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#### THE COLLECTION AND PRESERVATION OF BIOLOGICAL MATERIALS AND GENERAL PROCEDURE FOR TOXICOLOGICAL ANALYSIS

#### Sidney Kaye

Sidney Kaye (B.S. and M.S., New York University) was appointed Toxicologist in the newly organized Office of the Chief Medical Examiner, Commonwealth of Virginia, in October 1947. His present article was originally presented at the 1946 Annual Medicological Conference, St. Louis, which was held under the joint auspices of the St. Louis County Coroner's Office, the St. Louis County Medicologial Department, and the Washington University School of Medicine. At the time of these meetings and until he joined the Virginia Medical Examiner's Staff Mr. Kaye was a member of the Research Bureau of the St. Louis Police Department and of the Department of Pathology, Washington University School of Medicine.—EDITOR.

It is agreed generally that in areas where adequate toxicological facilities are provided, approximately ten per cent of all deaths investigated by medical examiners or coroners are likely to be the result of poisoning.<sup>1</sup> In a considerable number of violent deaths, poison may be found to be a contributing cause of death. Since the changes produced by poisons on various body tissues either are primarily biochemical rather than anatomical or are of such character that the causative agent cannot be identified precisely by pathological examination, it becomes the responsibility of the toxicologist to produce quantitative proof that a specific chemical agent has caused or contributed In clinical medicine where therapeutic agents may to•death. exhibit deleterious or fatal effects, the demand upon the toxicologist is less exacting, but in medico-legal investigations it is essential in many deaths that chemical agents be identified specifically and quantitatively determined or be completely excluded. Several case reports from personal files and the literature cited will serve to demonstrate this need.

(a) A young husband returned home unexpectedly one weekend to find that his wife had been unfaithful. Since this was not the first offense, he informed her of his intention to leave and sue for divorce. The wife persuaded him to remain with her for one last evening and celebration. Together they made the round of several taverns. The husband died shortly after they returned home. Chemical analysis disclosed a high concentration of alcohol in the brain and body fluids, but not an amount sufficient to account for death. Further analysis showed large amounts of chloral hydrate in the tissues. Upon interrogation and confronted with this evidence, the wife later confessed to poisoning her husband.

<sup>&</sup>lt;sup>1</sup>Alexander, L., Moore, M., and Leary, T., "Deaths from Poisoning," The American Journal of Medical Jurisprudence, 2, 1-14, 1940.

(b) An elderly couple were found dead in their hotel room. Autopsy revealed no adequate cause of death. Chemical analysis showed a faint trace of cyanide in the stomach. Special analysis of the lungs and blood resulted in the finding of large amounts of cyanide. Confronted with this evidence, the hotel management finally admitted fumigating the room directly below with cyanide gas without taking precautionary measures in regard to contigu-

(c) A woman was found dead in bed with all of the gas jets open. Chemical analysis failed to disclose the presence of carbon monoxide or any other poison. Under questioning, the husband admitted later that he had forced his wife's head onto the pillow until she died of suffocation. He confessed that then he had placed the body in a supine position on the bed and opened the gas jets in order to make it appear that his wife had committed suicide.<sup>2</sup>

Thus it may be seen how a toxicological laboratory may aid materially in the administration of justice. Assistance in the investigation of other types of death often results in significant findings as the following instance illustrates.

(d) A man was admitted to the hospital with severe injuries caused by an automobile accident. His condition required a number of blood transfusions. Death occurred several days later. Since there had been clinical evidence of nephritis, a toxicological analysis was performed. The kidneys showed a faint trace of mercury. Because of the circumstances of the illness, the analysis was repeated, however, with the same results. The quantity of mercury found was insufficient to account for death, but its presence was baffling. A thorough investigation of all treatment received at the hospital prior to death revealed that the decedent had received two transfusions of plasma that contained merthiolate (sodium ethyl mercuri-thiosalicylate) as a preservative which explained the presence of mercury in the kidneys.

#### PRESERVATION AND SHIPMENT OF SPECIMENS

It is essential that the toxicologist be supplied with an adequate amount of material placed in chemically clean glass covered containers. Intestinal contents, stomach contents, blood, urine, etc., should be placed in separate containers. Organs such as liver, kidney, lung, and brain may be placed in one container. All specimen jars should be sealed in a distinctive manner so that any tampering with the material will be evident

ous rooms.<sup>2</sup>

<sup>&</sup>lt;sup>2</sup>Gettler, A. O., "Why the Coroner System Has Broken Down," National Municipal Review, 13-10, 1924.

immediately. Specimens should be locked in a refrigerator until shipped or otherwise delivered to the toxicologist. The continuity of possession must not be broken. If the continuity of possession cannot be shown, the material becomes valueless for medicolegal purposes. A receipt with the date and hour indicated should accompany all transfers.

It is most satisfactory if the specimens are delivered shortly after first being obtained. The autopsy should be performed before embalming of the body since formaldehyde interferes with tests for cyanides, methyl alcohol, phenols, carbon monoxide, alkaloids, and hardens the tissue to such an extent that other analyses are difficult. When a chemical preservative must be used, 95% ethyl alcohol is to be preferred except, of course, where a determination for ethyl alcohol is indicated. In all cases, a sample of the preservative used should be submitted so that it can be analyzed for foreign substances.

It should be emphasized that where death is suspected to have been the result of poisoning, the amount of the drug, chemical, or other agent remaining in the gastro-intestinal tract at the time of death is inert insofar as the fatal dose is concerned. Examination of stomach or intestinal contents is often of material assistance in determining the mode of entry of the poison and the original form of the poison, but examination of the viscera or other body fluids is necessary to show that a fatal amount was absorbed. The demonstration of large amounts of a poison in the stomach contents and the failure to find the poison in the viscera or other body fluids is essential in deaths where efforts have been made to conceal homicide. In other instances where suicide, accident, or homicide by poison are in question, it is essential to show the quantity of the poison present either in blood or viscera.

Where accident or suicide by poison has been a matter of legal dispute, the records show that in many cases no effort has been made to show that poison was the actual cause of death!

Certain chemicals such as acids, alkalis, sulfides, chlorates, nitrates, and nitrites present to the toxicologist special difficulties in analysis because of rapid change. Where such chemicals are suspected, the toxicologist always should be provided with the stomach contents in addition to adequate amounts of various tissues.

Specimens from living cases should contain liberal samples of vomitus, stomach contents, urine, feces, blood for gas analysis, and suspected food, drink, or drugs. MATERIAL BEST SUITED FOR TOXICOLOGICAL ANALYSIS

Specimen	Minimum Amount	Poison For Which Best Suited
Urine	All available	In nearly all types of poisoning.
Stomach Contents	All available	In which poison is known or thought to have been taken by mouth within a few hours.
Intestinal Contents	All available	For cases in which poison was taken by mouth within one or two days.
Blood	At least 10 cc (preferably 200 cc)	All gas poisons, methemoglobin, sulfonamides, bromides, drowning test, and many other poisons.
Brain	500 grams	Volatile poisons, barbiturates, alkaloids, and acute alcohol- ism, etc.
Liver	300 grams	Metals, barbiturates, fluorides, oxalate sulfonal, and many other poisons.
Kidney	One kidney	Metals, especially mercury, sul- fonamides.
Bone	200 grams	Lead, arsenic, radium (espe- cially chronic).
Lung	One lung	For inhaled poisons (proof of entry).
Hair, Finger & Toe Nails	5 grams	Chronic arsenic poisoning.
Muscle	200 grams	In most acute poisoning, and when internal organs are badly putrefied.

#### LEADS AND CLUES

From the standpoint of the toxicologist it is indispensable that he have certain preliminary information for several reasons:

1. Attention can be concentrated on certain groups of poison,

2. Large numbers of time-consuming analyses can be avoided, and

3. Limited material can be more efficiently utilized. Lead and clues can be obtained from:

- 1. Circumstances surrounding the death or illness,
- 2. Symptoms and behaviour before death, and

3. Gross and microscopic autopsy findings.

It is essential to know the first appearance of symptoms or death after taking last food or drink, the nature and intensity of these symptoms, whether or not there was vomiting, deep sleep, tingling of skin and throat, convulsions or twitching of the muscles, delirium, dyspnea, contraction or dilatation of pupils, changes in vision or hearing, etc., in addition to any evidence found at the scene. The autopsy findings aid in eliminating or considering certain poisons with selective effects on the various tissues.

The symptoms and behavior of the patent may be of great help in excluding or taking into account large groups of poisons which probably would or would not be present. Many diseases produce symptoms which are similar to those caused by poisons. Unusual symptoms may be produced in an individual by various poisons, just as unusual symptoms may occur in any disease.

(e) A man was observed to be staggering down the street of an eastern city. The police stopped him for questioning and while doing so he collapsed. He was sent to the hospital for observation. An alcohol determination was performed, and it was negative. There was no evidence of diabetes mellitus nor of head injury. In the hospital he was observed to be comatose with shallow breathing and contracted pupils. A small sample of urine was submitted with a special request for the possible detection of morphine or its derivatives. No morphine was found. However, morphine is a particularly difficult alkaloid to isolate and identify, so the analysis was repeated, and again with negative results. The acid ether extraction which was made merely to remove coloring matter and other impurities before attempting to isolate alkaloids, was observed to contain a small crystalline formation, later identified as a barbiturate (amytal). This was least expected due to the alleged cardinal symptom of contracted pupils. However differentiation between contracted and pin point pupils due primarily to morphine was not made. Differentiation would have been simple by noticing reaction to either light or pain stimuli.

In instances where persons are found dead under obscure circumstances and autopsy discloses no significant changes grossly or microscopically, the toxicologist must have ample material with which to perform the large number of analyses that may be required. In such situations, a microscopic examination of the stomach and intestinal contents may provide a clue as to any chemical agents which may have been absorbed in lethal amounts. Carefully conducted qualitative tests of such material may assist in limiting the analysis of blood or various tissues to specific groups of poisons.

#### IMPORTANT SYMPTOMS OF POISONING

Vomiting, convulsions, coma, paralysis, changes in the size of the pupils, altered respiratory rate, delerium, and cyanosis are important features of poisoning by substances, but also accompany many diseases. Additional clues in differentiating poisoning from disease may be obtained from external staining of the skin, changes in the color of the urine, and alteration of skin color by absorbed poisons. In deaths by poisoning, characteristic odors may be encountered at autopsy. Following is a partial list of symptoms and signs produced by various poisons.

- *Vomiting:* Heavy metal salts; corrosive acids and alkalies; halogens; aconite; cantharides; croton oil; gelsemium; phosphorus; phenols; wood alcohol; veratrum; muscarine.
- Convulsions: Ammonium salts; aspidium; brueine, camphor, saponin; aconitine; picrotoxin; cyanides; strychnine.
- *Coma:* Opium; morphine; heroin; barbiturates; chloral hydrate; sulfonal; trional; paraldehyde; chloroform; ether; cyanide; carbon monoxide; carbon dioxide; atropine; nicotine; hyoscine; alcohols; phenols; scopolamine.
- General or Partial Paralysis: Cyanide; carbon monoxide; carbon dioxide; botulism.
- Dilatation of Pupil: Belladonna and derivatives; hyoscyamine; strammonium; alcohol; gelsemium; cocaine; nicotine.
- Contraction of Pupil: Opium and derivatives; physostigmine; pilocarpine; muscarine.
- Slow Respiration: Opium and derivatives; carbon monoxide; hypnotics.
- Rapid Respiration: Belladonna and derivatives; cocaine; carbon monoxide.
- Delirium: Belladonna and derivatives; cocaine; alcohols; camphor; solanine; marihuana.
- Dyspnea: Strychnine (during convulsions); cyanide; carbon monoxide; carbon dioxide; easily volatile organic liquids.
- Cyanosis: Nitrobenzene; aniline; acetanilid; opium; chlorates; amyl nitrite.
- Staining of the Skin: Iodine, black; bromine, deep brown; nitric acid and pieric acid, yellow; phenol, bleaching.
- Abnormal Coloring of the Urine: Phenol, salol, resorcinol, dark green; antipyrine, trional (after long use), red; pyrogallol, brown or black; picric acid, yellow; santonin, bright yellow changing to scarlet on adding caustic alkali.
- Abnormal Coloring of the Skin or Mucous Membranes: Carbon monoxide, nitrites; cyanide; cherry red or pink; silver salts, black blotches.
- Discoloration of Gums: Lead, mercury, bismuth (usually chronic poisoning).
- Abnormal Odor of Tissues at Autopsy: Phenol; creosote; chloroform; cyanide; ether; alcohol; nitrobenzene; phosphorus; crude opium.

### TOXICOLOGICAL ANALYSIS

The actual testing for poisons presents no great difficulty for the expert if the poison is present in moderate amounts, but considerable skill and experience are required in separating poisons from extraneous materials and in purification before specific tests can be applied.

It is impractical to describe here the various separation methods employed to isolate poisons since they vary with the nature of the materials. For most practical purposes the general scheme and modifications of Stas-Otto is still used for the isolation of many of the common poisons; for speed and conservation of material, some of the common poisons are divided into several groups, and the isolation is performed serially.

- 1. Volatile: Alcohols, acetone, aldehydes, camphor, aniline, benzene, nitrobenzene, phosphorus, ether, chloroform, cyanides, carbon disulfide, phenols, pyridine, chloral, etc.
- 2. Acid ether soluble: Barbiturate derivatives, salicylates and derivatives, benzoic acid, caffeine, cantharides, acetanilide, phenacetin, picrotoxin, sulfonal group, etc.
- 3. Alkaline ether soluble: Alkaloids in general such as atropine, cocaine, codeine, quinine, brucine, strychnine aconitine, etc.
- 4. Ammonia ether, chloroform (ethyl acetate) soluble: Morphine, theobromine, apomorphine, etc.
- 5. Heavy metals: Lead, bismuth, cadmium, antimony, arsenic, mercury, thallium, zinc, etc.

Testing for these five main groups is usually adequate for the isolation of many of the common poisons. However, it must be remembered that even some of these require special treatment (aniline, phosphorus, ether, chloroform, cyanides, methyl bromide, etc.), or they may be missed.

Still many others such as fluorides, carbon monoxide, radioactive agents, fluoroacetate, toxalbumins, metrazol, D.D.T., glucosides, benzedrine, etc., are analyzed by particular methods and techniques. A final word of caution: Toxicological examination of biological material is work for an expert and should never be undertaken except by those having specific training and experience. The rough qualitative testing sometimes attempted by those without instruction or experience in forensic chemistry is injudicious since it wastes irreplaceable material and may lead to an erroneous result.

Where poison is suspected of having caused or contributed to death, the demand on the part of integrated medicolegal investigation for scientific chemical proof can be met best by providing the toxicologist as soon as possible with full information and adequate amounts of the proper material suitably collected and preserved.

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