AN IMPROVED CALOMEL OINTMENT.*

BY E. E. VICHER, R. K. SNYDER AND E. N. GATHERCOAL.

INTRODUCTION.

Information to the effect that the calomel ointment of National Formulary VI produced an insufficient response to the F. D. A. Agar Plate Antiseptic Test to be considered antiseptic, led to an investigation of this product.

This ointment, prepared carefully after the N. F. VI formula, produces zones of inhibition up to 2 mm. in width. The N. F. V ointment, with white petrolatum as a base, instead of equal parts of hydrous wool fat and white petrolatum as in N. F. VI, gives inhibition zones almost identical with those produced by the N. F. VI ointment. Several samples of calomel ointment purchased on the open market yield inhibition zones from 1.0 mm. to 2.0 mm. in width.

It was considered undesirable to fortify calomel ointment with another antiseptic, even with a more easily soluble inorganic mercury compound. If any known antiseptic be added to the ointment its presence must be stated in the name of the ointment and on the label.

As certain insoluble salts or even elements acquire new or increased pharmacologic activity when reduced to the colloidal state, this process was attempted with calomel.

Precipitation of a newly formed salt in the presence of protective colloids tends to form colloidal suspensions of the insoluble salt. Acacia and gelatin are protective colloids commonly used for such a purpose.

SIZE OF CALOMEL PARTICLES.

Microscopic examination of various calomels indicates a variation in length of particles from 2 microns to 110 microns (1). U. S. P. Calomel shows a variation in length of from 2 microns to 50 microns, the average length of single crystals being about 7 microns.

In calomel suspensions prepared in this work, the maximum size of the visible calomel particles was 0.8 microns. Most of the visible particles were smaller and many particles were present that could not be seen under an oil-immersion lens; probably those invisible particles approach colloidal size. Ointments prepared from this new calomel developed much higher antiseptic power. Could the reduction in the size of the particles account for this?

PREPARATION OF THE NEW CALOMEL.

Pell Broady and C. B. Jordan (2) of Purdue University School of Pharmacy have developed a method for the preparation of what they termed "Colloidal Calomel." A solution of sodium chloride was added slowly to a 2 per cent solution of gelatin and to this was added a dilute solution of mercurous nitrate slowly with constant agitation. The resulting product was a stable suspension of calomel.

By calculation each 10 Gm. of mercurous nitrate requires 2.05 Gm. of sodium chloride to precipitate 8.41 Gm. of calomel.

Varying amounts of gelatin were used in the early precipitations (0.5%, 1.0%, 1.5% and 2.0%) prepared by us. It was found that the maximum size of the particles of calomel obtained

* National Formulary Research Project F 23 in progress at the University of Illinois School of Pharmacy.

in all cases was 0.5 microns. The stability of the suspensions, however, varied in direct proportion to the concentration of gelatin used, that containing 2% gelatin being the most stable.

The first lots of calomel were repeatedly washed by a process of centrifuging and decantation, but the constant cloudiness of the supernates despite long and continued centrifuging indicated that the smallest particles were being thrown away. This method was replaced by a dialysis of the suspension. Such a dialysis continued for 8 to 12 hours assured a completely neutral, pure suspension of the calomel in gelatin solution.

The following general method was eventually followed: Dissolve 3 parts of mercurous nitrate in 100 parts of acidulated water (nitric acid 1, water 100). Dissolve 2 parts of gelatin and 1.2 parts of sodium chloride in 100 parts of water. Add the mercurous nitrate solution drop by drop (about one drop per second) to the NaCl-gelatin solution which is under constant agitation by mechanical stirring. Wash the suspension by dialysis until free from acidity and soluble salts. Concentrate by evaporation in a vacuum pan until the concentrate contains 1 Gm. of calomel in 3 Gm. of the suspension. This concentrated suspension is permanent and suitable for incorporation into the ointment base.

PREPARATION OF THE CALOMEL OINTMENTS.

Calomel ointments, N. F. V and N. F. VI were prepared by the official method.

The new calomel used in the first ointments was centrifuged, washed, dried and resuspended in pure water. Later the ointments were made with calomel that had been centrifuged, washed and resuspended but not dried. The third group of ointments contained calomel suspension that had been dialyzed and concentrated. All of these ointments were made up with the official calomel ointment base.

The following formula was used for ointment made with the concentrated suspension: Into 70 parts by weight of ointment base, incorporate 90 parts by weight of suspension (containing 30 parts by weight of calomel) to make 160 parts by weight of ointment.

In reality this gives an ointment containing about 18 per cent of calomel (anhydrous).

ANTISEPTIC PROPERTIES OF CALOMEL OINTMENT.

Tests for the antiseptic power of ointments are usually run after the F. D. A. Agar Plate Technic (9).

In our work, 20 cc. of the specially prepared agar were used in each 100-mm. petri dish. The test organism was *Staph. aureus* A-209 grown in the special broth. After the melted agar had been inoculated with the test organism and the plates poured, a 2.0-Gm. sample of the ointment being tested was placed in intimate contact with a small area of the inoculated agar, using a suitable spatula for transfer from the covered sterile watch glass in which the ointment had been accurately weighed. The spatula was warmed before taking up the ointment sample to assure as complete a transfer as possible and to warm that portion of the ointment which was to be in contact with the agar surface so as to make that contact as intimate as possible. After incubation for 24 hours, the width of the inhibition zone was measured.

The following tabulation gives the results of the test on a wide variety of ointments:

Name of Ointment.	Number of Tests.	Width of Inhibition Zone.
Hydrous Wool Fat	2	None
White Petrolatum	2	None
N. F. VI Base	2	None
Calomel Ointment, N. F. V	3	1.5 to 2.0 mm.
Calomel Ointment, N. F. VI	7	0.5 to 2.0 mm.
Ointment made with a fine, precipitated calomel; particles average 2 microns	2	1.5 to 2.0 mm.

Ointment of an old sample of calomel; particles up to 100 microns long	1	0.5 to 1.0 mm.
Ointment with an English calomel; particles 9 to 110 microns long	1	0.5 to 1.0 mm.
Commercial calomel ointments in collapsible tubes	4	1.0 to 2.0 mm.
Ointment of Mercuric Chloride, 1–100	3	10.0 to 12.0 mm.
Ointment of Mercuric Chloride, 1–1000	3	5.0 to 6.0 mm.
Ointment of Mercuric Chloride, 1–10,000	3	1.5 to 2.0 mm.
Ointment with 10% of the new calomel (0.5%, 1.0%, 1.5%, 2.0% gelatin)	8	4.0 to 6.0 mm.
Ointment with 18% of the new calomel, centrifuged and in concentrated suspension	10	7.0 to 8.0 mm.
Ointment with 18% of the new calomel, dialyzed and in concentrated suspension	52	7.0 to 12.0 mm.
Ointment with 1.8% of the new calomel		4.0 to 6.0 mm.
Ointment with 0.18% of new calomel		2.0 mm.
Ointment with 0.018% of new calomel		1.0 mm.
Ointment with 0.0018% of new calomel		0.5 mm.

Results Obtained by Reddish and Wales (3).

Calomel Ointment	1.5 mm.
Ointment of Ammoniated Mercury	4.0 mm.
Ointment of Red Oxide of Mercury	4.0 mm.
Ointment of Yellow Oxide of Mercury	4.0 mm.
Mild Mercurial Ointment	7.0 mm.
Ointment of Mercurous Nitrate (Citrine Ointment)	13.0 mm.
Iodine Ointment	8.0 mm.
Ointment of Pine Tar	3.0 mm.

In an effort to determine the antiseptic value of the compound formed by mercurous nitrate and gelatin, the usual solution of mercurous nitrate was added to the 2 per cent gelatin solution from which the sodium chloride had been omitted. A precipitate was formed which remained in suspension and could be washed by dialysis as had been done previously with the calomel-gelatin suspension. The washed suspension was concentrated to contain the equivalent of 30 per cent of mercurous nitrate and this concentrate was then incorporated into an ointment after the regular formula for the new calomel ointment. This ointment gave by the F. D. A. Test a 4.0-mm. zone.

An 18% ointment was prepared using a calomel which had been precipitated in an NaCl-acacia solution instead of the NaCl-gelatin solution; *i. e.*, 2 per cent of acacia was substituted for the 2 per cent of gelatin. The size of the largest calomel particles in the suspension was found to be 0.8 microns. This ointment when tested for its inhibitory action by the F. D. A. Agar Plate Technic displayed a zone of 7.0 to 8.0 mm.

The antiseptic power of the suspension of the new calomel determined by the F. D. A. Antiseptic Test for liquids indicates no greater antiseptic power than that shown by a suspension of U. S. P. Calomel in a dilute gelatin solution.

A lot of the ointment containing the new calomel was put into porcelain jars and another lot into collapsible ointment tubes of one ounce capacity. The F. D. A. Agar Plate Test shows no decrease in antiseptic power of either lot of ointment up to three months of age. The testing will be continued to determine the extent of

deterioration, if any. Samples of the official ointment of a year or more in age apparently show some decrease in antiseptic power, as indicated in tests on commercial samples.

THE ASSAY OF CALOMEL SUSPENSION.

An assay for the calomel suspension and of the ointment containing the new calomel has been sought. The usual oxidation methods tended to volatilize the calomel. By first converting the chloride to the less volatile oxide, the following method gave results of 97 to 98 per cent of theoretical.

Place the accurately weighed calomel-gelatin suspension corresponding to about 0.5 Gm. of calomel into an acetylization flask. Add 50 cc. of a 10% solution of KOH in 80% alcohol and reflux on a water-bath for 3 hours; cool and filter (using a dry funnel and filter). Wash the flask and filter with 80% alcohol to remove excess chloride, dry the filter and contents and return to the flask. Add 10 cc. of sulfuric acid, and reflux over a direct flame until complete decomposition is effected (charring); cool, add 15 cc. of nitric acid and heat (without refluxing) until nitric acid fumes are driven off. Transfer the clear colorless liquid quantitatively to a 250-cc. volumetric flask, add distilled water to make 250 cc. of solution, and treat a 50-cc. aliquot, diluted with distilled water to 100 cc., with H_2S until complete precipitation of HgS has occurred. Filter through a tared Gooch crucible and repeatedly wash the HgS with distilled water, then three times with alcohol and followed with CS_2 until the latter washings when evaporated on a small watch glass leave no visible material. Wash the precipitate again about three times with alcohol to remove the CS_2, air dry for a short time and dry to constant weight at 110° C.

TOXICITY AND ABSORPTION OF CALOMEL OINTMENT.

A review of the literature (4, 5, 6, 7, 8) indicates that the official calomel ointment is absorbed through the skin of rabbits to produce diarrhea, and fatal poisoning. The diarrhea appears usually on the second day and death ensues in from 5 to 20 days. The fatal dose is at least 1 Gm. of calomel. Larger and repeated doses cause death more promptly.

The inunction of calomel ointment on man occasionally produces a mild stomatitis but no salivation.

Our work shows that 2- to 4-Gm. doses of the official and the "new" calomel ointments result in diarrhea on the first or second day after inunction and in death on the fourth to twentieth day.

Doses of 0.25 Gm. to 1.00 Gm. of ointment usually were not fatal.

The new calomel ointment exhibited but slightly higher toxicity than the official ointment.

SUMMARY.

1. The size of particles in U. S. P. calomel varies from 2 to 50 microns in length, and in certain old calomels the maximum size reaches 110 microns.

2. The new calomel, consisting of particles 0.8 micron or less in size, was prepared by slowly adding, with constant agitation, a 3% solution of mercurous

nitrate to an equal volume of a solution containing 2% of gelatin and 1.2% of sodium chloride. This suspension of calomel in gelatin is washed by dialysis and concentrated to contain 1 Gm. of calomel in 3 Gm. of the suspension. The suspension is permanent and can be incorporated directly into the ointment base.

3. Official calomel ointments were found to possess an inhibitory action which gave clear zones in the F. D. A. Agar Plate Test varying from 0.5 to 2.0 mm., whereas ointments prepared from the new calomel displayed zones of from 7 to 12 mm. in width.

4. Few ointments produce a broader zone in the F. D. A. Agar Plate Test than the new calomel ointment; of the official ones, only Citrine Ointment (Ointment of Mercurous Nitrate).

5. The official calomel ointment corresponds in antiseptic value to a 1 to 10,000 mercuric chloride ointment, but the new calomel ointment is more than equivalent to a 1–1000 mercuric chloride ointment.

6. A dilution of the suspension of calomel, containing 1 part of calomel (by weight) in 1000 parts of dilution (by volume), has a phenol coefficient about equal to that of a 1% phenol solution.

7. The new calomel ointment apparently is stable; no decrease in antiseptic power of the ointment is noted after three months' aging.

8. It was found that only slight differences exist in the toxicity on rabbits between the official ointment and the new calomel ointment, although the rate of absorption of the new calomel appeared to be more rapid than that of the official product.

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