A STUDY OF THE ANTISEPTIC PROPERTIES OF AMMONIATED MERCURY IN BENTONITE BASES GREASY BASES AND MIXTURES OF BENTONITE AND THE GREASY BASES

By

ELDON FRED HILL

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INTRODUCTION TO EXPERIMENTAL

Myers used the following procedure in his experimental work and obtained the following results:

> "In order to determine the antiseptic properties of medicaments in these new ointment bases, antiseptic tests were run as follows. An agar culture was inoculated with Staphlococcus Aureus (obtained from the U. of W. Agricultural Bacteriology Department). This culture was then transferred to a petri dish. After solidifying the culture was allowed to grow for 48 hours at 37° C. and at the end of this time three holes were made in the infected agar with a sterile cork borer. Into these holes was placed 10% Ammoniated Mercury ointment in the different base e.i. one in the official U.S.P. base, and one in 20% Bentonite.
>
> The results were as follows:

Medicinal Substance	Test organism Staphlococcus Aureus Zone of inhibition in mm. width
10% Ammoniated Mercury U.S.P. XI	1.0 mm.
10% Ammoniated Mercury in 20% Bentonite	NONE

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1 Myers.

INTRODUCTION

The object of this thesis is to present experimental data on the antiseptic properties of Ammoniated Mercury in bentonite bases, greasy bases, and mixtures of gentonite and the greasy bases.

The work was carried out to investigate the conclusions of Myers[1] in his thesis, in which he stated that: "Bentonite is not satisfactory as an ointment base for Ammoniated Mercury. The antiseptic and bactericidal properties of Ammoniated Mercury are reduced when incorporated in bentonite."

For the history, physical and chemical properties of bentonite, and characteristics of bentonite ointment base the reader is referred to the thesis of Myers.[2]

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1 Myers, D. J., "A Study of the Antiseptic Properties of Ammoniated Mercury in Bentonite and other Hydrophillic Bases", Thesis submitted for Bachelor of Science (Pharmacy) in 1940.

2 Ibid.

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The bentonite base when exposed to warm air will dry rapidly and as it dries it hardens and shrinks in volume. The base employed in the Official 10% Ammoniated Mercury will become soft in warm air. In the above procedure there is a tendency for the 20% bentonite base to harden and shrink in size causing the Ammoniated Merucry to lose contact with the agar. This fact prevents diffusion of the Ammoniated Mercury into the infected agar, and eliminates the possibility of the Ammoniated Mercury establishing a zone of inhibition. Conversely the Official Ammoniated Mercury ointment softens and establishes better contact with the agar. Therefore the following technique was employed.

Cultures of Staphlococcus Aureus and Escherichia Coli were obtained from the Unitersity of Wisconsin Agricultural Bacteriology Department. Transfers were made from these cultures to a media which was favorable for their growth.

This media was prepared by dissolving 8 gms. of Bacto Nutrient Broth in 1000 cc. of distilled water, and then sterilizing this solution (in 10 cc. quantities in test tubes with non-absorbent cotton plugs) in an autoclave.

The bacteria were allowed to incubate for 24 hours at 37° C., and then 0.1 cc. transfers were made to sterile petri dishes.

These sterile petri dishes had a definite amount of the ointment being tested, placed in the center of the dish. The amount and shape of the ointment was always the same in every

test. This was done by spreading the ointment on a pill tile and using a cork borer to cut out the sample. This circular sample was then put in the petri dish with a sterilized platinum wire.

The media used in the petri dishes for the further growth of the organisms was made by dissolving 10 gms. of Bacto Agar in a solution of 4 gms. of Bacto Nutrient Broth in 500 cc. of distilled water. This was placed in 8 oz. prescription bottles in 100 cc. quantities and sterilized in an autoclave.

This nutrient agar in approximately 15 cc. amounts and at a temperature of 45° C. was added to the petri dishes containing 0.1 cc. of the culture plus the ointment. Before this agar hardened the dishes were tipped slightly to assure an even mixture of the bacteria throughout the added agar.

Approximately 10 minutes was allowed for the agar to harden and then the dishes were inverted and incubated at 37° C. in an oven for 24 hours. At the end of this period the dishes were examined and the average zone of inhibition was noted. The distance, in mms., was measured from the edge of the ointment to the place in the nutrient agar where the bacteria were growing.

EXPERIMENTAL

Ammoniated Mercury was selected as the antiseptic agent to be used because of its effectiveness and also because there is an Official Ammoniated Mercury Ointment which gave a means of comparison between a recognized and a new product.

Rx 1. Ammoniated Mercury Ointment U.S.P. XI

Ammoniated Mercury	10 gms.
Wool fat	5
White wax	5
White petrolatum	80
	100

Melt the white wax in a suitable dish on a water bath, add the wool fat and the white petrolatum, and heat the mixture gently until it is liquified. Gradually add the warm liquid to the Ammoniated Mercury contained in a warm mortar, triturating thoroughly and stir the mixture until it congeals.

This gives a white greasy ointment which is soft and pliable.

For a comparison, an ointment made of 20% Bentonite and having the same percentage of Ammoniated Mercury (10%) was compounded.

Rx. 2. 10% Ammoniated Mercury in a 20% Bentonite base.

Ammoniated Mercury	3 gms.
Bentonite Base (20%)	27
	30

Moisten the Ammoniated Mercury with water to get a paste and then incorporate the Bentonite Base.

A 20% Bentonite Base was made according to the following formula.

Bentonite	20 gms.
Water	80 ccs.
	100 gms.

Heat the water and add the bentonite with constant stirring. Then rub on a pill tile with a large spatula until smooth.

The ointment is a smooth mass which will spread well on the skin leaving a light film that adheres when dry.

TABLE I

Comparison of the antiseptic properties of Official Ammoniated Mercury Ointment and a 10% Ammoniated Mercury in a 20% Bentonite Base

Medicinal Substance	Zone of inhibition in mm. S. Aureus	E.Coli
Rx 1		
10% Ammoniated Mercury	6	3
U.S.P. XI	6	3
	5	3.5
	6	3.5
Rx 2		
10% Ammoniated Mercury	12	5
Bentonite Base (20%)	10	5
	9	5.5
	11.5	5

The above table shows rather conclusively that Ammoniated Mercury exhibits greater diffusing properties in the Bentonite

Base than in the Official Ointment, as the zone of inhibition with the Bentonite Base is almost twice that of the zone created by the Official Ointment.

Although the Ammoniated Mercury was much more effective in the Bentonite Base, this preparation was not satisfactory pharmaceutically because of the tendency for the water to evaporate and a hard crust to form on the surface.

Various substances were incorporated into the Bentonite Base in order to retard the evaporation of water. The formulas of which are as follows:

Rx 3.

Ammoniated Mercury	1 gm.
Anhydrous Lanolin	4
Bentonite Base (13%)	5
	10

Rx 4.

Ammoniated Mercury	1 gm.
White Petrolatum	4
Bentonite Base (13%)	5
	10

Rx 5.

Ammoniated Mercury	1 gm.
Aquafor	4
Bentonite Base (13%)	5
	10

Make a paste of the Ammoniated Mercury with water and incorporate the Bentonite Base. Then add the greasy base and rub with a large spatula on the pill tile until the misxture is smooth and uniform.

The 13% Bentonite Base was compounded in the following manner.

Bentonite	6.5 gm.
Water	43.5 cc.
	50.0 gm.

Heat the water and add the bentonite with constant stirring. Then rub on a pill tile with a large spatula until smooth.

A 13% Bentonite Base was used because it was not quite as stiff as the 20% Bentonite Base, cheaper, more easily compounded, and theoretically would be stable longer than the 20% Bentonite Base since it could lose up to 7% of water before reaching the consistency of the 20% base.

These ointments when applied to the skin rub in well, are cooling, adhesive, smooth and do not leave a greasy film. Rx 5 more closely resembles the consistency of the Official Ammoniated Mercury Ointment and Rx 3 is of a harder consistency than the others.

TABLE II

A comparison of results of the antiseptic action of 10% Ammoniated Mercury when incorporated in the following vehicles or bases.

Medicinal Substance	Zone of inhibition in mm. Staph. Aureus	Escher. Coli
Rx 3		
10% Ammoniated Mercury	8	7.5
40% Anhydrous Lanolin	8.8	5.5
50% Bentonite Base (13%)	9	5.5
	8	6
	8.5	5
Rx 4		
10% Ammoniated Mercury	9.5	6
40% White petrolatum	10.5	6.5
50% Bentonite Base (13%)	10	7
	10	6.5
	9	8
Rx 5		
10% Ammoniated Mercury	11	10
40% Aquafor	14	10
50% Bentonite Base (13%)	13	9.5
	12	10
	11	9.5

When the above results were compared with those obtained from a 10% Ammoniated Mercury in a 20% Bentonite Base (Rx 2, Table I), it was observed that the 10% Ammoniated Mercury in a mixed base of Aquafor and the 13% Bentonite Base gave a larger zone of inhibition.

Therefore an ointment consisting of 10% Ammoniated Mercury in a 13% Bentonite Base was prepared.

Rx 6.

Ammoniated Mercury	1 gm.
Bentonite Base (13%)	9
	10 gms.

Make a paste of the Ammoniated Mercury with water and incorporate the Bentonite Base.

This percentage of Bentonite gave an ointment that was easily applied to the skin, did not rub off, but was easily washed off. In a closed ointment jar it was stable for more than three weeks.

TABLE III

The results of the antiseptic action of 10% Ammoniated Mercury incorporated in a 13% Bentonite Base

Medicinal Substance	Zone of inhibition in mm. Staph. Aureus	Escher. Coli
Rx 6		
10% Ammoniated Mercury	13	10
Bentonite Base (13%)	13.5	11
	14.5	9.5
	14	10

The above data seems to indicate that this ointment possesses greater bacteriostatic properties than the Ammoniated Mercury in the 20% Bentonite Base (see Rx 2., Table I). This also explains why a larger zone of inhibition was gotten from the ointment of 10% Ammoniated Mercury in a mixed base of aquafor and the 13% Bentonite Base (Table II), than from the ointment of 10% Ammoniated Mercury in a 20% Bentonite Base (Table I).

The work thus far had shown that the 13% Bentonite Base made the most effective base and that the greasy base (Official

Ammoniated Mercury Ointment) was the most stable. Then a successful attempt was made (see prescriptions 3, 4, and 5, Table II) to combine these two bases and get a combination of properties, i.e., a stable ointment which had an antiseptic action almost as great as that realized with the Bentonite Base.

It was also observed from the results obtained in prescriptions 3, 4 and 5 (See Table II) that the bases of anhydrous lanolin, white petrolatum and aquafor with the 13% Bentonite Base had shown greater effectiveness in that order. From the preceding data it seemed reasonable to assume that if ointments were made using them as bases that: 1. The zones of inhibition should be less than those gotten from a base consisting of a mixture of each with a bentonite base, and, 2. They should give ascending effectiveness in the same order, i.e., anhydrous lanolin, white petrotatum and aquafor respectively.

Therefore the following ointments were made:

Rx 7.
Ammoniated Mercury 1 gm.
Anhydrous Lanolin 9
10

Rx 8.
Ammoniated Mercury 1 gm.
White Petrolatum 9
10

Rx 9.
Ammoniated Mercury 1 gm.
Aquafor 9
10 gm.

Make a paste of the Ammoniated Mercury with water and incorpoate the base. Rub to smoothness on a pill tile with a large spatula.

TABLE IV

A comparison of the results of the antiseptic properties of 10% Ammoniated Mercury in Anhydrous Lanolin, White Petrolatum and Aquafor respectively

Medicinal Substance	Zone of inhibition in mm. Staph. Aureus	Escher. Coli
Rx 7	6	5
10% Ammoniated Mercury	7	4.5
in Anhydrous Lanolin	7	5.5
Rx 8	8.5	6
10% Ammoniated Mercury	8	5.5
in White Petrolatum	8.5	6
Rx 9	10	7
10% Ammoniated Mercury	9.5	6.5
in Aquafor	11	8

When these results are compared with those presented in Table II we find greater zones of inhibition from the 10% Ammoniated Mercury in the ointments of 40% greasy base and 50% of 13% Bentonite Base than in the ointments of the corresponding 90% greasy base. It is also indicated that a 10% Ammoniated Mercury ointment of Anhydrous Lanolin is less effective than a 10% Ammoniated Mercury ointment of White Petrolatum, and that a 10% Ammoniated Mercury ointment of Aquafor gives the greatest antiseptic action of the three.

CONCLUSIONS

An ointment of Ammoniated Mercury in a Bentonite Base has greater antiseptic action than the ointment of Ammoniated Mercury in the U.S.P. XI if tested within a week after preparation. Although the addition of a greasy base to an ointment of Ammoniated Mercury with a Bentonite Base lowers the bacteriostatic properties slightly, the resulting ointment is more desirable from the combined viewpoint of stability and effectiveness.

Of the mixed bases prepared Ammoniated Mercury shows the greatest diffusing properties in a mixed base of bentonite and aquafor. A mixed base of bentonite and white petrolatum was next in effectiveness, and a mixed base of bentonite and anhydrous lanolin was the least effective.

APPROVED BY Louis Wm Busse

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