J Forensic Sci, Jul. 1978, Vol. 23, No. 3

Case Report

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Drug Analyses of Skeletonizing Remains

A 47-year old white female was reported missing by her family after an argument with her husband and son. The arguments were of long and continuing nature with sharp differences as to the use of real properties. Her body was discovered by the family dog eight months later in a desolate area on the family ranch on the slope of a hill.

The body was virtually skeletonized and the remains were decomposed. The body, including the hands, was positioned in a reposed, supine manner. Near the body was found a paper sack containing a capped soft drink bottle with a small amount of clear, colorless fluid and an empty Styrofoam® cup.

The examination of the clothing of the decedent showed no scuffing or other damage to indicate that she could have fallen from the hill or suffered physical violence. In a pocket of the decedent's coat was a note enclosed in an envelope, which discussed in general the problems she had had with her husband and children and a reference to her burial wishes. However, according to her husband, the note was written a year prior to her disappearance. The husband also stated that his wife occasionally used diazepam when it was prescribed to him; however, no large amount of this drug was missing from the prescription bottle.

An autopsy was performed on the remains, but there was no indication of trauma. No fracture was revealed to indicate a precipitous fall from the hill. No foul play could be determined.

It was decided that toxicological examination be conducted on the remains. The matter of tissue specimen selection posed some problems. Blood was not available because of the decomposed condition of the body. There were remains of dried-up lung and muscle tissues and the skeleton. Analysis of the bone was made on the basis that it represented a blood repository and drugs present in blood may be contained in the bone marrow. In the recently deceased, the bone marrow is heavily laden with blood and compression yields oozing blood. In this case, the bone marrow was dry and it was reasoned that drugs present in the blood would be deposited in the marrow as dehydration proceeded.

Methods and Results

The vertebral column bone marrow was used. It was accumulated from the odontoid

Presented at the 29th Annual Meeting of the American Academy of Forensic Sciences, San Diego, Calif., 15-19 Feb. 1977. Received for publication 10 Nov. 1977; revised manuscript received 29 Dec. 1977; accepted for publication 4 Jan. 1978.

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processes of eight vertebrae beginning with the atlas bone and descending. The outer layer of bone of each odontoid process was removed with hand-held snips and discarded.

The accumulated bone marrow weighed 51.8 g. It was homogenized and then warmed in 200 ml of ethanol. The ethanol was filtered and one fourth of the recovered volume was used for analysis.

The ethanol was evaporated, and the residue was dissolved in 25 ml of distilled water. The internal standard, 100 μg of SKF-525-A (proadifen), was added to the water. To this was added 5 g of sodium bicarbonate. The solution was extracted with 250 ml diethyl ether. After settling and separating the ether layer was passed through filter paper into a separatory funnel. The ether was washed by shaking with 10 ml of 0.5*N* sodium hydroxide. After settling the aqueous layer was discarded. This wash was followed by a similar wash with 10 ml of distilled water. The ether was filtered through #2 Whatman filter paper, then extracted with 6 ml of 2*N* hydrochloric acid and the ultraviolet (UV) absorption spectrum of this acid was determined. There was considerable unrecognizable absorption in the 240-nm region.

The acid extract was made basic to pH 11 by the addition of 10*N* sodium hydroxide. Extraction with chloroform was done. The chloroform was separated and evaporated to dryness in a glass cone. One hundred millilitres of ethanol was used to dissolve the residue in the cone; a 2-μl sample of this ethanol solution was analyzed by gas chromatography (GC).

The GC analyses were conducted with a Hewlett-Packard Model 5700A equipped with a dual flame ionization detector. The columns used were 3% OV-1 and 3% OV-17, both on Chromosorb W-HP, and both maintained at 220°C in the oven. These columns had a helium flow of 20 ml/min. The temperature of the detector and the injection port was 280°C. On each column, the retention time of a prominent peak corresponded to that of amitriptyline. The UV absorption spectrum was not distinctive enough to identify amitriptyline specifically, so the bone marrow extract was further analyzed with the mass spectrometer. A gas chromatograph-mass spectrometer (GC-MS), Finnigan Model 3330, was used in the electron ionization mode. The mass spectrum of the substance in the sample believed to be amitriptyline showed favorably close agreement with the mass spectrum of standard amitriptyline; these spectra are shown in Fig. 1. In addition, the total ion GC from the appropriate peak and standard amitriptyline were identical. The GC-MS examination failed to detect diazepam.

Amitriptyline was quantitated by GC peak area ratio between the amitriptyline and the SKF-525-A internal standard. Amitriptyline was calculated to be 0.07 mg recovered from 51.8 g of bone marrow.

In addition to amitriptyline being found in the spinal cord bone marrow, it was also found in the remains of lung tissue and in leg muscle. The calculated amounts of amitriptyline in these two dry tissues were 20.5 mg/50 g lung and 0.85 mg/50 g leg muscle. The vertebral column bone marrow, lungs, leg muscle, oral cavity, and leg bone marrow were analyzed for barbiturate, narcotics, sedatives, hypnotics, and tranquilizers. No other drugs were detected.

Discussion

The drug finding in bone marrow has forensic value. The police did not expect the finding that a drug was present in the skeleton and immediately it led to a systematic search of pharmacies in the community in which the decedent lived. One of these pharmacies did in fact have in its files a prescription written for the decedent for amitriptyline.

The finding of amitriptyline in the bone marrow and other tissues in the remains of the deceased may be related to antemortem circulation of the drug. It is of interest to relate the amount of drug found in bone marrow with its blood level at the time of death. Many

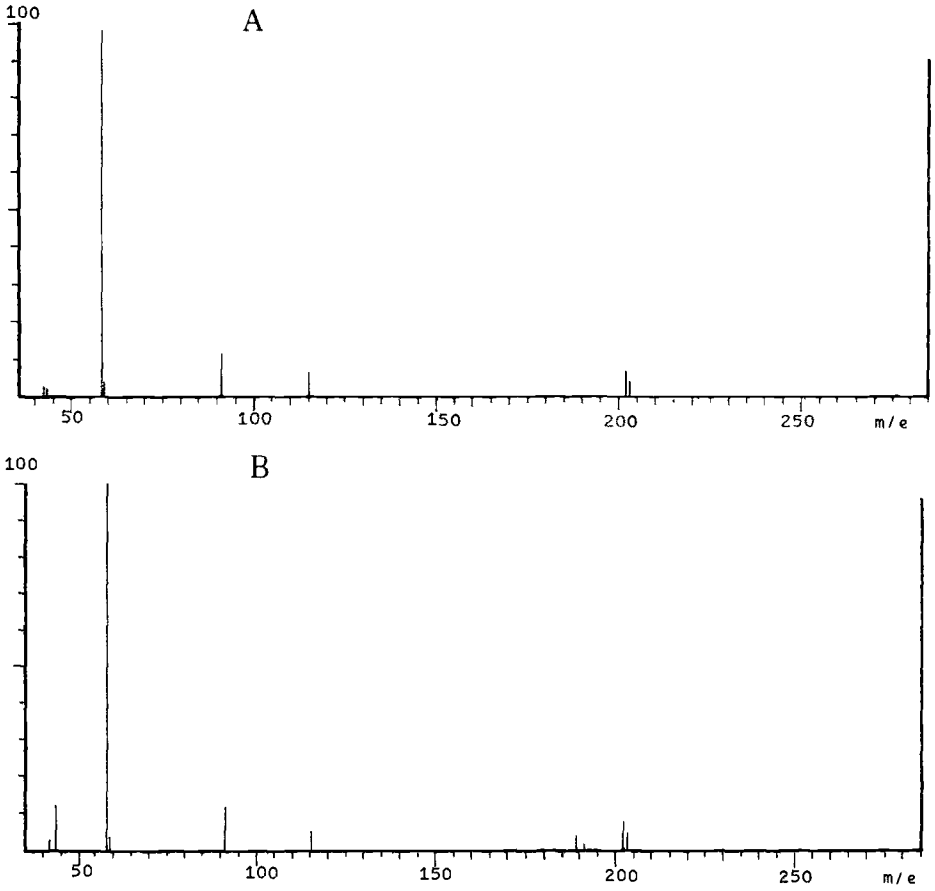

FIG. 1—*Mass spectra (electron impact) of amitriptyline of* (A) *bone marrow extract and* (B) *standard sample.*

difficult questions will have to be answered before such inferences can be made. For example, what is the distribution of the various drugs in bone marrow? How does the bone marrow drug concentration correlate with the concentration in systemic blood? What is the decay rate of drug in cadaverous bones?

Acknowledgment

We wish to thank T. C. Cairns, Food and Drug Administration, Los Angeles, Calif., for corroborative GC-MS work and L. Joiner and J. Campeau of this laboratory for analytical assistance.

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