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BIOTOXICOLOGY. I. METHODS.

BY JAMES C. MUNCH.*[1]

The usual methods for extraction of poisons from the viscera yield impure extracts, which often fail to give typical reactions with alkaloidal reagents. Elaborate systems of purification are required to separate the toxic principles from the fats, resins and degradation products simultaneously extracted from the tissues, and such purification is associated with significant loss of the poison.

The classical method of Stas was developed for the separation of Nicotine. Stas coagulated albuminoids with alcohol and oxalic acid, hoping to dissolve out the poison as the oxalate. After filtration from the tissues, the alcoholic solution was concentrated, and efforts made to purify the alkaloid, by making the solution alkaline with either the hydroxide, bicarbonate or carbonate. The free alkaloid was shaken out with ether, and evaporated to give the final product.

This Stas method was subsequently modified by J. and R. Otto in several particulars. Tartaric acid was used instead of oxalic, since the alkaloidal tartrates are more soluble in alcohol. The ether extraction of the acid alcoholic solution removed a large proportion of the fats, glucosides and organic acids. The aqueous solution, after evaporation of alcohol, was made alkaline with sodium bicarbonate, and the alkaloids extracted by ether. On evaporation the residue was much purer than that obtained by the Stas method.

Dragendorff macerated the minced viscera with water, acidulated with sulfuric acid to coagulate the albuminoids and to convert the alkaloids into sulfates. The aqueous solution of the alkaloidal sulfates is acidified and extracted, in turn, with petroleum ether, benzene and chloroform. The residual solution is made alkaline and extracted, seriatim, with petroleum ether, benzene, chloroform and amyl

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alcohol. Each of these solutions is filtered through dry paper, concentrated to a small volume on a water-bath, and tested.

Ogier, Douris and others have modified a few of the details of the Stas-Otto-Dragendorff method. Citric acid has been used instead of tartaric acid. Absolute alcohol is added to the viscera, then the material incubated for 12 to 24 hours for complete extraction. After filtration, the alcohol is removed by general heating on a water-bath, the residue taken up in water, and filtered. The aqueous solution is evaporated on the water-bath, taken up in absolute alcohol and filtered. This alternate treatment is continued until a colorless, or very lightly colored, aqueous solution is obtained (1–12, incl.). However, this modification still yields chemically impure residues for identification.

TABLE I.—SEPARATION OF COMMON POISONS FROM VISCERA.

I. VOLATILE POISONS:

Acidify the minced viscera with tartaric acid, and steam distill:

A. *Acid Distillate* may contain:

Alcohols (methyl, ethyl, amyl)	Chloroform
Aniline	Hydrocyanic Acid
Carbon Bisulfide	Nitrobenzene
Chloral	Phenol

B. *Residue* in flask:

Cool, add *N* NaOH until alkaline to litmus, and steam distill:

(1) *Distillate* may contain:

Coniine	Nicotine	Sparteine

(2) *Residue* may be tested for inorganic poisons.

II. NON-VOLATILE POISONS:

Acidify the minced viscera with tartaric acid and absolute alcohol to $p_H 3$, and reflux. Cool and filter through paper. Wash with absolute alcohol, adding washings to filtrate; add tartaric acid if necessary, until acid to litmus:

A. *Acid Filtrate:*

(1) *Extract* with petroleum ether:

Benzoic Acid	Phenol	Picric Acid
Camphor	Pilocarpine	Salicylic Acid

(2) *Extract* with ethyl ether:

Acetanilide	Colchicine	Picrotoxin
Aniline	Barbiturates	Salicylic Acid
Antipyrine	Phenacetine	Santonin
Caffeine	Picric Acid	Sulfonal
Cantharidin	Pilocarpine	Veratrine

(3) Make filtrate alkaline with NaOH or $NaHCO_3$; *extract* with ethyl ether:

Aconitine	Colchicine	Pilocarpine
Aniline	Codeine	Quinine
Antipyrine	Ergotoxine	Scopolamine
Atropine	Hydrastine	Strychnine
Brucine	Narcotine	Veratrum
Caffeine	Physostigmine	Yohimbine
Cocaine		

(4) Re-acidify filtrate with HCl, make alkaline with NH_4OH; *extract* with ethyl ether.

Apomorphine	Morphine

(5) *Extract* filtrate with chloroform:

Caffeine	Morphine
Colchicine	Narceine

B. *Residue:*

Combine with Residue I–B–2.

C. *Miscellaneous tests on chopped viscera:*

Arecoline	Glucosides
Bromine derivatives	Lobeline
Cocaine derivatives	Muscarine
Emetine	Saponins

Since the impurities are physiologically inert, it seems desirable to apply pharmacological information to the problem of developing methods of biotoxicology, that is, confirmation of chemical tests and suggestions of the nature of the toxic principles in these extracts. The methods of injection of frogs, mice, rats and guinea pigs, tests upon the cat's pupil, taste, and tests upon isolated tissues, have been reported (6). The general distribution of poisons most commonly used, or to be expected in toxicological procedures, is given in Table I. Subsequent papers in this series will consider biotoxicological tests upon these groups of poisons.

When special information indicates the presence of a poison not listed in Table I, special methods of purification and of testing may be employed.

CONCLUSION.

Tests of chemically impure toxicological extracts may be made upon animals to confirm or to orient chemical identification.

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