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Detection of Cocaine Metabolite in Perspiration Stain, Menstrual Bloodstain, and Hair

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ABSTRACT: Low nanogram and picogram quantities of cocaine metabolite equivalents were detected in extracts from perspiration stains, menstrual bloodstains, and hair using radioimmunoassay. The theory of drug inclusion in hair and its significance are discussed.

KEYWORDS: criminalistics, cocaine, radioimmunoassay, body fluids, hair

The radioimmunoassay (RIA) technique for benzoylecgonine (a cocaine metabolite) and structurally similar compounds that occur as congeners or metabolites of cocaine [*1-4*] has attained routine laboratory use as a drug use test of questioned body fluids. Its advantages over other methods (for example, thin-layer chromatography [*5,6*], gas liquid chromatography [*6, 7*], high performance liquid chromatography [*8*], and enzyme multiplied immunoassay technique [*9*]) include increased sensitivity, ability to be used with crude biological samples, and relatively inexpensive, rapid analysis of large numbers of samples. The use of a confirmatory chemical test is normally desirable in forensic science matters and gas liquid chromatography/mass spectrometry (GC/MS) [*10-14*] is often chosen because of its extreme sensitivity and ability to provide alternative information relating to the chemical structures of parent molecules, congeners, and/or metabolites detected. Unlike RIA, samples require pretreatments for GC/MS, and several samples cannot be analyzed simultaneously.

The applications of this technique to the detection of drugs in body fluids, stains, and hair [*15-21*] exemplify an increased awareness of the utility of RIA technology and point to a growing role of RIA in forensic science.

This report describes the analysis of extracts from perspiration-stained clothing, menstrual-bloodstained tampon, and hair which had been collected from an alleged sexual assault victim by law enforcement officials as part of their standard investigation procedures. While routine analyses for trace evidence associated with these items did not provide evidentiary links to incriminate the defendant, the detection of cocaine metabolite in these items was used to implicate the alleged victim of cocaine use, a fact that she denied in sworn testimony at trial but later admitted. The State's Attorney could have appealed the presentation of these findings by way of a petition for review, and he did not.

To the best of the authors' knowledge, this represents the first time that expert testimony

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regarding drug detection in hair was presented in court in the United States [*22*]. The challenges to this testimony were the questions of specificity of the RIA test for cocaine metabolite and of external contamination. The testimony maintained that, under the conditions of the test, only cocaine, its metabolites, and immunologically similar molecules could have caused the results observed and that their presence indicated cocaine use.

Materials and Methods

Sample Preparation

Perspiration stains were extracted and tested as previously described [*19*]. The bloodstained tampon was tested by first cutting a 1/4-in. (6.35-mm) section from the end. This section was further cut into small pieces, placed in a round-bottom glass culture tube, and eluted for seven days with 0.8 mL of 0.05% sodium dodecyl sulfate in 0.85% aqueous saline which had been stabilized with 1% sodium azide at 4°C. Subsequently, 0.1 mL of the eluate was assayed in duplicate by the RIA method. An equivalent-sized dried bloodstain on cloth served as a negative control and was tested in the same manner. Hairs (numbering 41 and weighing 58 mg) were washed six successive times with 100 mL 0.05% aqueous sodium dodecyl sulfate, cut into small segments, mechanically pulverized for 6 min in a dental amalgamator, and refluxed in 10 mL of ethanol for 2 h [*21*]. The liquid was filtered through glass wool filter paper, separated into equal aliquots, evaporated to dryness, to which 0.1 mL of negative control urine was added, and tested by the procedure described. Equivalent hairs from a drug-negative individual were used as negative controls and were tested by the same method as the questioned hairs.

Calibration Curve

A standard curve (Fig. 1) was prepared by testing, in duplicate, the designated quantities of cocaine corrected to benzoylecgonine equivalents according to the manufacturer's instructions [*23*]. This curve indicates that the procedure established for this series of experiments is capable of detecting 500 pg of benzoylecgonine equivalents. It also serves as a calibration curve for determining the concentration of benzoylecgonine equivalents in the samples, where "shirt" and "bloodstain" indicate the amounts detected from the underarm area of the shirt and the bloodstained tampon, respectively.

Results and Discussion

Table 1 shows that benzoylecgonine equivalents were detected in the amounts of greater than 10 ng (offscale reading) for the perspiration stain, 570 pg for the menstrual bloodstain, and 6.2 ng for the hair. All duplicates agreed within $\pm 14\%$ variation. Negative control samples were below the cutoff for the first positive standard, 500 pg, with the exception of the negative hair control, which was equivalent to this lowest standard. It has been reported that factors from pulverized hair may shift the standard curve slightly, possibly a result of matrix effects [*24*]. When this source of error is taken into account, the 6.2 ng reported in the questioned hair sample could have been underestimated by as much as 0.5 ng.

Cross-reactivity of chemical compounds sharing structural similarities to benzoylecgonine were cocaine (1.390), norcocaine (0.330), benzoylnorecgonine (0.466), pseudobenzoylecgonine (0.215), pseudococaine (0.0244), ecgonine (0.000 604), norecgonine (0.000 288), methylester of ecgonine (0.002 19) and thioridazine (0.000 481) [*23*]. The last drug exhibited so little cross-reactivity that its presence under normal circumstances would not cause a false positive. The varying degrees of cross-reactivity reported for cocaine, its metabolites, and its congeners enhance the sensitivity of the test for determining cocaine

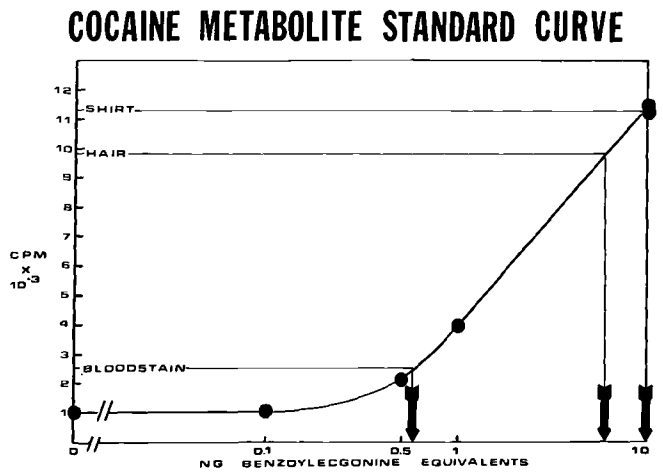

FIG. 1—*Standard curve for benzoylecgonine equivalents.*

TABLE 1—*Radioimmunoassay results from the analysis of evidence and control samples shown in radioactive counts per minute (cpm) and nanograms (ng) detected as extrapolated from the standard curve.*

Sample	cpm	ng
Perspiration stain (area on shirt)	11 339.0	>10
Menstrual bloodstain (tampon)	2 531.5	0.57
Hair	9 861.5	6.2
Negative bloodstain and fabric extraction control	1 433.5	0
Negative hair control	2 154.0	0
Negative urine control	1 795.0	0
Positive urine control	10 862.5	10.0

abuse because these structurally similar compounds are recognized to some extent by the antisera.

The practical concern that both perspiration stain and bloodstain could have been contaminated through handling by individuals with traces of cocaine on their hands could not be ruled out. On the other hand, the successive washings to which the hairs were subjected before analysis show that the substances detected were contained within the hair, not merely on the surface, and originated from an individual who used cocaine. This contention is supported by previous studies [*25-29*] which found that amphetamine, methamphetamine, phencyclidine, barbiturates, and opiates are found in hair after drug use.

The mechanism by which drugs become incorporated into hair is not known. Hair is composed of, predominantly, keratin microfibril proteins and matrix proteins [*30*]. The amino acid composition includes phenylalanine [*30*], which contains an aromatic system of pi-cloud electrons in its side chain. Other amino acids found in hair that contain conjugated systems or localized pi electrons or both are tyrosine, histidine, glutamic acid, aspartic acid, and arginine [*30*]. Given the regular, helical structure of the keratin microfibril proteins of hair, it is reasonable that these pi electrons would frequently occur on adjacent turns of the helix. The distance between turns of the helix (0.54 nm) [*30*] is great enough to allow the pi-cloud electrons found in cocaine-like structures to intercalate between the adjacent pi electrons of these amino acid side chains. Such bonds may act to extract cocaine metabolites from the blood that nourishes growing hair follicles. Subsequently, the drug metabolites could be solubilized from hair by the physical and chemical stresses of pulverizing and refluxing the hair in ethanol. Similarly, the drug substances may be bound by the matrix proteins that surround bundles of keratin microfibrils and released by the extraction procedure described. These questions, when resolved, will provide a deeper understanding of hair growth as well as models for predicting the avidity of growing hair to other circulating drugs.

Conclusion

RIA of drugs and hormones in body fluids and tissues is an established procedure in the practice of clinical medicine and forensic toxicology. It is our hope that communications such as this will lead to a greater awareness of the potential of RIA in forensic science, including new applications using RIAs for cannabinoids, certain prescription drugs, and lysergic acid diethylamide.

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