0.5 PER CENT EPHEDRINE SULFATE

Ephedrine sulfate	0.5 Gm.
Potassium phosphate monobasic	0.5 Gm.
Sodium phosphate dibasic	0.5 Gm.
Potassium chloride	0.15 Gm.
Sodium chloride	0.15 Gm.
Dextrose, anhydrous	0.9969 Gm.
Preserved water, a sufficient quantity, to make	100.00 cc.

If a one per cent solution of ephedrine sulfate is desired, the amount of dextrose in the above formula is reduced to 0.7867 Gm., and for a two per cent solution the dextrose is reduced to 0.3663 Gm.

The quantity for each component of the above formula must be weighed accurately on an analytical balance and made up to the indicated volume in a volumetric flask in order to obtain the desired tonicity and p_H values. In addition the chemicals used must be of the highest purity. The chemicals employed by the authors were all Merck's Reagent brand.

SUMMARY

1. Several improvements in the manufacture of nasal solutions have been developed. These include the substitution of water for the oily base and the adjustment of the osmotic pressure to approximate that of the blood.

2. Specifications for an ideal vasoconstrictive nasal preparation are advanced.

3. The method of manufacture, the calculations involved and the formula for such an ideal nasal preparation are submitted.

4. It is recommended that the formulas submitted for one-half, one and two per cent ephedrine sulfate intranasal solutions be included in the next edition of the National Formulary.

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"Read not to contradict and confute; nor to believe and take for granted; nor to find talk and discourse; but to weigh and consider".—Sir Francis Bacon

The Fungistatic Value of Certain Ointments*,†

By Francis J. O'Brien and William J. Bonisteel‡

Numerous bacteriological investigations have been made for the purpose of determining the fungicidal value of various substances in solution form. As a result of some of these investigations (1) the conclusion has been reached that there is a close relationship between the bactericidal and the fungicidal action of most substances but that there are exceptions to this rule. Copper salts, for example, are good bactericides but poor fungicides (2, 3). Thymol, oil of cinnamon and oil of clove are much more efficient as fungicides than as bactericides (4).

Other investigations have shown that a substance having a high bactericidal value in solution form may have little or no value when used in the form of an ointment or that its value may depend upon the base into which it has been incorporated (5, 6). Phenol, for example, although highly bactericidal in solution form, loses this value completely when incorporated into a base of petrolatum, but retains it when incorporated into a vanishing cream base.

The primary purpose of this investigation was to determine the fungistatic value of some of the official ointments and certain other non-official ointments prepared from substances which have known fungicidal value in solution form. The secondary purpose was to determine whether the base used in the preparation of the ointment had any effect on the fungistatic value.

The official ointments of iodine, ammoniated mercury, yellow mercuric oxide, phenol, sulfur and compound benzoic acid, and non-official ointments made from thymol, oil of cinnamon and chlorthymol were tested for fungistatic value.

EXPERIMENTAL

Method Used.—Since there is no standard in the literature for testing the fungistatic property of

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† An abstract of a thesis submitted to the Biology Department of Fordham University by Francis J. O'Brien in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ointments, the F. D. A. agar plate method was tried using two organisms frequently found in fungous infections, *Monilia albicans* and *Trichophyton interdigitale*. Both of these organisms were obtained from the American Type Culture Collection of Georgetown University, Washington, D. C. They were designated as *Endomycopsis albicans* A. T. C. C. 2091 and *Epidermophyton interdigitale* A. T. C. C. 4808. In the beginning, *Microsporum audouini*, strain M 31, was also used but was found to be the most sensitive of the three organisms to the action of the ointments and the tests with this organism were therefore discontinued.

Although the directions of the F. D. A. method were followed as closely as possible, the results varied so widely that certain modifications were deemed necessary. After a number of tests with *Monilia albicans* as the test organism, the following modifications were found necessary.

1. A definite quantity of ointment should be used in the test when comparative values are desired, since the width of the clear zone usually increased as the amount of ointment used was increased.

2. The ointment should be spread over a definite area of media. It was found that the same amount of ointment gave a greater zone of inhibition when the area of media covered was increased.

3. The ointment should be spread evenly. Uneven spreading of the ointment caused variations in the width of the clear zone, the greater width usually being in that part nearest to the greater thickness of ointment.

4. Since fungi grow more slowly than bacteria accurate readings could be made only after a period of about two weeks' incubation.

5. Incubation carried on at room temperature should not be allowed to vary more than 2° C. during the first 48 hours.

With these factors in mind the following method was devised and used with satisfactory results in all of the tests:

The spores and hyphæ of a 20-day-old culture of the organism were washed off with 10 cc. of sterile saline solution. The mixture was transferred to a test-tube and thoroughly shaken for five minutes with a few glass beads to break up any clumps. It was then strained through three layers of gauze and 5 cc. of the strained mixture added to 400 cc. of Sabouraud's maltose agar previously melted and cooled to about 45° C. Fifteen cc. of the inoculated agar were poured into a sterile petri dish and allowed to harden. Exactly 0.5 Gm. of the ointment to be tested was spread as evenly as possible with a glass rod over the surface of a circular cover glass having a diameter of 18 mm. After applying the ointment on the cover glass to the media, the cover glass was pressed down gently with a glass rod in order to spread the ointment as evenly as possible under but not beyond the cover glass. (Stiff ointments were warmed slightly before applying to secure better surface contact.) An incubation period of 14 days at room temperature was allowed. The result of the test was based on the width of the clear zone surrounding the ointment at the end of this period.

Determination of the Fungistatic Effect of Ointments.—Using the method previously described, five separate tests were made on each ointment during a period of three months. *Monilia albicans* and *Trichophyton interdigitale* were used as the test organisms. All ointments were freshly prepared at the beginning of the series of tests.

Table I shows the average results of the five tests made on each ointment with each organism.

Table I.—Fungistatic Effect of Certain Ointments

	Organism Used	
	Monilia albicans	*Trichophyton interdigitale*
Ointment Used	Clear Zone in Mm.	
Benzoic acid compound	7	10
Yellow mercuric oxide	0	0
Yellow mercuric oxide (double strength)	0	1
Iodine	1	5
Ammoniated Mercury	0	2
Sulfur	0	1
Phenol	0	0
Oil of cinnamon 2%*	11	No growth
Thymol 5%*	12	No growth
Thymol 5% and oil of cinnamon 2%*	12	No growth

* The base of these ointments was composed of petrolatum 90 parts, wool fat 5 parts and yellow wax 5 parts.

Discussion of the Results.—The ointments containing either 5 per cent of thymol or 2 per cent of oil of cinnamon were found to have a higher fungistatic value than any of the other ointments tested. Of the official ointments, benzoic acid compound was found to be the most effective. Ointments of yellow mercuric oxide and ammoniated mercury had no fungistatic value against *Monilia albicans* and had but slight value against *Trichophyton interdigitale*. Iodine was fairly effective against the latter organism but had little effectiveness against the former. It should be noted here that Reddish and Wales (7) and later Husa and Radin (8) found all three of these ointments to be highly bacteriostatic.

Phenol, although highly fungicidal in solution form, was found to have no inhibitory power in the form of the official ointment against either organism. Prout and Strickland (9) have reported that phenol ointment is without bacteriostatic value.

Figures 1, 2 and 3 show the photographic results of some of the tests.

Effect of Ointment Base on the Fungistatic Value of Certain Ointments Against Monilia Albicans.—In order to determine whether the base used had any effect on their fungistatic value, ointments of oil of cinnamon, thymol, chlorthymol and phenol were made with cold cream, vanishing cream, and petrolatum, wax and lanolin bases, and each ointment was tested against *Monilia albicans*. Thus each

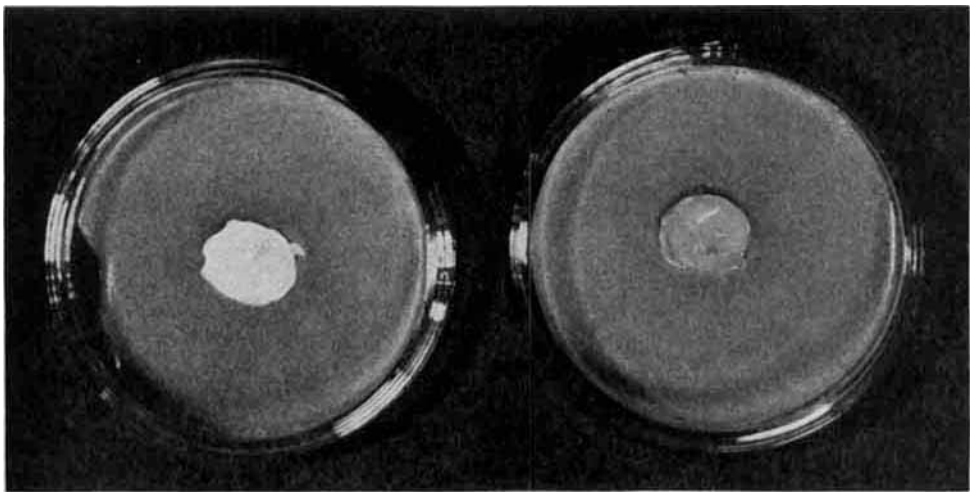

Fig. 1.—Ointment of Ammoniated Mercury (Left) and Ointment of Yellow Mercuric Oxide (Right) Showing no Fungistatic Property against *Monilia albicans*.

ointment was tested in three different bases which were prepared in the usual manner from the following formulas.

COLD CREAM

White wax	25.0 Gm.
Ceresin	12.5 Gm.
Mineral oil	120.0 cc.
Borax	1.2 Gm.
Water	50.0 cc.

VANISHING CREAM

Stearic acid	50.0 Gm.
Triethanolamine	2.0 cc.
Wool fat	5.0 Gm.
Glycerin	15.0 cc.
Water	130.0 cc.

PETROLATUM BASE

Yellow wax	5.0 Gm.
Wool fat	5.0 Gm.
Petrolatum	90.0 Gm.

In the preliminary tests, *Trichophyton interdigitale* was also used as a test organism. It was, however, so sensitive to thymol, oil of cinnamon and dichlorothymol that no growth appeared on any of the plates and comparison was impossible.

A test was made to determine whether the growth of *Trichophyton interdigitale* could be inhibited by the vapors only of the ointments of thymol, oil of cinnamon or chlorthymol. Maltose agar slants were inoculated with the organism and 1 Gm. of the ointment to be tested was placed on the tip of the cotton plug. The plug was then inserted into the

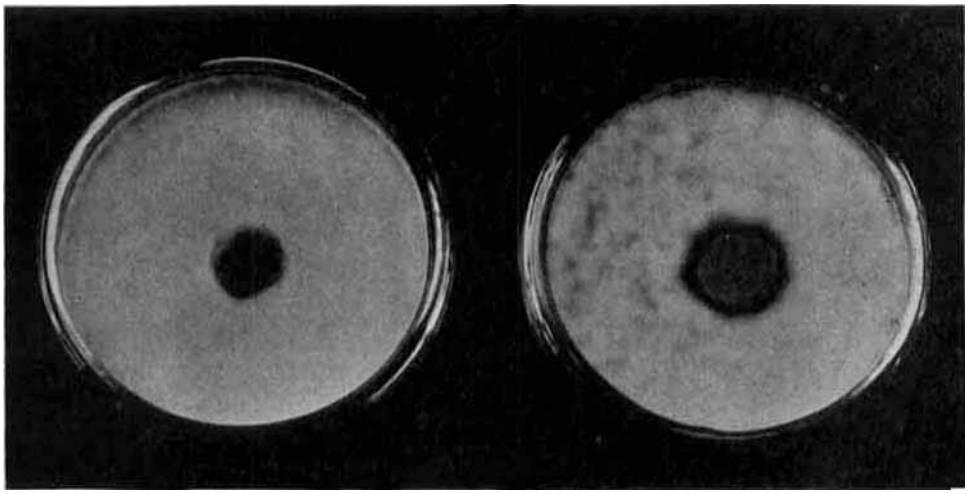

Fig. 2.—Ointment of Phenol (Left) Showing No Fungistatic Property against *Trichophyton interdigitale* and Ointment of Yellow Mercuric Oxide, 2 Per Cent (Right) Showing but Slight Value against the Same Organism.

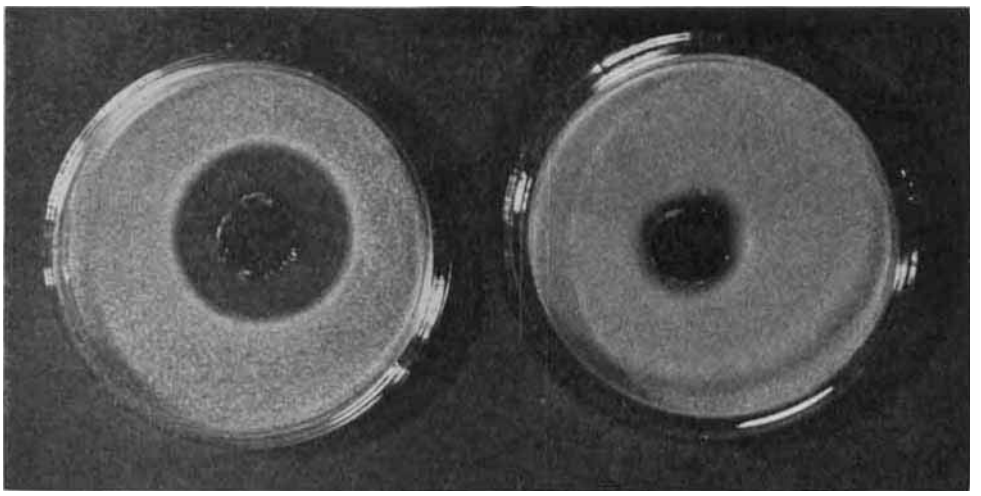

Fig. 3.—Iodine Ointment with 5 Per Cent of Thymol and 2 Per Cent of Oil of Cinnamon Added (Left) Showing Good Fungistatic Property against *Monilia albicans* and Plain Iodine Ointment (Right) Showing Only Slight Fungistatic Value against the Same Organism.

test-tube so that the ointment remained at least one inch away from the media. The tube was firmly corked, the cotton plug and the ointment being left in the tube. No growth was observed after 14 days of incubation at room temperature when ointments containing one per cent of oil of cinnamon or two and one-half per cent of thymol were used. Ointments made with two per cent of phenol or with two per cent of chlorthymol did not prevent growth.

In a similar test, *Monilia albicans* was not inhibited by any of the ointments.

Trichophyton interdigitale was therefore discontinued as a test organism and *Monilia albicans* which was much more resistant was used.

Five tests were made at different times with each ointment. In each test the ointments were identical except for the base used. Table II shows the variation in the width of the clear zone when the same ointment in a different base was tested against *Monilia albicans*.

Table II.—Fungistatic Value of Substances in Different Bases When Tested against *Monilia Albicans*

Substances Tested	Base Used: Cold Cream	Vanishing Cream	Petrolatum, Wax, and Wool Fat
	Clear Zone in Mm. (Average of 5 Tests)		
Cinnamon oil 2% Thymol 5%	16	20	12
Cinnamon oil 1% Thymol 2½%	14	16	9
Thymol 5%	15	17	12
Thymol 2½%	14	16	5
Cinnamon oil 2%	16	18	11
Cinnamon oil 1%	14	17	9
Chlorthymol 2%	4	10	3
Phenol 2%	4	5	0

Discussion of the Results.—In all of the ointments tested, the vanishing cream base permitted the greatest diffusion of the antiseptic as measured by the width of the clear zone around the ointment. Cold cream bases were only slightly inferior in this respect except in the case of chlorthymol ointment. The least diffusion was permitted by the base composed of petrolatum, wool fat and wax.

Ointments of oil of cinnamon or thymol were distinctly superior in fungistatic value to either ointments of chlorthymol or phenol. Phenol was without value in the petrolatum base, but was slightly effective in a base of either cold cream or vanishing cream.

The ointments containing 2½ per cent of thymol were just about as effective as those containing 5 per cent except in the petrolatum base. One per cent of oil of cinnamon was only slightly less effective than 2 per cent in any of the bases used.

It was observed that the growth of the organism outside of the clear zone was always less dense when ointments containing thymol were used than when any of the other ointments were used. This suggests the possibility that thymol may diffuse over a larger area than recorded by the clear zone in sufficient concentration to be slightly inhibitory.

None of the bases used had any inhibitory power in themselves, although cold cream did delay the growth of the organism for a period of about two days in a zone of 5-mm. width. It must therefore be concluded that vanishing cream and cold cream bases permitted a greater diffusion of the antiseptic into the media than did the petrolatum base.

Fungistatic Value of Some Commercial Ointments.—In order to check the relative efficiency of thymol and oil of cinnamon ointments with some of the commercial ointments used in treatment of fungus infections, a series of tests similar to those carried out previously was made. Table III shows the results of these tests in terms of the clear zone surrounding each ointment.

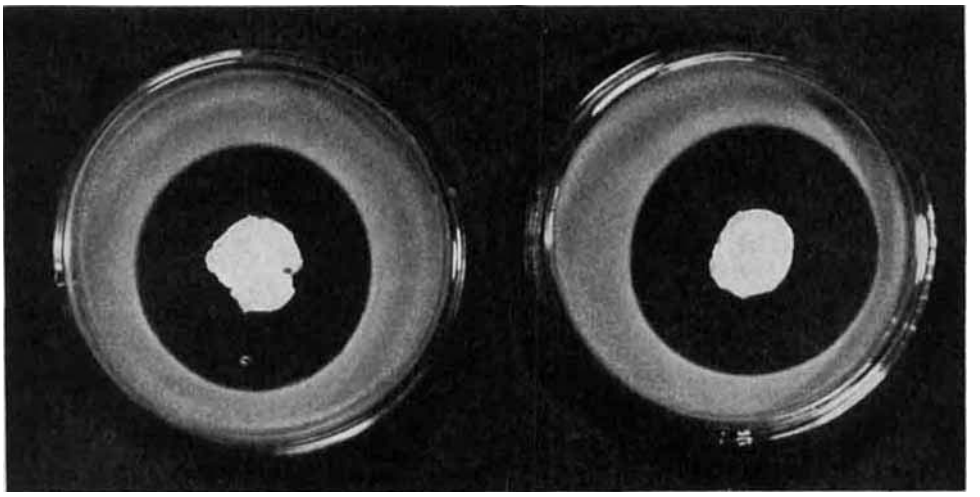

Fig. 4.—Showing Good Zones of Inhibition of *Monilia albicans* with a 2 Per Cent Ointment of Oil of Cinnamon in a Cold Cream Base (Left) and in Vanishing Cream (Right).

Table III.—Fungistatic Effect of Three Commercial Ointments

Ointment Used	Clear Zone in Mm. (Average of 5 Tests)
Merthiolate (1–2000)	11
Kerolysin	7
Fungi rex	7

Comparison with the preceding table shows that either a 2½ per cent thymol ointment or a 1 per cent oil of cinnamon ointment in either cold cream or vanishing cream bases is superior in fungistatic value to any of the commercial products tested when *Monilia albicans* is used as the test organism.

CONCLUSIONS

It is to be concluded that (1) a bacteriostatic ointment may or may not be fungistatic, (2) the fungicidal value of a substance in solution form is not an indication that it will have the same value or any value at all in ointment form, (3) the fungistatic effect of a substance in ointment form may depend upon the base into which it is incorporated.

In addition to the above general conclusions, it is also concluded that (1) the U. S. P. ointments of phenol, yellow mercuric oxide, ammoniated mercury, sulfur and iodine have little or no fungistatic effect when tested against *Monilia albicans* or *Trichophyton interdigitale*, (2) ointment of benzoic acid compound N. F. has decided

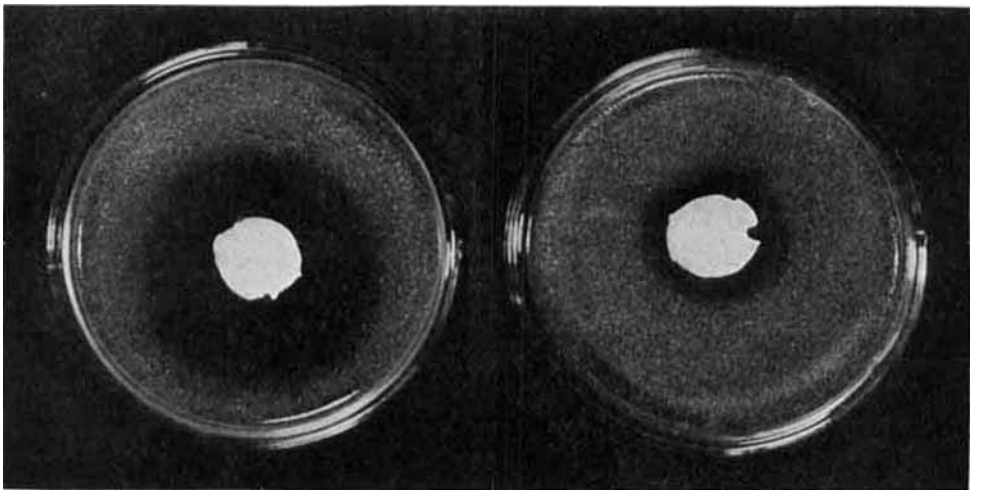

Fig. 5.—Showing the Wider Zone of Inhibition of *Monilia albicans* Produced by a 2½ Per Cent Thymol Ointment in a Base of Cold Cream (Left) than by a 2 Per Cent Chlorthymol Ointment in the Same Base (Right).

fungistatic property, (3) thymol and oil of cinnamon show excellent fungistatic action in any of the bases used but particularly in vanishing cream or cold cream, (4) ointments of thymol or oil of cinnamon or a combination of both in either cold cream or vanishing cream are superior to any of the ointments tested including three commercial ointments.

The favorable results of the *in vitro* reactions of thymol and oil of cinnamon seem to indicate that a clinical trial is warranted. The following specific formula and certain modifications of it are suggested.

Thymol	2.5 Gm.
Oil of cinnamon	1.0 cc.
Cold cream	
or	
Vanishing cream *q. s.*	100.0 Gm.

Cold cream should be used as the base when the soothing effect of an oily substance is desired or when the addition of salicylic acid is desired.

U. S. P. iodine ointment may be used as the base if the bacteristatic action of iodine is desired in addition to the fungistatic action of the thymol and oil of cinnamon.

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"To know things well, one must know them in detail, and as this is infinite, our knowledge is necessarily superficial."—La Rochefoucauld.

Ointments Prepared by Emulsification

Improvements and Advantages Gained; Choice of Emulsion Systems; Selection of Vehicles

*By A. J. Gibson, Ph.C., H. E. Parker, B.S.Pharm., and Anne Almus, A.B.**

For the purpose of this paper there are two basic methods of preparation of ointments regardless of the vehicles chosen in formulating the finished product. (1) They are those ointments made without water (non-emulsified) and (2) those made with water (emulsified). As will be shown, the second seems to offer the most practical approach to improvement in ointment compounding whether it be from the medical or pharmaceutical viewpoint. Recognition of the advantages of the emulsified type of ointment are becoming apparent in medical circles. Recently Fantus (1) pointed out that ointments are contraindicated in acute inflammatory conditions of the skin because of their "heating quality." He elaborates further and shows that the exception to this is in the use of cream ointments or emulsions. In fact, this clinician states that the success or failure of the treatment used may depend on the proper choice of the ointment base. Mumford (2), in discussing the role of emulsifying bases in dermatology, observes that the application of ordinary bases as petrolatum, lanolin or mixtures of these offers an objectionable barrier for the serous discharge of the skin. Contrasting this with the advantages of emulsions in overcoming this objection, he shows the superiority of emulsified ointments as carriers for both oil-soluble and water-soluble ingredients. Emphasis is placed on the lack of progress in prescribing ointments; in fact, Mumford goes so far as to state "progress in prescribing ointments has not proceeded far beyond the lanolin and vaseline of fifty years ago." Traub (3) calls attention to one of the prime reasons why patients do not follow instructions and quickly tire of the ointments prescribed—because

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