No. 16.—This specimen shows a variety of forms of particles. There are some isolated crystals (not acicular), a few rosettes, a number of crystalline aggregates and some very small particles. The range in size is from 0.05 to 0.1 $\mu$.

No. 17.—(*a*) and (*b*) is a specimen of the calomel extracted from the ointment which led to the investigation.

In these specimens large and pointed crystals, some of which appear to have been eroded, are in evidence.

None of the commercial calomels are identical with this specimen in appearance, and it is possible that some alteration in the physical condition of the crystals in the ointment sample may have resulted as a consequence of the action of the fat of the ointment base on the calomel after the ointment was prepared.

The results of the survey are interesting as showing the high quality to which the specimens conformed and also as illustrating the wide variance in size and type of particles as shown by the photomicrograph illustrations, all of which are on the same basis of magnification.

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# THE ASSAY OF CITRINE OINTMENT.*

## An Experimental Study.[1]

BY THOMAS G. WRIGHT.

### INTRODUCTION.

The Citrine Ointment of to-day is an ointment which is essentially a mixture of mercuric nitrate with a fatty base formed by the action of nitric acid upon lard. An ointment by this name first appeared in the London Pharmacopœia of 1650 and since that date there has been a wide difference of opinion as to its composition, stability and assay. The original preparation was a mixture of coral, limpet shells, white marble, white lead, quartz and tragacanth, incorporated with a base of hogs' lard, suet and hens' grease; but contained no mercury.

Nitrate of Mercury Ointment, approximating in composition the present Citrine Ointment, first appeared in the Edinburgh Pharmacopœia of 1722 and was introduced, according to Christison's Dispensatory, as a substitute for a then popular proprietary remedy, "Golden Eye Ointment," an ointment of yellow oxide of mercury. It was not until 1746 that a similar ointment was admitted to the London Pharmacopœia. A milder ointment, one made with twice the quantity of lard, was made official in the Edinburgh Pharmacopœia of 1792. An ointment composed of mercury, nitric acid, lard and olive oil was included in the Dublin Pharmacopœia of 1807. This ointment was admitted to the British Pharmacopœia of 1864, and one of similar composition was admitted to the French Pharmacopœia at a later date. In this country, the ointment received official recognition as early as 1820, when it appeared in the Pharmacopœia of the United States under the title of Unguentum Hydrargyri Nitratis. It was readmitted at each succeeding revision of the Pharmacopœia until that of 1920 when it was dropped. Its official status, however, was not changed as it was immediately given a place in the National Formulary.

From the date of its introduction into the Edinburgh Pharmacopœia to the present time, many different formulas have been suggested for the preparation of the ointment, the principal objectives being to simplify the preparation of the elaidin base and to improve the keeping qualities of the ointment. This phase of the subject, in so far as it pertains to the work reported in this paper, will be discussed in detail under the composition of the ointment.

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* Scientific Section, A. PH. A., Washington meeting, 1935.

[1] From the laboratory of A. G. DuMez, Professor of Pharmacy, School of Pharmacy of the University of Maryland. Compiled from a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science.

Considerable literature is available dealing with the preparation and stability of the ointment (1), some work on the composition of the ointment has been reported, but little has been published regarding its assay. Work along this line is therefore needed and particularly at this time when the National Formulary is being revised. It was primarily for this reason that the study reported in this paper was undertaken.

## COMPOSITION OF THE OINTMENT.

Before undertaking an analysis of any kind it is desirable that as much information as possible be obtained on the composition of the material to be analyzed. This is particularly true in the case of Citrine Ointment. While the component of importance, from a pharmaceutical standpoint, in this instance is mercury, the analytical method selected for the quantitative determination of the mercury will depend largely upon the form in which the latter occurs in the ointment.

As stated in the introduction, the composition of the ointment has been the subject of study by several investigators (2), and while the results obtained by them, particularly those obtained by the early investigators, were not in complete accord, the following general conclusions may be drawn. The ointment is essentially a homogeneous mixture of mercuric nitrate solution with a fatty base called elaidin. It is quite probable that it contains some basic oxides of mercury in additon to the normal nitrate and that a portion of the mercury is also present as mercuric elaidate, resulting from the interaction of elaidin with mercuric nitrate.

Citrine Ointment is prepared by first dissolving metallic mercury in a strong solution of nitric acid, a violent reaction occurring between these two substances with the formation of mercuric nitrate, as the chief product and probably some other compounds of nitrogen of little importance in this connection. Some investigators have expressed the view that this solution is a mixture of many chemical compounds. J. Laidley (3) believed the solution to be a mixture of mercurous and mercuric nitrates, while A. Astrug (4) considered the solution to be composed of mercuric nitrate, mercurous nitrate, nitrogen peroxide, nitrogen dioxide and free nitric acid.

The second step in the preparation of the ointment is that of preparing the base. The latter is the product resulting from the reaction occurring when nitric acid is added to lard. Lard is chiefly a mixture of the glyceryl esters of stearic, palmitic and oleic acids, the glyceryl ester of oleic acid predominating. When nitric acid is added to this mixture, it is reduced to nitrous acid and this in turn reacts with the oleic acid ester to form the isomer, elaidin, which is the ointment base. Some investigators have contended that the yellow color of the ointment is due to elaidin. H. C. Cook (5) held this view. Astrug and M. Donovan (6) on the other hand, reported the color to be due to basic mercuric nitrate, $Hg_2OHNO_3$, arising from the action of nitrogen dioxide on mercuric nitrate. A. Baumé (7) claimed that a "soap" having no yellow color is formed when nitric acid reacts with fat and that precipitation of mercury in the ointment is the cause for the color formation. The explanation of Baumé is now known to be fallacious since the fat turns yellow before the mercury solution is added, and is due to the action of the nitric acid alone.

The final step in the preparation of the ointment is that of incorporating the mercury with the base. This is accomplished by dissolving the required amount of mercury in nitric acid and stirring the solution into the fat. At this point in

the preparation of the ointment, most investigators believe that a series of reactions takes place with the formation of compounds in which mercury is organically combined. This belief is probably well founded, and the compounds of mercury most likely to be formed are the salts of the fatty acids liberated in the partial hydrolysis of the glyceryl esters. The ointment, therefore, probably contains some mercuric oleate, palmitate and stearate. Elaidate of mercury should also be present for the same reason.

## ASSAY METHODS.

Chemical assay methods, especially those designed for the determination of pharmaceutical preparations, should be methods that are practical from the standpoint of material and apparatus used, ease of carrying out the procedure, and time consumed; but should, nevertheless, be sufficiently accurate to give results which may be depended upon arriving at a fair evaluation of the substance assayed, even though other substances of a similar nature may be present.

The assay methods for ointments containing mercury compounds, reported in the literature, were reviewed to determine if they could be adapted to the assay of Citrine Ointment. In the main, these methods consisted of the decomposition of the ointment with acid and subsequent titration of the remaining mercury solution. Most of these were rejected without experimental trial because of their complexity and because the time required to complete the assays was too great to permit of their use in practice. The procedure of I. V. S. Stanislaus and E. A. Eaton for the assay of Citrine Ointment (8) was reviewed. The method consists of treating the ointment with a solution of potassium hydroxide, allowing the mixture to stand for 24 to 48 hours for complete subsidence of the mercury oxides, precipitation of the mercury as sulphides, addition of iodine solution, and subsequent titration with sodium thiosulphate solution. Although this method seemed to be satisfactory from a theoretical standpoint, it was deemed to be unsatisfactory because of the length of time required to carry it out, and was, therefore, not given an experimental trial. The method of Strickland (9) in which the ointment is decomposed by nitric acid and the remaining mercury solution titrated with potassium thiocyanate, seemed to meet most of the requirements of a good assay process. It was, therefore, used as a basis for beginning the work reported in this paper.

## EXPERIMENTAL PART.

For the purpose of providing material for assay, the mercury content of which was known, it was necessary to prepare a batch of ointment using mercury of known purity. A quantity of the ointment was therefore prepared according to the directions given in the National Formulary V, nitric acid (sp. gr., 1.42) was used and the fat was the best grade of lard obtainable. The lard was melted in a large porcelain evaporating dish at a temperature not exceeding 45° C. The nitric acid was added and the mixture was heated until the reaction between the lard and acid was complete and all of the olein was converted into the isomer elaidin. The specified quantity of mercury was then dissolved in nitric acid without the aid of heat and added to the elaidin base, which had been allowed to cool. The resulting product was stirred continuously with a glass rod until a uniform mixture of the consistence of an ointment was obtained. The ointment, when cool, was transferred to an amber-colored glass jar, which was covered tightly with a ground glass cover. No contact was made, at any point in the procedure, with metallic utensils.

The mercury used in the preparation of the ointment was assayed for purity by the method of the United States Pharmacopœia X. Twelve determinations were carried out, the results of which are given in the following table:

TABLE I.—ASSAY OF METALLIC MERCURY BY U. S. P. X METHOD.

| Sample* No. | 1. | 2. | 3. | 4. | 5. | 6. |
|---|---|---|---|---|---|---|
| Hg found in % | 99.29 | 99.04 | 99.74 | 98.75 | 99.82 | 99.91 |
| Sample* No. | 7 | 8 | 9 | 10 | 11 | 12 |
| Hg found in % | 99.85 | 99.98 | 99.93 | 99.96 | 99.95 | 99.90 |

* Description of Sample. The mercury used in all of the above assays was obtained from Merck & Co., and labeled triple distilled.

The potassium thiocyanate solution used in the foregoing determinations and in all other assays, was prepared according to the directions of the United States Pharmacopœia X, and was regularly standardized at intervals of about seven days against a $N/10$ solution of silver nitrate which was kept in a ground glass-stoppered bottle painted black, to prevent any deterioration which might be caused by the action of sunlight.

TABLE II.—DETERMINATION OF MERCURY LOST IN PREPARATION OF CITRINE OINTMENT.

| Sample No. | Wt. of Hg Taken. | Diluted to Cc. | Aliquot Taken, Cc. | Cc. KCNS. | Equiv. KCNS-Hg. | Wt. of Hg in Sample. | % of Theory. |
|---|---|---|---|---|---|---|---|
| 1A | 3.5124 | 100 | 10 | 30.4 | 0.01003 | 3.4941 | 99.48 |
| B | 3.5124 | 100 | 10 | 30.4 | 0.01003 | 3.4941 | 99.48 |
| C | 3.5124 | 100 | 10 | 30.4 | 0.01003 | 3.4941 | 99.48 |
| D | 3.5124 | 100 | 10 | 30.4 | 0.01003 | 3.4941 | 99.48 |
| 2A | 37.0703 | 1000 | 10 | 32.1 | 0.01003 | 36.8957 | 99.52 |
| B | 37.0703 | 1000 | 10 | 32.1 | 0.01003 | 36.8957 | 99.52 |
| C | 37.0703 | 1000 | 10 | 32.1 | 0.01003 | 36.8957 | 99.52 |
| D | 37.0703 | 1000 | 10 | 32.1 | 0.01003 | 36.8957 | 99.52 |
| 3A | 69.3710 | 2000 | 10 | 30.0 | 0.01003 | 68.9640 | 99.41 |
| B | 69.3710 | 2000 | 10 | 30.0 | 0.01003 | 68.9640 | 99.41 |
| C | 69.3710 | 2000 | 10 | 30.0 | 0.01003 | 68.9640 | 99.41 |
| D | 69.3710 | 2000 | 10 | 30.0 | 0.01003 | 68.9640 | 99.41 |

Summary: Sample No. 1 (0.52% Hg lost).
Sample No. 2 (0.48% Hg lost).
Sample No. 3 (0.59% Hg lost).

During the preparation of the ointment the reaction between the mercury and nitric acid becomes violent, with the evolution of considerable heat. Since mercury is a volatile substance, it was believed that there might be an appreciable loss, due to the heat thus generated, and that this condition was responsible for the low results obtained in the assay of the ointment by the different methods in use. To determine the accuracy of this belief three batches of mercuric nitrate solution were made using the approximate quantities of mercury and nitric acid necessary to prepare 50, 500 and 1000 Gm. of Citrine Ointment. The temperature seemed to increase directly with the size of the quantities of reacting materials used, hence, the preparation of the three batches of solution in the quantities stated. The mercury required was accurately weighed and dissolved in the nitric acid which was also weighed. No external heat was applied in these cases. The solutions were titrated with $N/10$ potassium thiocyanate solution in the usual manner, the results compared with the weight of mercury used, and the percentage loss of mercury computed. However, the loss in mercury, if any, was not over 0.59 per cent in any case and this loss was constant irrespective of the quantity of solution prepared as shown by the results presented in the foregoing table:

As previously stated, the method of Strickland (9) seemed to meet most of the requirements for a good assay process, and the analysis of the ointment by this method was therefore taken up. This method, as published, was originally intended for the assay of Mercurial Ointment, of the United States Pharmacopœia, but, according to the originator, it would also serve for the assay of Citrine Ointment.

About 10 Gm. of the ointment, accurately weighed, was heated under a reflux condenser with 100 cc. of nitric acid and the heating continued until all of the mercury had been dissolved and the fatty base completely broken down. The resulting solution was filtered through cotton into a 200-cc. graduated flask and a 3 per cent potassium permanganate solution was added until a permanent pink color was obtained. The solution was then decolorized with 3 per cent ferrous sulphate solution and the volume accurately made up to 200 cc. with distilled water. An aliquot of this solution was titrated with $N/10$ potassium thiocyanate solution. One cc. of potassium thiocyanate solution is equivalent to 0.01003 Gm. of mercury.

The method as outlined in the foregoing was subjected to test in the laboratory and was found to be unsatisfactory as the results obtained with it were low and variable, as shown in Table III.

TABLE III.—ASSAY OF CITRINE OINTMENT BY STRICKLAND'S METHOD.

| Sample No. | 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|---|
| Hg found in % | 4.93 | 5.37 | 2.41 | 4.21 | 6.79 | 4.97 | 2.79 |
| Hg theoretical in %* | 6.97 | 6.97 | 6.97 | 6.97 | 6.97 | 6.97 | 6.97 |

* The theoretical percentage of mercury present was computed from the quantity of triple distilled mercury used in the preparation of the ointment, and which assayed 99.67 per cent pure metallic mercury by the U. S. P. X method.

Many hours of refluxing were required to completely break down the organic matter present in the ointment, and volatilization, due to this continued heating, was probably the chief cause of the loss of mercury.

The addition of potassium permanganate solution also gave some difficulty as a large volume of the permanganate solution was often required to bring about the permanent pink coloration.

Various other methods of analysis were tested but the results obtained were the same as those of the above procedure in every case.

Due to the low results obtained it was believed that some of the organic compounds were not completely broken up and that the resulting solution did not, therefore, contain all of the mercury. A stronger oxidizing agent was thought to be essential for complete decomposition of these compounds. The oxidizing mixture selected for trial consisted of perchloric acid (sp. gr., 1.615), 1 part; fuming nitric acid (sp. gr., 1.49), 2 parts; and distilled water, 2 parts. This combination proved to be a more efficient oxidizing mixture than nitric acid or a mixture of nitric and sulphuric acids, and was satisfactory in other respects. A method of assay based on the use of this oxidizing mixture has therefore, developed. A description of the method follows:

Place about 5 Gm. of the ointment, accurately weighed, in a flask containing 50 cc. of the above acid mixture and reflux until a clear solution is obtained and the brown fumes are no longer distinguishable. Dilute the solution with about 20 cc. of distilled water, and pass it through a filter paper into a 100-cc. volumetric flask. Wash the funnel with sufficient distilled water to bring the volume up to 100 cc. Take a 20-cc. aliquot of this solution and titrate it with $N/10$ potassium thiocyanate solution, using ferric alum as the indicator, until a permanent reddish brown color is obtained. The condenser and flask used should be fitted with ground glass connections in order to avoid contamination and error which might occur from the action of the acid mixture upon either cork or rubber stoppers.

In the first trials, the assay was carried out by refluxing on a water bath and although the results obtained were all within a close range of the theoretical amount of mercury in the ointment and did not show a great variation, the time necessary for refluxing was about three and one-half hours. The results of these determinations are shown in Table IV.

TABLE IV.—ASSAY OF CITRINE OINTMENT BY PERCHLORIC-NITRIC ACID METHOD. HEAT FURNISHED BY WATER BATH.

| Sample No. | 1. | 2. | 3. | 4. | 5. | 6. | 7. | 8. |
|---|---|---|---|---|---|---|---|---|
| Hg found in % | 8.60 | 8.57 | 8.10 | 8.34 | 8.61 | 8.24 | 8.11 | 8.38 |
| Hg theoretical in % | 8.67 | 8.67 | 8.67 | 8.67 | 8.67 | 8.67 | 8.67 | 8.67 |

In order to reduce the time factor of the assay the water bath was eliminated and the ointment was refluxed over a low open flame. With this modification of the procedure, it was found that one hour was sufficient time to decolorize the solution in every case. Two batches of the ointment were prepared and were labeled, "batch A" and "batch B." Seven assays in which this procedure was followed were run on "batch A" and ten determinations were made with "batch B." The results obtained were not only constant but as nearly accurate as can be expected of a method of this type.

TABLE V.—ASSAY OF CITRINE OINTMENT BY PERCHLORIC-NITRIC ACID METHOD, "BATCH A." HEATED OVER OPEN FLAME.

| Sample No. | 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|---|
| Hg found in % | 8.51 | 8.45 | 8.65 | 8.58 | 8.63 | 8.63 | 8.64 |
| Hg theoretical in % | 8.67 | 8.67 | 8.67 | 8.67 | 8.67 | 8.67 | 8.67 |

TABLE VI.—ASSAY OF CITRINE OINTMENT BY PERCHLORIC-NITRIC ACID METHOD, "BATCH B." HEATED OVER OPEN FLAME.

| Sample No. | 1. | 2. | 3. | 4. | 5. |
|---|---|---|---|---|---|
| Hg found in % | 8.39 | 8.20 | 8.37 | 8.43 | 8.38 |
| Hg theoretical in % | 8.44 | 8.44 | 8.44 | 8.44 | 8.44 |

| Sample No. | 6. | 7. | 8. | 9. | 10. |
|---|---|---|---|---|---|
| Hg found in % | 8.34 | 8.34 | 8.34 | 8.33 | 8.29 |
| Hg theoretical in % | 8.44 | 8.44 | 8.44 | 8.44 | 8.44 |

## CONCLUSIONS.

From the results obtained in the experiments described above, the following conclusions are drawn:

1. Citrine Ointment is a homogeneous mixture consisting of mercuric nitrate and other mercury compounds which are uniformly distributed.

2. Most assay methods heretofore published are impractical for the determination of the mercury in this ointment because they are too complicated, too lengthy, and because they do not give constant results in the hands of different analysts.

3. Only a very slight amount of the mercury used is lost in the preparation of the ointment.

4. Citrine Ointment can be accurately assayed by the method proposed.

5. The method proposed is simple, rapid and practical.

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# THE PHARMACOLOGY OF GALINSOGA.*[1]

## A SERIES OF MICRO-RESPIROMETER STUDIES.

BY MARTIN A. YAVORSKY WITH EDWARD C. REIF.[2]

Preliminary experiments on the pharmacology of Galinsoga (1) indicate the presence of a principle or principles in the plant, which cause a drop in the blood pressure of the dog when certain preparations are injected intravenously

The chemistry of the plant has been studied by Dr. Karl Muller (2). No reference, however, has been made to the presence of a potent or active constituent.

The purpose of this paper is to describe certain experiments that were conducted to increase our knowledge of the pharmacology of Galinsoga and to add more information concerning the oxygen consumption by tissues, especially the influence upon the same by plant drugs.

It is a well-known fact that tissues removed from the body of a recently killed animal will, if suspended in a suitable medium, utilize oxygen for an indefinite period. Oxidations in animal tissues can be studied by means of a microrespirometer, various types of which are described by Warburg and his collaborators (3).

Extensive studies on the influence of certain compounds on the oxygen consumption of many tissues have been made by Voegtlin, Rosenthal and Johnson (4), (5).

The action of Vitamin C on the oxidations of tissue *in vitro* has been reported by Harrison (6).

Oxygen consumption by the tissues used in this series of experiments was

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[1] Submitted as partial requirement for B.S. in Pharmacy, School of Pharmacy, University of Pittsburgh.

[2] Professor of Materia Medica and Botany, School of Pharmacy, University of Pittsburgh.