Diana Garside,[1] Ph.D.; Jeri D. Ropero-Miller,[1] B.A.; Bruce A. Goldberger,[1] Ph.D.; William F. Hamilton,[2] M.D.; and William R. Maples,[3] Ph.D.

Identification of Cocaine Analytes in Fingernail and Toenail Specimens

REFERENCE: Garside D, Ropero-Miller JD, Goldberger BA, Hamilton WF, Maples WR. Identification of cocaine analytes in fingernail and toenail specimens. J Forensic Sci 1998;43(5): 974–979.

ABSTRACT: Fingernail and toenail specimens were obtained from 18 suspected cocaine users. The nails were cut, heated under methanolic reflux, and the methanolic extracts were purified by solid-phase extraction. Gas chromatography/mass spectrometry was utilized for the qualitative and quantitative analysis of nine cocaine analytes. Comparison of conventional postmortem analysis of blood and urine with nail analysis revealed a marked increase in the detection of cocaine use by nail analysis. Cocaine analytes were present in 14 (82.3%) subjects utilizing nail analysis. Out of those 14 subjects, only 5 (27.7%) were positive by conventional postmortem drug analysis. Cocaine and benzoylecgonine were the predominant analytes in all positive nail specimens. Anhydroecgonine methyl ester, ecgonine methyl ester, ecgonine ethyl ester, cocaethylene, norcocaine, and norbenzoylecgonine were detected in a limited number of specimens. The ratio of cocaine to benzoylecgonine ranged from 2–10:1. These findings suggest that nails may be a useful alternative matrix for the detection of cocaine exposure.

KEYWORDS: forensic science, forensic toxicology, cocaine, benzoylecgonine, cocaine metabolites, fingernail, toenail

Since the 1800s, nails have been used in the investigations of death associated with poisons. Historically, nails have been subjected to elemental analysis in a variety of applications, including arsenic in forensic investigations (1–4) and the transition metals for occupational (5–6) and environmental exposure (7–9). In addition, nail analysis has been applied to the clinical diagnosis of various disease states (10–12), and more recently, to therapeutic drug monitoring (13–15).

In 1984, Suzuki et al. (16) were the first to identify drugs of abuse in nails by successfully detecting amphetamine and methamphetamine in the nail clippings of methamphetamine abusers. Since then, Suzuki et al. (17) and Cirimele et al. (18) have pursued the analysis of nails for amphetamines, the latter group also detecting 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine. In 1994, preliminary findings by the Federal Bureau of Investigation's (FBI) Toxicology Laboratory demonstrated the presence of cocaine and benzoylecgonine in nails obtained from cadavers (19).

The literature, however, remains scant in the area of nail analysis for drugs of abuse. This report describes a sensitive and specific gas chromatographic/mass spectrometric (GC/MS) method for the isolation and detection of cocaine analytes in fingernail and toenail clippings. In addition, we discuss the possible mechanism of drug entry and incorporation, and describe the advantages and disadvantages of nail analysis for drugs of abuse.

Materials and Methods

Standards and Reagents

All organic solvents were high-performance liquid chromatography (HPLC) grade, and chemicals were reagent grade. Anhydroecgonine methyl ester, benzoylecgonine, d_3-benzoylecgonine, cocaine, d_3-cocaine, cocaethylene, d_3-cocaethylene, ecgonine ethyl ester, ecgonine methyl ester, d_3-ecgonine methyl ester, *m*-hydroxybenzoylecgonine, norbenzoylecgonine, and norcocaine were purchased from Radian International LLC (Austin, TX). *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was obtained from Pierce Chemical Company (Rockford, IL).

Phosphate buffer (0.1 M; pH 6.0 ± 0.1) was prepared from a mixture of potassium phosphate monobasic and deionized water. Acetate buffer (0.1 M; pH 4.0 ± 0.1) was prepared from a mixture of glacial acetic acid, sodium acetate, and deionized water. The solid-phase extraction (SPE) elution solvent, methylene chloride-2-propanol-ammonium hydroxide (80:20:2, *v*/*v*/*v*) was prepared daily.

Subjects

Fingernail (N = 17) and toenail (N = 15) clippings were obtained using cosmetic nail clippers from subjects examined at the 8th District Medical Examiner's Office (Gainesville, FL). Nail clippings from all digits were combined for each subject (approximately 7 to 50 mg). The selection of subjects was based upon the likelihood of drug use indicated by information gathered by the Medical Examiner's Office. Upon receipt in the laboratory, the nail specimens were stored at room temperature in griplock plastic bags. Epidemiologic data, and the cause and manner of death for each subject, are shown in Table 1. In addition, fingernail specimens were obtained as controls from 12 individuals who were

[1] Post doctoral fellow, graduate assistant, and assistant professor, respectively, Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL 32610.

[2] Medical examiner, Eighth District Medical Examiner's Office, Gainesville, FL 32601.

[3] The late distnguished service professor and founder of the C. A. Pound Human Identification Laboratory, University of Florida, Gainesville, FL 32611.

Received 1 Aug. 1997; and in revised form 15 Dec. 1997; accepted 30 Dec. 1997.

Copyright © 1998 by ASTM International

TABLE 1—*Subject history and results of conventional postmortem drug analysis.*

Subject Number	Age (Y)	Race	Gender	Manner of Death/Cause of Death*	Results of Conventional Postmortem Drug Analysis	
					Blood	Urine
1	41	Caucasian	F	Homicide/MBTI	Ethanol Diazepam Nordiazepam	Ethanol
2	21	Black	M	Vehicular Homicide/BTHI	Ethanol†	Ethanol
3	28	Black	M	Suicide/GSW	Benzoylecgonine	Benzoylecgonine Cannabinoids
4	36	Caucasian	M	Suicide/GSW	Ethanol	Not Analyzed
5	30	Black	M	Suicide/GSW	Benzoylecgonine Cocaine Diazepam	Not Analyzed
6	47	Caucasian	M	Suicide/GSW	Ethanol Diazepam Nordiazepam	Ethanol
7	38	Black	M	Acute Cocainism/Accidental Overdose	Ethanol	Benzoylecgonine
8	23	Caucasian	M	Suicide/GSW	Ethanol	Ethanol
9	24	Caucasian	M	MVA/MBTI	Ethanol†	Ethanol
10	32	Caucasian	M	Idiosyncratic Drug Reaction/Accidental Drug Overdose	Acetaminophen Lidocaine	None Detected
11	18	Caucasian	M	MVA/BTHI	Ethanol†	Ethanol
12	33	Caucasian	M	Suicide/GSW	Ethanol†	Ethanol
13	31	Caucasian	M	Suicide/Burns	Ethanol Cocaine Cocaethylene Benzoylecgonine Diphenhydramine	Ethanol Cocaine Cocaethylene Benzoylecgonine Diphenhydramine
14	27	Black	F	Suicide/GSW	None Detected	Cannabinoids
15	34	Caucasian	F	Vehicular Homicide/MMBTI	Ethanol	Not Analyzed
16	29	Caucasian	M	Idiopathic Dilated Cardiomyopathy	None Detected	None Detected
17	30	Caucasian	M	Suicide/Multiple Drug Overdose	Ethanol Cocaine Cocaethylene Benzoylecgonine Amitriptyline Nortriptyline	Ethanol Cocaine Cocaethylene Benzoylecgonine Amitriptyline Nortriptyline
18	31	Black	M	Natural/Ischemic Heart Disease	Lidocaine	None Detected

* GSW = gunshot wound
MVA = motor vehicle accident
MBTI = multiple blunt traumatic injury
MMBTI = multiple massive blunt traumatic injury
BTHI = blunt traumatic head injury
† These specimens were obtained antemortem. A complete toxicological screen was not performed.

unlikely to be cocaine users (i.e., laboratory personnel, law enforcement officers, children).

Conventional postmortem drug analyses using immunoassay, gas chromatography, and GC/MS were performed on blood and/or urine specimens by a commercial laboratory (SmithKline Beecham Clinical Laboratory, Leesburg, FL) or a university laboratory (University of Florida College of Medicine, Gainesville, FL). Testing of antemortem blood was performed by a hospital clinical laboratory. The results of these analyses are also shown in Table 1.

Solid-Phase Extraction

The solid-phase extraction of cocaine analytes from nails and subsequent analysis by GC/MS was adapted from the procedure previously reported by Cone et al. (20).

Nail clippings were cut into small pieces and approximately 7 to 25 mg aliquots were placed into disposable culture tubes. The samples were washed with methanol (3 mL) by vortexing for 10 s. The methanolic wash was decanted into a fresh tube and evaporated to dryness at 50°C under a stream of nitrogen (TurboVap®, Zymark, Hopkinton, MA). The washes were retained for further analysis. Control nail specimens and their washes were treated in the same manner as suspected cocaine user specimens. Methanol (3 mL) and trideuterated internal standards (5 ng/mg) were added to the washed nails, the tubes capped, and the mixture heated under reflux at 40°C for 16 h. After cooling, the methanolic extracts were decanted into fresh tubes and evaporated to dryness at 50°C under a stream of nitrogen.

After adding trideuterated internal standard (5 ng/mg) to the wash residues, both the nail extracts and wash residues were reconstituted in phosphate buffer (3 mL) prior to further extraction. The SPE cartridges (Clean Screen®, ZSDAU020, United Chemical Technologies, Horsham, PA) were conditioned sequentially with elution solvent (1 mL), methanol (3 mL), deionized water (3 mL), and phosphate buffer (2 mL). Vortexed samples were loaded onto the cartridges, and the cartridges were washed sequentially with deionized water (2 mL), acetate buffer (2 mL), methanol (3 mL), and acetonitrile (1 mL) before aspirating to dryness. Analytes were recovered from the columns with elution solvent (4 mL), and the

eluents were evaporated to dryness at 50°C under a stream of nitrogen. The extracts were derivatized with 30 μL of BSTFA with 1% TMCS at 60°C for 30 min and transferred to autosampler vials.

GC/MS Analysis

Analyses were performed with a Hewlett-Packard 5890A Series II gas chromatograph and 7673B automatic liquid sampler interfaced with a Hewlett-Packard 5972A Mass Selective Detector (MSD, Hewlett-Packard Company, Little Falls, DE). The gas chromatograph was equipped with an HP-5MS crosslinked 5% diphenyl, 95% dimethylpolysiloxane fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Hewlett-Packard Company). Automated injections (1 μL) were made in the splitless mode, and a 2-mm i.d. silanized borosilicate liner was utilized.

The MSD was operated in the selected ion monitoring mode. The following ions were monitored at a dwell time of 20 ms (ions in italics were used for quantitation): anhydroecgonine methyl ester: *m/z 152*, 166, and 181; d_3-ecgonine methyl ester: *m/z* 85, *99*, and 274; ecgonine methyl ester: *m/z* 82, *96*, and 271; ecgonine ethyl ester: *m/z* 83, *96*, and 285; d_3-cocaine: *m/z 185*, 275, and 306; cocaine: *m/z 182*, 272, and 303; d_3-cocaethylene: *m/z* 85, *199*, and 320; cocaethylene: *m/z* 82, *196*, and 317; d_3-benzoylecgonine: *m/z* 85, *243*, and 364; benzoylecgonine: *m/z* 82, *240*, and 361; norcocaine: *m/z 140*, 240, and 346; norbenzoylecgonine: *m/z 140*, 298, and 404; and *m*-hydroxybenzoylecgonine: *m/z* 82, 210, and *240*. Analytes were identified based upon comparison of retention times and ion ratios with the corresponding values of calibrators assayed in the same run. Ion ratios were calculated by dividing the ion peak-area of the confirming ion by the ion peak-area of the quantitative ion. Quantification of analytes was based upon the ratios of the integrated ion peak-areas to the corresponding trideuterated standard analogues.

Calibration curves were constructed with a minimum of six calibrators prepared at a concentration range of 0.1–10.0 ng/mg utilizing 25 mg of pre-washed drug-free nails. The calibrators were fortified with standard solutions of anhydroecgonine methyl ester, ecgonine methyl ester, ecgonine ethyl ester, cocaine, cocaethylene, benzoylecgonine, norcocaine, norbenzoylecgonine, *m*-hydroxybenzoylecgonine, and their respective trideuterated cocaine analogues. Cocaine and ecgonine methyl ester analytical controls (10 ng/mg) were included in all batches in order to monitor the hydrolysis of cocaine during extraction and GC/MS analysis, and the formation of anhydroecgonine methyl ester during GC injection, respectively.

Results

Methanolic extracts of fingernail and toenail clippings were purified by SPE and assayed by GC/MS for the presence of cocaine analytes. The results of the nail analyses are listed in Table 2 and summarized in Table 3. The limit of detection (LOD) of the assay was approximately 0.10 ng/mg (signal-to-noise ratio ≥ 5 for the quantitative ion) for all cocaine analytes, except for ecgonine ethyl ester and norbenzoylecgonine which had an LOD of approximately 0.25 ng/mg. The limit of quantitation (LOQ) of the assay was 0.25 ng/mg for all cocaine analytes, except for ecgonine ethyl ester and norbenzoylecgonine which had an LOQ of 0.50 ng/mg. The calibration curves were linear over a concentration range of 0.1 ng/mg to 10 ng/mg; the correlation coefficients of all standard curves were ≥ 0.993.

Hydrolysis of cocaine was monitored in all batches and was less than 5%. All analytical controls fortified with either cocaine or ecgonine methyl ester alone were negative for anhydroecgonine methyl ester. These controls verified that the degradation of these analytes during the analytical process was insignificant.

Cocaine and benzoylecgonine were the predominant cocaine analytes in all postmortem nail specimens. Cocaine analytes were present in 14 (82.3%) subjects utilizing nail analysis. Out of those 14 subjects, only 5 (27.7%) were positive by conventional postmortem drug analysis. Anhydroecgonine methyl ester, ecgonine methyl ester, ecgonine ethyl ester, cocaethylene, norcocaine, and norbenzoylecgonine were found in a limited number of specimens. Results of the 12 control nail specimens were negative for all cocaine analytes.

Analysis of the wash fractions revealed the presence of cocaine analytes in 5 subjects (no. 3, 5, 7, 13, and 17). Cocaine analytes present in the washes included cocaine, benzoylecgonine, anhydroecgonine methyl ester, ecgonine methyl ester, and cocaethylene. The wash concentrations ranged from less than 0.10 ng/mg to greater than 10.0 ng/mg. Results of the wash fractions for the control nails were negative for all cocaine analytes.

Discussion

Description of the Nail and Mechanism of Drug Incorporation

Nails are derived from the same cells as the epidermis and hair and consist of hard, dead keratinous cells. Nails are generally colorless, but appear pink due to the blood supply in the underlying dermis. The anatomy of the nail, with the relevant anatomic features indicated, is shown in Fig. 1.

The mechanism of drug entry and incorporation into the nail matrix is unknown. However, it is assumed that the dividing cells responsible for nail formation also incorporate drug. During formation, drug may be incorporated continuously or as a single event. It has been reported that drugs gain rapid access to the distal nail plate during nail production by incorporation into cornified cells of the nail bed. Incorporation of drug by diffusion from the nail bed to the ventral portion of the nail plate is thought to be minimal (21,22). Studies have also shown that nail production and drug incorporation occurs in the lunular germinal matrix as the nail plate grows distally from the base of the nail (23,24).

Other potential mechanisms of drug entry is through exposure to environmental contamination and biological fluids including sweat, sebum, saliva, and urine. In addition, processes previously identified in hair may also influence incorporation (25). These include the chemical nature of the drug analyte (e.g., lipophilicity and state of ionization), the metabolic profile of the drug, and the composition of the matrix.

The degree to which each of these proposed routes contribute to the overall drug content of nails is unknown.

Cocaine Analysis of Nails

In the present study, fingernail and toenail analysis greatly increased the detection of drug use. While conventional postmortem drug analysis of blood and urine identified recent cocaine use in 27.7% of the subjects, nail analysis detected cocaine exposure in 82.3% of the cases. In addition, positive test results were observed in five of six subjects known to authorities as cocaine users based upon conventional postmortem analysis, whereas all six subjects were positive by nail analysis. Moreover, the nails of these subjects contained minor cocaine analytes not seen in other subjects.

TABLE 2—*Results of nail analysis.*

Subject Number	Specimen*	GC/MS Results (ng/mg)†							
		AEME	EME	EEE	COC	CE	BE	NCOC	NBE
1	FN	ND	ND	ND	<0.25	ND	ND	ND	ND
2	FN	ND	ND	ND	<0.25	ND	ND	ND	ND
3	FN	7.71	>10.0	ND	>10.0	ND	>10.0	6.36	2.59
	TN	0.53	0.80	ND	8.19	ND	3.71	0.38	ND
4	FN	ND	ND	ND	<0.25	ND	ND	ND	ND
	TN	ND	ND	ND	ND	ND	ND	ND	ND
5	FN	>10.0	7.09	ND	>10.0	ND	>10.0	4.65	1.22
	TN	1.63	1.18	ND	>10.0	ND	>10.0	0.87	<0.50
6	FN	ND	ND	ND	0.51	ND	Trace	ND	ND
	TN	ND	ND	ND	2.83	ND	0.27	ND	ND
7	FN	2.69	0.87	ND	>10.0	ND	>10.0	0.52	0.61
	TN	Trace	Trace	ND	2.13	ND	1.75	Trace	ND
8	FN	ND	ND	ND	Trace	ND	ND	ND	ND
	TN	ND	ND	ND	ND	ND	ND	ND	ND
9	TN	ND	ND	ND	ND	ND	ND	ND	ND
10	FN	ND	ND	ND	0.25	ND	Trace	ND	ND
	TN	ND	ND	ND	Trace	ND	Trace	ND	ND
11	FN	ND	ND	ND	ND	ND	ND	ND	ND
12	FN	ND	ND	ND	<0.25	ND	ND	ND	ND
	TN	ND	ND	ND	ND	ND	ND	ND	ND
13	FN	Trace	ND	ND	16.1	<0.25	1.84	Trace	ND
	TN	Trace	0.29	<0.50	4.19	1.03	1.42	Trace	ND
14	FN	ND	ND	ND	<0.25	ND	ND	ND	ND
	TN	ND	ND	ND	Trace	ND	ND	ND	ND
15	FN	ND	ND	ND	<0.25	ND	ND	ND	ND
	TN	ND	ND	ND	ND	ND	ND	ND	ND
16	FN	ND	ND	ND	ND	ND	ND	ND	ND
	TN	ND	ND	ND	ND	ND	ND	ND	ND
17	FN	ND	ND	ND	13.1	ND	0.91	Trace	ND
	TN	ND	ND	ND	1.18	ND	0.55	ND	ND
18	FN	ND	ND	ND	ND	ND	ND	ND	ND
	TN	ND	ND	ND	ND	ND	ND	ND	ND

* FN = fingernail
TN = toenail
AEME = anhydroecgonine methyl ester
BE = benzoylecgonine
CE = cocaethylene
COC = cocaine
EME = ecgonine methyl ester
NCOC = norcocaine
NBE = norbenzoylecgonine
ND = none detected

† Trace refers to a concentration less than the limit of detection. Concentrations have been weight-corrected based upon a sample weight of 25 mg.

TABLE 3—*Comparison of postmortem drug analysis and nail analysis.*

Subject Number	Conventional Postmortem Drug Analysis	Fingernail Analysis	Toenail Analysis
1	−	+	NS*
2	−	+	NS
3	+	+	+
4	−	+	−
5	+	+	+
6	−	+	+
7	+	+	+
8	−	+	−
9	−	NS	−
10	−	+	+
11	−	−	NS
12	−	+	−
13	+	+	+
14	−	+	+
15	−	+	−
16	−	−	−
17	+	+	+
18	−	−	−
Total Positive (%)	5 (27.7)	14 (82.3)	8 (53.3)

* NS = no specimen was available for analysis.

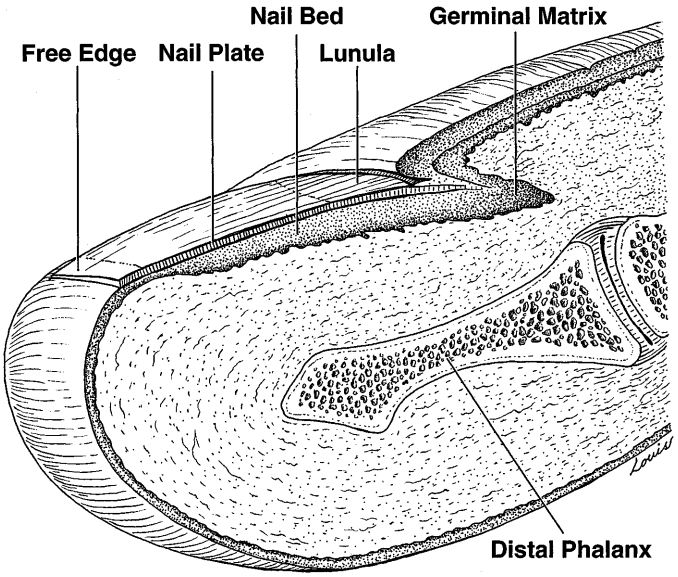

FIG. 1—*The anatomy of a nail.*

The concentration of cocaine analytes in fingernails was generally greater than toenails. In addition, cocaine analytes were not detected in the toenails of several subjects (no. 4, 8, 12, and 15) with positive fingernail analysis. There are several factors that may contribute to this finding including potential differences in the degree of environmental contamination, personal hygiene, rate of nail growth, and blood supply to the nail bed.

Cocaine and benzoylecgonine were the predominant cocaine analytes detected in nails, with cocaine present at a concentration approximately 2 to 10 times greater than benzoylecgonine. This is similar to the well established cocaine-to-benzoylecgonine ratio observed in hair (26). However, the ratio is contrary to that previously observed by the FBI Toxicology Laboratory (19) which reported the presence of cocaine and benzoylecgonine in nails to be approximately in equal amounts.

The detection of anhydroecgonine methyl ester, a pyrolysis product of cocaine, in nails indicates exposure to ''crack'' cocaine (27). Furthermore, the presence of anhydroecgonine methyl ester in toenails suggests that the individual actively inhaled ''crack'' smoke since toenails are much less likely to be externally contaminated than fingernails. While cocaine, benzoylecgonine, and anhydroecgonine methyl ester can be incorporated into the nail through environmental contamination, the presence of metabolic markers such as norcocaine and norbenzoylecgonine strongly support cocaine ingestion followed by metabolism and incorporation into nails.

Our study demonstrated that several cocaine analytes are less frequently detected in nails than are cocaine and benzoylecgonine. Ecgonine methyl ester, ecgonine ethyl ester, and cocaethylene were detected in the nails of one subject at low to moderately low concentrations. Cocaethylene, a cocaine metabolite commonly observed following the coingestion of cocaine and ethanol, was not detected in the nails of the subjects with other reliable evidence indicating concomitant use of these drugs. For example, cocaethylene was surprisingly absent in the nails of subject no. 7, who was a known alcohol and cocaine user and whose conventional postmortem toxicology tests indicated high concentrations of both ethanol and benzoylecgonine. Finally, *m*-hydroxybenzoylecgonine was the only cocaine analyte not found in the nails analyzed.

Cocaine analytes were present in the fingernail washes of subjects found to contain high quantities of cocaine analytes in their nails, but were found to a lesser extent in the corresponding toenail washes. Generally, the pattern of cocaine analytes in the nail washes was similar to that observed in the nail extracts. As expected, cocaine analytes that are present in the environment including cocaine, benzoylecgonine, and anhydroecgonine methyl ester, were the predominant analytes found in the washes. Although ecgonine ethyl ester and cocaethylene were detected in the nails, these analytes were not found in the corresponding washes.

Advantages and Disadvantages of Nail Analysis

The principal advantage to nail analysis may be its ability to provide a long-term measure of drug use representing several months to years. Conventional matrices, such as blood and urine, can provide only recent accounts of drug exposure. In addition, nails are a relatively noninvasive specimen to collect, probably even more so than hair. Although not evaluated in this study, we believe the likelihood for racial bias is minimal since nails lacks significant pigmentation which has been attributed as a source of bias in hair (28). The concentration of cocaine analytes found in nails has proven to be high enough to readily detect drug use, even when small quantities (< 25 mg) of nail are available for analysis.

Like hair, nail specimens are easy to store and the drug analytes are presumably stable within the nail matrix. The stability of drugs in nails makes their analysis a valuable tool for postmortem investigations. For example, in cases having decomposed remains, conventional toxicological analysis could potentially produce negative test results due to the instability of the drug in the body fluids, or alternatively, cannot be performed due to lack of body fluids.

Clearly, the most significant disadvantage of nail analysis is the limited work that has been conducted in this area, particularly with drugs of abuse. With nail analysis, recent drug use may not be readily detectable and the potential for environmental contamination leading to positive test results exists. If drugs are incorporated through the nail bed and along the entire nail following drug use, then nail analysis cannot determine time and magnitude of drug exposure through sectional analysis such as that previously reported for hair analysis. Given the numerous factors that may affect nail growth such as age, sex, heredity, environment, and the difference in nail growth for individual digits, the interpretation of nail analysis is currently speculative (29,30). In addition, the unknown mechanism of drug incorporation further complicates the interpretation of nail analysis data.

Conclusions

Nail analysis can successfully detect drug exposure in situations where conventional postmortem analysis has proved unsuccessful, corroborating prior drug-use history as well as potentially reducing false negative test results. The presence of several unique cocaine analytes in nails contributes additional information regarding the subject's drug exposure. For example, the presence of unique cocaine analytes reveals information regarding concomitant cocaine and ethanol use (cocaethylene), route of cocaine administration (anhydroecgonine methyl ester), and the likelihood of drug ingestion and subsequent metabolism (norcocaine and norbenzoylecgonine).

The potential use of nail analysis in drug testing applications, including the workplace, clinical, forensic, epidemiological, and historical, necessitates further investigation. Currently, interpretation of nail analysis is challenging and should be considered semiquantitative at best. Future study, including paired hair and nail analysis, may prove the utility of nail analysis as an alternative biological matrix for the testing of drugs of abuse.

Acknowledgments

This research was supported in part by a grant from The Forensic Sciences Foundation's Lucas Grant Program received in 1995. The authors wish to thank Jason Byrd (University of Florida) for his technical assistance, and Albert Isaac and Pete Zeller for their assistance with data collection (8th District Medical Examiner's Office). In addition, the authors wish to acknowledge all individuals who generously participated as control subjects by donating nail specimens.

References

1. Lander H, Hodge PR, Crisp CS. Arsenic in the hair and nails. Its significance in acute arsenical poisoning. J Forensic Med 1965;12: 52–67.
2. Shapiro HA. Arsenic content of human hair and nails. Its interpretation. J Forensic Med 1967;14:65–71.

3. Pounds CA, Pearson EF, Turner TD. Arsenic in fingernails. J Forensic Sci 1979;19:165–73.
4. Pirl JN, Townsend GF, Valaitis AK, Grohlich D, Spikes JJ. Death by arsenic: a comparative evaluation of exhumed body tissues in the presence of external contamination. J Anal Toxicol 1983;7: 216–9.
5. Gerhardsson L, Englyst V, Lundstrom NG, Nordberg G, Sandberg S, Steinvall F. Lead in tissues of deceased lead smelter workers. J Trace Elem Med Biol 1995;9:136–43.
6. Gerhardsson L, Brune D, Lundstrom NG, Nordberg G, Wester PO. Biological specimen bank for smelter workers. Sci Total Environ 1993;139–40:157–73.
7. Wilhelm M, Hafner D, Lombeck I, Ohnesorge FK. Monitoring of cadmium, copper, lead and zinc status in young children using toenails: comparison with scalp hair. Sci Total Environ 1991;103: 199–207.
8. Alexiou D, Koutselinis A, Manolidis C, Boukis D, Papadatos J, Papadatos C. The content of trace elements (Cu, Zn, Fe, Mg) in fingernails of children. Dermatologica 1980;160:380–2.
9. Kasperek K, Iyengar GV, Feinendegen LE, Hashish S, Mahfouz M. Multielement analysis of fingernail, scalp hair, and water samples from Egypt (a preliminary study). Sci Total Environ 1982;22: 149–68.
10. Sunderman Jr. FW. Atomic absorption spectrometry of trace metals in clinical pathology. Hum Pathol 1973;4:549–82.
11. van Noord PAH, Maas MJ, van der Tweel I, Collette C. Selenium and the risk of postmenopausal breast cancer in the DOM cohort. Breast Cancer Res Treat 1993;25:11–9.
12. Bank HL, Robson J, Bigelow JB, Morrison J, Spell LH, Kantor R. Preparation of fingernails for trace element analysis. Clin Chem Acta 1981;116:179–90.
13. Schatz F, Brautigam M, Dobrowolski E, Effendy I, Haberl H, Mensing H, et al. Nail incorporation kinetics of terbinafine in onchomycosis patients. Clin Exp Dermatol 1995;20:377–83.
14. De Doncker P, Decroix J, Pierard GE, Roelant D, Woestenborghs R, Jacqmin P, et al. Antifungal pulse therapy for onchomycosis. A pharmacokinetic and pharmacodynamic investigation of monthly cycles of 1-week pulse therapy with itraconazole. Arch Dermatol 1996;132:34–41.
15. Uematsu T, Sato R, Suzuki K, Yamaguchi S, Nakashima M. Human scalp hair as evidence of individual dosage history of haloperidol: method and retrospective study. Eur J Clin Pharmacol 1989;37: 239–44.
16. Suzuki O, Hattori H, Asano M. Nails as useful materials for detection of methamphetamine or amphetamine abuse. Forensic Sci Int 1984;24:9–16.
17. Suzuki S, Inoue T, Takako I, Hori H, Inayama S. Analysis of methamphetamine in hair, nail, sweat, and saliva by mass fragmentography. J Anal Toxicol 1989;13:176–8.
18. Cirimele V, Kintz P, Mangin P. Detection of amphetamines in fingernails: an alternative to hair analysis [letter to the editors]. Arch Toxicol 1995;70:68–9.
19. Miller M, Martz R, Donnelly B. Drugs in keratin samples from hair, fingernails and toenails. Second International Meeting on Clinical and Forensic Aspect of Hair Analysis [Abstract]; Genoa, Italy; 6–8 June 1994;39.
20. Cone EJ, Hillsgrove M, Darwin WD. Simultaneous measurement of cocaine, cocaethylene, their metabolites, and ''crack'' pyrolysis products by gas chromatography-mass spectrometry. Clin Chem 1994;40:1299–305.
21. Johnson M, Shuster S. Continuous formation of nail along the bed. Br J Dermatol 1993;128:277–80.
22. Johnson M, Comaish JS, Shuster S. Nail is produced by the normal nail bed: a controversy resolved. Br J Dermatol 1991;125:27–9.
23. Zaias N. The movement of the nail bed. J Invest Dermatol 1967; 48;402–3.
24. Norton LA. Incorporation of thymidine-methyl-H^3 and glycine-2-H^3 in the nail matrix and bed of humans. J Invest Dermatol 1971; 56:61–8.
25. Cone EJ. Mechanisms of drug incorporation into hair. Ther Drug Monit 1996;18:438–43.
26. Garside D, Goldberger BA. Determination of cocaine and opioids in hair. In: Kintz P, editor. Drug testing in hair. Boca Raton: CRC Press, 1996;151–80.
27. Jenkins AJ, Goldberger BA. Identification of unique cocaine metabolites and smoking by-products in postmortem blood and urine specimens. J Forensic Sci 1997;42:824–7.
28. Cone EJ, Joseph R Jr. The potential for bias in hair testing for drugs of abuse. Kintz P, editor. Drug testing in hair. Boca Raton: CRC Press, 1996;69–94.
29. Hamilton JB, Terada H, Mestler GE. Studies of growth throughout the lifespan in Japanese: growth and size of nails and their relationship to age, sex, heredity, and other factors. J Gerontol 1955;10: 401–15.
30. Zook EG, Van Beek AL, Russell RC, Beatty ME. Anatomy and physiology of the perionychium: a review of the literature and anatomic study. J Hand Surgery 1980;5:528–36.

Additional information and reprint requests:
Bruce A. Goldberger, Ph.D.
University of Florida College of Medicine
P.O. Box 100275
Gainesville, FL 32610-0275