THE ANTISEPTIC VALUE OF PHENOL OINTMENTS.*,**

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INTRODUCTION.

For many years phenol has been considered a standard for the evaluation of antiseptics. Furthermore, various pharmaceutical preparations of phenol have been extensively used in medical practice. One of the standard preparations of phenol is Ointment of Phenol, U. S. P. X. It has generally been supposed that this ointment had an antiseptic action, but Reddish (1) has shown by laboratory tests that it has no antiseptic value.

It is known that the effect of ointments frequently is dependent to a large extent on the nature of the base. Hence, it was thought that by variations in the base it might be possible to produce a phenol ointment having definite antiseptic properties. The purpose of the present investigation was to make such a study of the effect of variations in the base on the antiseptic properties of phenol ointments.

HISTORICAL REVIEW.

As far back as 1881 Robert Koch (2) made the statement that phenol when dissolved in oil or alcohol does not exhibit any antiseptic properties. This statement was based on the following experiments: Sterile test-tubes were filled with various phenol solutions. Silk fibres impregnated with spores of *Bacillus anthracis* were immersed in the solutions and the test-tubes tightly stoppered. After definite periods the fibres were taken out with the aid of a platinum loop and transferred into gelatin, blood serum or bouillon broth, and the growth and development of the organism observed. Aqueous solutions of phenol of 1% and 2% strength were ineffective, as growth was observed even after immersion for 15 days and 7 days, respectively. Growth was completely inhibited by 3% phenol in 7 days, by 4% in 3 days and by 5% in 2 days. In similar tests using the bacilli themselves instead of spores no growth was observed even after immersion in 1% aqueous phenol solution for only 2 minutes, while the controls showed abundant colonies. Koch next tried 5% solutions of phenol in oil and in alcohol. The inefficiency of such oily or alcoholic solutions was shown by the fact that the spores were not killed in 110 days, and with the bacilli the growth was completely inhibited only after 6 days.

G. Wolfhügel and G. von Knorre (3) explained the behavior of phenol in oil on the basis of the difference in diffusibility of oily and aqueous solutions. A 5% solution of phenol in oil was brought into direct contact with water, and the amount of phenol which diffused into the water was determined by the Landolt and Koppen-

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schaar method (phenol is changed into tribromphenol and the excess of bromine is titrated). The amount of phenol which diffused into the water from the oil was negligible compared with that which went over from an aqueous solution into oil.

Gottstein (4), in 1889, experimented with 5% of phenol in a lanolin base. Small amounts of the ointment were inoculated with *Bacillus prodigiosus* (*Serratia marcescens*) and *Bacillus fluorescens liquifaciens* and a drop of the mixture transferred into nutrient gelatin, and the development of the organism observed. Growth was not inhibited. Gottstein made the general statement that oil-soluble antiseptics when incorporated in an ointment base do not possess any antiseptic properties, but that water-soluble antiseptics do not lose their antiseptic properties when incorporated in oily bases.

In 1895, E. Breslauer (5) carried out a series of experiments with the object of determining to what extent antiseptic substances, when incorporated in ointments, exert their germicidal action and whether the antiseptic action can be influenced by varying the bases. Cultures of the test organisms were grown on agar for two days at 22° C. The colonies were then transferred into 2 cc. of bouillon, vigorously shaken and filtered through glass wool. A number of glass platelets were then introduced into the bouillon suspension, removed after a short time and dried on a Petri dish. The dried platelets were next introduced into the ointment to be tested, and after a definite period of time they were taken out, washed with ether to remove the ointment, transferred into sterile bouillon tubes, and observed for growth of the organism. Ointments containing 5% of phenol in various bases were tested against *Bacillus prodigiosus* (*Serratia marcescens*) and the time after which growth was inhibited was as follows: oil, 3 days; vaseline, 90 minutes; fat, 45 minutes; lanolin (anhydrous), 20 minutes; lanolin, 5 minutes; cold cream, 5 minutes. The same ointments were tested against *Staphylococcus aureus* and the time after which growth was inhibited was: oil, growth continued: vaseline, 1 day; fat, 4 hours; lanolin (anhydrous), 2 hours; lanolin, 45 minutes; cold cream, 20 minutes. Breslauer concluded that lanolin and cold cream are the best bases for antiseptics.

During the World War, Cheyne (6) experimented with various antiseptics and antiseptic ointments with the hope of finding one that would prevent sepsis in wounds. As a result of his work the British adopted the plan of forwarding two packages to each man in the navy, one a paste of 20% phenol in a lanolin base (Cheyne recommended a base of 6 parts of lanolin and 1 part of white wax) and the other a powder of equal parts of boric and salicyclic acids. Keilty and Packer (7) adopted Cheyne's technique in testing antiseptics. Ointments spread on cover slips were placed in Petri dishes under agar slabs, the ointment being next to the agar. The upper surface of the slab was inoculated by gently stroking with two loopfuls of a bouillon suspension of bacteria, such as *Staphylococcus aureus*, *Streptococcus pyogenes* or *Bacillus coli*. After incubation for 24 hours, the plates were examined for growth and such points were observed as extent of growth, whether or not growth was directly over the ointment, how close the growth was to the margin of the ointment and the size and numbers of the colonies in relation to their distance from the ointment. In classifying antiseptics, Keilty and Packer rated 10 to 20% phenol as markedly active.

Another method involving the spreading of the ointment on an inoculated

agar medium is that devised by Reddish (8) for the Food, Drug and Insectide Administration, U. S. Dept. of Agriculture. In this test the culture of *Staphylococcus aureus* is thoroughly mixed with the melted nutrient agar, which is then poured into a sterile Petri dish and allowed to cool at room temperature. As soon as this inoculated agar has hardened, the ointment is spread over a small portion of the surface with a sterile glass rod and the plates are incubated for 24 or 48 hours. If an ointment has an antiseptic action there is a clear zone around the streak. Ointment of Phenol, U. S. P. X, did not show antiseptic properties by this test.

EXPERIMENTAL PART.

Method Used.—The Reddish method (8) was used, with some slight modifications. *Staphylococcus aureus* was grown at 37° C. in broth containing 0.5% Bacto beef extract, 0.1% Bacto peptone and 0.5% sodium chloride, the p_H being adjusted to 6.8. The culture was transferred into this medium for three consecutive days. The third transfer was used after 24 hours' incubation. One-tenth cc. of this culture was thoroughly mixed with 10 cc. of melted nutrient agar (composed of 3 Gm. Bacto beef extract, 5 Gm. Bacto peptone, 15 Gm. agar and 1000 cc. distilled water, and the p_H adjusted to 6.8) at 45° C., the mixture poured into a sterile Petri dish and allowed to solidify at room temperature. The ointment, warmed to 37° C., was spread over a small portion of the surface of the inoculated agar with a sterile glass rod, the plate inverted and incubated at 37° C. for 48 hours. A separate Petri dish was used for each individual test.

Antiseptic Value of Some U. S. P. and N. F. Ointments.—A number of U. S. P. X and N. F. V ointments were tested in duplicate to determine how the results would compare with those reported by Reddish (1) for the same ointments.

TABLE I. ANTISEPTIC ACTION OF SOME U. S. P. AND N. F. OINTMENTS.

Ointment.	Width of Zone in Mm. Test A.	Test B.	Reported by Reddish.
Ointment of Phenol	No zone	No zone	No zone
Ointment of Yellow Mercuric Oxide	6	6	4
Iodoform Ointment	No zone	No zone	No zone
Iodine Ointment	6	6	8
Chrysarobin Ointment	No zone	No zone	No zone
Ointment of Mild Mercurous Chloride	4	4	1.5
Ointment of Boric Acid	No zone	No zone	No zone
Sulphur Ointment	No zone	No zone	No zone
Calamine Ointment	3	3	2
Tar Ointment	3	3	3
Ointment of Zinc Oxide	No zone	No zone	No zone
Ointment of Ammoniated Mercury	8	8	5

Table I indicates that our results agreed closely with those reported by Reddish.

Variations in Method of Smearing.—The ointments listed in Table I were also tested by placing the ointment on the bottom of the dish and pouring the melted agar over it. The results were the same as by the other method except in the case of Ointment of Ammoniated Mercury, which when smeared on the bottom of the dish gave a zone that extended to the edge of the dish. Although Reddish smeared the ointments both above and below the agar, he did not report any difference in

the zones obtained by the two methods. In the present investigation it was felt that the method of smearing on the surface of the agar was more trustworthy and in all further experiments this method was the only one used.

It was thought that the amount of ointment used in the test might influence the width of the zone. Tests were carried out on this point by using varying amounts of ointment smeared on a definite area in the center of the dish.

TABLE II.—EFFECT OF VARYING THE AMOUNT OF OINTMENT USED.

Ointment of Yellow Mercuric Oxide.		Ointment of Ammoniated Mercury.		
			Width of zone in mm.	
Amount of ointment in Gm.	Width of zone in Mm. when smeared on 2 cm. square.	Amount of ointment in Gm.	On 2 cm. square.	On 3 cm. square.
0.25	6	0.25	7	7
0.50	7	0.45	8	8
0.75	8	0.65	8	8
1.00	8	0.75	9	9
1.15	9	0.95	9	9
		1.15	9	9

As indicated in Table II, the zone increased slightly in width with increasing amounts of ointment. From these results it was concluded that it would be best to smear a definite amount of ointment over a definite area. For the present work a weight of 0.5 Gm. on an area 2 cm. square was adopted. The area was marked on the under side of each dish with a colored wax pencil.

Varying Percentages of Phenol in Petrolatum.—Since Ointment of Phenol, U. S. P. X, containing 2% of phenol, showed no antiseptic properties, tests were carried out to determine what percentage of phenol was necessary to show antiseptic properties when incorporated in petrolatum. As indicated in Table VIII, when the proportion of phenol was 5% or less there was no zone. Ten per cent of phenol gave a 4-mm. zone and for higher percentages the width of the zone increased with increasing percentages of phenol.

Effect of Water in Phenol Ointments.—Breslauer (5) reported that the addition of water to a base increased its value as an antiseptic carrier. For this reason it was thought desirable to determine the effect of incorporating water in phenol ointments.

According to St. Onge (9) petrolatum is capable of absorbing 10% of water. A number of ointments containing 10% of water and various percentages of phenol and petrolatum were prepared by fusion on a water-bath. The mixtures were uniform while hot, but on cooling there was a separation into solid and liquid portions. Due to the separation no tests of antiseptic power were made.

Since phenol ointments containing 10% of water could not be prepared using petrolatum, similar ointments containing 5% of phenol were prepared in which approximately one-fourth of the petrolatum was replaced by anhydrous lanolin. As a control on the effect of the water a 5% phenol ointment in which the base consisted of 25% anhydrous lanolin and 75% petrolatum was prepared. This base, without phenol, was also tested as a control on any possible antiseptic action of the base; this base alone gave no zone.

The results in Table III indicate that a 6-mm. zone was obtained in the test of 5% phenol ointment in which the percentage composition of the base was 75% petrolatum and 25% anhydrous lanolin. This result was rather surprising in view

of the fact that it had previously been proven that an ointment containing 5% of phenol in petrolatum gave no zone. The addition of 5% and 10% of water, respectively, resulted in a zone of 7 mm., or practically the same as that obtained in absence of water. Therefore, it was apparent that the presence of small proportions of water was not of particular importance, but that the addition of anhydrous lanolin to the ointment was the essential factor in producing an ointment showing antiseptic properties.

TABLE III.—EFFECT OF WATER IN OINTMENTS CONTAINING 5% PHENOL IN BASES OF VARIED COMPOSITION.

Composition of Base. Lanolin anhydrous, %.	Petrolatum, %.	Water, %.	Width of Zone. Sample A, mm.	Sample B, mm.
25	75	0	6	6
22.5	72.5	5	7	7
20	70	10	7	7

Variations in Proportion of Anhydrous Lanolin and Petrolatum.—Tests were next conducted on 5% phenol ointments in which the proportions of anhydrous lanolin and petrolatum were varied over wide limits.

TABLE IV.—EFFECT OF 5% PHENOL IN BASES CONSISTING OF MIXTURES OF PETROLATUM AND ANHYDROUS LANOLIN IN VARIOUS PROPORTIONS.

Composition of Base. Lanolin anhydrous, %.	Petrolatum, %.	Width of Zone.	Composition of Base. Lanolin anhydrous, %.	Petrolatum, %.	Width of Zone.
100	0	No zone	20	80	No zone
75	25	No zone	17	83	No zone
50	50	No zone	15	85	No zone
29	71	No zone	13	87	No zone
27	73	No zone	10	90	No zone
26	74	No zone	9	91	No zone
25.5	74.5	Entire plate clear	8	92	No zone
25	75	6 mm.	7	93	No zone
24.5	75.5	Entire plate clear	5	95	No zone
24	76	Entire plate clear	4.5	95.5	No zone
23	77	Entire plate clear	4	96	No zone
22	78	No zone	3.5	96.5	No zone
			3	97	3 mm.
			2.5	97.5	No zone
			2	98	No zone
			1	99	No zone

The results in Table IV confirmed the idea that the relative proportion of anhydrous lanolin and petrolatum was a deciding factor in the antiseptic action of the ointment. When the percentage of anhydrous lanolin in the base was between 23 and 25.5% very good zones were obtained. With a base containing 3% of anhydrous lanolin a small zone resulted but when the percentage was increased to 3.5% or reduced to 2.5% there was no zone. In the case of all other proportions tested there was no evidence of antiseptic action.

Further Variations in Technique.—Up to this point the glass rods used in smearing the ointments were sterilized in a flame just before using. In some experiments on the effect of variations in temperature at which the media were solidified and at which the ointments were smeared, it appeared that the temperature of the rod might be a distributing factor. For this reason, in all further tests, the

rods were sterilized in an autoclave at 15 lbs. pressure for 40 minutes and allowed to cool to room temperature before use. In all further work the ointments were not warmed to 37° C. before smearing, but were smeared at room temperature. A final change in technique was the adoption of a circular area equivalent in area to the 2-cm. square previously used. This was done because smearing on a circular zone was easier and since the antiseptic zone was circular, the smearing of a circular area facilitated the measurements of the zone.

Further Test of Variations in Proportions of Anhydrous Lanolin and Petrolatum.—Another set of experiments was carried out with 5% of phenol in bases consisting of mixtures of anhydrous lanolin and petrolatum.

TABLE V.—EFFECT OF 5% PHENOL IN BASES CONSISTING OF MIXTURES OF PETROLATUM AND ANHYDROUS LANOLIN IN VARIOUS PROPORTIONS.

Composition of Base. Lanolin anhydrous, %.	Petrolatum, %.	Width of Zone.	Composition of Base. Lanolin anhydrous, %.	Petrolatum, %.	Width of Zone.
20	80	5 mm.	25	75	7 mm.
20.5	79.5	6 mm.	25.5	74.5	6 mm.
21	79	7 mm.	26	74	7 mm.
21.5	78.5	8 mm.	26.5	73.5	2 mm.
22	78	4 mm.	27	73	No zone
22.5	77.5	8 mm.	27.5	72.5	No zone
23	77	Entire plate clear	28	72	No zone
23.5	76.5	Entire plate clear	28.5	71.5	No zone
24	76	Entire plate clear	29	71	No zone
24.5	75.5	7 mm.	29.5	70.5	No zone
			30	70	No zone

Table V shows that with percentages of anhydrous lanolin running from 20 to 26.5% good zones were obtained, the most favorable percentage of anhydrous lanolin being between 23 and 24%.

Similar tests were made with ointments containing 2% of phenol, thus corresponding in phenol content to the U. S. P. X Ointment of Phenol.

TABLE VI.—EFFECT OF 2% PHENOL IN BASES CONSISTING OF MIXTURES OF PETROLATUM AND ANHYDROUS LANOLIN IN VARIOUS PROPORTIONS.

Composition of Base. Lanolin anhydrous, %.	Petrolatum, %.	Width of Zone.	Composition of Base. Lanolin anhydrous, %.	Petrolatum, %.	Width of Zone.
20	80	No zone	25.5	74.5	5 mm.
20.5	79.5	No zone	26	74	No zone
21	79	No zone	26.5	73.5	No zone
21.5	78.5	2 mm.	27	73	No zone
22	78	No zone	27.5	72.5	No zone
22.5	77.5	3 mm.	28	72	No zone
23	77	2.5 mm.	28.5	71.5	No zone
23.5	76.5	No zone	29	71	No zone
24	76	2.5 mm.	29.5	70.5	No zone
24.5	75.5	3 mm.	30	70	No zone
25	75	5 mm.			

As shown in Table VI, with a 2% concentration of phenol, the widest zones were obtained with bases containing 25% and 25.5% of anhydrous lanolin, al-

Since Ointment of Phenol, U. S. P. X, contains 5% of yellow wax, the effect of this ingredient on the antiseptic action was considered. A series of ointments was prepared containing 2% of phenol and 5% of yellow wax with the remainder consisting of various proportions of anhydrous lanolin and petrolatum.

TABLE VII.—2% OF PHENOL IN BASES CONSISTING OF MIXTURES OF YELLOW WAX, ANHYDROUS LANOLIN AND PETROLATUM IN VARIOUS PROPORTIONS.

Composition of Ointment.				
Phenol, %.	Yellow wax, %.	Lanolin anhydrous, %.	Petrolatum, %.	Width of Zone.
2	5	18.6	74.4	No zone
2	5	19.065	73.935	No zone
2	5	19.530	73.470	No zone
2	5	19.995	73.005	No zone
2	5	20.460	72.540	No zone
2	5	20.925	72.075	No zone
2	5	21.390	71.610	No zone
2	5	21.855	71.145	No zone
2	5	22.320	70.680	No zone
2	5	22.785	70.215	No zone
2	5	23.250	69.750	No zone
2	5	23.715	69.285	No zone
2	5	24.180	68.820	No zone
2	5	24.645	68.355	No zone
2	5	25.110	67.890	No zone
2	5	25.575	67.425	No zone
2	5	26.040	66.960	No zone
2	5	26.505	66.495	No zone
2	5	26.970	66.030	No zone
2	5	27.435	65.565	No zone
2	5	27.900	65.100	No zone

The results in Table VII indicate that none of the ointments containing yellow wax showed any antiseptic properties. Repetition of the experiment led to the same results, thus showing definitely that incorporation of yellow wax, in ointments of this composition, reduces the antiseptic properties to zero on the basis of the Reddish test.

Relative Value of Various Bases.—The next set of experiments was carried out to determine the relative value of different bases as antiseptic carriers. The following percentages of phenol were incorporated in each base by fusion: 1, 2, 5, 10, 15, 20, 25 and 50%. Petrolatum and benzoinated lard were miscible with as much as 25% of phenol but when 50% of phenol was used in each of these two bases some of it crystallized out on standing. Anhydrous lanolin was miscible with 15% of phenol but the higher percentages crystallized out. Hydrous lanolin produced homogeneous ointments with 1, 2 and 5% of phenol, but with percentages of 10% and higher the ointment separated into two layers, a solid layer and a liquid layer. Aquaphor was miscible with all proportions of phenol mentioned above. In all cases only the ointments showing no crystallization or separation were tested for antiseptic properties.

As shown in Table VIII, hydrous lanolin, Aquaphor and benzoinated lard showed antiseptic properties with lower concentrations of phenol than were required to give a zone in the case of petrolatum and anhydrous lanolin. It is worth noting that none of the individual ointment bases gave a zone with 2% of phenol,

whereas certain mixtures of petrolatum and anhydrous lanolin gave good zones with 2% of phenol.

TABLE VIII.—EFFECT OF VARYING PERCENTAGES OF PHENOL IN INDIVIDUAL OINTMENT BASES.

Phenol, %	Lanolin Anhydrous.	Lanolin Hydrous.	Width of Zone in Mm. Aquaphor.	Petrolatum.	Benzoinated Lard.
1	No zone	No zone	No zone	No zone	No zone
2	No zone	No zone	No zone	No zone	No zone
5	No zone	3	2.5	No zone	4.5
10	5	*	6	4	7.5
15	8	*	6.5	5	10
20	*	*	8	5	12
25	*	*	9	8	13
50	*	*	9.5	*	*

* Not miscible in this proportion.

Theoretical Aspects.—The theoretical aspects of the action of antiseptic ointments appear to be as follows. In order for an ointment to exert an antiseptic effect it is necessary that the antiseptic substance shall leave the base and enter the underlying tissues which may harbor pathogenic bacteria. In the case where the ointment and the culture medium or tissues are immiscible, the distribution of the antiseptic substance between the ointment base and the culture medium or tissues would be in accordance with the Van't Hoff partition law, which states that, if the solvents are immiscible, there is a constant ratio of concentration of the solute in the two solvents as long as the molecular weight of the solute is the same in both. Thus, in the case of the Reddish test using phenol ointments the distribution of phenol between the ointment base and the agar medium at equilibrium would be proportional to the partition coefficient. Accordingly, if the ointment base were changed in composition in such a way that it became a poorer solvent for phenol, the partition coefficient would be altered in a direction favorable to increased antiseptic action. Another factor that might enter is the rate at which equilibrium is approached, *i. e.*, the rate of diffusion of phenol from the ointment into the medium. For example, certain concentrations of cholesterol or similar substances in lanolin or other bases might increase the rate of diffusion. In order for the antiseptic action to be apparent in the Reddish test it is necessary that the phenol reach a sufficient concentration to prevent growth within the length of time necessary for colonies to develop. A complete test of the above hypothesis has not yet been made, but it is presented here as a tentative explanation of the results.

Practical Applications.—Although the U. S. P. directs that Ointment of Phenol shall contain 2% of phenol, higher concentrations are sometimes used, for example, the British Pharmacopœia Ointment of Phenol contains 3% of phenol. It has also been reported (7) that the British Navy used phenol ointments of 20% strength. However, reports have been made (10) that the continuous use of concentrations of phenol of 5% or higher causes gangrene. For this reason the development in the present investigation of a 2% phenol ointment showing definite antiseptic properties is of interest in making available the antiseptic action of phenol at a low concentration, thus avoiding any possible bad effects of a higher concentration.

The following formula is recommended for inclusion in the U. S. P. XI:

OINTMENT OF PHENOL.

Phenol	2.0 Gm.
Wool Fat	24.5 Gm.
Petrolatum	73.5 Gm.
To make	100.0 Gm.

Melt the phenol on a water-bath, add the petrolatum, stir until the mixture is uniform, then add the wool fat. Stir, and allow the mixture to congeal.

SUMMARY.

1. A study was made of the effect of variations in the base on the antiseptic value of phenol ointments as measured by the Reddish test.

2. Tests made on 5% phenol ointments containing water, anhydrous lanolin and petrolatum showed that the presence of small proportions of water is unimportant, but that the relative proportion of anhydrous lanolin and petrolatum is the deciding factor in determining the antiseptic properties.

3. In 5% phenol ointments with a base of petrolatum and anhydrous lanolin good zones were obtained when the proportion of anhydrous lanolin in the base was between 23% and 25.5%. In similar 2% phenol ointments 25% and 25.5% of anhydrous lanolin gave the best results. In such ointments, the incorporation of 5% of yellow wax reduced the antiseptic value to zero.

4. Tests with individual bases showed that hydrous lanolin, Aquaphor and benzoinated lard gave antiseptic ointments with lower percentages of phenol than in the case of anhydrous lanolin or petrolatum.

5. An ointment of the following composition is recommended for inclusion in the U. S. P. XI: Phenol, 2 Gm.; Wool Fat, 24.5 Gm.; Petrolatum, 73.5 Gm. This ointment shows definite antiseptic properties in the Reddish test, although the U. S. P. X Ointment of Phenol shows no antiseptic value by the same test.

REFERENCES.

(1) G. F. Reddish and H. Wales, JOUR. A. PH. A., 18 (1929), 576–578.
(2) R. Koch, *Mitt. Kaiser. Gesundheit.*, 1 (1881), 234–282.
(3) G. Wolfhügel and G. von Knorre, *Ibid.*, 1 (1881), 635.
(4) A. Gottstein, *Therap. Monatsh.*, 3 (1889), 698.
(5) E. Breslauer, *Ztschr. f. Hyg.*, 20 (1895), 165.
(6) W. W. Cheyne, *Lancet*, London, Feb. 27, 1915; cited by reference (7).
(7) R. A. Keilty and J. E. Packer, *J. Amer. Med. Assn.*, 64 (1915), 2123.
(8) G. F. Reddish, JOUR. A. PH. A., 16 (1927), 654; see also reference (1).
(9) A. St. Onge, Merck's Rept., 6 (1897), 601.
(10) Sollman, "Action of Drugs," 2nd Edition, page 129.

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Aqua Sterilisata is a new official of the British Pharmacopœia, 1932. The sterilization is by autoclave or by boiling for thirty minutes. Storage in suitably closed vessels is limited to one month.