2. The ammonia chloroform method has been critically studied and it is shown that a large excess of ammonia is necessary for complete liberation of the alkaloids.

3. A demonstration of physiological assay suggests another method for confirming the chemical methods used.

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THE QUANTITY OF ALKALOID IN HYOSCYAMUS AND A NEW METHOD FOR ITS EVALUATION.

BY H. R. WATKINS AND S. PALKIN.

In a recent paper on automatic devices for extracting powdered materials, striking results are shown with hyoscyamus.[1] Further work has shown that in some cases the new method gives yields of alkaloid in this drug more than three times as great as those obtained by Pharmacopœial (IX and X) procedures. The data on samples of hyoscyamus in Table I illustrate this point.

TABLE I.

TOTAL ALKALOID IN HYOSCYAMUS BY U. S. P. AND AUTOMATIC EXTRACTOR METHODS.

Sample.	Total alkaloid. U. S. P. method. Per cent.	Total alkaloid. Automatic extractor method. Per cent.	Sample.	Total alkaloid. U. S. P. method. Per cent.	Total alkaloid. Automatic extractor method. Per cent.
H-N	0.026	0.118	4A	0.104	0.200
	0.023	0.123		0.102	0.202
		0.121	5A	0.059	0.230
		0.124		0.056	0.234
C-9031	0.042[a]	0.172	6A	0.068	0.198
	0.038	0.177		0.084[a]	
P-11216	0.099[a]	0.164	7A	0.061	0.191
	0.103	0.164		0.052	0.191
1A	0.056	0.177	8A	0.175	0.315
	0.064	0.181		0.142	0.321
2A	0.069	0.215	9A	0.055	0.177
		0.210		0.050	0.181
3A	0.123	0.200			
	0.122	0.200			

[a] Assays by U. S. P. X Method; all other assays in this column by U. S. P. IX Method, prior to January 1, 1926.

[1] Palkin and Watkins, *Ind. Eng. Chem.*, 19, 535 (1927).

Although the results in Table I are not to be ascribed entirely to the use of the extracting device, but are in a measure due to the manner of treatment of the powdered material preparatory to extraction, as is shown by experiments to be described later, the fact remains that Pharmacopœial methods of evaluation of total alkaloids in hyoscyamus are seriously defective in that only a fraction of the alkaloid present is extracted and determined.

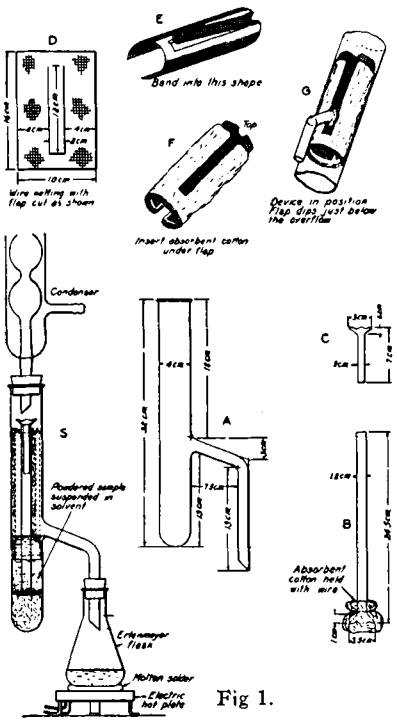

Fig 1.

The essential steps in the new procedure are as follows: (1) Treatment of the mass of crude drug with an alkaline medium to liberate the alkaloid; (2) continuous extraction by means of the device described,[1] using mainly ether as an extracting solvent; (3) purification process of the alkaloidal residue, in which chlorophyl, tarry and other plant extractives are removed by precipitation in acid solution; (4) extraction of the alkaloid from aqueous medium, using another automatic device,[1] and (5) titration of the alkaloid obtained.

All treatments, purifications, extractions, etc., are carried out in such a manner as to insure the stability of the hyoscyamine, as described in a previous publication.[2]

The steps in this experimental study were in a general way taken up in the reverse order of the process described under "Method," to make certain that while the effect of varying conditions in any one step was being studied no errors or additional variables were being introduced into subsequent steps. Although some 12 samples of hyoscyamus have been examined and reported on in Table I, the experimental study was in the main carried out on one sample (C 9031), a large quantity of which was available.

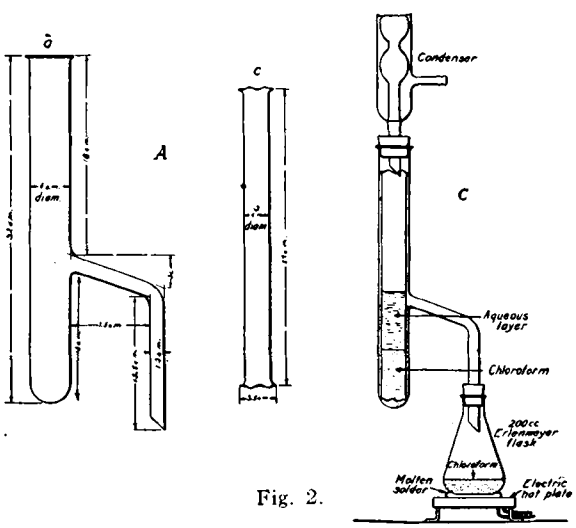

Fig. 2.

Steps 4 and 5.—The extraction of alkaloids from aqueous medium and their titration were carried out as previously described in a paper on automatic extraction

[1] Palkin, Murray and Watkins, *Ind. Eng. Chem.*, 17, 612 (1925).

[2] Palkin and Watkins, JOUR. A. PH. A., 16, 21 (1927).

devices for liquid preparations,[1] due precaution being observed in the extraction and titration to insure the stability of the hyoscyamine.[2]

Step 3: Purification of the Alkaloidal Residue.—The ethereal extract obtained in the continuous extraction of the crude drug contains, in addition to the alkaloid, a large quantity of chlorophyl and tarry-like plant extractives. It does not lend itself to purification by immiscible solvents in the usual way because of difficult emulsions. It was found that the bulk of the impurities could be thrown out in a finely divided state from the acid solution by a procedure similar to that used in the dealcoholization and purification treatment for nux vomica and belladonna fluidextract described in another publication.[3] Certain modifications are necessary here to insure complete removal of alkaloidal salt by repeated treatments with acid. Since the complete removal of the alkaloidal salt from the tarry residue involves repeated treatments of this residue in chloroformic solution with acid and repeated removals of the solvent to precipitate this tarry residue in a finely divided state, the question arose as to the optimum acidity for such a procedure. To reduce the conditions of experiment to the one variable, the combined ethereal extract from several charges was diluted to 500 cc. and 50-cc. aliquots were subjected to this purification treatment, using varying concentrations of acid. The extraction and titration of alkaloid were carried out uniformly, as given in Steps 4 and 5, under "Method." The results (Table II) show virtually no variation over an acid range from 20th normal to normal.

TABLE II.

EFFECT OF "ACID PURIFICATION" TREATMENT ON YIELD OF ALKALOID.

Variable—Acid concentration.

Acid used. (10 cc.).	Yield of alkaloid. Mg.	Per cent.
0.05*N*	12.96	0.154[a]
	14.70	0.175
0.1*N*	14.12	0.168
	14.70	0.175
0.2*N*	14.12	0.168
	14.12	0.168
N	14.12	0.168
	14.70	0.175

[a] Error, probably due to partial hydrolysis.

TABLE III.

EFFECT OF AMMONIA CONCENTRATION ON YIELD OF ALKALOID.

Ammonia. Quantity, cc.	Normality.	Alkaloid. Mg.	Per cent.
5	5	19.09	0.159
		16.49	0.138
4	16	20.54	0.171
		21.12	0.176
7	16	20.54	0.171
		20.54	0.171
9	16	20.54	0.171
		21.12	0.171

Steps 1 and 2: Treatment of the Crude Drug for Liberation of the Alkaloid Preparatory to Extraction.—The ratios of aqueous ammonia, alcohol and ether determined by a few preliminary experiments were adopted because they yielded a homogeneous medium and did not produce two layers when diluted with more ether during the extraction process. It then became necessary to determine the optimum ammonia concentration for the liberation of the alkaloid, bearing in mind the fact that straight aqueous ammonia for the maceration (without admixture of alcohol) is not feasible because of the instability of hyoscyamine under those conditions.

[1] Watkins and Palkin, JOUR. A. PH. A., 14, 1099 (1925).

[2] Palkins and Watkins, *Ibid.*, 16, 21 (1927).

[3] Palkin and Watkins, *Ibid.*, 13, 694 (1924).

It was not inconceivable that a too low ammonia concentration would be ineffective in liberating the alkaloid, and that a very high concentration of ammonia might be destructive of alkaloid.

In this series of experiments the alcohol-water (from ammonia-water) ratio was kept constant while the ammonia concentration was varied and all other conditions, such as maceration period, extraction, purification and determination, were carried out uniformly. An increased alkaloid yield is evident when the ammonia concentration is raised from 5 cc. 5 *N* (equivalent to 25 cc. *N*) to 4 cc. 16 *N* (equivalent to 64 cc. *N*) and practically no difference is observed when the concentration is further increased (Table III). All the titrated alkaloidal solutions were found to be of full hyoscyamine mydriatic strength when tested by the cat-eye method.[1]

Maceration Period.—Preliminary experiments had shown a variation in the yield of alkaloid, with variation in time of soaking of the crude drug with the ammoniacal medium. The alkaloid yield varies markedly with periods of from 10 minutes to 16 hours, slightly from 16 hours to 48 hours and virtually not at all for longer periods (Table IV).

TABLE IV.

EFFECT OF PERIOD OF CONTACT WITH AMMONIACAL MEDIUM ON YIELD OF ALKALOID.

Period of maceration. Hours.	Yield of alkaloid. Mg.	Per cent.
1/6	15.27	0.127
	15.27	0.127
2	15.85	0.132
	15.27	0.127
16	19.32	0.161
	19.32	0.161
48	21.70	0.180
	21.40	0.178
	21.40	0.178
	21.12	0.176
120	21.12	0.178
	21.12	0.178

TABLE V.

EFFECT OF MACERATION AT ELEVATED TEMPERATURE ON YIELD OF ALKALOID.

Variable { Composition of macerating medium. Temperature.

Macerating medium. Ammonium hydroxide. Cc.	Alcohol. Cc.	Ether. Cc.	Yield of alkaloid. Mg.	Per cent.
5	10	20	18.80	0.157
..	..	..	13.60	0.113
..	..	..	13.60	0.113
5	70	..	16.49	0.138
10	65	..	17.07	0.142
15	60	..	17.07	0.142

In view of the marked effect of time of maceration, it is thought possible that soaking at a somewhat elevated temperature might have an accelerating effect on alkaloid liberation. In view of the wide difference in boiling point of the constituents of the maceration medium, the series of experiments on maceration temperature variation was not entirely satisfactory.

Maceration at Elevated Temperature.—The charges of hyoscyamus in the extractor jackets were given the ammoniacal soaking medium, the whole apparatus was connected to the condenser as for automatic extraction, and the lower end of the jacket containing the charge was immersed in hot water and allowed to reflux for one-half hour, thus keeping the composition of the soaking liquid approximately uniform during the heating process.

Different ratios of alcohol, aqueous ammonia and ether were used as soaking media, thus permitting different heating temperatures. All were heated for the

[1] Report on Bio-Assay of Drugs, *J. Assocn. Official Agri. Chem.*, 10, 3, 383 (1927).

same length of time, however, and all subsequent operations to the point of alkaloid titration were carried out as described under "Method." In no case was the alkaloid yield equal to that obtained where "cold soaking" was applied over an extended period, and the process of liberation of alkaloid from the crude drug could evidently not be hastened by elevation of temperature during maceration (Table V).

If the alkaloid yield depended entirely upon the efficacy of the process entailing the alkaloid-liberation treatment of the crude drug and the manner of extraction were not significant, then simple digestion of the hyoscyamus with aqueous acid or acid alcohol solutions, filtration, washing, etc., and subsequent extraction of the alkaloid from the filtrate should yield a full return of the alkaloid.

To test this hypothesis, charges of hyoscyamus were digested on the steam-bath with varying concentrations of aqueous acid and alcoholic acid solutions for varying periods, filtered by suction, washed, etc., and the filtrate was subjected to purification before alkaloidal extraction as in the other experiments. Although apparently slightly more alkaloid was obtained in some instances, the increased quantities of plant extractives, particularly tarry material, rendered the purification process difficult, and the ultimate alkaloidal residue sufficiently contaminated to make the titration values doubtful.

There was some evidence that the crude drug has been exhausted by the method (Table IV), but further evidence obtained by repeated extraction of the marc and by the use of weak alcoholic hydrochloric acid solutions as the extracting medium in conjunction with the automatic device showed that only very small additional quantities of alkaloid were obtained by repeated treatment of the marc.

As digestion of the crude drug with hot alcoholic solution of hydrochloric acid without the aid of the automatic extracting device gave an appreciably lower alkaloid yield than the method here described, further experiments in which the extraction with alcoholic hydrochloric acid was made, using the automatic extractor, were conducted.

These experiments appeared to give an appreciably higher yield of alkaloid on prolonged extraction of about four hours (up to approximately 0.2 per cent). Here, too, however, larger quantities of tarry impurities were extracted, rendering the purification process difficult and titration so doubtful as to make a reëxtraction of the titrated solutions necessary. The final results were about the same as, or slightly lower, than those obtained by the ammoniacal alcohol-ether method.

In view of the materially higher yield of total alkaloid in hyoscyamus by the new method, it would appear desirable that the U. S. P. Revision Committee consider a new standard for this drug in the coming revision.

METHOD.

Treatment of Crude Drug.—Place 15 Gm. of the drug (No. 60 powder) in a Type S[1] extractor and pour enough ether through the center tube to moisten the drug. Add a mixture of 5 cc. of stronger ammonia water (26 per cent), 10 cc. of alcohol and 20 cc. of ether. Stir with a glass rod, so that the ammoniacal liquid is intimately mixed with the drug, washing the rod with a little ether. Stopper the apparatus securely and allow the drug mixture to macerate over night.

Extraction Process (Solid).—Stir the drug again with the glass rod and wash

[1] See Figure 1.

the rod with a little ether. Insert the filtering gauze and place the funnel in the upper end of the center delivery tube. Pour ether into the funnel in the tube until the liquid flows over into the Erlenmeyer flask to the extent of about 75 cc. Connect the extractor with a long reflux condenser, allowing the Erlenmeyer flask to rest on a steam-bath. Boil briskly for 2 hours. (If any channeling in the powdered mass is observed during the extraction, the extractor should be removed and the mass again stirred with the glass rod to break up the channels.)

Purification Treatment.—Remove the extractor and drain the solvent remaining in the jacket into the Erlenmeyer flask. Evaporate the ether extract to about one-third its volume, using a current of air. Add 10 cc. of 0.05 *N* sulphuric acid and 5 cc. of water and evaporate until the odor of ether is entirely gone. (The aqueous liquid remaining must be acid but not excessively so.) Pour the acidified liquid into a 50-cc. volumetric flask. Take up the resinous material remaining in the Erlenmeyer flask with a few cc. of chloroform and add 5–10 cc. of water and 3 drops of 0.05 *N* sulphuric acid. Heat this material on the steam-bath to expel the chloroform. Pour the acidified liquid into the 50-cc. volumetric flask, cool to room temperature, and make up to the mark with water. Mix thoroughly and filter through a 9-cm. filter paper, rejecting the first few cc. of the filtrate. Use for extraction.

Extraction (Liquid Extractor).—Place an aliquot of 40 cc. of the filtrate in a Type C[1] extractor, make alkaline with ammonia and extract with chloroform until the solution is exhausted of alkaloid (2 hours is more than sufficient).

Titration.—Evaporate the chloroform on the steam-bath to about 5 cc. Add a measured excessive volume of 0.02 *N* sulphuric acid and continue the evaporation until the odor of chloroform has disappeared. Cool the solution and titrate back with *N* 0.02 sodium hydroxide, using 1 drop of methyl red test solution as indicator.

Each cc. of *N* 0.02 sulphuric acid used is equivalent to 0.005786 Gm. of the alkaloids of belladonna.

SUMMARY.

Evaluation of total alkaloid in hyoscyamus niger by a new process of treatment and automatic extraction shows that in general the alkaloid content of this drug as determined by U. S. P. methods has been under-estimated.

A new method is described for the determination of total alkaloids in hyoscyamus which yields from two to three times as much alkaloid as that obtained by the U. S. P. IX and X methods. Physiologic tests show that all of the alkaloid so obtained is at least equivalent to hyoscyamine in mydriatic strength.

PREPARED REMEDIES FOR ANIMAL AND POULTRY DISEASES, ETC.

The United States Department of Agriculture has published recently a series of pamphlets on the above subject. They are referred to as "Poultry Short Courses," No. 5, No. 6, etc. These and other bulletins released by the United States Department of Agriculture devoted to the subject of diseases, ailments and abnormal conditions in poultry, swine and other domestic animals should be of interest to the manufacturer of animal remedies. In this connection the attention of manufacturers of these preparations is called to the foreign export market for veterinarian medicines, sheep and cattle dips, remedies for domestic pets, anti-hog cholera serum, etc., notice of which appears in the Chemical Division's bulletins from time to time.

[1] See Figure 2.