contained therein. Since this investigation has shown that B. P. and N. F. Aqueous Extracts of Ergot prepared by the processes specified do not contain significant amounts of the ergot alkaloids, it is believed that these preparations are practically without value with respect to ergot therapy. The appearance of these aqueous extracts on the market has provided for their use by clinicians and physicians, usually with little or no success, resulting in a subsequent undue condemnation of all types of ergot preparations and a search for a suitable substitute.

(To be continued)

HAZINESS OF FINAL CHLOROFORMIC EXTRACTIONS IN ALKALOIDAL ASSAYING.*

BY GEORGE E. ÉWE.

In estimating alkaloids, as a rule, the alkaloids are eventually segregated in an ammoniacal, aqueous liquid containing large quantities of ammonium sulphate. The alkaloids are then "shaken out" with an immiscible solvent, chloroform usually being the choice. These chloroformic extractions, instead of being brightly clear, are often hazy. This haziness, when not due to the presence of solid matter, is due to an almost colloidal suspension of a trace of the aqueous liquid from which the alkaloids were extracted. Since this aqueous liquid contains ammonium sulphate, and since, under the influence of heat and moisture, alkaloids tend to liberate ammonia from ammonium sulphate (and are thereby proportionately neutralized by the sulphate radicle), it seems important to ascertain the possible effect of this latter type of haziness of final chloroformic extractions upon the results of alkaloidal assays.

The following experiments illustrate the effect of alkaloid upon ammonium sulphate under several varied conditions: When a grain or two each of strychnine alkaloid and ammonium sulphate are placed in a 100-cc. volumetric flask along with about 15 cc. of water, or water-saturated chloroform, and a piece of moistened red litmus paper is placed between pledgets of cotton in the neck of the flask and the mixture then boiled, the litmus paper turns blue, due to liberation of ammonia from the ammonium sulphate by the alkaloid. When the alkaloid and ammonium sulphate are dried by heat and the chloroform is essentially deprived of moisture by boiling down to half its volume, and the above test is repeated, a very doubtful alkaline reaction is obtained, if any. No reaction is obtained in this test from mixtures of ammonium sulphate and water-saturated chloroform, or strychnine alkaloid and water-saturated chloroform, or dried ammonium sulphate and dried chloroform or dried alkaloid and dried chloroform.

To ascertain if haziness of the final chloroformic extracts has any influence upon results of alkaloidal assays, the final chloroformic extracts, from alkaloidal assays which happened to be hazy, were brought to a definite volume and divided into three equal aliquots. One aliquot was evaporated to 5 cc. excess tenth-normal sulphuric acid added, the chloroform boiled off completely and the excess of acid titrated back with fiftieth-normal sodium hydroxide, using methyl red as indicator. The second aliquot was either washed with 10 cc. of water in a separator

* Scientific Section, A. PH. A., Rapid City meeting, 1929.

(the wash water being extracted with three 10-cc. portions of fresh chloroform and the chloroformic washings then combined with the main bulk of alkaloidal solution), or the aliquot was clarified by shaking it with a pinch of powdered tragacanth and filtering; the wash or clarified chloroformic extractions then being evaporated to 5 cc., excess standard acid added and so on. The third aliquot was evaporated to 10 cc., a 10-cc. portion of water added, the chloroform boiled off, a drop of hydrochloric acid added, the mixture cooled, filtered and treated with 2 drops of barium chloride test solution as a test for ammonium sulphate. The following are:

TYPICAL RESULTS ON VARIOUS HAZY, FINAL CHLOROFORMIC EXTRACTIONS.

Drug Product.	Degree of Haziness.	Sulphate Test.	Difference in *N*/50 Alkali on Titration.
Fluidextract Belladonna leaves	Slight	Distinct opalescence	None within a drop or two.
Powdered Hyoscyamus	Quite hazy	Distinct opalescence	Washed aliquot showed less alkaloid to extent of 0.05 cc.
Powdered Extract Nux Vomica	Very hazy	Immediate turbidity	Aliquot clarified by tragacanth showed more alkaloid to extent of 0.1 cc.
Powdered Nux Vomica	Almost turbid	Strong turbidity	Washed aliquot showed less alkaloid to extent of 0.05 cc.
Ground Belladonna leaves	Very hazy	Immediate turbidity	Washed aliquot showed more alkaloid to extent of 0.05 cc.
Ground Hyoscyamus	Very hazy	Immediate turbidity	No difference (Tragacanth used)

These results would seem to indicate that the influence of the ammonium sulphate present in hazy final chloroformic extracts obtained in alkaloidal assays is negligible. Possibly the free ammonia, with which these extractions are saturated, tends to prevent the action of the alkaloid upon the ammonium sulphate, until boiling has rendered the mixture substantially free from water, in the absence of which the action is hindered or becomes negligible. That this is possible appears to be shown by the following experiments: When U. S. P. Chloroform is saturated with ammoniacal water, filtered perfectly clear and boiled in a flask, a ring of droplets of moisture is seen to gradually rise on the sides of the flask as boiling commences, so that this ability of chloroform to force out water upon boiling should enable it to essentially deprive itself of moisture quite readily. At this stage, a piece of red litmus paper, moistened with alcohol, held in the vapor is instantly blued. The ability of the vapors to turn the color of red litmus paper continues long after the ring of moisture has been dissipated, so that it appears that some ammonia remains in chloroform saturated with ammoniacal water long after the water is dissipated by boiling, which would account for the negligible action of ammonium sulphate upon the alkaloid in boiling down hazy chloroformic extractions obtained in alkaloidal assaying. When the preceding experiment is conducted in an evaporating dish, in order to prevent the ring of moisture from

refluxing into the boiling chloroform, the same results are obtained; that is, some of the ammonia remains long after the moisture is apparently completely dissipated.

In order to ascertain something of the quantitative effect of this apparent ability of ammonia to prevent the action of alkaloid upon ammonium sulphate in the presence of water, two empirical solutions of strychnine alkaloid in chloroform saturated with ammonia water were prepared and the following series of experiments applied to these solutions:

Experiment No. 1 was designed to determine the strychnine content of the empirical solution and was conducted as follows: a 15-cc. aliquot was boiled down to 5 cc., 10 cc. tenth-normal sulphuric acid added; the chloroform completely boiled off and the excess of acid titrated back with fiftieth-normal sodium hydroxide, using methyl red as indicator.

Experiment No. 2 was intended to determine the effect of ammonium sulphate in the absence of ammonia and water. This experiment was conducted by boiling down a 15-cc. aliquot of the strychnine solution to 5 cc.; adding 25 cc. U. S. P. chloroform, boiling down to 15 cc.; adding 0.02 Gm. ammonium sulphate, boiling down to 5 cc.; adding 10 cc. tenth-normal sulphuric acid, etc., as in the first experiment.

Experiment No. 3 simulated conditions met with in alkaloidal assay processes and was conducted by adding 0.02 Gm. ammonium sulphate to 15 cc. of the strychnine solution, boiling down to 5 cc., adding 10 cc. standard acid, etc., as in preceding experiments.

Experiment No. 4 also simulated another set of conditions met with in alkaloidal assay work and was conducted by adding 0.02 Gm. of ammonium sulphate to a 15-cc. aliquot of the strychnine solution, followed by 30 cc. of chloroform previously saturated with ammoniacal water and filtered; boiling down to 10 cc., adding another 30-cc. portion of the ammoniacal, water-saturated chloroform; again boiling down to 10 cc.; repeating the addition of ammoniacal water-saturated chloroform twice more; boiling down after each addition; finally, boiling down to 5 cc. and adding 10 cc. standard acid, etc., as in preceding experiments.

Experiment No. 5 was intended to determine the effect of excessive water, corresponding to inadvertently drawing off some of the aqueous layer, containing ammonia and ammonium sulphate, along with the chloroformic layer, in making an alkaloidal assay. This experiment was made by adding 0.02 Gm. ammonium sulphate to a 15-cc. aliquot of the strychnine solution, boiling down to 5 cc., adding 1 cc. distilled water and 15 cc. U. S. P. chloroform; boiling down to 5 cc., adding 10 cc. standard acid, etc., as in preceding experiments.

In all of these experiments the evaporation to dryness of the solutions containing alkaloids was avoided in order to prevent the possible influence of a temperature higher than that of boiling chloroform, in effecting a partial reaction between the alkaloid and ammonium sulphate, and to avoid possibility of error due to partial destruction of the alkaloid by overheating.

The results of these experiments upon the two empirical strychnine solutions are given in terms of strychnine alkaloid in the tables on next page.

The results of these experiments seem to again indicate that the effect of ammonium sulphate is practically negligible under the ordinary conditions of

Strychnine Solution No. 1.		Strychnine Solution No. 2.	
Experiment No. 1	0.0936 Gm.	Experiment No. 1	0.1009 Gm.
Experiment No. 2	0.0936 Gm.	Experiment No. 2	0.1002 Gm.
Experiment No. 3	0.0949 Gm.	Experiment No. 3	0.1009 Gm.
Experiment No. 4	0.0949 Gm.	Experiment No. 4	0.1002 Gm.
Experiment No. 5	0.0929 Gm.	Experiment No. 5	0.0976 Gm.

analysis; although, in the presence of a great excess of aqueous solution, lower results may be obtained through prolongation of the action of the alkaloid upon the ammonium sulphate as shown by the results yielded by the experiments designated as No. 5 in the foregoing tables.

Another factor, possibly operating to prevent reaction between alkaloid and ammonium sulphate in an ammoniacal aqueous chloroformic solution, resides in the peculiar selective solvent action of chloroform for certain components of such a solution; for, whereas water is soluble in chloroform and ammonium sulphate is soluble in water, yet an aqueous solution of ammonium sulphate is not soluble in chloroform and the latter selects only water from such a solution and excludes the ammonium sulphate. Thus, it seems possible for the chloroform to mechanically isolate the alkaloid from the ammonium sulphate, although it is likely that some degree of reaction may result in the presence of water, and under the influence of heat and prolonged and intimate contact, as shown by the lower results obtained under the conditions outlined in Experiment No. 5 in the preceding series of experiments.

In spite of the apparently negligible influence of haziness of final chloroformic extracts, due to inclusion of aqueous, ammoniacal ammonium sulphate solution, upon the results of alkaloidal assays under ordinary conditions, good analytical chemical practice dictates that these extracts be perfectly clear before evaporating for titration. This clarity can often be attained by allowing the extracts to stand for some time or by filtering them. Washing the hazy extracts in a second separator with a little water is also effective (however, the aqueous washings should be extracted, in turn, with small portions of fresh solvent and the latter then combined with the main bulk of alkaloidal solution). Another satisfactory method is to agitate the hazy extracts in a second separator with a trifle of powdered tragacanth followed by filtration.

In an article entitled "On the Estimation of Alkaloids in Admixture with Vegetable Drugs," published by the writer in the Jour. A. Ph. A., Vol. XVIII, No. 3, 1929, an attempt was made to ascertain if the basicity of the ammoniacal, chloroformic extracts of several vegetable drugs was due, at least in part, to the presence of the ammonium radicle, by boiling the extracts with strychnine alkaloid. It was presumed that since "strychnine alkaloid readily liberates ammonia from even 'strong' acids," loss of ammonia would result from this treatment, if the ammonium radicle was present, and a lower total basicity would consequently result. Since the total basicity was found to check up closely with the calculated total it was concluded that the ammonium radicle was not present in the extracts. However, in the light of the results obtained in the present study the results reported in the former paper must be qualified to the extent that the amount of ammonia liberated, if present, would have likely been negligible under the conditions outlined in the recorded experiments, and so the results were not conclusive evidence that the

ammonium radicle was not present in the various drug extracts used in the experiments. The experiments have, therefore, been repeated, employing the more satisfactory method of boiling the extractive with an aqueous suspension of magnesium oxide in testing for ammonia. The ammoniacal, chloroformic extract was boiled down in a 100-cc. volumetric flask to 5 cc., the remainder being evaporated without heat by a current of air; some of the magnesium oxide suspension was added through a funnel with a long stem, so as to avoid contact of the magnesium oxide with the neck of the flask; a piece of moistened red litmus was inserted in the neck of the flask between pledgets of cotton and the contents of the flask then boiled. In the case of Cannabis and Capsicum, distinct, but not strong, alkaline reactions were obtained, while in the case of Podophyllum, Jalap and Gentian very strong reactions were obtained, the litmus paper turning deep blue as soon as reached by the steam. It would seem then that the basicity of these ammoniacal, chloroformic extracts of vegetable drugs is due, in part at least, to the presence of the ammonium radicle. However, the proportion of ammonium radicle must be quite small, since even the strong reactions on litmus paper were rendered neutral by the escaping steam from the mixture in the flask within a minute of continued boiling, just as moistened red litmus paper blued by ammonia fumes and then held in a jet of steam is soon rendered to a neutral shade.

SUMMARY.

Haziness of final chloroformic extracts, obtained in alkaloidal assaying, from ammoniacal, aqueous solutions containing ammonium sulphate, when not due to the presence of solid matter, is due to an almost colloidal suspension of a trace of the aqueous liquid from which the alkaloid was extracted. This type of haziness thus results in contamination of the chloroformic extract of the alkaloid with traces of ammonium sulphate, ammonia and water.

Under ordinary conditions, this type of haziness has but a negligible effect, if any, upon the results of titration of the alkaloid. However, in the presence of excessive water (corresponding to the inadvertent drawing-off of a portion of the aqueous layer along with the chloroformic layer), lower results may be obtained, due to liberation of ammonia from the ammonium sulphate by the alkaloid, during the evaporation process, with corresponding proportionate neutralization of the alkaloid by the sulphate radicle.

Means of insuring clarity of (absence of ammonium sulphate from) the final chloroformic extracts, preparatory to evaporation for titration, are outlined.

DISCUSSION.

BY JOHN URI LLOYD.

In connection with the foregoing paper, read by title at the meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION, at Rapid City, I shall presume to make a few remarks, stating in connection therewith that at my request, the privilege of reading this paper was awarded me,[1] I having from the title concluded that to me it would be very serviceable.

The estimation of alkaloids, by whatever method is adopted, is yet, in my opinion, in some directions involved in unknown complications, or rather I might put it, little known complications. Mr. Ewe has touched one of these in connection with the presence of ammonium sulphate, which, as shown by him, has a tendency to liberate ammonia during chloroformic alkaloidal extraction.

[1] By the Editor, JOUR. A. PH. A.

The paper calls attention to the haziness throughout the liquid, which Mr. Ėwe finds to be "an almost colloidal suspension of a trace of the aqueous liquids from which the alkaloids are extracted." In this connection, which is one of the problems I have met in studying the meniscus between liquids, I have referred to as a characteristic of semi-physical relationships. This is touched upon by Professor Ostwald and Mr. Walter Haller, as follows:

"A phenomenon observed years ago by the senior author (John Uri Lloyd), with acetone-glycerol and other pairs of liquids, namely, the lack of sharpness of the menisci, is very remarkable."

It will be noted that this lack of sharpness of the menisci does not exactly parallel the haziness described by Mr. Ėwe as follows:

"The chloroformic extractions, instead of being brightly clear, are often hazy. This haziness, if not due to the presence of solid matter, is due to an almost colloidal suspension of a trace of the aqueous liquid from which the alkaloids are extracted."

My opinion was that the haziness shown about the menisci of acetone and glycerin resultant, was by me unexplainable, nor did I attempt to do more than record the fact of observation.

Mr. Ėwe has, in an independent direction, referred to a haziness, not alone a meniscus complication, but of the chloroformic liquid as a whole, indicating, as he expresses it, a disturbance of alkaloidal assaying exactness. Let me venture to hope that he will continue his research in the direction of colloidal disturbances.

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NOTES ON CASCARA SAGRADA.*

BY MILFORD HARRIS AND EDWARD D. DAVY.

The varying results reported on Cascara Sagrada, the lack of positive evidence as to the active constituents, the effect of alkalies and oxidation, led us to try fractionation of the active constituents by varying solvents only.

All of the common immiscible solvents were tried either in the extraction of the drug, or in subsequent efforts to fractionate the active material from aqueous and alcoholic extracts. Water, Ether and Ether-Alcohol mixtures were found more satisfactory than the others.

The references listed indicate the wide interest in this subject and we have consulted them freely, using such help as might apply to any particular case.

The cascara used for this work was approximately six years old and solvents used for the initial extractions were water and alcohol.

(*A*) *Water Extract.*—A preliminary extraction of twenty-five grams of drug was made in a Soxhlet with water, using an air condenser, so that the water reaching the drug was just short of boiling. When the extraction was complete the drug was dried and reëxtracted with alcohol. The alcoholic extract yielded considerable emodin, a small amount of resin, but no glucoside.

Five pounds of drug were then extracted with boiling water, and after reducing to extract by evaporating in an open container, alcohol was added to precipitate the alcohol-insoluble extractive. This was filtered and the precipitate washed thoroughly with alcohol, the filtrate and washings combined and reduced to an extract, the extract then diluted to fluidextract strength with water. A portion

* Scientific Section, A. PH. A., Rapid City meeting, 1929.