A POSSIBLE SOURCE OF ERROR IN SOME ALKALOIDAL ASSAYS.*

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A short time ago, as the result of an accident, my attention was directed to the interaction which, in favorable circumstances, may take place between free alkaloids and ammonium salts, and to the error which may arise from this cause in alkaloidal assays where the alkaloid is titrated. Although there does not appear to be any probability that a capable and experienced analyst ever falls a victim to this possible source of error, yet it appears quite worthy of notice, since it is certainly not generally recognized, while, at the same time, it may easily produce serious inaccuracies in the work of any one who is somewhat inexperienced in alkaloidal determinations.

That certain of the alkaloids have the power of expelling ammonia from its salts is fairly well known, but it does not appear to be equally well known that probably this power is possessed—to some extent and in suitable circumstances—by nearly all alkaloids. Ammonia, it is true, is a much stronger base than most fixed alkaloids, but its great volatility largely counterbalances this fact, for it is a matter of common knowledge that a feeble base or acid is capable—in conditions allowing interaction—of expelling from combination a much stronger member of its class, if the former is fixed and the latter volatile, at the temperature employed. A good example of this is given by the process of glazing earthenware, where, at the high temperature used, the extremely feeble acid, silicic acid, is able to decompose sodium chloride, with formation of sodium silicate, and expulsion of the very strong (but volatile) acid, hydrochloric acid.

Now, in an alkaloidal assay, if the alkaloid is liberated by *ammonia* and the volatile solvent employed in the final shaking-out is not washed with water, but is run directly into a dish and evaporated, there is considerable danger of a small volume of the aqueous layer (containing an ammonium salt) being carried into the dish with the alkaloid. This may happen in two ways: in the first place, by carelessless in the separation of the two layers of liquids, and, secondly, by a little of the ammoniacal solution clinging to the sides of the separator being carried down mechanically by the friction of the issuing stream of solvent; in either case, during the final stage of the evaporation, we have in the dish free alkaloid in contact with a solution of ammonium salt. The natural result is that, as pointed out above, a certain amount of interaction takes place (the extent of this being dependent on several circumstances), with consequent loss of ammonia and formation of an alkaloidal salt. The residue finally obtained, therefore, consists of a mixture of free alkaloid and alkaloidal salt, and the titration, of course, only gives the amount of the former.

It is interesting to note that here the result obtained is *too low*, whereas when the alkaloid is not titrated, but *weighed*, the introduction of ammonium salt causes the result to become *too high*. The error in the case of titration may,

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however, be much more serious than when the alkaloid is weighed, since owing to the high molecular weights of all alkaloids, a very small quantity of a salt of ammonia is capable of causing the neutralization of a comparatively large weight of free alkaloid if interaction is complete. Thus one part of ammonium chloride will interact with 12 parts of aconitine, 5.4 parts of atropine, or 6.3 parts of strychnine.

In order to ascertain the degree of error which may result from the cause under consideration, in alkaloidal assays, a number of quantitative experiments were made, with the following results:

In the first series of experiments, weighed amounts of pure alkaloids were dissolved in a little chloroform or ether, a small quantity of a weak solution of ammonium chloride added, and the whole evaporated to dryness on a water-bath and titrated in the usual way. In each case a similar experiment was performed, in which the ammonium chloride was omitted, in order to eliminate any other error and check the purity of the alkaloid.

The results obtained, together with fuller details of each individual experiments, are given in Table I.

It will be seen from the Table that the interaction was greatest in the case of atropine, the reaction being practically complete in both experiments. With aconitine the larger amount of apparent loss in the second experiment was probably due to the use of ether causing better contact between the reacting substances than when chloroform was employed, a result which might very well be expected. Strychnine gave least interaction, this no doubt being due to its extreme insolubility in water rendering contact with the ammonium chloride very difficult. In all the experiments, however, considering the minute quantities of ammonium chloride used, the apparent loss of alkaloid was comparatively large.

TABLE I.

Alkaloid Used	Details of Experiment	Amount Taken	Amount Found by Titration	Apparent Loss
Atropine ...	Alkaloid dissolved in 5 mils of ether, and about 4 milligrammes of ammonium chloride in 1 mil of water added.....	0.0189 gm.	0.0243 gm.	0.0246 gm.
Atropine ...	Alkaloid dissolved in 5 mils of chloroform, and about 2 milligrammes of ammonium chloride in 0.5 mil of water added	0.0451 gm.	0.0333 gm.	0.0118 gm.
Aconitine ..	Alkaloid dissolved in 3 mils of chloroform and about 4 milligrammes of ammonium chloride in 1 mil of water added	0.0925 gm.	0.0839 gm.	0.0086 gm.
Aconitine ..	Alkaloid dissolved in 10 mils of ether and about 2 milligrammes of ammonium chloride in 0.5 mil of water added.....	0.0502 gm.	0.0336 gm.	0.0166 gm.
Strychnine..	Alkaloid mixed with 3 mils of chloroform and about 8 milligrammes of ammonium chloride in 2 mils of water added	0.0956 gm.	0.0809 gm.	0.0147 gm.
Strychnine..	Alkaloid mixed with 10 mils of ether and about 2 milligrammes of ammonium chloride in 0.5 mil of water added.....	0.0479 gm.	0.0451 gm.	0.0028 gm.

A few assays of belladonna leaves were next carried out, using the method of the present Pharmacopœia (Br.), but in some experiments washing the chloroform used in the final extraction, with water, and in others allowing small quantities of the aqueous layer to pass into the dish with the alkaloids. The method adopted for the purpose of eliminating any possibility of variation in the first stages of the assay, in comparative experiments, was as follows:

Fifty grammes of the powdered leaf were taken, the extraction with ether-chloroform and shaking-out with acid performed with five times the pharmacopœial quantities of all the reagents, and the acid extract then made up to 250 mils; 50 mils of this liquid were taken for the final stage of each separate experiment, and the exact volumes of chloroform prescribed by the Pharmacopœia used in shaking-out. Three samples of leaf were employed, and the circumstances were varied, as described in Table II.

In experiments (3) and (4) the separation of the chloroform was done with great care, the only aqueous liquid carried into the dish being that unavoidably removed from the sides of the separator by friction, as explained above; the results are, however appreciably low. In experiments (6), (7), and (9), small quantities of the aqueous layer (containing, of course, some ammonium sulphate) were added intentionally, the other circumstances being the same as in experiments (3) and (4), and in all three cases the errors produced were very serious. It is worthly of note that in this assay the fact that ether is added to the alkaloidal residue and then evaporated off again appears to increase any error due to the presence of ammonium salt by promoting better contact, since the apparent loss in experiment (9), where no ether was used, was comparatively much smaller than in experiment (6).

TABLE II.

Number of Sample	Number of Expermt.	Circumstances of Experiment	Percentage of Alkaloid Found	Error
I.	1	Chloroformic solution of alkaloid washed with water	0.295	—
	2	Chloroformic solution of alkaloid washed with water	0.289	—
	3	Chloroform run very carefully into dish without washing	0.278	0.014
	4	Chloroform run very carefully into dish without washing	0.278	0.014
II.	5	Chloroformic solution of alkaloid washed with water	0.402	—
	6	As expts. (3) and (4), but about 0.3 mil. of aqueous layer run into dish in addition	0.197	0.205
	7	As expt. (6), but 0.5 mil. of aqueous layer added	0.191	0.211
III.	8	Chloroformic solution of alkaloid washed with water	0.278	—
	9	As expt. (6), but 0.25 mil. of aqueous layer added, and alkaloid not treated with ether finally	0.208	0.070

It is therefore quite evident, from the figures obtained in both sets of experiments, that in any alkaloidal assay in which the alkaloidal residue is titrated great

care must be exercised to avoid the introduction into the latter of even as little as 1 milligramme of an ammonium salt—that is to say, it is extremely advisable to wash the solution of alkaloid in volatile solvent with water.

From this point of view it is somewhat unfortunate that in the pharmacopœial assay processes for belladonna leaf and tincture and dry extract of belladonna no directions are given for washing the chloroformic solution of alkaloid before evaporation and titration, while it is certainly very difficult to understand why the precaution which was omitted in these cases should have been taken in the assay of liquid extract of belladonna. With regard to aconite and its preparations, while the prescribed filtration of the ether, if properly carried out, appears to render any appreciable error unlikely, yet great care must be taken that none of the aqueous layer passes through the filter, otherwise owing to the high molecular weight of aconitine, a very serious error may result.

THE VARIATION CLAUSE OF THE FOOD AND DRUGS ACT.*

SOME REASONS FOR THE EXISTENCE OF THE CLAUSE AND AGAINST ITS REPEAL.

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Section Seven of the Federal Food and Drugs Act declares that a drug shall be deemed to be adulterated if, when "sold under or by a name recognized in the United States Pharmacopœia or National Formulary, it differs from the standard of strength, quality or purity, as laid down in the United States Pharmacopœia or National Formulary official at the time of investigation."

To this declaration the so-called variation clause is attached in the form of a proviso which reads, *"Provided,* That no drug defined in the United States Pharmacopœia or National Formulary shall be deemed to be adulterated under this provision if the standard of strength, quality, or purity be plainly stated upon the bottle, box or other container thereof, although the standard may differ from that determined by the test laid down in the United States Pharmacopœia or National Formulary."

The meaning of the foregoing somewhat involved pharaseology is, in brief, that when a title found in the United States Pharmacopœia or National Formulary is used without qualification or explanation, the article sold thereunder must be of strictly U. S. P. or N. F. quality, but that such a title may be used (under the proviso) upon an article of a different standard if the label plainly indicates the standard to which it conforms.

Identical or very similar provisions are found in many of the state food and drug acts, so that arguments for or against the existence of the variation clause of the Federal law will have equal application to state laws.

In view of the fact that the repeal of the variation clause has been demanded upon the ground that it permits the sale of inferior and adulterated products under official titles, it may be profitable to consider some of the reasons which

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