BIOTOXICOLOGY. II. VOLATILE POISONS.

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A method for separation of the more common poisons from viscera has been reported (2, 3). Under that method, distillation with steam from the acidified viscera, and subsequently from alkalinized viscera, gave distillates which might contain volatile poisons. Because of the presence of various impurities in these distillates, chemical tests may give inconclusive results. Purification of these distillates is time consuming, and much of the toxic material may be lost.

Distillates from viscera of unpoisoned animals, while containing substances which reacted with many alkaloidal reagents, failed to produce characteristic effects upon animal tests. To assist in confirming chemical identity reactions, as well as to orient chemical methods of purification, certain biotoxicological procedures have been developed.

ACID DISTILLATE.

The volatile poisons in the distillate from the acid viscera possess characteristic odors which often furnish valuable information regarding the poison sought. However, some distillates especially from putrefied tissues have strong odors which interfere with organoleptic detection. Make up the distillate from acid viscera to a definite volume (10–25 cc.) and inject 1 cc. intraperitoneally into each of three mice weighing 10 to 15 Gm. (heavier mice may be used, but are less susceptible). Inject 0.1 cc. into each of three additional mice. Store the six animals in individual containers (1000-cc. beakers), and observe their behavior over a period of half an hour. Methyl, ethyl or isoamyl alcohol, or any of their alkyl esters, such as ethyl acetate, produce transitory stimulation, followed by depression, hypnosis, narcosis, coma and death, depending upon the quantity of the product injected. Chloroform, chloral and ether usually produce depression without preliminary stimulation. Aniline, nitrobenzene, phenol and carbon bisulfide produce marked stimulation with evidences of irritation, but rarely lead to narcosis. If animals die, the abdominal walls should be examined for evidence of irritation. Hydrocyanic acid tends to produce a vivid scarlet-red color in the venous as well as the arterial blood.

While positive animal findings must be confirmed by chemical tests, since they are not specific, negative results will suggest the absence of this group of poisons.

ALKALINE DISTILLATE.

The distillate from the alkaline viscera may show the "mouse odor" attributed to coniine.

(A) Mount a strip of muscle of the leech (Hirudo medicinalis) in 10 cc. of oxygenated frog Ringer's solution, immobilizing one end and connecting the other to a writing lever in the same manner as for the assay of pituitary extract on the isolated guinea-pig uterus. The tip of the writing lever is brought in contact with smoked paper on a slowly moving kymograph drum. After the strip relaxes to

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write a definite horizontal base line, with or without small spontaneous contractions, replace the immersion fluid with 10 cc. of frog Ringer's solution containing a concentration of 5 mg. of nicotine per liter, and allow to stand for 10 minutes. Drain off the nicotine solution and immerse the strip in frog Ringer's solution containing 4 per cent of alcohol until the writing lever has returned to its original level. By further tests on this sensitized muscle strip determine the threshold, which is usually about 0.25 mg. per liter. After the threshold has been determined, immerse the strip in a solution containing 1 cc. of distillate with 9 cc. of frog Ringer's solution. If a positive contraction is obtained, repeat with a more dilute solution; if no effect develops, repeat with a mixture of 5 cc. of distillate and 5 cc. of frog Ringer's solution. Continue with various dilutions until the threshold is determined. By this method 5 gamma of nicotine in 10 cc. (0.5 mg. per liter) may be detected and determined. Coniine requires one hundred times this concentration, while sparteine is inert.

To confirm the presence of nicotine, repeat the test on a new strip of leech muscle. A concentration of 1 mg. of nicotine per liter produces a definite sustained contraction, which is not relieved by washing with Ringer's solution, but is promptly relieved by immersion in a solution of 100 mg. of strychnine sulfate per liter. The contractions produced by coniine are shorter in duration, and are not so quickly counteracted by strychnine.

If desired, the dilutions of distillate in frog Ringer's solution may be combined, and steam distilled, or shaken out with ether, thus recovering the product originally taken for the test. In many toxicological examinations this economy of material is vitally necessary.

(B) Mount the sciatic nerve and gastrocnemius muscles of a frog weighing about 15 Gm. in the usual manner. Apply the distillate directly to the muscle, collecting the overflow for eventual recovery. Stimulate the nerve at 1-minute or 2-minute intervals, and observe the character of contractions produced. Nicotine, in a concentration of 5 mg. per liter tends to produce a persistent tonic contraction, which is quickly relieved by strychnine. Sparteine tends to depress the muscle fiber, large doses producing a curare-like paralysis (1, 5). The coniine contraction is somewhat similar to that produced by nicotine, but may be differentiated by strychnine. Exposure of the gastrocnemius muscle to concentrations of 100 mg. of strychnine sulfate per liter for 5 minutes tends to prevent subsequent coniine contractions, but does not affect subsequent tonic nicotine contractions.

(C) The injection of 0.1 to 0.2 mg. of nicotine into the lymph sac of a 15-Gm. frog causes decreased respiration and extension of the hind legs in a sitting position but the heels are not brought together. This reaction is characteristic for nicotine. It should be conducted on several frogs using a standard solution of nicotine before testing the distillate. Intraspinal injection of strychnine was 250 times as sensitive as lymph sac injections for the detection of strychnine (4). Intraspinal injections of nicotine appear to be more effective than lymph sac injections.

SUMMARY.

The following animal tests have been developed for volatile poisons:

1. Leech Muscle: Counteracted by strychnine: Nicotine.
   Less completely counteracted by strychnine: Coniine.
   Inert: Sparteine.

2. Frog Nerve-Muscle Preparation:
   Depression: Alcohol, ether, chloroform.
   Contraction: Relieved by strychnine: Nicotine.
   Prevented by strychnine: Coniine.
   Paralysis: Sparteine.
3. Frog Lymph Sac:
   Characteristic position: Nicotine.
   Negative: Coniine.
4. Mice, Intraperitoneal Injection:
   Depression: Alcohol, ether, chloroform, chloral.
   Stimulation: Phenol, aniline, carbon bisulfide.
   Blood changes, red color: Hydrocyanic acid.

CONCLUSION.

Methods of testing common poisons in the acid and the alkaline distillate from minced viscera have been developed to orient and to confirm chemical toxicological identification.

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A NEW ENTERIC COATING AND A LABORATORY METHOD FOR ITS CONTROL.*

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INTRODUCTION.

For many years enteric coatings of one kind or another have been applied to pills, capsules and tablets. In 1884, Unna (1) used keratin which was considered to be the first real enteric coating. Thirteen years later Hausmann and Weyland (2) created a new wave of interest when they developed formalin-gelatin. In 1915, Toplis (3) suggested stearic acid, and a few years later Freeman (4) supported its use as an efficient coating. Ammoniated white shellac was used by both Hilton (5) and Wruble (7), the former applying it in combination with salol. In 1932, Husa and Magid (6) reported the use of a mixture of salol, stearic acid and shellac. The most recent work was reported in June 1937 by Mills (9) who used a combination of cetyl alcohol and mastic, for which she claimed an efficiency of 98 per cent.

Generally, commercial enteric coatings have not been applied in a manner giving consistently efficient results. Also, there have been many different interpretations of a satisfactory coating. However, during the last several years radiography has been of great value in determining the behavior of tablets in the gastrointestinal tract. Bukey and Rhodes (10) in 1935 reported a wide variation in the efficiency of commercial enteric coatings, after having tested, radiographically,

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