicate that the antiseptic power will not disappear in time."

Prout and Smith observed in their work that when phenol ointment is made according to U.S.P. method, as much as 25% of the phenol may be lost; consequently when Burnside and Kuever made their study of phenolic ointments, they used a modified method in which the phenol was added just before the base was congealed. Even though this modified method was used, no zones of inhibition were obtained with the U.S.P. ointment base.

With an emulsion type ointment base containing 2% gardinol, 0.24% propylene glycol, 1.88% water, and 90% white petrolatum; 2% phenol showed a zone of inhibition of 6.0 millimeters. They found that an addition of waxes to the emulsion base had a deleterious effect by reducing the bacteriocidal potency.

A microscopic study of the U.S.P. phenol ointment showed that the phenol had crystallized whereas in the emulsion type base the phenol had remained in solution in the outer aqueous phase.

U.S.P. phenol ointment was also tested at various pH's. At a pH of 3.0 it showed a small zone of inhibition, but at any pH higher than 3.0 no zones of inhibition were observed.

While these studies are somewhat conflicting in nature, one definite conclusion can be drawn, namely, that a petrolatum base is not an effective vehicle for phenol.

EXPERIMENTAL

A. Preparation of Ointment Bases

Five ointment bases were prepared containing 2% phenol.

Base no. I

Bentonite	70 gm.
Water	sufficient quantity to produce proper consistency.

Base no. II

Bentonite	60 gm.
Water	sufficient quantity to produce proper consistency. To this 5 gm. of petrolatum was added.

Base no. III

Cetyl alcohol	15%
Propylene glycol	10%
White wax	1%
Sodium lauryl sulfonate	2%
Water	72%

Oil phase is melted and added to the aqueous phase. Both phases are at a temperature of 65° C. This produces an oil in water emulsion as determined by testing with an electric current.

Base no. IV

Stearic Acid	15 gm.
Expressed oil of almond	14 cc.
Spermaceti	1 gm.
Cetyl alcohol	1 gm.
White wax	1 gm.
Lanolin	1 gm.
Glycerin	1 gm.
Distilled water	90 cc.
Ammonia water	2 cc.

The oil phase is melted and heated to about 80° C. The water, glycerin, and ammonia water are mixed together and heated to the same temperature. Ammonia water is added just before addition to the oil phase. This is an oil in water emulsion.

Base no. V

U.S.P. Phenol Ointment

Phenol	2%
Yellow Ointment	98%
Wool fat	5%
Yellow wax	5%
Petrolatum	90%

Before the bacteriological tests were carried out, the ointments were assayed for their phenol content. Standard sodium thiosulfate solution was prepared and standardized. Normality of the sodium thiosulfate was .1043. Koppescharrs solution was also prepared and standardized. Normality of the Koppescharrs solution was .1034. The assays were carried out according to the U.S.P. XII method in which the phenol is obtained by steam distillation. Samples weighing approximately 2 grams were used. The following percentages were obtained:

Base no. I	1.73%
Base no. II	1.66%
Base no. III	2.17%
Base no. IV	1.98%
Base no. V	1.50%

The low percentage of phenol found in bases no. II and V may be due to the strong affinity which phenol has for petrolatum, thereby not volatilizing out of the base when subjected to steam distillation.

B. Bacteriological Tests:

Two strains of Staphlococcus aureus obtained from the American Pure Culture Society were used, 209 P and 9194. Later tests proved strain 209P to be a much more resistant strain than 9194. At the time these tests were started the ointments had aged for approximately one month and a half.

Various methods were first tried to introduce the ointment into the petri dish. A syringe was used, but the amounts and shape of the ointments on the plates did not produce even results. Secondly porous porcelain cylinders were tried. No zones of inhibition were obtained due to lack of diffusion through the porcelain because of the small surface exposed to the agar. Thirdly, cylinders were prepared from stainless steel screen made up largely of nickel. These cylinders were 15 mm. in diameter and 12 mm. in height. Ointments placed in these cylinders produced satisfactory and uniform zones of inhibition.

Since the cylinders were made using solder, they could not be sterilized by heat therefore alcohol was used. These were then filled with the ointment base with a sterile forceps and spatula. The ointment filled cylinders were then placed in the petri dishes and agar-medium poured around these cylinders. This produced and excellent interface between the ointment and the agar which consequently produced good diffusion.

Twenty-four hour cultures in beef broth were used and 0.1 cc. was inoculated into 20 cc. of the agar medium before it was poured into the petri dishes. This was done when the agar was at a temperature of 47° C.

TABLE I

Zones of Inhibition in Millimeters

Staphlococcus aureus 209P

Bases	11/30/46	12/6/46	12/13/46	12/20/46
I	8	7	7	6.5
II	7.5	6.5	6.5	7
III	4.5	3.5	4	4
IV	4	3.5	3.5	4
V	0	0	0	0
Control	0			

Incubation for 24 hours at 37.5° C.

TABLE II

Zones of Inhibition in Millimeters

Staphlococcus aureus 9194

Bases	11/30/46	12/6/46	12/13/46	12/20/46
I	8	10	10	10
II	8	10	8.5	9.5
III	4.5	7.5	6.5	5.5
IV	5	7	6	5
V	2	2	1.5	0.5
Control	0			

Another reading was taken on the plates after 48 hours to determine whether there was any appreciable change in the size of the zone after that length of time. No appreciable change was noted.

CONCLUSIONS

1. The bentonite base produced the largest zones of inhibition. The bentonite and petrolatum base II was second in the size of zones produced. Since there was little appreciable difference between the size of zones of the two bases, Base II may be considered to be the more desirable of the two. The base containing bentonite alone will dry out rapidly on the skin and the powder would flake off whereas the base to which petrolatum was added would not dry out as rapidly and after drying would still tend to remain on the skin. Another point in favor of the second base is its stability. A much longer period of time is required to dry out the Base II than the Base no. I.

2. Bases III and IV contained from 15 to 20% more fat or oil than the second base and a 100% more than the first. The zones from III and IV were about one-half of that produced by the first two. This might indicate that the amount of fat, oil, or wax in a base has an appreciable effect on the germicidal action of a base and bears out the theory which Koch advanced.

3. The amount of water in a base has little effect on the germicidal action. The amount of fat, wax, or oil is a much greater factor overpowering any effect the amount of water present may have had in increasing the germicidal action.

4. This study also bears out, that U.S.P. XII Phenol Ointment has little or no antiseptic action after standing for a short period of time.

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