

disinfectants (2), which is one of the accepted methods of the United States Food and Drug Administration. Briefly, the procedure is as follows: 20 cc. of nutrient agar which had been melted and cooled to 42–45° C. and to which had been added 0.1 cc. of a 24-hour broth culture of *Staphylococcus aureus*,<sup>1</sup> were poured into a sterile petri dish and allowed to harden at room temperature. By means of a sterile cork borer, a disk, 1.5 cm. in diameter, was cut out of the solidified agar, leaving a cup with an approximate depth of 0.2 cm. Into this cup was placed 0.5 Gm. of the ointment to be tested, and the plate was then incubated under an unglazed porcelain top for 24 hours. At the end of the period of incubation, the zones of inhibition were measured by means of a vernier caliper. Each ointment was prepared *secundum artem*, maintaining the pharmacopœial strengths of the active constituents and employing the bases previously mentioned.

The accompanying graphs show the differences between the zones of inhibition produced by the germicidal agents when incorporated in the silica gel bases and the same germicidal agents when incorporated in the pharmacopœial base. It will be noted that the zones produced by any one of the antiseptic medicaments are approximately the same with all six silica gel bases; that there are significant differences between the individual antiseptic medicaments, which would be expected inasmuch as the germicidal power of the medicaments *per se* varies greatly; that the corresponding ointments prepared with the pharmacopœial base failed, with the exception of ammoniated mercury, to exhibit any zones of inhibition.

#### DISCUSSION

Earlier investigation of two of the authors (3) has shown that these same antiseptic medicaments when prepared with other bases failed (again with the exception of ammoniated mercury) to exhibit any zones of inhibition when tested in a similar manner. At present the explanation of these observed differences is theoretical and is based upon the apparent constitution of the bases employed.

An ointment that has any antiseptic value owes that characteristic to the fact that the medicament, in sufficient concentration to destroy microorganisms, is capable of diffusing from the base to the surface upon which it is applied. Any factor or factors which have a tendency to influence the diffusion of the medicament from the ointment base will, in turn, affect the antiseptic prop-

erty of the ointment. It would appear as though silica gel, due to its high water content, permits a greater diffusion of the medicament from the ointment. On the other hand, the pharmacopœial base having a relatively low water content and holding securely what little water is present, apparently does not afford a very good carrier for certain antiseptic substances.

#### CONCLUSIONS

1. Silica gel, when combined with such substances as glycerin, expressed oil of almond, liquid petrolatum, castor oil, olive oil and cottonseed oil, respectively, appears to react satisfactorily as a carrier for certain antiseptic medicaments.

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## Phenolic Ointments\*

By Carl B. Burnside† and Rudolph A. Kuever‡

#### INTRODUCTION

Phenol Ointment has long been used as an antiseptic application. It has been official in the United States Pharmacopœia (1) since 1873 at which time it first appeared in the fifth revision under the title, Ointment of Carbolic Acid. This ointment contained 12.5% of phenol. In six revisions of the United States Pharmacopœia the formula for the official ointment was altered as many times. Its phenol content has been gradually reduced until at present it contains 1.8 to 2.2%. The title was changed from Ointment of Carbolic Acid to Ointment of Phenol in the eighth revision and in the eleventh revision it takes the name Phenol Ointment.

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<sup>1</sup> No. 209, obtained from the United States Department of Agriculture.

The base has been changed at various times and these changes may be noted in the accompanying Table I.

Table I.—Changes in Composition of Phenol Ointment, 1873–1936

U. S. P. Year	Title	Phenol Content, %	Base, %
V 1873	Ointment of Carbolic Acid	12.5	Yellow wax 17.5 Lard 70.0
VI 1882	Ointment of Carbolic Acid	10.0	Yellow wax 18.0 Lard 72.0
VII 1893	Ointment of Carbolic Acid	5.0	Yellow wax 19.0 Lard 76.0
VIII 1905	Ointment of Phenol	3.0	White Petrolatum 97.0
IX 1916	Ointment of Phenol	2.25	White wax 19.55 Benzoinated lard 78.20
X 1925	Ointment of Phenol	2.0	Yellow wax 5.0 Petrolatum 93.0
XI 1936	Phenol Ointment	1.8–2.2	Yellow wax 5.0 Petrolatum 93.0

#### HISTORICAL

In view of the work that has been done with phenol and phenolic derivatives, as it applies to their use in oily base ointments, it is obvious that their antiseptic potency is quite unpredictable. When tested for bactericidal potency the results obtained are usually unsatisfactory. In 1881 Koch (2) advanced the theory that when phenol is dissolved in oil or alcohol it shows no antiseptic properties. This statement has subsequently been upheld by many workers. Prout and Strickland (3), experimenting with various oleaginous bases, found no bactericidal potency when phenol was incorporated into oily bases. Reddish and Wales (4) tested U. S. P. Ointment of Phenol along with some 40 other ointments and found it to possess no antiseptic potency. By testing various phenol ointments Bryan (5) found that at least 10% of phenol was required to produce bactericidal potency. Gottstein (6), in 1889, made the statement that oil-soluble antiseptics, when incorporated into an oily ointment base, possess no antiseptic potency. Cheyne (7), during World War I, suggested an ointment of 20% phenol for use in the British Navy as an antiseptic application. Husa and Radin (8) experimented with Ointment of Phenol and suggested a formula which was later found to be only feebly bacteriostatic by Gershenfeld and Miller (9) who also concluded that

2% phenol in petrolatum or a mixture of petrolatum and anhydrous wool fat displayed no bactericidal action.

A later report by Gershenfeld and Miller (10) suggested the use of water-miscible vanishing cream bases for bactericidal substances. Clark (11) found phenol to be non-antiseptic in oily bases but suggested the use of lauryl sodium sulfate as an emulsifying agent in the preparation of Phenol Ointments. He observed evidence of increased bactericidal action with a base of petrolatum containing 2.5% glycerin, 2.5% water and 2% of lauryl sodium sulfate, when he prepared ointments of thymol and chlor-thymol. Gershenfeld and Brillhart (12) suggested the inclusion of emulsion bases in ointments of the United States Pharmacopœia and National Formulary because greater bactericidal action could thus be obtained.

Mention should be made of work done by Prout and Smith (13) who observed that when Phenol Ointment is made according to the United States Pharmacopœia as much as 25% of its phenol content may be lost by evaporation.

A great deal of work has been done on ointment bases containing emulsifying agents. Formulas are given. Numerous writers have suggested the use of cholesterol and its derivatives (12, 14, 16, 18, 20). Other investigators mention the use of various alcohols for emulsifying purposes (15, 17, 18, 19). Use of hydrogenated castor and peanut oils is suggested by Lesser (16) and Fiero (24). Triethanolamine is suggested by Mumford (15), an English writer.

#### PURPOSE

Phenol Ointment U. S. P. has no bactericidal potency when tested by the Standard F. D. A. Method (23) using *Staphylococcus aureus* as the test organism. Since phenol is antiseptic it is obvious that an improper base is employed. The purpose of this work is to prepare a Phenol Ointment with bactericidal potency. Since bases like that of U. S. P. Phenol Ointment have proved to be ineffective for phenol, emulsion types of ointment bases were created. Ointments were made with phenols and phenolic de-

rivatives, including phenol, thymol, chlor-thymol, resorcinol, hexylresorcinol, chlorcarvacrol, trinitrophenol, *o*-hydroxy diphenyl, *m*-hydroxy diphenyl, *p*-hydroxy diphenyl, 3-chlor-4-hydroxy diphenyl and betanaphthol.

A study was first made of the antiseptic properties of these phenols in U. S. P. Phenol Ointment Base. The same phenols, in the proposed emulsion ointment base, were prepared and their bactericidal potency determined. Considering the fact that U. S. P. Phenol Ointment has a single phase oleaginous base, this affords an opportunity of comparing the action of these same phenols in the two types of bases.

EXPERIMENTAL

Melting points of the various bases employed were determined by using the method devised by Li and Kuever (14).

A tabulation of the melting points of all the U. S. P. Phenol Ointments and of the proposed emulsion ointment base is given in Table II.

Table II.—Melting Points of Phenol Ointments

U. S. P.	Melting Point, Deg. C.
V	49
VI	50
VII	53
VIII	49
IX	53.5
X	47
XI	47
Emulsion ointment base	47

Since Phenol Ointment U. S. P. displays no bactericidal potency, a study was made to determine how the other phenols would behave in U. S. P. Phenol Ointment Base.

Phenol Ointment U. S. P.

Phenol	2 Gm.
Yellow wax	5 Gm.
Petrolatum	93 Gm.

Melt the yellow wax and the phenol on a water-bath, add the petrolatum and stir the mixture until it congeals.

Since Prout and Smith (13) observed a marked loss of phenol by this method, the directions were altered slightly in making these phenolic ointments using the U. S. P. Phenol Ointment Base. In each case two per cent of the phenolic substance used was incorporated into the base using the following directions:

Melt the yellow wax and the petrolatum on a water-bath and stir until nearly congealed before adding the phenolic substance. This method eliminates undue loss of phenols by evaporation.

Table III gives the bacteriological results obtained by testing these ointments using the Standard F. D. A. Method, *Staphylococcus aureus* as the test organism.

Table III.—Bactericidal Potency of Phenolic Ointments (U. S. P.) Made with Phenol Ointment Base

Ointment	Per Cent	Base, %	Zone, Mm.
Hexylresorcinol	2	Yellow wax 5	9.0
		Petrolatum 93	
Chlorcarvacrol	2	Yellow wax 5	5.0
		Petrolatum 93	
<i>m</i> -Hydroxy diphenyl	2	Yellow wax 5	5.0
		Petrolatum 93	
Chlorothymol	2	Yellow wax 5	4.5
		Petrolatum 93	
Thymol	2	Yellow wax 5	3.0
		Petrolatum 93	
3-Chlor-4-hydroxy diphenyl	2	Yellow wax 5	3.0
		Petrolatum 93	
<i>o</i> -Hydroxy diphenyl	2	Yellow wax 5	2.5
		Petrolatum 93	
Betanaphthol	2	Yellow wax 5	1.5
		Petrolatum 93	
<i>p</i> -Hydroxy diphenyl	2	Yellow wax 5	No zone
		Petrolatum 93	
Resorcinol	2	Yellow wax 5	No zone
		Petrolatum 93	
Trinitrophenol	2	Yellow wax 5	No zone
		Petrolatum 93	
Phenol	2	Yellow wax 5	No zone
		Petrolatum 93	

*Procedure for Testing Ointments.*—The Standard F. D. A. Agar Cup Method (23) was used in each case. The amount of ointment used and the area of contact with the agar in this method are more nearly constant than in the Agar Plate Method. A twenty-four-hour broth culture of *Staphylococcus aureus* in nutrient agar, containing peptone, Leibig's beef extract and salt, as suggested by the F. D. A. Method, was used.

*Emulsion Ointment Bases.*—Phenol Ointment U. S. P. has a single-phase oleaginous base. This appears to be the reason for its non-antiseptic properties. Oily, single-phase ointment bases, generally are unsatisfactory for phenols. From the results

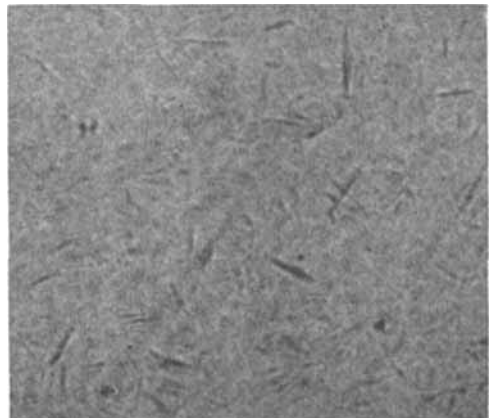


Fig. 1.—Phenol Ointment U. S. P. XI. Crystals of Phenol May Clearly Be Seen. × 160.

obtained it appears that single-phase oleaginous bases impair the antiseptic power of all the phenols.

Figure 1 is a microphotograph of U. S. P. Phenol Ointment showing that the phenol has crystallized. While undoubtedly the phenol was in solution when the ointment was warm, upon cooling and standing the phenol crystallized as the microphotograph clearly reveals.

Other phenolic ointments displayed similar characteristics with U. S. P. Phenol Ointment base. Ointments employing the proposed emulsion ointment base do not show this crystallization. This ointment base has an internal oleaginous and an external aqueous phase. It is evident that the phenols are in solution in the aqueous phase.



Fig. 2.—Product: Phenol Ointment U. S. P. XI.  
Test organism: *Staphylococcus aureus* of 24-hour broth culture.  
Method: Agar Cup.  
Period of Incubation: 48 hours at 37° C.  
Width of Zone: No zone.

After some experimentation, the following base was devised as a satisfactory emulsion ointment base.

Gardinol*	0.25 Gm.
Propylene glycol	6.00 Gm.
Water	1.92 Gm.
White petrolatum	91.83 Gm.
To make	100.00 Gm.

\* A mixture of neutralized, sulfated, higher alcohols such as lauryl sodium sulfate and oleyl sodium sulfate sold in commerce as Gardinol.

The ointments of the various phenols may be made with the proposed emulsion ointment base according to the following formula:

Phenol	2.00 Gm.
Gardinol	0.24 Gm.
Propylene glycol	5.88 Gm.
Water	1.88 Gm.
White petrolatum	90.00 Gm.
To make	100.00 Gm.

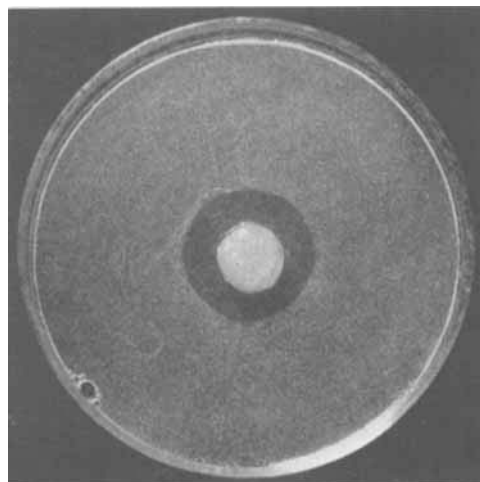


Fig. 3.—Product: Phenol Ointment with Proposed Emulsion Ointment Base.  
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.  
Method: Agar Cup.  
Period of Incubation: 48 hours at 37° C.  
Width of Zone: 6.0 mm.

Dissolve the gardinol in the propylene glycol and the water, warming gently if necessary. To this solution add the phenol and agitate or triturate until dissolved or dispersed. Gradually add this phase to the white petrolatum with trituration. A homogeneous emulsion ointment is readily formed.

Resorcinol, *o*-hydroxy diphenyl, phenol, *m*-hydroxy diphenyl, thymol, chlorcarvacrol, chlor-

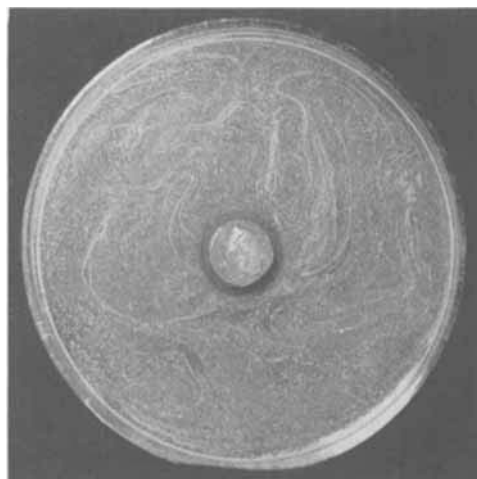


Fig. 4.—Product: Thymol Ointment with U. S. P. Phenol Ointment Base.  
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.  
Method: Agar Cup.  
Period of Incubation: 48 hours at 37° C.  
Width of Zone: 3.0 mm.

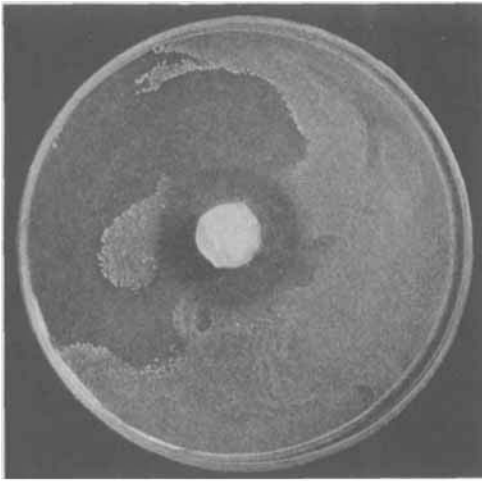


Fig. 5.—Product: Thymol Ointment with Proposed Emulsion Ointment Base.  
 Test Organism: *Staphylococcus aureus* of 24-hour broth culture.  
 Method: Agar Cup.  
 Period of Incubation: 48 hours at 37° C.  
 Width of Zone: 7.0 mm.

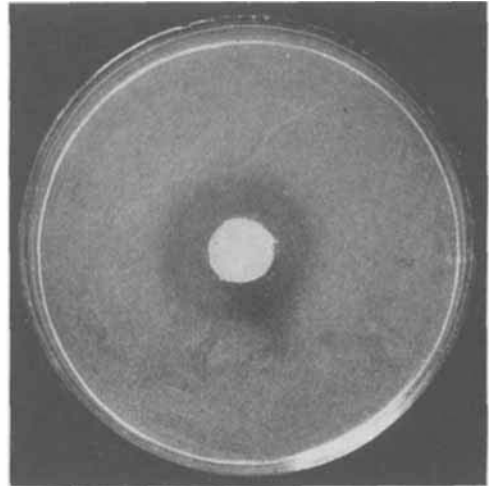


Fig. 7.—Product: Chlorthymol Ointment with Proposed Emulsion Ointment Base.  
 Test Organism: *Staphylococcus aureus* of 24-hour broth culture.  
 Method: Agar Cup.  
 Period of Incubation: 48 hours at 37° C.  
 Width of Zone: 8.0 mm.

thymol and hexylresorcinol were all completely soluble; 3-chlor-4-hydroxy diphenyl was partially soluble; trinitrophenol, *p*-hydroxy diphenyl and betanaphthol were sparingly soluble in the aqueous phase.

As a matter of comparison twelve ointments were prepared with the proposed emulsion ointment base containing the phenols and phenolic derivatives mentioned in Table III. Their bactericidal potency was determined by the Standard F. D. A. Method. Table IV shows the results.

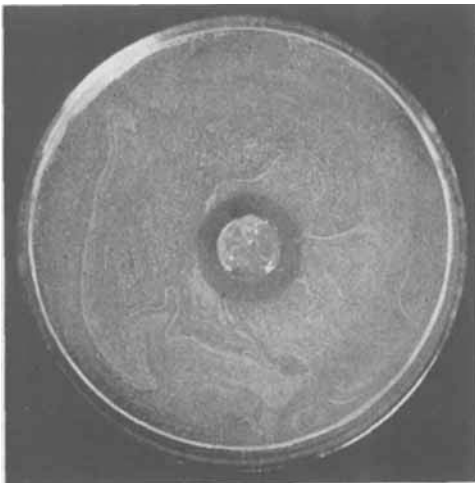


Fig. 6.—Product: Chlorthymol Ointment with U. S. P. Phenol Ointment Base.  
 Test Organism: *Staphylococcus aureus* of 24-hour broth culture.  
 Method: Agar Cup.  
 Period of Incubation: 48 hours at 37° C.  
 Width of Zone: 4.5 mm.

Table IV.—Bactericidal Potency of Phenolic Ointments with Proposed Emulsion Ointment Base

Ointment	Per Cent	p <sub>H</sub>	Zone, Mm.
Hexylresorcinol	2	4.8	12.0
Chlorthymol	2	5.1	8.0
Chlorcarvacrol	2	4.6	7.0
Thymol	2	5.0	7.0
<i>m</i> -Hydroxy diphenyl	2	4.9	7.0
Phenol	2	4.6	6.0
Betanaphthol	2	4.8	6.0
3-Chlor-4-hydroxy diphenyl	2	4.8	3.0
<i>o</i> -Hydroxy diphenyl	2	5.2	4.0
Resorcinol	2	4.9	3.0
<i>p</i> -Hydroxy diphenyl	2	5.2	2.5
Trinitrophenol	2	Acid	1.0

*p<sub>H</sub> Indices.*—Many antiseptics are activated by acid media. Goedrich (21) and Bittenbender, Degering and Tetrault (22) have shown that antiseptics are more potent when they are in a solution with a *p<sub>H</sub>* index below seven. They have established definite relationships between *p<sub>H</sub>* indices and bactericidal potency.

Three phenol ointments with emulsion ointment bases were prepared, the external phase of which was oleaginous and the internal aqueous. Each ointment contained 2% phenol. The aqueous phase of one was made acid with benzoic acid to a *p<sub>H</sub>* of 3. The aqueous phase of another was left with its natural acidity, a *p<sub>H</sub>* of 4.6; the aqueous phase of the third was made alkaline with sodium borate, to a *p<sub>H</sub>* of 8. Table V shows the relative potency of these three ointments.

Table V.—Bactericidal Potency of Phenol Ointments with Adjusted *p<sub>H</sub>*

Ointment, %	p <sub>H</sub>	Zone, Mm.
Phenol 2	3.0	2.0
Phenol 2	4.6	No zone
Phenol 2	8.0	No zone

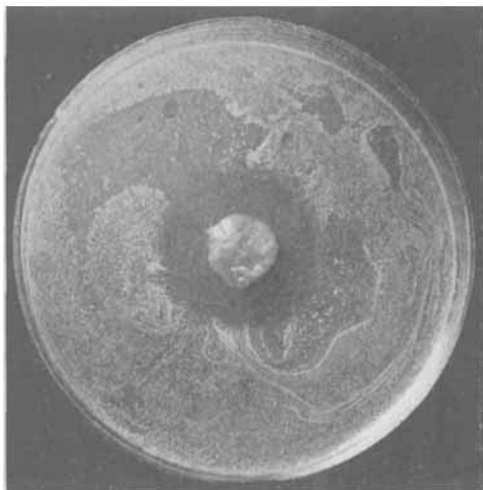


Fig. 8.—Product: Hexylresorcinol Ointment with U. S. P. Phenol Ointment Base.  
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.  
Method: Agar Cup.  
Period of Incubation: 48 hours at 37° C.  
Width of Zone: 9.0 mm.

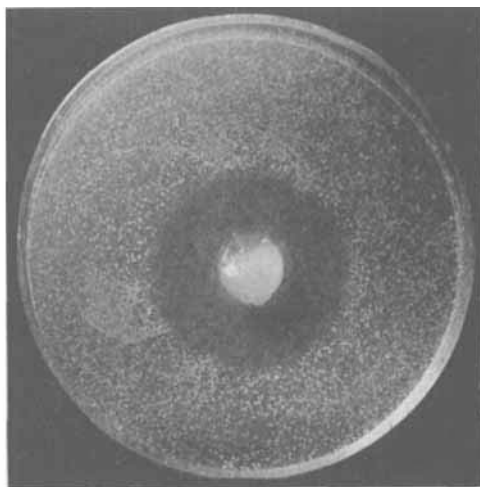


Fig. 9.—Product: Hexylresorcinol Ointment with Proposed Emulsion Ointment Base.  
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.  
Method: Agar Cup.  
Period of Incubation: 48 hours at 37° C.  
Width of Zone: 12.0 mm.

The aqueous phases of all the phenols and phenolic derivatives employed in the ointments given in Table IV were acidic in themselves. Their  $pH$  indices are given.

*Addition of Waxes to Ointment Bases.*—The proposed emulsion ointment base contains no waxes. Its consistency is such that waxes are not required. Incidentally, the addition of waxes to ointments exerts an inhibitory effect on their bactericidal potency. Several waxes including hydrogenated castor oil (Opal Wax), paraffin, white wax, yellow wax, spermaceti and carnauba wax were investigated.

Three ointments of chlorthymol in a base of white petrolatum proved this inhibitory effect clearly. By varying the amounts of white wax and spermaceti in the ointment base, the zones of inhibition varied inversely with the amount of waxes present. Table VI gives the percentages of waxes in these bases and their antiseptic potencies.

Table VI.—Bactericidal Potency of Ointments with Wax Added to the Base

Base, %	Chlor-thymol, %	Zone, Mm.
White petrolatum, 90		
White wax, 5	2	1.5
Spermaceti, 5		
White petrolatum, 95		
White wax, 2.5	2	5.0
Spermaceti, 2.5		
White petrolatum, 98		
White wax, 1	2	7.5
Spermaceti, 1		

Previously Husa and Radin (8) and Clark (11) had observed the deleterious effect of waxes. It now becomes apparent how directly and extensively they influence bactericidal properties of ointments.

Figures 2 to 9 show the difference in bactericidal potency of ointments made with U. S. P. Phenol Ointment base and the proposed emulsion ointment base. The width of inhibited zone, measured in millimeters, denotes the antiseptic power of the ointment.

#### SUMMARY

Phenol Ointment has repeatedly been proved to be non-antiseptic when tested by the Official F. D. A. Method using *Staphylococcus aureus*. This fact has again been confirmed.

Ointments of hexylresorcinol, chlorcarvacrol, *m*-hydroxy diphenyl, chlorthymol, thymol, 3-chlor-4-hydroxy diphenyl, *o*-hydroxy diphenyl and betanaphthol show varying antiseptic potency in the U. S. P. Phenol Ointment base.

Like phenol, *p*-hydroxy diphenyl, resorcinol and trinitrophenol show no antiseptic potency in the U. S. P. Phenol Ointment base.

The twelve phenols and phenolic derivatives all show substantial antiseptic potency in the proposed emulsion ointment base.

Waxes in ointment bases are deleterious by reducing the bactericidal potency of the ointment.

Phenol Ointment U. S. P. XI is devoid of antiseptic potency while the proposed oint-

ment produces a clear zone of inhibition 6 mm. wide.

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## Establishment of the Drug Laboratory in the Bureau of Chemistry, United States Department of Agriculture\*

By Lyman F. Kebler†

At various times, it has been suggested that the establishment and the early work of the Drug Laboratory of the Bureau of Chemistry of the U. S. Department of Agriculture ought to be written up and that I, being the only living person who is in possession of the necessary information, should write the story. This I have consistently hesitated to do heretofore for the simple reason that it necessarily brings me prominently into the picture. Of late I have, however, decided to put aside my personal feeling in the matter and write up the founding of this laboratory, including some of its early work, some of the prior activities of Congress and the lack of action on the part of Government officials in the field of pure and safe drugs for the suffering sick.

The first Congress of the United States in the second tariff act (1) included "Medicinal Drugs," among the imported articles to pay duty. Drugs used for dyeing were not included under this head. It also provided for the inspection and testing of all kinds of wines. The alcohol content was the chief factor to be considered. Determining the strength of alcohol was included in the first tariff act and all later general tariff acts. During the third session of the same Congress, an extensive tariff law was passed (2) containing 62 sections. Drugs as heretofore were included. A large proportion of the drugs in the early years of our country were imported. Cosmetics and perfumes were covered in the 1794 tariff act. General tariff laws were passed at irregular intervals by more than a score of Congresses. But, excepting the alcoholics, there was apparently a dearth of activity as to the character

\* Read by Author in condensed form before Section on History of Pharmacy, Atlanta meeting, 1939.

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