CASE REPORT

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Insect Larvae Used to Detect Cocaine Poisoning in a Decomposed Body

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ABSTRACT: Insect larvae are often found on human remains long after disappearance of the usual toxicologic specimens. It is important for forensic pathologists and toxicologists to recognize the potential of this unique specimen when routine specimens are not available.

Cocaine and benzoylecgonine was extracted from Calliphorid larvae found on a badly decomposed body of a man who had been missing 5 months and was also identified in the decomposing skeletal muscle. This toxicologic information combined with the autopsy findings and the circumstances of the death and disappearance was essential in the determination of cocaine poisoning as the cause of death.

KEYWORDS: toxicology, cocaine poisoning, insect larvae

Forensic pathologists frequently examine decomposed and skeletonized human remains. In such situations, traditional toxicologic specimens, such as blood, urine, and solid organs, are often unavailable. Fly larvae have recently been used as toxicologic specimens to detect such diverse substances as bromazepam, levomepromazine [*1*], malathion [*2*], phenobarbital [*3,4*], trazolam, oxazepam, alimemazine, and clomipramine [*3*]. Mercury has even been recovered from live flies associated with an individual forensic autopsy case [*5*]. The postmortem intervals in these cases have ranged from 8 days to 6 months. In addition, morphine [*6*] and cocaine [*7*] have been recovered from larvae under experimental circumstances. This report details the first forensic-science case where cocaine and benzoylecgonine was recovered from fly larvae and where this information was valuable in determining the cause of death.

Case History

Almost completely skeletonized human remains were discovered by walkers in a wooded area of Connecticut in February. The remains were identified as a 29-year-old male

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intravenous drug abuser who had been reported missing the previous September, one week after he was last seen alive. His last contact was with his girlfriend who thought he had injected cocaine, become agitated, and then disappeared.

The body was found prone, lightly covered with snow, and partially embedded in frozen earth in a densely wooded area approximately one quarter mile from where he was last seen. The skull and mandible were located 25 ft and 8 ft from the body respectively. No drug paraphernalia was discovered.

At autopsy, the clothing had no unusual cuts, tears, or holes. The head and trunk were completely skeletonized, and both arms were mostly skeletonized (Fig. 1). Both legs and feet were completely fleshed with decomposed and desiccated skin and soft tissue (Fig. 2). Other than some small animal disruption, no injury was seen. Many dead, partially decomposed, and intact small and large maggots, in addition to pupal cases, were found on the body surface and within the body cavities and the clothing. Neither projectiles nor projectile fragments were seen on complete body radiographs. The body was identified by comparison of antemortem and postmortem radiographs.

Skeletal muscle from the legs, fly larvae, and pupal cases were collected at autopsy and frozen at −4°C. Rearing larvae to the adult stage was impossible as none of the collected specimens were alive. Preserved larvae were subsequently identified, based on

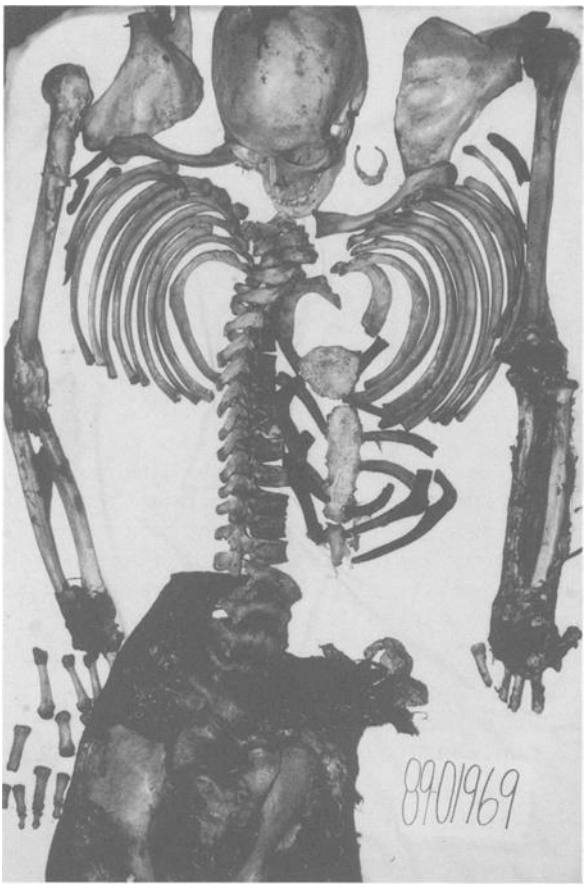

FIG. 1—*Completely skeletonized head and trunk and almost completely skeletonized arms of decedent.*

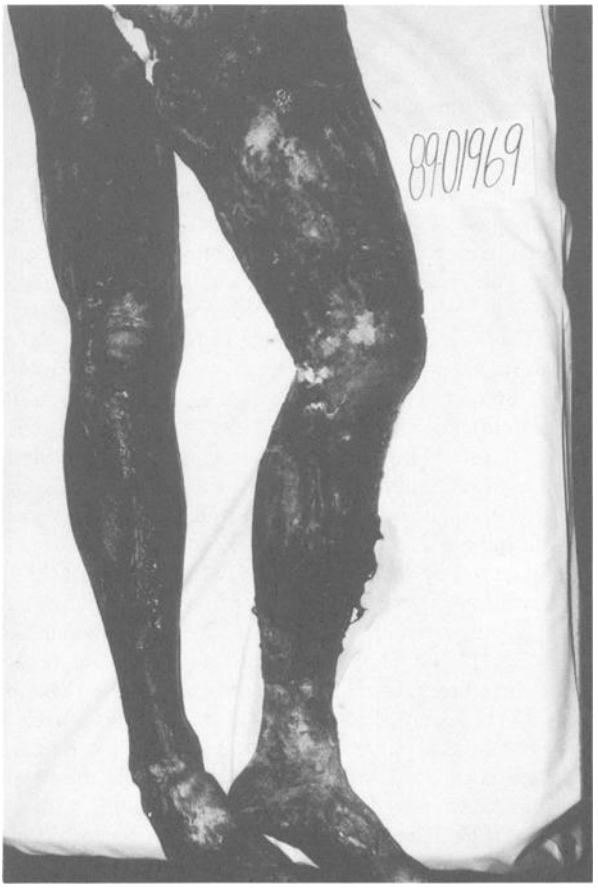

FIG. 2—*Legs of decedent fleshed with decomposed and desiccated skin and soft tissue.*

morphological characteristics, as postfeeding, third instar stages of the blue bottle fly, *Calliphora vicina* (Robineau-Desvoidy).

Analytical Procedures

Initial screening for benzoylecgonine and cocaine was performed using radioimmunoassay. Both muscle and larvae were homogenized with approximately three times their weight of deionized water. The homogenates then were pipetted and processed according to the procedure specified for urine by the assay manufacturer.[4] The positive reference standard contained 300 ng/mL benzoylecgonine.

Confirmation of the presence of cocaine in muscle was accomplished by full spectrum gas chromatography/mass spectrometry (GC/MS) (Hewlett Packard, model 5970A). A 4 g sample of the muscle homogenate was extracted with 8 mL n-butyl chloride at pH 9.8; backextracted into 2 mL 1N H_2SO_4; re-extracted into 4 mL n-butyl chloride; taken to dryness and reconstituted with 50 μL ethyl acetate. One microliter of the extract was injected, using the splitless mode, onto a 5% phenyl, methyl silicone capillary column

[4]Abuscreen Radioimmunoassay for cocaine metabolite, Roche Diagnostics Systems, Inc., Nutley, NJ, Dec. 1988.

(25 m by 0.2 mm by 0.33 μ). The carrier gas, helium, was set at a flow rate of 0.59 mL/min (measured at 200°C). Column temperature was programmed at the following rate: 50°C (2 min hold) raised at 40°C/min to 200°C (4 min hold) raised at 10°C/min to 300°C (15 min hold). These conditions resulted in a retention time of 16.3 min for cocaine.

Quantitative evaluation of cocaine in muscle and larval specimens was performed with gas chromatography (GC) (Perkin Elmer, model 8500) using a nitrogen-phosphorus detector. Both muscle and larval homogenates were handled in an identical manner. Two g of each specimen homogenate were spiked with 0.4 mL of a 0.5% solution of prazepam (internal standard) in methanol. This mixture was buffered to pH 9.8 and extracted with 2 mL of butyl acetate. The organic layer was carefully removed, transferred to a clean conical tube, and taken to dryness. The dry residue was reconstituted with 100 μL of methanol. One-half μL of the extract was injected, using the splitless mode, onto to a 50% phenyl, methyl silicone capillary column (25 m by 0.32 mm by 0.25 μ). Column temperature was programmed at the following rate: 240°C (2 min hold) raised at 8°C/min to 280°C (0 min hold) raised at 10°C/min to 310°C (2 min hold). Column head pressure was set at 12.2 psi. Under these conditions, the retentions for cocaine and prazepam were 4.3 min and 8.5 min respectively.

Fourteen months after the specimens were collected, a GC/MS procedure for cocaine was performed on the larvae. No cocaine was detected. Therefore, the presence of benzoylecgonine was pursued by full spectrum GC/MS (Finnigan Mat ITD, model 800) in both muscle and larval homogenates. Aliquots of the larval and muscle homogenates equivalent to 2 and 4 g, respectively, were added to 50 mL silanized tubes containing 2.5 μg ketamine, 2 mL 0.1N NaOH, 200 mg NaCl and 20 mL chloroform:isopropanol (4:1). The tubes were mixed on a rotator for 15 min, centrifuged and an 18 mL aliquot of the organic phase was transferred to a clean 15 mL tube and back extracted into 3 mL of 1N HCl. The pH of the acid phase was adjusted to 8.5 with concentrated ammonium hydroxide and extracted with 5 mL methylene chloride. The methylene chloride was evaporated to dryness and derivatized with pentafluoropropionic anhydride (PFFA) and hexafluoroisopropanol (HFP). The derivatized extract was taken to dryness and reconstituted with 25 μL ethyl acetate. Five μL of the solution was injected into the GC/MS fitted with a 30 m by 0.25 mm fused DB-5 capillary column. Column temperature was programmed at the following rate: 100°C (5 min hold) raised at 10°C/min to 280°C (5 min hold). Under these conditions, the retentions for ketamine and the hexafluoroisopropyl derivative of benzoylecgonine were 11.13 and 12.57 min respectively.

Benzoylecgonine was quantitated using the Finnigan automated quantitation program.[5] The program integrates the GC peaks for the internal standard ketamine and the derivatized benzoylecgonine, transfers the data into a quantitation file, and calculates the concentration based on a calibration file prepared from standards.

Results

Positive findings are summarized in Table 1. No ethanol or other volatile hydrocarbons were detected in a muscle specimen screened by gas chromatography. No opiates, benzodiazepines, or barbiturates were detected in a muscle specimen screened by radioimmunoassay. In addition to cocaine (Table 1), lidocaine and caffeine were qualitatively identified in a muscle specimen analyzed by GC/MS. No other basic drugs were found.

Discussion

This is the first report identifying cocaine recovered from insect larvae associated with a decomposed body. The skeletal muscle also contained cocaine. Since the in vivo half-

[5]Ion Trap Detector Service Manual, Quantitation chapter, Finnigan Mat, 1985, pp. 125–165.

TABLE 1—*Analytical results on muscle and insect larvae.*

	RIA (C[a] & BE[b])	GC (C)	GC/MS (C)	GC/MS (BE)
Muscle	positive	*[c]	positive	0.33 mg/Kg
Larvae	positive	0.49 mg/Kg	negative[d]	0.03 mg/Kg

[a]Cocaine.
[b]Benzoylecgonine.
[c]Interference in assay.
[d]14 months after specimen obtained.

life of cocaine is short and ranges from 0.8 to 1.25 h depending on the route of administration [*8*], the presence of cocaine in both larvae and skeletal muscle compellingly indicates cocaine use in the immediate few hours before death.

Several potentially fatal manifestations of cocaine toxicity have been described during the past decade. These have included adverse cerebral, cardiac, obstetrical, intestinal, and pulmonary effects [*9*]. In the case described, knowledge of the circumstances, combined with the absence of violent injury and the presence of cocaine in larvae and skeletal muscle, allowed a reasonable determination of "cocaine poisoning" as the cause of death. Toxicologic results from maggots have been useful in the determination of the cause of death in two other instances involving suicidal ingestion of phenobarbital [*4*] and malathion [*2*].

Flies of the genus *Calliphora* are large, metallic blue, bristly insects commonly encountered in and around decomposing materials [*10*]. These flies enjoy a wide geographic distribution and are particularly abundant in urban areas and other habitats closely associated with people [*11*]. *Calliphora* populations frequently dominate vertebrate carrion communities during cooler seasons of the year [*12*].

In regions with hot summers, the blue bottle fly (*Calliphora vicina*) has a bimodal population distribution with adult abundance peaking in the spring and fall [*13*]. In the late fall, *Calliphora vicina* adults generally cease activity and seek protected sites in which to overwinter. *Calliphora vicina* eggs deposited on decomposing remains in late fall undergo a slowed development due to seasonal environmental conditions. Similarly, larvae hatching from these eggs undergo a prolonged maturation process, enduring the winter months either within the remains or in the substrate beneath. With the onset of spring, *Calliphora vicina* metamorphosis is completed and a new generation of adult flies emerges [*10*]. The prolonged hiemal development characteristic of late fall populations of *Calliphora vicina* and other carrion-frequenting insects enhances the availability of analyzable insect tissues long after conventional toxicological specimens have disappeared. In the case reported, the postmortem interval was 5 months. Cocaine has been shown to alter the developmental rate of fly larvae [*7*]. When these rates are used to estimate postmortem interval, it may be important to toxicologically analyze larval specimens.

Although skeletal muscle contained cocaine, quantitation by gas chromatography was not possible because of interference by tissue-decomposition products. The larvae provided a more suitable specimen without decomposition interference, and quantitation was possible. It is not known whether larvae bioaccumulate or eliminate cocaine. Therefore, the interpretation of cocaine concentrations in larvae would require controlled studies. Since homogenates of whole larvae and pupal cases were prepared, it is unclear if the cocaine is localized in the chitinous exterior, the soft viscera or both regions of these insects.

Cocaine and other drugs have been identified in the protein matrix of human hair of drug abusers [*14,15*]. Even 167 years after his burial, opiates were found in the hair of

the poet John Keats, who used laudanum for pain control [*15*]. Benzoylecgonine has been found in the hair of pre-Columbian South American mummies approximately 3000 years after death.[6] If cocaine or other drugs are similarly deposited in the protein matrix of growing pupal cases toxicologic evaluation may be possible several years after death and indeed may even be possible with paleopathologic specimens. It is important for forensic pathologists to make use of insect larvae as toxicologic specimens when human specimens are not available. Additional case reports and controlled toxicological studies will eventually delineate the full value of insect-larvae specimens.

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