

being unfavorable. The stage was set for lime versus U. S. P. and no one could see the shaking-out process.

In conclusion, I think I have proved that:—

First:—The lime method is inferior in every sense, except shorter time of operation, to the U. S. P. method, and is not worthy of adoption in the pharmacopœia of this country.

Second:—That the shaking-out method devised by us is superior in every sense, will eventually be adopted in the U. S. P. and should be adopted now. If not adopted now, then the modified U. S. P. should be adopted by the Revision Committee.

Third:—That the shaking-out process is adapted to determining morphine in practically all kinds of mixtures as well as practically all forms of medication.

*Analytical Laboratory, Sharpe & Dohme, August, 1914.*

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### THE LIME ASSAY OF OPIUM.

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Of the numerous methods that have been proposed for the morphimetric assay of opium, two only seem to have found favor with the authors or compilers of national Pharmacopœias.

In one, the drug is exhausted with water, the solution concentrated to a small volume, alcohol and ether added together with water of ammonia, the mixture shaken well and allowed to stand for a specified time for separation of the morphine in crystals.

In the other, the powdered opium is mixed with lime and a certain proportion of water, allowed to macerate, with occasional stirring during half an hour, the solution filtered and an aliquot portion of the filtrate treated with ammonium chloride which causes the morphine to separate in crystalline form.

The advantages claimed for the second method are (1) rapidity of execution; (2) superior purity of the morphine obtained, owing partly to the fact that lime combines with morphine forming a very soluble compound—a property not shared with it by narcotine or most of the other alkaloids of opium—partly because the lime throws out of solution certain organic acids and other compounds which otherwise are liable to be thrown down with the morphine; (3) alleged uniformity of results.

Against the lime method it is urged: (1) It involves unavoidably the principle of the aliquot part; (2) crystallization of the morphine is from a more dilute solution than in the first general method, hence more of the morphine is held in solution so that an arbitrary correction is generally prescribed to compensate this loss. (It is generally admitted that there is also loss of morphine in the first assay method, in which no correction factor is generally prescribed;) (3) the assay requires that the opium be in the form of a powder, whereas

opium is imported in a moist condition, so that it must be dried (the loss of weight noted) and reduced to a powder—operations which greatly lengthen the time required for an assay.

Before entering into any discussion of these claims and counter claims, it is necessary to enter more in detail into the particular method of carrying out the respective assays. A typical lime assay process is that of the French Codex. Somewhat simpler and more familiar to American pharmacists is the Stevens assay. In the latter four grammes of powdered opium are mixed with two grammes of calcium oxide, the powder made into a paste with 10 cc. of distilled water, 19 cc. more of distilled water are added and the mixture is stirred occasionally during half an hour. The magma is then transferred to a filter and exactly 15 cc. of filtrate is taken for the assay, assumed to represent 2 grammes of the opium. To this is added 4 cc. of alcohol, 10 or 15 cc. of ether and 0.5 grammes of ammonium chloride. The mixture is shaken frequently during 30 minutes, then allowed to stand over night to complete the crystallization. The crystals of morphine are then collected in a small funnel loosely plugged with absorbent cotton, washed with morphinated water, transferred to the flask in which the crystallization process was carried out, the morphine being finally determined by titration with volumetric acid and alkali.

The first question that arises is as to the accuracy of the aliquot part taken. This will depend on the increase in volume of the fluid, due to the dissolved extract and lime. A number of experiments made with several different samples of opium have showed that where slaked lime is used, the increase in volume over 29 cc. is about 0.6 cc. covering a range from 0.5 to 0.75 cc. In the assay, the increase is taken as 1 cc. and the allowance thus made for incomplete exhaustion of the drug, amounting to from 0.8 to about 1.7 *per cent.* of the total may be considered reasonable. It is considerably less than the allowance in the assay process of the French Codex, and in other official processes. It is to be noted, however, that if calcium oxide is used instead of calcium hydroxide, this allowance is increased to 3 *per cent.* or more, since the oxide combines with and fixes one equivalent of water.

It is sometimes difficult to obtain a filtrate measuring as much as 15 cc. In such case, the filter containing the residue may be enclosed in a small piece of cheese cloth or muslin, and the residual fluid pressed out and then filtered through a small filter.

Measurement of the 15 cc. of filtrate must be made as accurately as possible—best with a measuring pipette. Of course, exposure of the filtered solution to air, particularly to the air exhaled from the lungs, must be minimized, by receiving the filtrate in a small flask, keeping the funnel covered with a watch glass and conducting the filtration as rapidly as possible to guard against any precipitation of calcium carbonate.

It is a question whether addition of any alcohol to the filtrate is advisable. Larger and whiter crystals of morphine are formed if the alcohol is added, but the separation of the morphine by crystallization under the ordinary conditions of the assay, is certainly less complete than if the alcohol is omitted. A careful study ought to be made of the influence (a) of alcohol, (b) of temperature,

(c) of the amount and kind of shaking, and (d) of the quantity of ether added. It should be understood that when morphine is set free in presence of ether or of a mixture of ether and alcohol, the alkaloid is largely transferred in the shaking to these solvents, and that at the end of the crystallization, the residual morphine will be found chiefly in the ethereal fluid.

I have found by experiment that when ether alone is used the residual morphine, after 14 hours, is almost a negligible quantity. When alcohol is present a notably larger quantity is held in solution. Whether it is possible to determine to a close approximation just how much of the alkaloid will be held in solution under prescribed conditions of shaking, temperature and time, can only be ascertained by a series of experiments. Inasmuch as the loss of morphine, where only ether is used, is inconsiderable, it seems to me unwise to use the alcohol—unless it can be shown that other alkaloids are thus prevented from crystallizing with the morphine, of which there seems to me practically no possibility.

Stevens' method of collecting the morphine and determining it by titration is neat, rapid, and I believe beyond criticism.

When the lime assay is carried out, as above detailed, the possible sources of fallacy are reduced to the following: (a) possible failure to extract the whole of the morphine by the half hour maceration; (b) possible presence in the morphine of other alkaloids; (c) possible inexactness of the aliquot part of the lime solution; (d) possible inexactness in the *titre* of the volumetric solutions used.

(a) It is not unlikely that in inexperienced hands there may be failure to extract the whole of the morphine in the initial maceration. It is my belief that if the directions for extracting the opium are carried out with reasonable care, practically the whole of the morphine will go into solution, but it is not easy to determine this experimentally. Results of comparative assays by the lime and aqueous extraction methods are not conclusive, particularly when such results have been reached in coöperative investigations.

In case it should be made to appear that powdered opium is not always completely exhausted in the prescribed routine a different procedure might be adopted in which the opium would be first exhausted with water, as in the U.S.P. assay. This, however, would sacrifice the great advantage of economy of time in the lime assay. It would, on the other hand, make the lime assay applicable to moist crude opium and with only slight modifications to all galenical preparations of opium, except the camphorated tincture.

(b) Presence in morphine separated in the lime assay of by-alkaloids could hardly be expected considering the manner in which the alkaloid is separated. At all events, alkalimetric titration indicates a high degree of purity in the crystals. The French Codex directs to wash the crystals with benzene (benzol) to remove such possible contamination. I do not think the precaution is necessary, but I have not tested the question experimentally whether benzene will reduce the weight of the crystals.

(c) The question of exactness of the aliquot, I have already considered. Of course, it is important that measurements of the water and the lime solution

should be made with exactness, and that the temperature at which these measurements are made should be practically the same, though not necessarily 25° C., the official standard temperature

Greater exactness can, of course, be secured by weighing the fluids instead of measuring them. If this is done a certain arbitrary allowance must be made for dissolved lime and extractive. It would be safe to make this allowance one-third the weight of the opium present, i. e., for a quantity of solution representing 2 grammes of opium, 0.667 gm. If 4 grammes of dry opium have been treated with 29 grammes of water, the aliquot representing 2 grammes of opium will be  $14.5 + 0.667 = 15.167$  grammes of the lime solution.

(d) To secure exactness in the result of the titration, it is only necessary to standardize the volumetric acid used on pure recrystallized morphine, dried over sulphuric acid. The conditions of the titration as to dilution of solution, etc., should be practically the same as in the assay; it is particularly important that the same indicator be used. For the professional chemist whose laboratory equipment is complete, there is no difficulty about so simple a matter as making an alkaloid determination by titration. In absence of such advantages a routine may be adopted which insures exact results in spite of difficulties.

One must be sure in the first place of the exact neutrality of the distilled water to be used. Put into a small flask a sufficient quantity of the water—which, although distilled, may have absorbed traces of ammonia, or if acid vapor—add a few drops of the indicator, and if necessary bring to exact neutrality by adding very dilute acid or alkali. To dissolve the morphine use this neutral distilled water with the addition of the requisite quantity—say 10 cc.—if decinormal hydrochloric acid, very accurately measured. For alkali, use half strength lime water, i. e., lime water diluted with an equal volume of distilled water (which need not be strictly neutral), and filtered if necessary. Ascertain the exact strength of this solution by titrating with it 10 cc. of the volumetric acid, diluted with some of the neutral water previously prepared. If it requires 46.5 cc. of the diluted lime water, then  $46.5 \div 10 = 4.65$  cc. of the alkali correspond with 1 cc. of decinormal acid. If the titration of the excess of acid after solution of the morphine, has consumed 38.45 cc. of the diluted lime water, deduct this figure from 46.5 and divide the remainder by 4.65. The quotient (1.73) represents the value in decinormal terms of the excess of acid. Hence, the morphine has required for neutralization  $10.00 - 1.73 = 8.27$  cc. of decinormal acid, of which each cc. represents 30.3 milligrams of morphine, and  $8.27 \times 30.3 = 250.581$  mg. is the quantity of morphine indicated by the titration.

If an aqueous solution (from any form of opium, or from a galenical preparation of that drug) is to be assayed by the lime method, it is to be brought to such a concentration that 30 cc. of it will represent 4 grammes of opium, 2 grammes of dry slaked lime are to be added, the mixture allowed to stand with occasional shaking 20 minutes, filtered and 15 cc. of the filtrate taken for the assay, to be conducted exactly as above described. In case the opium preparation contains alcohol, this must be driven off by evaporation on the water-bath. The official tincture may advantageously be diluted with twice its volume of water, the mixture, after standing 20 or 30 minutes, filtered and an aliquot part of the

filtrate, representing 4 grammes of opium evaporated to a volume of 30 cc. Otherwise 40 cc. of the tincture, after addition of about 20 cc. of water, may be evaporated until alcohol is dispelled, the concentrated solution transferred to a cylindrical graduate, the container rinsed with successive small portions of water to bring the volume of the fluid to exactly 30 cc.

Experiments should be made to ascertain whether at a low temperature (15° C. or below) crystallization of morphine in the final operation is practically complete in six hours, or in four hours even, provided the mixture is shaken continuously during 10 minutes, or at frequent intervals during 30 minutes after the ammonium chloride is added. (Note that the quantity of the latter salt must not exceed 0.5 gm.; 0.3 gm. should be sufficient.)

Just how the assay would be affected by the accidental presence of a notable quantity of sugar, is an interesting query. Of course, it would increase the amount of lime taken into solution and would therefore, call for a larger quantity of ammonium chloride, which again might cause a larger proportion of morphine to be held in solution.

Advocates of the Squibb assay method argue that that process should be preferred to the lime method for the following reasons: (1) The yield of morphine is greater; (2) the process is not complicated by the use of an aliquot part; (3) crystallization of the morphine is from a more concentrated solution, and hence should be more complete; (4) no arbitrary correction of the result is required; (5) the morphine is obtained in bold crystals, which, by titration, are shown to be free from any considerable impurity; (6) the assay is free from the sources of possible error to which the lime assay is subject—particularly,

Advocates of the Squibb assay method argue that that process should be maceration in the initial steps.

In rejoinder, advocates of the lime process say:

(1) Deficiency in morphine yield is not unavoidable in a lime assay. It has resulted chiefly from remedial defects in the details of the lime assay as generally practiced. Two main causes have been the use of alcohol, as well as ether, to facilitate crystallization and the prescribing of too large a quantity of ammonium chloride. Besides this, due attention has not been given to the temperature during crystallization and to the amount and method of shaking prescribed. It is well known that in the Squibb method, emphasis is laid on vigorous and prolonged shaking, and results are greatly influenced by neglect of this detail.

I am sure that the same opium solution assayed by the lime method, as herein-before described, yields practically the same quantity of morphine as by the Squibb assay. I am not equally positive that the lime method applied to opium in powder will give results correspondingly close to those of the Squibb assay, although it is my belief that they will. If so, the great saving of time and labor demand giving preference to the lime assay.

(2) The aliquot part, which is freely used in other assays, does not condemn an assay process. I have shown that for practical purposes, the aliquot of the Stevens assay (if calcium hydrate is used in place of calcium oxide) may be accepted as reasonably exact. If greater accuracy is wanted, it is easy to weigh the fluids instead of measuring them.

(3) It is apparently true that crystallization in the lime assay is from a more dilute solution than in the Squibb assay, but the conditions for crystallization are more favorable after all, in the former case. It is to be remembered, too, that the crystallization takes place in either case rather from the ethereal than from the aqueous solution. Experiments have shown that residual morphine, under proper conditions, is likely to be less in the former case than in the latter.

(4) If the last statement is true, an arbitrary factor is as necessary in one assay as in the other, the difference in amount being likely to be in favor of the lime process, and compensated at that by a negative correction in the Squibb assay for impurities in the crystals of morphine.

For practical purposes, the small correction may be ignored as immaterial, provided one or the other of the methods in their most approved form be taken as a basis for the commercial valuation of the drug.

(5) Where determination of the morphine is to be made by titration rather than by weighing, the smaller crystals are preferable, as being more quickly dissolved.

(6) The sources of possible error in the lime assay have already been shown to be inconsiderable and largely avoidable by slight modifications in the detail of the assay process. Loss of water by evaporation need not occur to any appreciable extent if obvious precautions are taken.

Confessedly neither of the assay processes under discussion are capable of yielding results of a high degree of exactness. The problem of singling out one from a number of alkaloids which exist in combination with a complex mixture of organic substances, and separating this in a reasonable state of purity by a simple and easily executed process is a baffling one indeed. The lime assay gives the neatest solution we are likely to reach.

Otherwise we may remove, by suitable solvents, the other alkaloids and then by some other solvent to take out the morphine in a state of purity. More than one process involving this principle has been devised—notably the Gordin-Prescott method, in which the solvents employed are successively benzole and acetone, and several shaking-out methods that have been strongly recommended in recent years, in which by-alkaloids are removed from a lime solution by shaking with chloroform, the morphine being afterwards set free by addition of ammonium chloride and separated by shaking out with alcohol, chloroform or some other solvent.

Presumably the results obtained are more nearly exact than those by the process now official, but until they have been put to the test of actual use under varying conditions, it is best to use them only tentatively and as confirmation of results more easily reached by the more familiar process.

#### DISCUSSION.

HARRY M. GORDIN, of Chicago:—There are two difficulties in the lime method. One is that the final liquid is so deeply colored that the end-point in titration cannot be clearly observed. This, however, can be eliminated by a method which I shall publish in the near future.

The second difficulty consists in that the vacuum distillation usually is accompanied by such violent bumping that some of the liquid may be lost by being thrown out of the distilling flask. How did you avoid this difficulty, Dr. Engelhardt?

DR. ENGELHARDT:—As I pointed out already this latter difficulty can easily be overcome

by first distilling the chloroform under ordinary pressure and then distilling the isobutyl-alcohol under diminished pressure. While doing so bumping will never occur.

MR. CHARLES E. CASPARI:—With reference to these methods for the isolation of morphine from opium. The lime method does not yield all the morphine which is present in the opium. While it may be all right for comparative purposes, it does not give absolute knowledge of the actual amount of morphine which is present, either in opium, or opium preparations, which ever may be under examination.

I have had some experience with the method of shaking out with chloroform and alcohol, a modification of Dr. Engelhardt's method, as presented here, and I have found it to yield immediate and certain results, and I have been very much pleased with it. I have used it particularly on tablets,—morphine mixed with other ingredients—and found that it was very satisfactory.

From my knowledge of the methods, I would say that I like the shaking-out method best. It is the simplest, and quickest, and as far as I can determine, the most accurate.

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## MICROSCOPY OF CINCHONA BARKS AND ADULTERANTS.

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The question of the identity, both macroscopic and microscopic of the various species and varieties of cinchona, has been one of great importance for many years. The difficulties in the way of proper identification, are increased greatly, by the necessary admission of many hybrids of the official species. Of course, the drug is to great extent sold upon assay, but this does not relieve the pharmacognosist of the task of deciding, whether a sample submitted, is official or recognized, as there is always the possibility of the addition of non-official barks to a lot of genuine, and the more likely is such addition, when the drug is in powdered form. The chemistry of cinchona has been treated at great length in the proceedings of this Association, and, likewise, several papers have been submitted upon the macroscopic or gross appearance and structure of the drug, but, in a search of the publications, I fail to find much reference to the microscopy.

The results, in many cases, are not quite as definite and satisfactory as I would desire, and this lack of absolute uniformity, is undoubtedly due to hybridization in cultivation. An excellent article upon the subject of cinchona is given by Vogl, in his text book on Pharmacognosy (German), but this is not accessible to all, as I do not know of an English translation of the work. For this reason, I append a useful classification of the histological characteristics of the various cinchonas taken from this text book. (Dr. A. Vogl, "*Pharmacognosie*," Carl Gerold's Sohn, Vienna, 1892.)

Macroscopic characteristics are treated at length by so many authorities that no reference will be made to them, excepting, if necessary, to more fully explain microscopic structure. I append a list of the literature available at the library of Columbia University College of Pharmacy upon the subject. A further list, and one which is complete up to the date of publication, is given in the English translation of Fluckiger's "Cinchona Barks," by F. B. Power (Blakiston, 1884). In this connection, I would make special mention of a folio volume in French, "*Historie Naturelle des Quinquinas*," M. H. A. Weddell, 1849, which contains excellent illustrations in natural color of many specimens.