A three-hour distillation period is sufficient for most drugs.

The method can be used for determining moisture in some drugs containing a volatile constituent.

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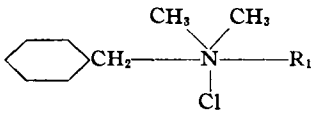
ANTISEPTIC PROPERTIES OF ALKYL-DIMETHYL-BENZYL-AMMONIUM CHLORIDE.*

BY PAUL G. HEINEMAN.[1]

INTRODUCTION.

The preparation investigated is of special interest because it is related to a group of chemical compounds, whose germicidal properties are apparently little understood. The chemical structure of the preparation is



(1)

It represents a mixture of high molecular alkyl-dimethyl-benzyl-ammonium chlorides, in which the high molecular alkyl residue (R_1) is composed of the alkyl radicals C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$ and $C_{18}H_{37}$. The source of these radicals is the mixture of fatty acids of coconut oil in original proportion. Inasmuch as these fatty acids in coconut oil occur in constant proportion, this relationship holds in the final product resulting in a preparation of uniform composition.

The product is freely soluble in water, forming a clear, almost colorless solution. It is soluble also in acetone and in alcohol, but insoluble in ether and only slightly soluble in benzol. The aqueous solution is slightly alkaline to litmus. It possesses an aromatic odor and foams like soap on shaking. Aqueous solutions have an acrid taste, which disappears as dilutions increase.

Studies of antiseptic properties of the ammonium chloride compound have been carried on for a period of more than two years. The following standard procedures were applied:

1 Phenol coefficient determinations.
2 Inhibition and penetration tests.
3 Tests for disinfection of the human skin.
4 Destruction of spores of bacteria and fungi.

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Results of this investigation will be grouped under the aforementioned four headings.

1. PHENOL COEFFICIENT DETERMINATIONS.

The method of the Food and Drug Administration (2) was followed with one slight modification. Tests for bacteriostasis according to official directions were made by carrying over a small amount of the first subculture to a tube containing ten cc. of F. D. A. broth. In this work, the second subculture was incubated for seven days at 37° C. because prolonged contact of bacteria with an antiseptic tends to retard growth. Exhaustive tests for bacteriostasis appear to be imperative to avoid excessively high coefficient figures. Numerous additional tests were made by adding one-half cubic centimeter of sterile horse serum to five cubic centimeters of antiseptic, before adding the culture, in order to test its potency in the presence of organic matter.

Phenol controls were made with each test and when the resistance of the organism did not meet requirements the tests were disregarded. Such conditions were minimized by preparing daily cultures of the test organism.

Presence of bacteriostasis was determined by preparing subcultures of all negative cases, which approached the concentration used for calculation of coefficients. Separate tests were made at 20° C. and at 37° C.

The following organisms were used: *Eberthella typhi*, Hopkins strain; *Staphylococcus aureus*, Government strain 209; *Streptococcus pyogenes;* and *Escherichia Coli*. The first two types were obtained from the bacteriological laboratories of the Food and Drug Administration, while the other two types were isolated in our laboratories. In the following table the number of tests made are recorded and average figures appear in Column 5.

SUMMARY OF PHENOL COEFFICIENTS OF ALKYL-DIMETHYL-BENZYL-AMMONIUM CHLORIDE.

Organism.	Temperature of Medication.	Organic Matter.	Number of Tests.	Average Coefficient.
		None	12	271
		Serum	6	72
Eberthella typhi	37° C.	None	7	176
	20° C.	Serum	5	51
		None	13	293
Staphylococcus aureus	37° C.	Serum	6	154
		None	9	275
	20° C.	Serum	5	104
Streptococcus pyogenes	37° C.	None	7	272
		Serum	5	129
Escherichia coli	37° C.	None	3	318

The figures stated in Column 5 of the table show that coefficients are reduced by addition of 0.5 cc. horse serum to 5 cc. of antiseptic. The figures further show that the efficacy of the compound increases with the temperature of medication.

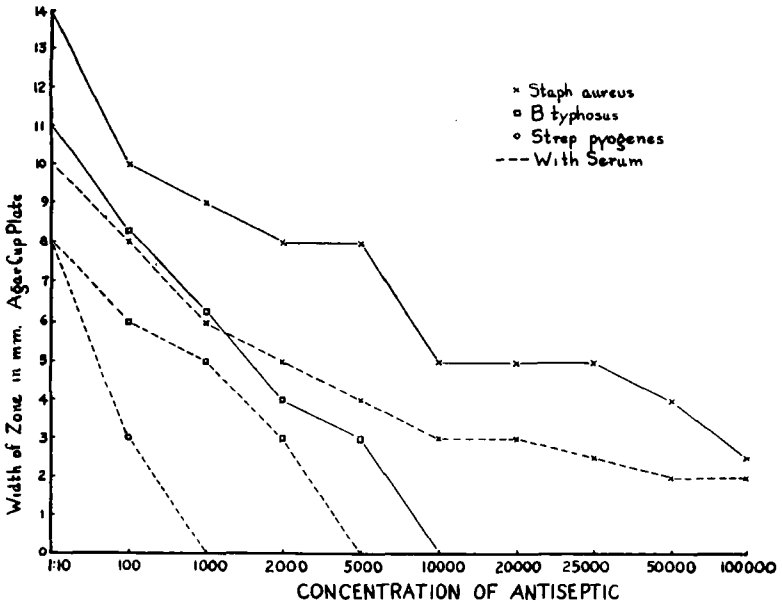
Tests were also made with a ten per cent solution of the compound in 95 per cent ethyl alcohol. Concentrations required for carrying out the tests were made by using distilled water as diluent, in accordance with recognized phenol coefficient technique. Therefore the amount of ethyl alcohol contained in dilutions was reduced to a negligible quantity. The temperature of medication was 20° C.

The average of three tests with *Eberthella typhi* was 176 and for three tests with *Staphylococcus aureus* was 275. Both tests virtually agreed with the results obtained, when the aqueous solution was used. It should be stated that a number of the determinations which appear in the table were made with the same stock solution. This solution was tested within two weeks of preparation. Later tests were made with the same solution after 3, 5, 9 and 14 months. Inasmuch as the results showed no decrease in coefficient the assumption appears justified that the aqueous solution remains stable for at least fourteen months.

2. PENETRATION AND INHIBITION TESTS.

Penetration and inhibition tests were made according to the Agar Cup-Plate method of the Food and Drug Administration. A sterilized rubber stopper of

The chart shows the following facts:



Inhibitory effect of the ammonium chloride compound in decreasing concentrations on the test organisms.

suitable diameter was placed in the center of a Petri dish before pouring the agar. The stopper is removed readily after the agar has solidified in the Petri dish, and by this means the chance of contamination from the air is reduced.

A large number of such tests was made with cultures of *Eberthella typhi*, *Staphylococcus aureus* and *pyogenes*. In all cases duplicate plates were prepared, one with the addition of ten per cent horse serum, the other without serum.

The most comprehensive series will be given in some detail.

Ten solutions of the ammonium chloride compound were prepared in distilled water, *i. e.*, 1 in 10, 1 in 100, 1 in 1000, 1 in 2000, 1 in 5000, 1 in 10,000, 1 in 20,000, 1 in 25,000, 1 in 50,000 and 1 in 100,000. One plate of each concentration was prepared with plain agar and the other plate with plain agar with the addition of ten per cent horse serum. For *Streptococcus pyogenes* serum agar only was used, because it was not possible to produce abundant growth on plain agar

consistently. Accompanying chart shows the results graphically, abscissæ showing the concentration of the solutions and ordinates giving the widths of the clear zones in mm.

The largest zones were formed by *Staphylococcus aureus*, with *Eberthella* as a close second, and *Streptococcus pyogenes* had the smallest zone. In both *Eberthella typhi* and *Staphylococcus aureus* the zones were consistently smaller in serum agar than in plain agar. *Staphylococcus aureus* was inhibited in a concentration of 1:100,000 both in plain agar and in serum agar, although the latter inhibited in lesser degree than the former. *Eberthella typhi* was not inhibited in a concentration of 1:10,000, but was inhibited in a concentration of 1:5000. The limit of inhibition is therefore somewhere between the two concentrations. *Streptococcus pyogenes* was inhibited in a concentration of 1:100, but not in 1:1000.

A five per cent solution of phenol was prepared and tested as control. For *Eberthella typhi* it was 11 m./m. in plain agar and 10 m./m. in serum agar. For *Staphylococcus aureus* it was 9 m./m. in plain agar and 7 m./m. in serum agar. For *Streptococcus pyogenes* it was 5 m./m. in serum agar.

The Agar Cup-Plate method was applied to test inhibitory action of a solution of the ammonium chloride compound on the spores of *Trichophyton gypseum*. A seven-day culture on Sabouraud's honey agar was scraped off and suspended in a sterilized bottle containing physiologic sodium chloride solution and some glass beads. The mixture was shaken in a machine designed for preparing bacterial vaccines. The suspension was strained through sterile cheese cloth and the number of spores adjusted to 10,000 per cubic millimeter, using a Hausser counter to determine the number of spores. From this spore suspension agar cup plates were prepared with Sabouraud's honey agar, both plain agar and serum agar. In plain agar a large clear zone of 20 m./m. width developed, while in serum agar the width of the clear zone was 14 m./m. These tests illustrate the inhibitory effect of the solution.

Efficacy of the ammonium chloride compound in oil was determined by the official Agar Cup-Plate method both with serum and without serum, using *Staphylococcus aureus* as test organism. The following solutions were prepared:

1. 0.05 Gm. of the compound in 50 cc. cotton seed oil. (0.1%.)
   A clear zone of 5 m./m. width appeared on plain agar, and a zone of 3 m./m. on serum agar.
2. 0.1 Gm. of the compound was dissolved in 100 cc. of mineral oil. (0.1%.)
   A clear zone of $5\frac{1}{2}$ m./m. width formed in plain agar, and a zone of 3 m./m. in serum agar.
3. 0.1 Gm. of the compound in a mixture of 50 cc. each of mineral oil and cotton seed oil. (0.1%.)
   A clear zone of 4 m./m. width was formed in plain agar, and a zone of 3 m./m. in serum agar.
4. 0.1 Gm. of the compound in a mixture of 5 Gm. lanolin and 95 cc. of mineral oil. (0.1%.)
   A clear zone of 4 m./m. width formed in plain agar and a zone of 3 m./m. in serum agar.

Clear zones, which appear in agar cup plates, show the ability of the solution to penetrate agar jelly. The clear zones may be caused either by inhibition of growth or by actual destruction of the organism. In the series of tests, which has been described, small amounts of the clear agar were transferred to tubes contain-

ing ten cubic centimeters of F. D. A. broth, but in all cases growth failed to appear. Therefore the organisms were actually destroyed. In phenol coefficient tests, aqueous solutions are used and penetration is readily accomplished. In agar cup-plate tests the solution meets with some resistance from the agar jelly, and when serum is added resistance increases. Solutions in oil naturally meet with greater resistance than do aqueous solutions. It is clear, therefore, that concentrations in agar cup-plate tests do not necessarily yield results comparable with those obtained in phenol coefficient tests.

In high concentrations of the ammonium chloride compound an opaque zone appeared at the edge of the central cup and this opaque zone was surrounded by a clear zone. After planting small pieces of the opaque zone in tubes containing broth, and incubating these tubes, no growth appeared, showing that the organisms were actually destroyed. Therefore, the opacity was probably the result of precipitation of agar.

If results obtained in agar cup-plate tests with *Eberthella typhi*, for example, are compared with those of phenol coefficient tests with the same organism, it is found that there was no clear zone around a concentration of 1:10,000, while in phenol coefficient tests the organism was destroyed in ten minutes in a concentration of 1:40,000. The difference is really greater than these figures indicate because in the agar cup plate, destruction may continue for the incubation period of 48 hours, while in the phenol coefficient test the time limit was ten minutes.

3. SKIN DISINFECTION TESTS.

Skin disinfection tests were made with two solutions each containing ten per cent of the dry compound using 95% ethyl alcohol for one of the solutions and distilled water for the other. A total of four tests was made on two individuals.

The hands were scrubbed for eight minutes with a brush, using hot water and soap, after which they were dried with a sterilized towel. The fingers were then immersed for five minutes in the test solution and were washed in sterile water. Agar was liquefied and, after cooling to 45° C., was poured over the fingers and collected in sterilized petri dishes. This part of the work was carried out in a booth used for bacteriological work.

The plates were incubated at 37° C. and observations recorded after one, two and five days. In order to detect small colonies the plates were examined under a microscope as well as with a hand lens.

No colonies could be detected.

A more sensitive method for making skin disinfection tests is described by Allen, Moorhead and Edgerley (3).

The same individuals submitted to this test and the same solutions were used as stated above. The technique described by the authors was rigidly followed. The inoculated broth tubes and the agar plates were incubated at 37° C. for five days. At the termination of the period of incubation the broth tubes and agar plates showed no growth.

It might be stated that neither of the test subjects showed immediate or deferred evidence of skin irritation by application of a ten per cent solution of the ammonium chloride compound. Similar skin disinfection tests should be made with lower concentrations of the compound.

4. DESTRUCTION OF SPORES OF BACTERIA AND MOLDS.

A series of tests was made with the object of determining the destructive effect of a solution of the ammonium chloride compound on spores of *Bacillus subtilis*, *Trichophyton gypseum* and a *Mucor*, which was isolated from milk. *Bacillus subtilis* was grown in broth for three days. There was abundant growth, and microscopic examination showed the presence of profuse spore forma-

tion. One four-millimeter loopful of the well-shaken culture was placed in five cc. of various concentrations of the compound. From the mixture one four-millimeter loopful was transferred to tubes containing ten cc. of F. D. A. broth, at certain time intervals. These tubes were incubated for seven days at 37° C.

The molds were grown on Sabouraud's honey agar for seven days at room temperature. The growth was scraped off and shaken in physiological sodium chloride solution in a shaking machine. The number of spores was adjusted to 10,000 per cmm. From these spore suspensions one-tenth of a cc. was introduced into tubes containing ten cc. of the test solution. Finally, after definite time intervals, one four-millimeter loopful was planted in tubes containing ten cc. of Sabouraud's honey broth. The inoculated honey broth tubes were incubated at room temperature in a dark place for thirty days. Controls were prepared with phenol solutions and bacteriostasis was checked in the usual manner. No transfers were made in less than five-minute intervals.

The results showed that in a concentration of one in 500 (distilled water) the ammonium chloride compound destroyed the spores of *B. subtilis* and those of the two types of fungi in 48 hours. The spores of *Trichophyton gypseum* were destroyed by phenol solution 1:120 in five minutes, while the spores of *Mucor* required 48 hours to show evidence of destruction by phenol 1:120. Spores of *B. subtilis* were not destroyed by 5% phenol solution in 24 hours.

SUMMARY AND CONCLUSIONS.

A new chemical compound, alkyl-dimethyl-benzyl-ammonium chloride, has been studied to determine its antiseptic and bacteriostatic properties. The work has been carried out according to methods which are practiced in the laboratories of the Food and Drug Administration. In addition, some methods for which no standard has been adopted, have been applied in order to extend the work. Results may be summarized as follows:

1. Phenol coefficient tests carried out according to the method of the Food and Drug Administration at a medication temperature of 37° C. and 20° C. gave the following results: For *Eberthella typhi* the average was 271 at 37° C., and 176 at 20° C. With addition of serum the figures were 72 and 51, respectively. For *Staphylococcus aureus* at 37° C. the coefficient was 293, and 275 at 20° C. With addition of serum the figures were 154 and 104, respectively. With our strain of *Streptococcus pyogenes* the coefficient at 37° C. was 272 and with serum 129. Three tests with *Escherichia coli* at 37° C. yielded an average coefficient of 318.

The reduction of coefficient values at the lower temperature of medication and in the presence of organic matter is clearly shown and is in accord with similar results, which were obtained with other antiseptic agents. However, even the lower figures prove a high germicidal value. These differences are less in proportion when *Staphylococcus aureus* is the test organism than when *Eberthella typhi* is used.

2. Solutions in 95% ethyl alcohol yielded virtually the same results as were obtained with aqueous solutions. Inasmuch as the method for determining phenol coefficients demands the use of distilled water for preparing the required concentrations, the alcohol content is reduced to a negligible quantity. In high concentrations of alcoholic solutions the effect of the alcohol may prove to be appreciable.

3. A concentration of 1:100,000 still retains inhibitory potency for *Staphylococcus aureus* as shown by the Agar Cup-Plate method. Some inhibition is also apparent in the presence of horse serum in the same concentration. For *Eberthella*

typhi the limit of inhibition is somewhat less than 1:10,000 without serum and somewhat less than 1:5000 with serum.

4. Spores of *Trichophyton gypseum were readily inhibited, which fact was shown in* agar cup-plate tests by the formation of a clear zone of 20 m./m. width without serum and a zone of 14 m./m. when ten per cent serum was added.

5. Solutions in various mixtures of oils, using the spores of *Trichophyton gypseum*, gave clear zones of 4 and 5 mm. width according to the kinds of oil used. This fact demonstrates that solutions in oil may penetrate and inhibit growth of microörganisms. It should be noted in this connection that in agar cup-plate tests the size of the area of inhibition depends not only on activity of the antiseptic, but also on its ability to penetrate the agar jelly.

6. Spores of *Bacillus subtilis* were destroyed in five minutes or less in a concentration of 1 Gm. of the ammonium chloride compound in 500 cc. of aqueous solution.

7. Spores of two types of fungi were destroyed in five minutes or less by the same concentration 1:500.

8. A limited number of tests for disinfecting the human skin demonstrated that ten per cent solution in distilled water or in ethyl alcohol actually destroyed microbial life with no immediate or deferred irritation of the skin observed. Manifestly, this work should be extended in order to determine the exact concentration required to yield satisfactory results.

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p_H STUDIES OF MILK OF MAGNESIA WITH THE GLASS ELECTRODE.

BY J. A. C. BOWLES* AND E. C. MERRILL.[1]

It has been known for some time that Milk of Magnesia develops an unpleasant taste after standing in ordinary glass bottles for several months. This was thought to be due to the Milk of Magnesia attacking the silicates of the glass causing an increase in hydroxyl ions. Billheimer and Nitardy (1) found that the addition of a small amount of citric acid to Milk of Magnesia yielded a buffer of sufficient capacity to prevent, for a considerable time at least, any objectionable increase in hydroxyl ions. In their investigation they used only Milk of Magnesia made (private communication) by the double decomposition method.

The purpose of this work was (1) to study the change in p_H of Milk of Magnesia made by the direct hydration and double decomposition methods on standing in ordinary glass bottles; (2) to determine the p_H's of Milk of Magnesia made by

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