Two unique metabolites of cocaine, cocaethylene and norcocaine, were identified by GC/MS in the hair of cocaine users. Their presence cannot be explained by environmental contamination; thus, their presence together with cocaine provides convincing evidence that cocaine is excreted in hair after active cocaine administration. The amount of cocaine in hair predominated over all metabolites generally by a factor of 5-10. Two washing procedures were evaluated for their efficiency in removal of cocaine from environmentally contaminated hair. Neither procedure completely removed cocaine, suggesting that false positives can result from environmental contamination. Analysis of the methanolic wash of the hair of cocaine users also revealed the presence of cocaine metabolites, indicating that washing removes cocaine from the interior as well as from the exterior surface of hair during decontamination procedures.

Introduction

Hair testing for drugs of abuse offers the potential for detection of drug exposure over an extended period of time. Because hair grows at an average rate of 1–1.5 cm/month (1), it may be possible to test hair lengths that represent months to years of potential drug exposure. With the epidemic spread of cocaine and “crack” use in the United States in the 1980s, attempts have been made to take advantage of this ostensibly longer detection time of hair testing compared to urine testing as a means of identifying cocaine users. Early studies employing immunounassay indicated that hair testing was effective for the detection of cocaine or cocaine metabolite (benzoylcegonine) in hair segments of known users (2–4). As interest grew, more specific analytical methods were needed and assays were developed for cocaine and benzoylcegonine in hair by gas chromatography/mass spectrometry (GC/MS) (5–8) and MS/MS (8,9).

Despite these early studies on hair testing for cocaine, there remains general uncertainty regarding the actual form of cocaine found in hair and its mode of entry. Most immunounassays cross-reacted with cocaine and cocaine metabolites, and consequently failed to distinguish cocaine from benzoylcegonine, the major metabolite of cocaine (2–4). Also, some methods employed extraction with strong acids and bases that could convert cocaine to benzoylecgonine during sample work-up (2). In addition, the information from the early GC/MS and MS/MS studies was limited because controls that would have distinguished environmental contamination from active drug use were not included (5,7,8).

Detection of a unique cocaine metabolite in hair, whose presence could not be explained by hydrolysis or environmental exposure, would unequivocally establish that internal cocaine exposure had occurred in an individual. Cocaethylene, a recently identified metabolic conversion product of cocaine and ethanol co-administration (10), would appear to meet these criteria as a unique metabolite. Cocaethylene is only formed when cocaine and ethanol are used concurrently. Because cocaethylene is not found in illicit cocaine samples (11), it would not be present in hair as a result of environmental contamination; thus its presence in hair could be considered a marker of cocaine exposure. Another unique metabolite which might serve as a marker of cocaine use is norcocaine, a metabolic oxidation product of cocaine (12). Studies by Cook et al. (13) have indicated that norcocaine is not formed during the pyrolysis of cocaine.

The goal of the present study was the development and application of a GC/MS assay for cocaine, cocaine metabolites, and cocaethylene in hair samples obtained from active drug users. Stringent controls were included to evaluate whether wash procedures prior to assay could eliminate heavy environmental contamination. Ten hair samples from heavy cocaine users and 10 drug-free control hair samples were examined by the developed method. The presence of cocaethylene or norcocaine was considered to be a potential marker of cocaine exposure and evidence that cocaine excretion occurs in hair after active use. Relative amounts of cocaine, benzoylecgonine, and ecgonine methyl ester were examined to determine the actual chemical form of cocaine excreted in hair.

Materials and Methods

Chemicals

Cocaine hydrochloride was obtained from Mallinckrodt, Inc. Benzoylecgonine tetrahydrate, ecgonine methyl ester HCl, ben-
Hair samples

Hair samples were obtained from subjects who previously had been enrolled in a 180-day outpatient maintenance and detoxification study at the Addiction Research Center. During the detoxification study, subjects received either methadone or buprenorphine. Three times per week, subjects provided urine specimens, which were tested by immunoassay (Emit d.a.u.™, Syva Co.) for opiates (300-ng/mL cutoff) and cocaine metabolite (300-ng/mL cutoff). After completion of the detoxification study, subjects received either methadone or buprenorphine. Three times per week, subjects provided urine specimens, which were tested by immunoassay (Emit d.a.u.™, Syva Co.) for opiates (300-ng/mL cutoff) and cocaine metabolite (300-ng/mL cutoff). After completion of the detoxification study, subjects were requested to provide hair samples for study of their drug usage patterns. Ten heavy cocaine users were selected based on their high frequency of positive urines for cocaine (Table I). The interval between completion of the detoxification study and the 10 subjects' return visits to provide hair samples ranged from 4-15 months with a mean ± S.E.M. of 10.2 ± 1.1 months. Hair trimmings, varying in lengths from 3-10 cm, were collected from the ends of the hair shafts.

Solid-phase extraction (SPE) columns were washed with 0.5 mL of deionized water, 1 mL of 0.1M hydrochloric acid and 2x1 mL of methanol and aspirated to dryness. Analytes were eluted from the column with 3x 1 mL of methanol and aspirated to dryness. The eluates were evaporated to dryness under nitrogen and reconstituted with 0.02 mL of acetonitrile. The samples were transferred to 0.1-mL autosampler vials and 0.02 mL of BSTFA (with 1% TMCS) was added. Sample vials were sealed and heated at 60°C for 30 minutes. Immediately after derivatization, the samples were analyzed by GC/MS.

Acid extraction of hair samples

After the methanolic wash procedure, hair samples remained in the reservoirs. They were thoroughly dried and the reservoir tip was resealed. One mL of 0.05M sulfuric acid and internal standards (100 ng) were added, and a small stirring bar was placed in the reservoir. The top was sealed with parafilm and the reservoir was placed in a heated water bath equipped with a magnetic stirrer. The specimens were incubated overnight at 37°C with stirring. After incubation, the cooled acid extract was collected in a glass tube and saved for further extraction.

Extraction and derivatization procedures

Aliquots of the acid extract were removed and neutralized with 1.0M NaOH solution. The pH of the extract was adjusted to pH 4.0 with 1 mL of 2M sodium acetate in preparation for SPE extraction. SPE columns were mounted on an extraction vacuum manifold and conditioned with 1 mL of methanol (x2) and deionized water (x2), respectively. Sodium acetate buffer (0.2 mL) was aspirated onto the column followed by sample. Vacuum was applied to produce a column flow of 1-2 mL/min. The columns were washed with 0.5 mL of deionized water, 1 mL of 0.1M hydrochloric acid and 2x1 mL of methanol and aspirated to dryness. Analytes were eluted from the column with 3x 1 mL of a solution of methylene chloride–2-propanol (8:2) containing 2% ammonium hydroxide. The eluates were evaporated to dryness under nitrogen and reconstituted with 0.02 mL of acetonitrile. The samples were transferred to 0.1-mL autosampler vials and 0.02 mL of BSTFA (with 1% TMCS) was added. Sample vials were sealed and heated at 60°C for 30 minutes. Immediately after derivatization, the samples were analyzed by GC/MS.

GC/MS assay

Derivatized extracts were analyzed on a Hewlett-Packard 5890A gas chromatograph coupled to a 5970B mass selective detector equipped with a 7673A automatic liquid sampler. An HP-1 cross-linked fused-silica capil-
chloride were prepared by soaking bundles of control hair exposure) were prepared by suspending bundles of control hair (approximately 100 mg) in solutions of cocaine hydrochloride samples were prepared by soaking in drug-free tap water. After (1 mg/mL, tap water) at room temperature for 48 h. Similar hair bundles were suspended at a distance of 10 in. above the heated which cocaine base (10 mg) was heated at 200°C. The hair samples were stored in individual plastic bags and frozen until analysis.

Preparation of environmentally exposed cocaine hair samples

Hair samples exposed to cocaine vapor (simulated "crack" exposure) were prepared by suspending bundles of control hair (approximately 100 mg) inside a plexi-glass box (2×3×3 ft.) in which cocaine base (10 mg) was heated at 200°C. The hair bundles were suspended at a distance of 10 in. above the heated cocaine for 1 h. Similar hair samples suspended outside the box were used as drug-free controls. After exposure, the samples were stored in individual plastic bags and frozen until analysis.

Hair samples exposed to aqueous solutions of cocaine hydrochloride were prepared by soaking bundles of control hair (approximately 100 mg) in solutions of cocaine hydrochloride (1 mg/mL, tap water) at room temperature for 48 h. Similar hair samples were prepared by soaking in drug-free tap water. After soaking, the samples were blotted dry, air dried, and stored in individual plastic bags and frozen until analysis.

Results

Identification and quantitation of cocaine and metabolites in cocaine-users’ hair

Ten hair samples obtained from cocaine users (Table II) were washed with methanol, extracted in sulfuric acid solution, and analyzed for cocaine and cocaine metabolites by GC/MS. The presence of cocaine, benzoylecgonine, ecgonine methyl ester, norcocaine, and cocaethylene was established by comparison of the mass spectral patterns with authentic standards. An example of the mass spectral identification of cocaethylene in the acid extract of Subject B compared to standard cocaethylene is shown in Figure 1. A small amount of coeluting impurity (m/z 73, 117, and 296) is present in Subject B’s spectrum (Figure 1B). An additional metabolite, norcocaethylene, was detected by SIM analysis in most samples that contained cocaethylene. However, the abundance was not sufficient for full scan identification. An example of the SIM tracings for cocaethylene, benzoylecgonine, norcocaine, and norcocaethylene is shown in Figure 2. Responses are evident for the respective nor-metabolites of cocaine and cocaethylene at m/z 140 and 360 in the extract fraction (Panel 2B) and in the methanolic wash fraction (Panel 2D) from Subject B. The ion for benzoylecgonine, m/z 240, also serves as a confirming ion for norcocaine (Panel 2A). Subject A’s hair did not contain cocaethylene and consequently shows a lack of response at m/z 196 for cocaethylene and at m/z 360 for norcocaethylene.

The quantitative analysis of cocaine and cocaine metabolites in hair was performed by first washing the samples with methanol, then extraction in sulfuric acid solution, further extraction, and GC/MS assay. All samples, calibrators, and controls were processed in the same manner. The assay had a limit of detection of approximately 0.1 ng/mg with a 50-mg hair sample for all analytes. Recovery of cocaine that was added to control hair (after the wash step) was approximately 90% with a 10% conversion to benzoylecgonine. The efficiency of cocaine recovery

<table>
<thead>
<tr>
<th>Subject</th>
<th>Wash (ng/mg)</th>
<th>Extract (ng/mg)</th>
<th>Wash (ng/mg)</th>
<th>Extract (ng/mg)</th>
<th>Wash (ng/mg)</th>
<th>Extract (ng/mg)</th>
<th>Wash (ng/mg)</th>
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<td>6.8</td>
<td>2.0</td>
<td>0.6</td>
<td>T</td>
<td>0.6</td>
<td>T</td>
<td>T</td>
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</tr>
<tr>
<td>B</td>
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<td>9.3</td>
<td>0.4</td>
<td>0.9</td>
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<td>9.7</td>
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<td>1.3</td>
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<td>T</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>10.0</td>
<td>6.4</td>
<td>0.5</td>
<td>0.9</td>
<td>T</td>
<td>0</td>
<td>0</td>
<td>T</td>
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<td>0.7</td>
<td>0.8</td>
<td>0.4</td>
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<tr>
<td>SEM</td>
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<td>0.1</td>
<td>1.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Some analytes were detected in trace (T) amounts below the GC/MS calibration curve.
from cocaine user's hair was not determined, although re-extraction indicated that recovery was incomplete.

Quantitative results from the analysis of the wash fraction and the acid extract fraction from 10 cocaine users are summarized in Table II. The amount of cocaine and metabolites found in the wash fraction was generally equal to or higher than that found in the acid extract. Amounts of cocaine in the acid extract ranged from 6.4 to 19.2 ng/mg with a mean ± SEM of 10.8 ± 1.4 ng/mg. Benzoylecgonine ranged from 0.3 to 2.5 ng/mg with a mean ± SEM of 1.2 ± 0.2 ng/mg. The ratio of cocaine to benzoylecgonine in the acid extract (uncorrected for chemical conversion of cocaine to benzoylecgonine) ranged from 5.2 to 22.0 with a mean ± SEM of 10.5 ± 1.5. Ecggonine methyl ester ranged from 0.6 to 1.9 ng/mg. Norcocaine was assayed in 4 samples in amounts ranging from 0.5 to 0.7 ng/mg. Cocaethylene was measured in 6 samples in amounts ranging from 0.4 to 2.6 ng/mg. Traces of norcocaethylene were detected in those specimens containing cocaethylene.

Ten drug-free control samples were processed in the same manner as the cocaine users' samples. The acid extracts from these samples were negative for cocaine and metabolites.

**Analysis of environmentally exposed cocaine hair samples**

Hair samples (ca. 100 mg) exposed to cocaine vapor were prewashed by two methods in an attempt to remove surface contamination. The amount of cocaine remaining on hair was measured by SIM at m/z 182; D3-cocaine was monitored at m/z 185 (Figure 3). Standard cocaine appeared at a retention time of 6.28 min. (Figure 3A). As shown in Figure 3B, control hair exposed to drug-free room air was negative for cocaine. Samples exposed to cocaine vapor remained heavily contaminated with cocaine after washing with methanol (Figure 3C). The total amount of cocaine remaining on the hair samples ranged from 510–3,430 ng by the methanol procedure and 1,851–9,485 ng by the Baumgartner method (Table III). Washing with methanol (1 mL x 3) was effective in removing more than 90% of the cocaine from hair, whereas use of the alternate procedure reported by Baumgartner et al. (6) removed an average of 81%.

Hair samples soaked in 1 mg/mL of aqueous cocaine hydrochloride remained grossly contaminated with cocaine after both wash procedures. No attempt was made to quantitate the amount of cocaine remaining in hair because all GC/MS responses were saturated (Figure 3D).

**Discussion**

Hair analysis for cocaine has been stated to be an efficient means of providing "...useful information on cocaine consumption by cocaine addicts ..." (2). Accordingly, Baumgartner reported finding a linear response between cocaine metabolite (presumably benzoylecgonine) in mouse hair and the administered dose (6). In the same article, he also reported finding a "reasonably good correlation" between cocaine metabolite in the hair of patients, probationers, and parolees and their self-reported amounts of cocaine use (6). In contrast, Kidwell (9) reported finding a large excess of intact cocaine over benzoylecgonine and ecgonine in the hair of users by tandem MS analysis under pyrolysis conditions. In the present study, we sought to clarify the nature of the chemical species that is excreted in hair.
after cocaine use. This required the development of a sensitive and specific GC/MS assay for cocaine and metabolites for use with small amounts of hair samples. Controls were included to evaluate the potential role of environmental contamination of cocaine in hair analysis. Analysis of hair samples from the 10 documented cocaine users provided evidence for the presence of cocaine in each subject’s hair, whereas negative findings were obtained for the 10 drug-free controls. The mean ratio of cocaine to benzoylecgonine was 10.5. Only traces of egeonine methyl ester were detected. These findings agree with Kidwell’s findings (9) that cocaine is the principal analyte in hair and occurs in much higher abundance than other cocaine metabolites.

The detection of norcocaine and cocaethylene along with cocaine by GC/MS in the present study provided convincing proof that cocaine is excreted in hair and rules out the possibility of environmental contamination as an alternate explanation for cocaine’s occurrence. Neither of these compounds occur in appreciable amounts in illicit cocaine and their presence can only be explained by human biotransformation of cocaine to these unique metabolites. Cocaine is oxidatively metabolized to norcocaine (12) and cocaethylene has been shown to arise as a result of the concurrent use of cocaine and alcohol (10). It is known that alcohol is often abused along with cocaine. A recent report indicates that approximately 85% of cocaine users polled in the 1985 National Survey on Drug Abuse used ethanol at the same time or within a few hours (14). Hence, the detection of cocaethylene in 8 of 10 heavy cocaine users’ hair is not surprising. The detection of traces of norcocacethylene, the N-demethylated metabolite of cocaethylene, in the hair samples containing cocaethylene further supports the current finding of cocaethylene.

The washing procedure was included in an attempt to eliminate potential surface contamination from environmental cocaine. Human head hair could be exposed to a variety of sources of cocaine free base and cocaine hydrochloride from the environment. Smoking “crack” involves the vaporization of base cocaine. Small amounts of “sidestream” crack vapor can be released into the atmosphere. This allows the possibility of adsorption of cocaine onto an individual’s hair located nearby. Certainly, the smoker’s hair would be contaminated in the same process. Alternately, cocaine hydrochloride dust particles in the air and on articles of clothing and money would present the opportunity for hair contamination from the powder and aqueous solutions. Although the environmentally contaminated controls utilized in this study simulated examples of extreme exposures from cocaine base and aqueous solutions of cocaine hydrochloride, other investigators have employed even more exaggerated conditions. Baumgartner and Hill (15) produced contaminated hair specimens for study by suspending locks of hair in a 1-L glass flask filled with vapor from 500 mg of cocaine freebase.

Table III. Efficiency of Wash Procedures for Removal of Cocaine from Control Hair Specimens That Were Contaminated with Cocaine Vapor*  

<table>
<thead>
<tr>
<th>Wash Method</th>
<th>Total Cocaine Recovered (ng)</th>
<th>Percent cocaine removed by washing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wash fraction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alcohol</td>
<td>Buffer</td>
</tr>
<tr>
<td>Methanol</td>
<td>11,052</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30,954</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>118,320</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol-phosphate buffer (6)</td>
<td>9,238</td>
<td>1,057</td>
</tr>
<tr>
<td></td>
<td>31,160</td>
<td>7,396</td>
</tr>
<tr>
<td></td>
<td>17,208</td>
<td>4,978</td>
</tr>
</tbody>
</table>

* Triplicate drug-free hair specimens (Caucasoid, brown, ca. 100 mg each) were analyzed by two wash procedures as described in Methods section. The data indicate the total amount of cocaine recovered in ng by each procedure.
The methanol washing procedure used in the present study was more than 90% effective in removing vaporized cocaine, but sufficient cocaine residue remained to produce false positive results. After soaking in an aqueous solution of cocaine, the methanol wash procedure was totally inadequate in removing cocaine; hair samples remained extremely contaminated. The washing procedure reported by Baumgartner et al. (6) fared no better in removing cocaine contamination from these samples. Additional wash-out kinetic criteria have been proposed by Baumgartner and Hill (15) as a means of distinguishing external cocaine contamination from active drug use; however, these criteria remain untested by other investigators. Other studies have included a variety of conditions in attempts to remove drug contamination. Marigo et al. (16) reported that serial washings with ethyl ether and dilute HCl successfully removed "loosely bound morphine." In contrast, Kidwell (9) reported the failure of a variety of wash solutions in removing phencyclidine from contaminated hair.

Analysis of the methanolic wash for cocaine metabolites produced evidence that the wash procedure removed more than just external cocaine contamination. The presence of norcocaine and cocaethylene along with cocaine and benzoylecgonine in amounts generally exceeding those found in the extract indicated that interior drug components were removed in the wash. Clearly in the present procedure, the "wash" procedure could more aptly be called an extraction. The depth of penetration by the "wash" solution is likely to be dependent upon the condition of the hair undergoing testing. Highly damaged hair is likely to be more porous and yield greater amount of interior drug during washing. If this is true, the amount of drug remaining in the extract is likely to be distorted and bear little relationship to dosing patterns. Based on the present data, it seems unlikely that there is a clear demarcation in hair between environmental drug exposure and drug residues resulting from active use.

The elimination of surface contamination from hair prior to its analysis for drugs of abuse is important, given the possibilities of environmental contamination. Unfortunately, in the present effort, the techniques employed were not effective. It may be necessary to adopt an alternate approach in the development of hair analysis technology by requiring the identification of a unique metabolite whose presence can only be explained by internal drug exposure with subsequent biotransformation. The presence of cocaine, benzoylecgonine, and ecgonine methyl ester could potentially be explained by environmental contamination with subsequent hydrolysis. In sharp contrast, the presence of either norcocaine or cocaethylene in an individual's hair would appear to constitute sufficient evidence of internal cocaine exposure.

**References**