over night. By allowing the whole reaction mixture to stand over night we obtain the total yield with one filtration. The crude product is pure enough not to need recrystallization, but may be recrystallized once from hot ethylene dichloride if desired.

REFERENCES.

(1) *Zt. f. Chem.*, 4, 358 (1868).
(2) *Ber.*, 36, 1010 (1903).
(3) *Ber.*, 40, 2862 (1907).
(4) *Ber.*, 32, 3533, footnote (1899).

EVALUATION OF LIME METHODS FOR DETERMINING MORPHINE IN OPIUM.*

BY V. H. WALLINGFORD AND AUGUST H. HOMEYER.**

The literature on the determination of morphine in opium prior to 1920 was reviewed by Jermstad (1) who compared experimentally 16 official and 14 non-official methods. The early work was frequently unscientific and self-contradictory. Recent investigators have viewed the problem more critically and attempted to uncover the errors in the most widely used procedures. Hollman (2) investigated certain features of lime and ammonia methods. Reimers (3) made a critical review of the literature from 1920 to 1930.

Our research leads us to conclude that no method developed so far is satisfactory and that the precision obtainable varies with the type of opium tested. The behavior of pure morphine when subjected to many of the operations typical of lime methods has been studied in detail and the effects of various added substances have been quantitatively determined. Procedures have been devised for evaluating the errors of the principal methods now in use and the method proposed by the Commission of the League of Nations.

"Ammonia Methods" such as Jermstad's (1) are considered unsound in principle because, as pointed out by Reimers (3), the separation of narcotine from morphine by fractional precipitation with ammonia is not sharp. The lime method of the United States Pharmacopœia, Tenth Edition (U. S. P. X), the method of Dr. Joseph Rosin (4)[1] and "ammonia methods" use water to extract morphine from opium. In our experience and that of other workers (2), (5), (6), (7) this extraction is sometimes incomplete due to insufficient natural acidity of the opium.

In the methods of the United States Pharmacopœia X, British Pharmacopœia of 1932 (B. P.) and Rosin empirical aliquot parts are used which do not take into account variable factors of the opium which affect the solution. Our work has shown this practice to be inaccurate; in a particular case a U. S. P. X assay was

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* The subject matter of this paper was submitted privately to H. J. Anslinger, United States Commissioner of Narcotics, and members of the Commission of Experts of the League of Nations engaged in standardizing opium assay methods, in two progress reports dated April 2 and June 25, 1935. Acknowledgment was made in the Quarterly Bulletin of the Health Organization of the League of Nations, Vol. IV, Extract No. 16, page 816.

** Contribution from the Organic Research Laboratory of the Mallinckrodt Chemical Works, St. Louis, Mo., submitted for publication July 24, 1935.

[1] Since this paper was prepared Dr. Rosin's method has been adopted in the U. S. P. XI and becomes official on June 1, 1936.

2 per cent too high on account of this error. Recent workers (1), (2), (8), (9), (10) have recognized this source of error, and the lime method reported by van Itallie (11) for the Commission of the League of Nations (hereafter referred to as the L. N. method) provides for determination of moisture and extractibles and precise calculation of the aliquot.

Since the method reported by van Itallie is being proposed by the League of Nations as an international method, it should be pointed out that this procedure is subject to several errors inherent in all lime methods. We shall discuss these errors briefly at this point before presenting the experimental evidence on which our conclusions are based.

Morphine to the extent of 2 or 3 per cent of the total is carried down into the marc with the precipitate of calcium meconate. Our results are corroborated by Mallory and Valaer[1] who isolated similar amounts of "insoluble" morphine from thoroughly washed lime marcs by a different experimental procedure. Hollman (12) was also of the opinion that adsorption of morphine by the insoluble residue can cause a loss, and Marden and Elliott (13) observed coprecipitation of morphine with barium carbonate. Baumgarten (14) believed that practically none of the alkaloid remained in an insoluble form in the lime residue.

The assay morphine has been found to be contaminated by several per cent of titratible impurities such as by-alkaloids, calcium carbonate, etc. Baggesgaard-Rasmussen, Jackerott and Jespersen (15) found the optical rotation of L. N. assay morphine to be lower than that of the pure substance and methoxyl groups were detected by Zeisel's method. Rakshit (16) isolated 5.65 per cent benzene soluble impurities, including 3.6 per cent codeine, from B. P. assay morphine. By-alkaloids have been found in assay morphine[2] by Smith (17), Hollman (2), (12) and Jermstad (1). Marden and Elliott (13) have shown that water saturated with ether is as good a solvent for codeine as ether saturated with water. Therefore, an ether layer does not remove codeine from the water layer or prevent its coprecipitation with morphine. We have shown that lactose (and probably other substances) raises the assay[3] due, perhaps, to the formation of a titratible calcium complex. This explains in part the high results and impure assay morphine obtained by Jermstad (1) with lime methods. This error was not eliminated by extracting the assay morphine with methanol.

The solubility of morphine in the mother liquor depends in part on the kind and amount of gums, etc., present in the solution. This point was investigated carefully by Hollman (2). The correction adopted by the League of Nations Commission amounts to 28.5 mg. per 25 Gm. of lime filtrate taken in the assay, or 1.14 mg. per Gm., and is admittedly arbitrary. Our own work indicates that the solubility is 0.55 mg. per Gm. of lime solution when the starting material is pure

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[1] Private communication from Messrs. G. E. Mallory and Peter Valaer, Jr., of the Bureau of Internal Revenue, Washington, D. C. Cf. Mallory and Valaer, *Am. J. Pharm.*, 107, 515 (1935).

[2] Recently Rosin and Williams (22) reported finding about 3 per cent by-alkaloids in U. S. P. assay morphine, and that extraction with methanol eliminated approximately 2 per cent of titratible impurities.

[3] Damas (18) found that large amounts of saccharose reduced the assay.

morphine. In the case of one type of opium the solubility was found to be 16 mg. per L. N. assay or 0.66 mg. per Gm. of lime extract.

A method of purifying assay morphine published by Thoms (19) has been used by Jermstad (1) and Hollman (2), and Smith (17) described a method of determining certain impurities. For the purpose of this investigation a method was developed for purifying assay morphine by repeated precipitations under arbitrary but carefully standardized conditions. The morphine could be accounted for quantitatively by the application of a suitable solubility correction. The precision and reproducibility of the method is shown by our experimental work on pure alkaloid and assay morphine.[1] The method proved valuable in the study of lime assays and particularly in determining the purity of assay morphine when no other procedure seemed applicable. Although the process is tedious it has proved valuable in research work. Recently Mannich (20) has developed a method of determining morphine as the 2,4-dinitrophenyl ether which may prove useful.

EXPERIMENTS ON PURE MORPHINE.

It seemed logical to investigate first the behavior of pure morphine when subjected to many of the operations typical of lime methods. The effects of various substances such as lactose, meconic acid and pseudomorphine in the lime method of assay were likewise studied using pure morphine in place of opium. The significance of these results is pointed out later in connection with the experimental data.

Since the reprecipitation procedure is repeatedly referred to in the later discussion it will be described in some detail.

Reprecipitation of Pure Morphine.—In Table I are given the results of reprecipitation of highly purified morphine. The samples of crystal alkaloid were weighed accurately and dissolved in a slight excess of 0.1*N* sulphuric acid and the excess titrated with 0.1*N* sodium hydroxide using methyl red indicator. The average titration of nine samples indicated 93.44% anhydrous base with an average deviation of 0.2%. The percentages given in the third column are based on the average titration.

The solutions were adjusted to the net weights indicated in the fourth column. U. S. P. alcohol and peroxide-free ether were added in the ratio of 2 cc. and 15 cc., respectively, per 30 Gm. aqueous solution. Then 1*N* ammonia was added, series A, 4 cc.; series B, 8 cc.; and series C, 11 cc. After keeping at room temperature with occasional shaking for 4 hours the samples were kept in a refrigerator at 5° C. over night. The morphine was filtered off, washed, dried, dissolved in neutral absolute methanol and titrated as in the L. N. method. The difference in mg. between the anhydrous morphine taken (based on weight and average titration) and the amount titrated after precipitation is tabulated as the loss by the first reprecipitation.

The titrated solutions were concentrated by evaporation on the steam-bath, the residual liquor was transferred quantitatively by several small portions of hot water to a flask, and the morphine was reprecipitated again under exactly the same conditions. The difference between the amounts of morphine recovered from the first and second reprecipitations is given as the loss in the second reprecipitation.

In the last column is given the percentage (based on original weights and average titration) of morphine accounted for by the final titration and a correction for solubility of 0.5 mg. per Gm. of aqueous solution.

In the working range, series A and B, the amounts of alkaloid accounted for by titration and solubility correction are in good agreement with the quantities taken. At high dilution, as in series C, a slight increase in solubility loss is indicated.

[1] Professor R. Eder of the Institute of Pharmacy, Zürich, reported similar results by a related method to the Commission of Experts in July 1935. The information was submitted to us privately.

TABLE I.—REPRECIPITATION OF PURE MORPHINE.

No.	Morphine Taken, Gm. (93.44% Anhydrous Alkaloid).	Morphine by Titration, %.	Aqueous Solution, Gm.	Loss by Reprecipitation, Mg. 1st.	2nd.	% Accounted for by 0.5 Mg. per Gm. Correction.
A1	0.6000	99.5	30	11.6	14.2	100.7
2	0.6000	100.0	30	10.2	14.2	100.9
3	0.6000	100.2	30	13.0	14.3	100.4
B1	1.2000	100.1	60	24.8	..	100.5
2	1.2000	100.2	60	26.2	31.3	100.2
3	1.2000	100.3	60	23.3	34.2	100.2
C1	1.0000	99.8	120	74.5	69.9	97.4
2	1.0000	99.9	120	63.2	75.5	98.0
3	1.0000	99.9	120	67.4	69.9	98.2

The precision of the method is borne out further by the results obtained in reprecipitating L. N. and U. S. P. assay morphine where impurities were removed by the first and second reprecipitations and no loss occurred thereafter.

Solubility of Morphine in Mother Liquor from Lime Process.—In order to determine the solubility of morphine in the mother liquor from lime methods two sets of experiments were made using highly purified samples of morphine alkaloid.

Three 1.2000-Gm. samples of morphine alkaloid crystals A were placed in tared flasks, dissolved in a slight excess of 0.1*N* sulphuric acid, diluted and the excess acid titrated with 0.1*N* sodium hydroxide using methyl red indicator. The solutions were adjusted to 60.0 Gm. and treated with 2 Gm. calcium hydroxide. After shaking for 1 hour the solutions were filtered and 30.0 Gm. of the filtrate (one-half aliquot) was treated with 2 cc. U. S. P. alcohol, 15 cc. peroxide free ether and 1 Gm. ammonium chloride A. R. After keeping at room temperature for 3 hours with occasional shaking and in a refrigerator at 5° C. over night, the precipitated morphine was filtered off, washed, dried, dissolved in neutral absolute methanol and titrated as in the L. N. method. The difference between one-half the original amount of morphine taken and the final amount recovered was the loss due to solubility in 30 Gm. of lime filtrate.

In a second group 1.2000-Gm. samples of crystal alkaloid B were subjected to the same procedure except that the amounts of water and all other reagents were doubled. In this case the loss due to solubility was calculated for 60 Gm. of lime filtrate.

The data are presented in Table II. In the final column is given the solubility in mg. per Gm. of lime filtrate used for precipitation.

TABLE II.—SOLUBILITY OF MORPHINE IN LIME MOTHER LIQUOR.

No.	Morphine Taken, Gm. (by Titration).	Morphine Recovered, Gm. (by Titration).	Loss, Gm.	Solubility Mg. per Gm.
A1	1.1165	0.5419	0.0163	0.55
2	1.1166	0.5390	0.0193	0.64
3	1.1180	0.5405	0.0185	0.62
B1	1.1222	0.5305	0.0306	0.51
2	1.1222	0.5319	0.0292	0.49
3	1.1208	0.5319	0.0285	0.48
			Average	0.55

Effect of Dissolving Assay Morphine in Methanol.—In order to determine the effect of drying the assay morphine and dissolving it in methanol as in the L. N. procedure two series of parallel experiments were made.

Method I. One-Gm. samples of pure morphine alkaloid crystals were dissolved in an equivalent amount of sulphuric acid, made up to 50 cc. in a volumetric flask and shaken thoroughly. The solution was mixed with 2 Gm. calcium hydroxide, shaken mechanically for 30–45 minutes, filtered and 25 cc. of filtrate (one-half aliquot) taken for precipitation. Two cc. U. S. P. alcohol, 15 cc. peroxide-free ether and 1 Gm. ammonium chloride A. R. were added. After shaking for 15 minutes and keeping about 18 hours at 5° C., the assay morphine was filtered off on a

3G4 sintered glass funnel, washed, dissolved in excess 0.1*N* sulphuric acid and titrated. The morphine found in 6 determinations, calculated on the basis of anhydrous alkaloid used, averaged 96.33% with an average deviation of 0.27%.

Method II. The above experiments were repeated with the modification of drying the assay morphine on the funnel at 100°, dissolving in neutral absolute methanol and titrating as in the L. N. method. A slight insoluble residue remained on the filter. The morphine found in three determinations averaged 94.92% with an average deviation of 0.34%. This was 1.4% lower than by Method I showing contamination of the assay morphine by basic, methanol insoluble impurities, probably calcium carbonate.

Effect of Meconic Acid.—By adding meconic acid to the morphine and carrying out the assay as in Method I, losses up to 3.4% were experienced probably due to coprecipitation of the alkaloid with calcium meconate which forms a gelatinous, voluminous precipitate.

TABLE III.—COPRECIPITATION OF MORPHINE WITH CALCIUM MECONATE.

Blanks by Method I Averaged 96.33% ± 0.27%.

Meconic Acid Added, Gm.	Morphine Found (by Titration), %.	% Loss by Coprecipitation.
0.5	92.96	3.4
0.5	93.14	3.2
0.2	92.97	3.3
0.2	94.20	2.1

Effect of Lactose.—Since lactose is used in diluting opium to pharmacopœia strength its effect on the assay was determined by Methods I and II.

TABLE IV.—EFFECT OF LACTOSE IN RAISING THE ASSAY.

Blanks by Method I Gave 96.33% ± 0.27%.

Lactose Added, Gm.	Morphine Found (by Titration), %.	Increase Due to Lactose, %.
0.2	97.60	1.3
0.5	97.77	1.4
0.5	96.89	0.6
1.0	98.26	2.0
1.0	99.91	3.6
1.5	98.85	2.5
2.0	98.91	2.6
	Average 98.33	2.0

Blanks by Method II Gave 94.92% ± 0.34%.

Lactose Added, Gm.	Morphine Found (by Titration), %.	Increase Due to Lactose, %.
2.0	96.51	1.6
2.0	96.85	1.9
2.0	96.68	1.8
	Average 96.68	1.8

The results in Table IV show that the presence of lactose raises the assay, perhaps due to formation of a basic complex with calcium which contaminates the assay morphine. The error is not avoided by dissolving the morphine in methanol. Probably other substances behave similarly.

Oxidation and the Effect of Pseudomorphine.—In order to determine whether morphine is oxidized appreciably in lime water by oxygen a stock solution of morphine sulphate was prepared and 50 cc. portions were assayed by Method II. The mixtures with lime were shaken and allowed to stand for various lengths of time in atmospheres of nitrogen and oxygen. As shown in Table V, it was found that no loss occurred in an atmosphere of nitrogen and the solutions remained light in color, but in an atmosphere of oxygen there was a marked loss of morphine, the solution

became dark and the precipitated alkaloid contained a dark, methanol insoluble impurity. The percentage recovered was figured on the basis of the average of the first and second determinations.

TABLE V.—OXIDATION OF MORPHINE BY OXYGEN IN LIME SOLUTION.

Total Time in Lime, Hrs.	Time Shaken, Hrs.	Atmosphere.	Color of Solution.	Morphine Recovered, % (by Titration).
0.5	0.5	Air	Light	99.9
0.5	0.5	Air	Light	100.3
24.0	2.0	Nitrogen	Light	99.9
24.0	2.0	Oxygen	Dark	90.3
24.0	2.0	Oxygen	Dark	90.3
48.0	2.0	Nitrogen	Light	100.3
48.0	2.0	Oxygen	Dark	60.3
48.0	2.0	Oxygen	Dark	63.6
48.0	2.0	Oxygen	Dark	58.8

It is concluded, therefore, that prolonged exposure of alkaline morphine solutions to oxygen results in loss by oxidation. The loss during an ordinary assay, where the time of exposure is less than one hour, is probably not significant.

Since pseudomorphine is the most common oxidation product of morphine, its fate in a lime assay was investigated. Pseudomorphine was prepared by oxidation of morphine with potassium ferricyanide (21). In one experiment 0.5 Gm. pseudomorphine sulphate was treated as in Method I, morphine being absent. Only a trace of precipitate was obtained by adding ammonium chloride to 25 cc. of filtered lime solution, and its titre, calculated as morphine, amounted to 26 mg. In another experiment by Method I using 1 Gm. morphine alkaloid, 0.5 Gm. pseudomorphine alkaloid was added to the lime solution. The recovery of morphine was 96.66% compared to blanks containing no pseudomorphine of 96.33%. It is concluded, therefore, that pseudomorphine contaminates the assay morphine from a lime process only to a limited extent.

INVESTIGATION OF THE LEAGUE OF NATIONS METHOD.[1]

The sample was a mixture of Jugoslavian and Turkish opiums which lost 11.74% moisture on drying at 60–70° and was ground to pass a 50-mesh sieve.

The moisture of the ground opium was determined by drying 1-Gm. samples at 105° in an electric oven[2] (Table VI).

TABLE VI. DETERMINATION OF MOISTURE IN OPIUM.

	Sample No. 1.	Sample No. 2.
Loss after 3 hours	4.22%	4.12%
Loss after 4 hours	4.49%	4.59%
Loss after 24 hours	4.78%	4.87%
Loss after 48 hours	5.36%	5.32%

Extractives were determined on three occasions by the method prescribed in the L. N. method (Table VII).

TABLE VII.—DETERMINATION OF EXTRACTIVES IN OPIUM.

Set No. 1	46.1%	46.1%
Set No. 2	44.9%	44.6%
Set No. 3	47.0%	47.1%
Average 46.2%		

[1] A copy of the method, dated Leyden, October 17, 1933, was received from H. J. Anslinger, United States Commissioner of Narcotics.

[2] According to Baggesgaard-Rasmussen and Co-Workers (15) the loss of weight is most reproducible by heating at 100° at 1–2 mm.

Nine determinations of morphine were made in groups of three by the method prescribed by the Commission. In order to adhere to conditions used throughout this work the precipitation was carried out by keeping the mixture at room temperature for about 4 hours with occasional shaking and then in a refrigerator at 5° C. over night. The results in Table VIII, given as per cent anhydrous morphine in the ground opium sample, were calculated by the L. N. formula, which includes a solubility correction of 28.5 mg., using 5.3% as the moisture and 46.2% as the extractives in the opium. The amount of morphine indicated by titration, calculated as anhydrous base, is tabulated in the next column.

The titrated solutions of assay morphine were saved for investigation of the purity. The amounts of extractives being known from previous determinations, all the lime extract of opium in excess of 25.0 Gm. used for precipitation of morphine was saved with the marc, acidified and worked up as described in a later paragraph. The filtered mother liquor and ether washings from the assay morphine were kept separate from washings with morphinated water, acidified and saved for determination of unprecipitated morphine.

Purity of the Assay Morphine.—The purity of the assay morphine was determined by reprecipitation. The titrated solutions of assay morphine, containing 5 drops excess acid, were combined in groups of three and evaporated to a small volume on the steam-bath under an electric fan. The concentrate was transferred to a tared Erlenmeyer flask and made up to 45 Gm. The transfers were made quantitatively by use of several small portions of hot water. Three cc. U. S. P. alcohol, 22.5 cc. peroxide-free ether and 5.5 cc. 1*N* ammonia were added. The process was completed as previously described under reprecipitation of pure morphine. Further reprecipitations were made in the same way. The amount of morphine accounted for by titration and solubility correction (0.5 mg. per Gm. of aqueous solution) is given in Table VIII expressed as a percentage of the original morphine indicated by titration. Experience has shown, and it is indicated by the data, that after two or three reprecipitations the assay morphine is pure and no further loss occurs in subsequent precipitations.

TABLE VIII.—PURITY OF L. N. ASSAY MORPHINE.

No.	Results of L. N. Assays.	Morphine Isolated (Original Titration).	Purity of Isolated Morphine by Reprecipitation. 1st.	2nd.	3rd.	4th.
1	16.43%	0.3619 Gm.				
2	16.41%	0.3614 Gm.	98.3%	96.6%	96.2%	96.4%
3	16.53%	0.3642 Gm.				
4	16.31%	0.3591 Gm.				
5	16.31%	0.3591 Gm.	97.5%	96.3%	96.0%	..
6	16.20%	0.3563 Gm.				
7	16.31%	0.3591 Gm.				
8	16.37%	0.3605 Gm.	97.3%	96.3%	96.3%	..
9	16.43%	0.3619 Gm.				
Average	16.37% ± 0.07%	0.3604 Gm.				96.2%

Average amount of pure morphine isolated per assay = 0.3604 × 0.962 = 0.3467 Gm.

Unprecipitated Morphine.—In order to determine the amount of morphine remaining in the mother liquor, for which a correction of 28.5 mg. is allowed in the L. N. procedure, the filtrates and ether washings from the original nine L. N. assays were kept separate from the washings with morphine water, combined in groups of three and acidified with sulphuric acid. The calcium sulphate was filtered off on a sintered glass funnel and washed several times with small portions of water. The by-alkaloids were separated and the morphine isolated by applying the "shake-out" method of Stucki (8). The acid solution was extracted twice with 50 cc. portions of a 3 to 1 mixture of chloroform and isopropanol which removed some gums. The extract was washed twice with 10 cc. of 0.1*N* sulphuric acid to recover any morphine and the washings were added to the main portion. This was made alkaline to phenolphthalein with 10*N* sodium hydroxide and 2 cc. were added in excess. The by-alkaloids were extracted with 75-, 75- and 50-cc. portions of chloroform-isopropanol, the extracts being washed twice with 10 cc. of 0.1*N* sodium hydroxide and the washings returned to the main portion. Three grams ammonium sulphate were added and

the morphine was extracted with 75, 75, 75 and 50 cc. of chloroform-isopropanol. These extracts were filtered and evaporated to dryness. The residue was a dark, varnish-like gum which was dissolved in absolute methanol and titrated; a precipitate formed near the neutral point. The amounts of crude morphine found are given in Table IX.

The titrated solutions were combined, evaporated and the morphine reprecipitated from 40 Gm. of aqueous solution as described for pure morphine. The amounts of morphine accounted for (by titration and solubility) after each reprecipitation are given in Table IX.

TABLE IX.—MORPHINE UNPRECIPITATED FROM MOTHER LIQUOR.

No.	Crude Morphine Isolated, Gm.	Pure Morphine by Reprecipitation. 1st.	2nd.	3rd.
1–3	0.100			
4–6	0.093	0.214 Gm.	0.177 Gm.	0.148 Gm.
7–9	0.100			

The precipitates from the first and second reprecipitations were deeply colored, slimy and left black residues when dissolved in absolute methanol. The final product was granular and fairly light in color. Although the precision of this determination was probably affected somewhat by the nature and amounts of impurities, the result is believed to be approximately correct. The final quantity, 0.148 Gm., was the anhydrous morphine dissolved in the mother liquors from nine L. N. assays. Therefore, the solubility per assay was 0.0164 Gm. compared to 0.0285 Gm. allowed as an arbitrary correction in the Commission's method. The amount found is equivalent to a solubility of 0.66 mg. per Gm. of aqueous solution which is in fair agreement with the loss of 0.55 mg. per Gm. suffered when pure morphine was subjected to a lime assay. As shown by Hollman (2) the presence of gums and other substances in the solution affects the solubility, so the amount of unprecipitated morphine may vary with different opium extracts.

Morphine in the Marc.—For the purpose of determining whether or not all morphine was in solution at the time the aliquot was taken in the L. N. method, the lime marc and all liquor in excess of the 25 Gm. used for precipitating the morphine were washed into a beaker and acidified to bromphenol blue with sulphuric acid. The volume was about 200 cc. After digesting for 1 hour at 80° it was filtered on a Büchner funnel by suction and the residue was extracted twice more with 100-cc. portions of acidified water. The quantitative isolation and purification of this morphine proved difficult, the marcs from the first six assays being used in working out a satisfactory method. It was finally accomplished by clarifying the solution by a method similar to that devised by Buchbinder[1] and removing the by-alkaloids and isolating the morphine according to the method of Stucki (8).

The acid extract, amounting to about 400 cc., was evaporated to 100 cc. and transferred quantitatively with hot water to a tared 200-cc. volumetric flask. Forty grams of powdered sodium chloride was dissolved in the liquor. The solution was neutralized with 10% sodium hydroxide and 20 cc. was added in excess. Twenty cubic centimeters saturated barium chloride solution were added. Then the flask was filled to the mark with water, shaken vigorously and weighed. The solution was filtered and 150 cc. were used for isolation of the morphine. Complete data were taken for calculation of the aliquot which was found to be 0.760 and 0.755 for two samples when precipitated solids were taken into account. The average aliquot was applied to the third sample. It is recognized that coprecipitation of morphine may occur in the clarification step, hence the amount of morphine found in the marc should be considered a minimum figure.

By-alkaloids were removed by extracting the alkaline filtrate three times with 75-cc. portions of a 3 to 1 mixture of chloroform and isopropanol. The organic layers were washed with two 10-cc. portions of 0.1*N* sodium hydroxide to recover any morphine and returned to the main aqueous layer. This was acidified with hydrochloric acid, made alkaline with ammonia and the morphine extracted with 75, 75, 50 and 50 cc. of chloroform-isopropanol. The solvent layers were filtered, evaporated to dryness and the residue dissolved in absolute methanol and titrated. The amounts of crude morphine isolated from the marcs, taking into account the aliquot, are given in Table X. The titrated solutions were evaporated together and reprecipitated as previously described. The purity, based on the original amount found by titration, is given in Table X.

[1] Private communication from the Bureau of Narcotics.

Using the data from Table X the average amount of morphine remaining in the excess liquor and marc was 0.297 × 0.883 = 0.2623 Gm.

TABLE X.—MORPHINE EXTRACTED FROM LIME MARC.

No.	Crude Morphine in Marc.	Purity by Reprecipitation. 1st.	2nd.	3rd.
7	0.302 Gm.			
8	0.289 Gm.	89.8%	88.6%	88.3%
9	0.301 Gm.			
Average	0.297 Gm.			

The total amount of solution originally employed in the L. N. assay is the sum of the water, moisture in the opium and extractives:

Water	40.0 Gm.
Moisture in opium = 5.3% × 4 =	0.2 Gm.
Extractives = 46.2% × 4 =	1.9 Gm.
Total Solution	42.1 Gm.

Twenty-five grams of this, the aliquot, yielded 0.3467 Gm. pure morphine by precipitation and the mother liquor contained 0.0164 Gm. or a total of 0.3631 Gm. Then the excess liquor, 42.1 − 25.0 = 17.1 Gm. would contain $\frac{17.1 \times 0.3631}{25.0} = 0.2484$. Gm. Since the excess liquor and marc yielded 0.2623 Gm. pure morphine, the marc held 0.2623 − 0.2484 = 0.0139 Gm. morphine in the undissolved state, probably coprecipitated with calcium meconate. This amount, which is 2.2% of the total morphine in the opium, is in good agreement with the loss by coprecipitation (2–3%) when a solution of pure morphine and meconic acid was subjected to a lime assay.

Check of Solubility Corrections.—In order to check the precision of solubility corrections applied in the foregoing reprecipitations of morphine isolated from various parts of the L. N. process, the mother liquors from reprecipitation of the assay morphine and that obtained from the original L. N. mother liquors were acidified and saved.

The amounts of morphine which had been allowed were:

4 Mother Liquors from assay morphine 1–3	0.0900 Gm.
3 Mother Liquors from assay morphine 4–6	0.0675 Gm.
3 Mother Liquors from assay morphine 7–9	0.0675 Gm.
Morphine isolated from L. N. Mother Liquors 1–9, and mother liquors from its reprecipitation	0.1484 Gm.
Total morphine allowed	0.3734 Gm.

These liquors were combined, made ammoniacal and extracted 4 times with 100-cc. portions of chloroform-isopropanol. The extract was concentrated to about one-half its volume and the morphine was extracted with two 50-cc. portions of 0.1*N* sodium hydroxide and washed twice more with 30 cc. of water. The combined alkaline extracts were treated with 1 Gm. ammonium sulphate to liberate the morphine which was extracted with four 100-cc. portions of the immiscible solvent. The extracts were filtered and evaporated to dryness. The residue was a black, varnish-like gum, the titration of which was only approximate due to the color of the solution. The titre indicated 0.448 Gm. morphine. The first reprecipitation accounted for 0.379 Gm. morphine and the second for 0.347 Gm. of alkaloid of good quality. This is in good agreement with the quantity (0.3734 Gm.) assumed to have been present.

Summary of Results on League of Nations Method.—The pure morphine found in the various parts of the L. N. assay may be summarized as follows:

Precipitated from 25.0-Gm. filtrate	0.3467 Gm.
Dissolved in mother liquor	0.0164 Gm.
In excess liquor	0.2484 Gm.
Undissolved in marc	0.0139 Gm.
Total from 4 Gm. opium	0.6254 Gm.
Per cent morphine in opium	15.63

The difference of 0.74% between this figure and 16.37%, the average result of the original 9 assays, is made up of errors due to impurities in the assay morphine, incorrect solubility correction and coprecipitation of morphine in the lime marc.

EXPERIMENTS ON U. S. P. X AND ROSIN METHODS.

The U. S. P. X and Rosin methods are closely related and are subject to the same inherent errors as the improved lime method proposed by the League of Nations Commission as regards coprecipitation of morphine in the lime marc and impurities in the assay morphine. No correction is applied in these methods for the solubility of morphine in the mother liquor, which is probably of the same magnitude as in the L. N. procedure. The opium is extracted (exhaustively) with water which is sometimes incomplete due to insufficient natural acidity to form salts with all alkaloids. The concentrated extract is treated with lime and an empirical aliquot is taken by volume in the U. S. P. method and by weight in the Rosin method without taking into consideration the solids precipitated by lime. This last-mentioned error and the purity of U. S. P. assay morphine have been examined experimentally.

Precision of the Aliquot.—Four 8-Gm. samples of opium were extracted and evaporated as in the U. S. P. X method. The concentrates were combined in a 250-cc. volumetric flask and made up to the mark. In this solution the concentration of extractives was essentially the same as in the solution used for treatment with lime in the U. S. P. X and Rosin methods.

Three-gram samples of this solution were evaporated and the residue dried at 105° to determine solids. Fifty-cubic centimeter samples were measured into tared flasks and weighed. Four grams powdered calcium hydroxide were added, the mixture shaken for 1 hour and filtered. Twenty-five-cubic centimeter portions of filtrate were measured into tared flasks and weighed. Further 3.00-Gm. samples of filtrate were evaporated and dried to determine solids. From this data, given in Table XI, the actual aliquot was calculated and compared with the empirical aliquots of the U. S. P. X and Rosin methods.

TABLE XI.—PRECISION OF EMPIRICAL ALIQUOTS.

	Sample No. 1.	Sample No. 2.
(*a*) Solids from 3.00-Gm. liquor	0.2313 Gm.	0.2261 Gm.
(*b*) Wt. of 50.00-cc. liquor	50.98 Gm.	50.97 Gm.
(*c*) Wt. of 25.00-cc. lime filtrate	25.42 Gm.	25.44 Gm.
(*d*) Solids from 3.00-Gm. filtrate	0.1646 Gm.	0.1650 Gm.

$$\text{Actual Aliquot} = \frac{c - d\left(\frac{c}{3}\right)}{b - a\left(\frac{b}{3}\right)}$$

0.5107 0.5101

Average 0.5104

$$\text{Aliquot by volume (U. S. P. X)} = \frac{25}{50} = 0.5000$$

$$\text{Aliquot by weight (Rosin)} = \frac{c}{b}$$

0.4986 0.4991

Average 0.4989

Therefore, the error introduced by taking the aliquot by volume would be

$$\frac{0.5104 - 0.5000}{0.5104} \times 100 = 2.04\%$$

Similarly the aliquot taken by weight would be in error 2.26%. It is evident that considerable solids were precipitated by lime, the percentage being 7.63 before and 5.49 after lime treatment.

The above experiment on precision of the aliquot was repeated on another sample of opium which yielded an extract containing 7.32% solids before and 5.81% after lime treatment. The actual aliquot was 0.5072 instead of 0.5000 as assumed in the U. S. P. X aliquot by volume. It is obvious that other opiums may contain considerably different amounts of water extractible and

lime precipitable materials. Then the magnitude of the error would be changed, but it would always raise the assay since solids are thrown out of solution by the lime.

Experiments on the empirical aliquot used in the B. P. 1932 assay showed that 52 cc. of lime filtrate represented 5.011, 5.012, 5.009, 5.012, average 5.011 Gm. opium instead of the assumed 5.000 Gm. or an error of 0.22%. It must be emphasized, however, that the error would vary markedly with moisture and extractives in the opium.

Purity of U. S. P. Assay Morphine.—Seven U. S. P. X assays were carried out in the official manner up to the filtration of the assay morphine. This was collected on a sintered glass funnel, washed as usual, dried at 100° and weighed. The amounts obtained were 0.700, 0.690, 0.696, 0.707, 0.672, 0.670 and 0.702 Gm. These were combined, thoroughly mixed and portions examined by the following methods.

Three 0.5-Gm. samples (Table XII) were titrated and purified by reprecipitation in the presence of alcohol and ether as previously described. Corrections were applied for loss due to solubility and the purity was calculated on the basis of the original titration.

TABLE XII.—PURITY OF U. S. P. X ASSAY MORPHINE BY REPRECIPITATION.

Wt. Taken.	Anhydrous Morphine, Indicated by Titration, Gm.	Purity by Reprecipitation. 1st.	2nd.
0.5000	0.4663	94.1%	92.1%
0.5000	0.4677	94.4%	..
0.5000	0.4663	93.5%	90.6%

Two 1-Gm. samples were dissolved in absolute methanol as in the L. N. method and titrated. The solutions were evaporated and transferred to a separatory funnel; added 2.0 cc. 10*N* sodium hydroxide and extracted with 40-, 30- and 30-cc. portions of a 3 to 1 mixture of chloroform and isopropanol to remove by-alkaloids. The extracts were washed counter-currentwise with a little water to recover any morphine. The chloroform layers containing non-phenolic alkaloids were combined, evaporated, the residue dissolved in neutral methanol and titrated. The morphine was precipitated from the aqueous layers in the presence of alcohol and ether, determined in the usual manner, and a correction of 0.5 mg. per Gm. of solution added for solubility. The results were calculated as percentage of the original titration and are presented in Table XIII. Further loss in titratible material due to water-soluble impurities is indicated in the last column.

TABLE XIII.—IMPURITIES ACCOMPANYING U. S. P. ASSAY MORPHINE.

Wt. Taken Gm.	Anhydrous Morphine, Indicated by Titration, Gm.	Non-Phenolic Alkaloids.	Morphine Recovered.	Water-Soluble Impurities.
1.0000	0.9298	3.83%	91.5%	4.67%
1.0000	0.9326	3.52%	92.3%	4.18%

It is concluded, therefore, that the titration of U. S. P. assay morphine from this opium included 8–9% impurities of which 3.6% were non-phenolic alkaloids and 4.4% were water-soluble, basic substances.

U. S. P. assay morphine was prepared from another sample of opium and purified by reprecipitation. The purity was calculated as a percentage of the original titration (Table XIV).

TABLE XIV.—REPRECIPITATION OF U. S. P. X ASSAY MORPHINE.

Wt. Taken Gm.	Anhydrous Morphine, Indicated by Titration, Gm.	Purity by Reprecipitation, %. 1st.	2nd.	3rd.
1.0000	0.9168	96.8	96.6	96.6
1.0000	0.9154	97.0	96.8	96.7
1.0000	0.9154	97.5	96.8	96.8

In this case the assay morphine was much purer (96.7%) than that yielded by the previous sample of opium (92%) by the same assay method.

SUMMARY AND CONCLUSIONS.

The behavior of pure morphine in a lime assay has been studied and procedures have been described for investigating methods of opium analysis. The principal lime methods, including the one proposed by the Commission of the League of Nations, have been thoroughly investigated. The sources of error, and their magnitude in particular cases, have been pointed out. It has been found that:

(*a*) Morphine is coprecipitated with calcium meconate.

(*b*) The solubility correction used in the League of Nations method is approximately double the amount of morphine that can be extracted from the mother liquor, and is double the loss suffered by pure morphine when it is substituted for opium in the assay.

(*c*) The assay morphine is contaminated by basic impurities, including byalkaloids and calcium salts.

(*d*) Substances such as lactose raise the assay.

(*e*) Empirical aliquots are inaccurate.

The errors of lime methods vary depending upon the type of opium. It has been our experience that the results are generally too high. It is concluded that the ultimate solution of the problem does not lie in the direction of lime methods.

We are now engaged in the study of methods based on extraction with immiscible solvents which we expect to make the subject of a later paper. Such methods offer many desirable features but in their present form appear to be unsatisfactory in certain details such as size of sample, the nature of the immiscible solvent and the purity of the assay morphine.

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THE BIOASSAY OF VERATRUM VIRIDE.*,[1]

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Although the U. S. P. X recognizes no chemical or biological assay for *Veratrum viride*, it is a well-known fact that the preparations of this drug vary greatly in potency (1, 2). This variation in potency and the lack of a satisfactory assay method seem to be the only explanation for the fact that this drug has been almost completely discarded.

A chemical assay has been suggested (3, 4), but since the activity of *Veratrum viride* is due to a number of alkaloids which differ qualitatively and quantitatively in action (5) it would appear that a chemical assay would not be sufficient. This has been pointed out by several workers (1, 6).

Houghton and Hamilton (7) reported a biological assay of *Veratrum viride* based upon the M. L. D. per Gm. body weight of frog. Pilcher (2) did further work with this method and concluded that for all practical purposes the frog assay appeared to be satisfactory.

Rowe (8), in reporting a biological assay based upon the M. L. D. for white mice, made the following comment on the indefinite end-point of the frog method and the length of time required: "A whole series of frogs given graded doses may be found fifteen hours later to be more dead than alive, but still they are not dead and even a skilled technician hesitates about drawing any conclusions." In our experience the mouse method has the same objection. The advantages claimed for the mouse method were that fewer animals were needed and that the assay required less time. Pilcher (2) used an M. L. D. for guinea pigs but preferred the frog method.

Veratrum viride is a centrally acting cardiac depressant. In therapeutic doses it has a selective stimulating action on the vagus nerve (9). It has long been known that large doses cause emesis. Some workers claim that the emesis is central, while some claim that it is local. Hanzlik (10) states that the emesis of Veratrum is the result of local irritation, and that it is produced by intraperitoneal but not by intravenous injections.

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