FINAL REPORT

"HAIR ANALYSIS FOR DRUGS OF ABUSE"

Grant Number NIJ 90-NIJ-CX-0012

Prepared

for

National Institute on Justice

National Institute on Drug Abuse

Prepared

by
Gary L. Henderson, Ph.D.
Martha R. Harkey, Ph.D.
Department of Medical Pharmacology and Toxicology
School of Medicine
University of California, Davis 95616

Reese Jones, MD
Langley Porter Institute
University of California, San Francisco

September, 1993

ABSTRACT

Isotopically-labeled cocaine (benzoyl-d5-cocaine-HCl, pentadeuterated cocaine, d5-C) was administered intravenously and/or intranasally to 25 human volunteers under controlled clinical conditions. Sequential blood and sweat were collected for up to 3 days and hair samples were collected for up to 10 months. All samples were then analyzed by gas chromatography/mass spectrometry for d5-C and its metabolite d5-benzoylecgonine (d5-BE).

The use of isotopically-labeled cocaine allowed us to distinguish between the administered drug and any cocaine used by the subjects either before or during the study. In both hair and sweat the predominant analyte was the parent drug, d5-C. In contrast, d5-BE was the major analyte in blood, especially at later time periods. The seven-fold range of cocaine doses used in the study (0.6 - 4.2 mg/kg) resulted in from 0.1 - 5 ng of d5-C per hair sample and approximately 1/6th that amount of d5-BE.

Sweat contained very high and persistent levels of d5-C (more than 50 mg/ml at 1 hr decreasing to less than 1 mg/ml by 48 hr). The d5-C to d5-BE ratio ion sweat was similar to that found in hair, approximately 6:1.

In plasma, following IV administration d5-C concentrations peaked at 5 min (400-900 ng/ml), then decreased rapidly to less than 100 ng/ml by 3 hr. Conversely, d5-BE concentrations rose steadily and by 2 hr reached plateau levels of approximately 400 ng/ml. The elimination of d5-C from plasma, sweat, and hair appeared to be exponential with half-lives estimated to be 66 min, 15 hr, and 2 months respectively.

Hair analysis using GC/MS was found to be a very sensitive means for detecting cocaine ingestion. The threshold dose using this method was estimated to be approximately 25 - 35 mg drug IV. This is the amount of drug typically found in a single "line" of cocaine. However, subjects given the same dose differed considerably in the amount of d5-C incorporated into their hair. Non-Caucasians, in particular, incorporated considerably more (from 2 to 12 times as much depending upon how it was measured) d5-C into their hair than did Caucasians. These interindividual differences could not be explained by differences in their plasma pharmacokinetics. Also, there was little correlation between the time since drug administration and the position of drug along the hair shaft. Segmental analysis of the hair samples revealed that some subjects who received only a single dose had d5-C distributed along most of the hair shaft, while some subjects who received multiple doses had the drug confined to a much smaller area. In addition, d5-C could be detected in hair as early as 8 hr after drug administration. Considered together, these results suggest cocaine incorporation into hair may occur by mechanisms other than that usually proposed – simple diffusion from the bloodstream into the growing hair cells. It is more likely that cocaine is incorporated into hair through multiple mechanisms – through sweat for example – and at various times in the hair growth cycle.

In a series of *in vitro* experiments, control (drug-free) hair was exposed to cocaine in an aqueous solution or in vapor form ("Crack Smoke"). Hair could be extensively contaminated through this external exposure. Washing with various washing solutions (detergents and organic solvents) or treatment with cosmetic agents (bleach, perming, or straightening solutions)removed most, but not all, of the externally applied drug. In fact, drug-free hair could be extensively contaminated simply by being held in the hand of an individual who received

d5-C intranasally a few hours earlier. Again, washing could remove some, but not all the external contamination.

In summary, our studies show that hair analysis with a sensitive and specific method like GC/MS can be used to detect cocaine use or exposure. However, it is our opinion that the mechanism(s) for cocaine incorporation into hair appear to be more complex than previously thought. Thus, there is not, at present, the necessary scientific foundation for hair analysis to be used to determine either the time or amount of cocaine use. Further, because external contamination may be a possible source for evidentiary "false" positives for cocaine (i.e., drug is present, but not due to ingestion), all hair testing procedures for cocaine must be designed to rigorously guard against any inadvertent contamination of the sample during collection or analysis and external contamination must be ruled out when interpreting hair analysis results.

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

HISTORICAL BACKGROUND

The use of hair analysis to determine exposure to drugs and other chemicals has been an area of active debate for over twenty years. Hair analysis has been used in forensic and environmental toxicology to evaluate cases of heavy metal poisonings and the exposure of populations to environmental contaminants such as mercury. There is no question that metals can be found in hair and that excessive levels of arsenic, lead or mercury in hair have been used successfully to document cases of poisoning by these metals. However, because there is great variability in testing methods and a wide range of values reported for these metals in "normal" populations, there is less agreement on the value of hair analysis in identifying cases of sub-chronic poisoning or evaluating the health risk of populations exposed to environmental contaminants (Chatt, Secord *et al.* 1980).

One of the most controversial uses of hair analysis has been trace element analysis. In the early 1980's, some laboratories began offering hair analysis for nutritional analysis, often through beauty shops and health food stores. After analyzing a hair sample, some laboratories would provide a report listing the concentrations of trace elements found and often suggested dietary changes that included supplements and in some cases, chelation therapy. Hair analysis lost much of its credibility during this period because of the unscientific and often irresponsible commercialization of hair analysis for nutritional analysis. As a result, hair analysis for trace elements is now severely regulated or prohibited in most states.

It was into this rather negative climate that hair analysis for drugs of abuse was introduced. One of the earliest reports of hair analysis was in a study of drug-induced dermatitis. Because of their structural similarity to melanin, it was suggested that drugs such as l-DOPA, alpha-methyl DOPA, isoproterenol and amphetamine might be incorporated along with melanin into hair pigments. Although the objective of these studies was to study drug uptake and metabolism by skin rather than detecting drug use, it was noted that "hair may be a useful and sensitive indicator of past consumption by humans of amphetamines and related drugs" (Harrison, Gray *et al.* 1974a; Harrison, Gray *et al.* 1974b).

In 1979, Baumgartner *et al.* reported that a radioimmunoassay could be used to detect nanogram levels of morphine in the hair of heroin abusers (Baumgartner, Jones *et al.* 1979). In addition, they claimed that the position of drug along the hair shaft correlated with the time of drug use. Shortly thereafter other investigators demonstrated that morphine could be detected in the hair of heroin overdose victims and suggested that hair analysis could be used to reconstruct the drug use history of the deceased (Klug 1980). Since then, hair has been used by an increasing number of forensic laboratories throughout the world as a specimen to detect drugs of abuse such as: cocaine (Arnold and Puschel 1981; Baumgartner, Black *et al.* 1982; Michalodimitrakis 1987; Parton, Warburton et al. 1986; Smith and Liu 1986a; Smith and Liu 1986b; Valente, Cassini *et al.* 1981), methamphetamine (Ishiyama, Nagai *et al.* 1983; Niwaguchi, Suzuki *et al.* 1983; Suzuki, Hattori et al. 1984), phencyclidine (Baumgartner, Jones *et al.* 1981; Sramek, Baumgartner *et al.* 1985), and nicotine (Haley and Hoffman 1985; Ishiyama, Nagai *et al.* 1983).

Unfortunately, many of the early studies on hair analysis used immunoassay screening tests that were not confirmed by more specific methods such as gas chromatography-mass spectroscopy (GC/MS). In addition, these studies relied on self-reported drug use to determine drug dosage rather than controlled dose administration. As a result, most published reports have not shown a good correlation between drug dose and concentration in hair. More recent studies, which used controlled doses of drugs and more specific methods, have shown a better relationship, but the numbers of subjects were small (Cone 1990).

In summary, numerous studies over the last decade have shown that hair analysis can be used to detect prior drug use. However, much remains to be learned about this emerging technology and it must rest on a solid scientific foundation before it can be used routinely in the detection of drugs of abuse.

GOALS AND OBJECTIVES

PROGRAM GOAL

The overall goal of the program was to develop the necessary scientific informational base to support the use of hair analysis for drug detection and monitoring.

PROGRAM OBJECTIVES

The objectives of the program, as specified in the research grant, were as follows:

Objective 1

To determine the relationship between amount (i.e., the dose) of the drug used and the amount of drug found in hair.

Objective 2

To develop methods for distinguishing drug administered during the experimental procedures from any drug taken surreptitiously during the study or any drug retained in the body from previous use.

Objective 3

To measure the amounts of drug and metabolites present in sweat and assess any contribution this may have on the drug and metabolite concentrations ultimately found in hair.

Objective 4

To assess the extent that external contamination (through exposure to smoke or direct contact with drugs) can occur and the impact this may have on inferring drug usage from the results of hair analysis.

Objective 5

To identify and evaluate laboratory methods (e.g., washing procedures and/or evaluation of relative ratios of drug to metabolite) and handling procedures to meet evidentiary challenges based on claims of environmental contamination versus ingestion.

Objective 6

To identify and evaluate the extent to which clients may reduce the drug content of hair through intentional evasion tactics based on washing or other hair treatments.

Objective 7

To develop methods for evaluating the extent to which drug absorption and retention by hair may vary with age, race, sex differences between sub-populations.

OVERALL RESEARCH STRATEGY

Our research program was composed of two major components: a set of *in vivo* studies in which the incorporation of cocaine into hair was studied using human volunteers and a set of *in vitro* studies in which cocaine was applied directly to drug-free hair.

The strategy of the *in vivo* studies was to administer precise doses of cocaine to human volunteers in a controlled clinical setting and determine the amount of cocaine absorbed and retained by hair.

The strategy of the *in vitro* studies was to determine whether hair can be contaminated by exposure to smoke or direct contact with the drug. A series of *in vitro* studies were performed in which the uptake of cocaine in hair was measured following exposure to cocaine in vapor form (simulating "crack smoke") or in an aqueous solution.

Experiments were also conducted to determine whether hair can be contaminated by cocaine excreted in sweat. Finally, various washing solutions or hair cosmetic preparations were studied to determine whether they could reduce or remove any externally applied cocaine.

PHARMACOKINETIC STUDIES WITH HUMAN VOLUNTEERS

OVERVIEW

Precise doses of deuterium-labeled cocaine were administered to human volunteers under controlled laboratory conditions. Sequential plasma, urine, sweat, and hair samples were collected then analyzed by gas chromatography/mass spectrometry (GC/MS). The resulting data were then analyzed to determine 1) the relationship between the amount of cocaine used

and the concentration of parent drug and metabolites present in plasma, urine, sweat, and hair; 2) the reliability of segmental analysis, i.e., the relationship between the time of drug use and the position of drug along the hair shaft, and the time interval between drug use and appearance of drug in hair; 3) the minimum dose of cocaine required to produce a positive result in hair; 4) *in situ* stability of cocaine in hair, i.e., how long hair will remain positive; and 5) how factors such as sex, age, race and hair type affect drug incorporation and retention.

EXPERIMENTAL METHODS

Research Subjects

Selection Criteria

A total of 31 volunteers were recruited into the study through newspaper advertisements asking for volunteers to participate in studies on cocaine effects. In general, moderate users of cocaine were sought. Moderate use is defined as use from once or twice every six weeks to weekly. Frequent users are defined as individuals who use greater than weekly. Light users are individuals who use once or twice a year. We find that moderate users usually provide a more reliable subject group and are less likely to be concurrently using multiple other psychoactive agents. Users of multiple psychoactive agents not only present significant confounding variables, they may be less reliable as well, in terms of following research protocols.

All the subjects reported they commonly used the drug under study, i.e., cocaine, but none were cocaine–dependent as judged by DSM III-R criteria or had a history of cocaine dependence in the past. No subjects were accepted into the study who engaged in seemingly out of control psychoactive drug use. Subjects were healthy as judged by medical, laboratory and psychiatric evaluation and all were able to give adequate informed consent. Female volunteers were tested before any drug administration to ensure that they were not pregnant. Subjects must have had experience with the route of drug administration being studied and, in so far as could be determined, with the appropriate dose ranges used.

Potential subjects were carefully and conservatively screened to eliminate those with significant medical or psychiatric illness. Evaluation included a comprehensive history and physical examination, CBC, SMA–12, dibucaine number determination, EKG, pregnancy screen and HIV test. All HIV testing results were kept confidential and in a separate file from all other data. If subjects were HIV–positive, they were excluded from the study and were informed of their results by the medical director of the program. HIV–positive individuals were excluded because of the profound effects of cocaine on NK cell activity (Van Dyke, Stesin et al, 1986). Appropriate medical referral was made if HIV positive.

Written informed consent was obtained from all subjects and all protocols were approved by the Human Subjects Review Committee at the University of California campuses at San Francisco and at Davis.

Subjects were paid $9.00 per hour for the time they spent in the laboratory and, when multiple sessions were involved, they were given a 20% bonus for successfully completing the series of sessions. For the return visits to provide hair samples, subjects were paid $25 to $100 per visit depending on the time from original testing. Prospective subjects had the opportunity to visit

the Drug Dependence Research laboratories and always had at least a few days to think about the various aspects of the study before participating.

Subjects were tested as outpatients in the Drug Dependence Research Center at Langley Porter Institute. The laboratory consists of approximately 1300 square feet and has two subject testing areas. A temperature and humidity controlled room with an upper temperature range to 40°C was available in the laboratory for the sweat studies. Cardiac resuscitation equipment was available in the laboratory and excellent medical support facilities (Code Blue team, etc.) were immediately available.

Procedures Used to Protect Against or Minimize Risks to the Human Subjects

The likelihood of serious risk to the subjects was minimized by carefully screening the subjects, selecting appropriate cocaine doses, and carefully monitoring during the experiments. Cardiac resuscitation equipment and personnel trained in its use and in CPR techniques were immediately available during all of our experiments. Most laboratory staff have had firsthand experience with the management of drug toxicity. All have been trained to terminate a drug experiment rather than risk worsening of a suspected problem. All studies were done in a general medical hospital environment. The doses of cocaine studied were well within the range of those used previously in this or other laboratories.

Subject confidentiality was maintained by coding all forms, files, etc., with identification numbers, keeping all data in secure files or disks with the identifying information, and limiting access only to limited research personnel. Subjects with any concern about being identified as someone who has used illicit drugs were urged not to participate. We had no problems with confidentiality.

No subject was sent home from the laboratory while any significant degree of intoxication was evident. Follow-up treatment and support were made available to the subjects. When they finished any experiment, they were counseled about the dangers of illicit drug use and asked if they wanted a referral for more information about treatment alternatives.

Finally, throughout our studies we were very sensitive to the potential risk that our experiments might introduce a new or more dangerous pattern of illicit drug use to the volunteers. No subject who seemed unable to control their behavior (drug use or otherwise) was allowed to enter the study. Although a pattern of increased compulsive drug-seeking after experimental exposure in experiments like ours is theoretically possible, it has never been demonstrated. In humans, the determinants of compulsive drug use (addiction) are so complicated and depend so much on multiple variables (availability, setting, associations, alternate activities, etc.) that it would be difficult, probably impossible, to tease out the specific contribution added by participation in a laboratory study such as ours. Our experience (and that of many other investigators) with cocaine, cannabis, nicotine/ tobacco, LSD, morphine, caffeine and ethanol studies is that drug administration in a medical/ research setting is viewed by subjects as an experience different from their use of the same drug and dose in a social setting.

Subject Demographics

The demographics of the subjects recruited for our studies are shown in Table 1.

Table 1. Demographics of the human volunteers participating in the studies.

Age	Sex	Race	Weight (lb.)	Height	Hair Color	Hair Pattern	Hair Texture	Cosmetic Treatments	Cocaine Use
30	M	NC	163	6' 2"	black	curly		straightened	heavy
26	F	C	135	5' 7"	brown	straight			moderate
21	M	C	125	5' 4"	brown	straight			moderate
26	M	C	178	5' 8"	brown	straight			moderate
31	M	C	160	5' 5"	red	curly			heavy
32	M	C	140	5'	brown (graying)	curly			heavy
39	M	NC	155	5' 7"	black	curly			moderate
36	M	C	135	5' 11"	brown	wavy (thin)	thin		light
35	M	C	150	5' 8"	brown	wavy			moderate
38	M	C	155	6' 1"	brown (graying)	straight			moderate
21	M	C	160	6'	brown	straight		dyed black	heavy
23	M	C	170	6'	brown	straight			moderate
34	M	C	160	5 '9"	brown (graying)	straight			heavy
26	M	C	155	5' 9"	black	wavy			moderate
37	M	C	140	6'	brown	straight			heavy
38	M	C	147	6' 1"	brown (graying)	wavy			heavy
24	M	C	194	6' 4"	blonde	straight		bleached	light (iv)
28	M	NC	148	5' 7"	black	straight			heavy (iv)
24	M	C	130	5' 5"	brown	curly			moderate
30	F	C	120	5' 5"	black	straight			heavy
23	F	C	114	5' 6"	brown	straight		tinted red	heavy
30	F	C	130	5' 5"	brown	straight		dyed black	moderate
32	M	C	170	5' 9"	brown	wavy			heavy
33	M	NC	165	5' 10"	black	straight			moderate
28	M	C	150	6'	blonde	straight		bleached	heavy (iv)
37	M	A	196	6'	black	curly			heavy
31	M	C	160	5' 7"	brown	straight			heavy
27	F	C	114	5' 6"	brown	straight			light
38	M	C	143	5' 11'	brown	wavy (thin)	thin		moderate
23	M	C	135	5' 9"	brown	straight			light (iv)
34	M	C	157	5' 9"	brown	straight			moderate
29	M	C	137	5' 9"	brown	curly (thick)	thick		moderate

A total of 32 subjects were recruited into the study. Four of the subjects participated in early pharmacokinetic and range-finding studies in which the doses of d5-cocaine used were too low to be detected in hair. In addition, two subjects withdrew from the study before providing any post-dose hair samples. Thus, successive hair samples with detectable amounts of drug were collected from a total of 25 volunteers. This group included four females and four non-Caucasians. Their ages ranged from 21 - 39 years with a mean of 30. Although most of the subjects had straight brown hair, there was a range of hair colors, types and textures. The hair of four subjects was described as "graying" and six of the subjects had treated their hair with cosmetic products such as bleach, dyes, and straighteners.

Synthesis of Deuterium Labeled-Cocaine.

Cocaine hydrochloride labeled with five deuterium atoms on the benzoyl group [deuterated cocaine hydrochloride, d5-cocaine, (benzoyl-d5)-cocaine-HCl] and suitable for intravenous and intranasal use was synthesized according to the general method of Bell and Archer (Bell and Archer, 1960) shown in Figure 1.

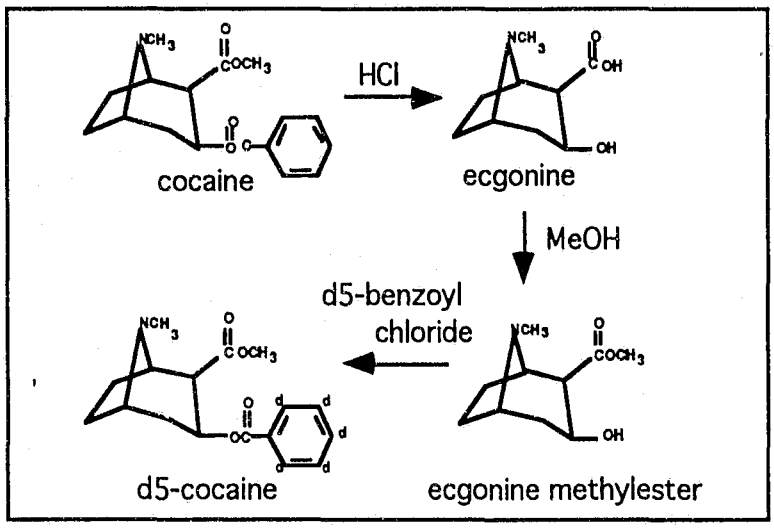

Figure 1. Synthesis of d5-cocaine.

The starting material, unlabeled cocaine-HCl, was hydrolyzed to ecgonine-HCl by refluxing in dilute hydrochloric acid, then purified by recrystallization from methanol. The ecgonine-HCl was subsequently esterified with methanol and anhydrous hydrogen chloride to form ecgonine methyl ester-HCl and was used without further purification. Ecgonine methyl ester-HCl was converted to the free base with aqueous potassium carbonate and benzoylated with d5-benzoylchloride to yield crude (benzoyl-d5)-cocaine free base. Column chromatography of the crude material yielded pure, crystalline (benzoyl-d5)-cocaine free base. This was converted to (benzoyl-d5)-cocaine-HCl with one equivalent of concentrated aqueous hydrogen chloride in 2-propanol/diethylether. The crystalline product was collected by filtration and, under sterile conditions, dried under a high vacuum. Chemical purity was certified by GC-MS, TLC, melting point, and elemental analysis. There were no impurities detectable by GC-MS or TLC. The melting point was identical to the melting point of unlabeled USP cocaine obtained from Malinckrodt Corporation. Sterile solutions of labeled cocaine for

intravenous injection were prepared by dissolving the labeled cocaine in sterile saline and then filtering the solution through a Millipore filtration unit. Fresh cocaine solutions were prepared for each administration from a stock solution replaced each month.

Deuterated cocaine is pharmacologically and metabolically identical to cocaine; however, it will produce a unique response in a mass spectrometer. The benzoyl site was chosen for deuteration because the benzoyl moiety remains intact during metabolic conversion to the primary metabolite benzoylecgonine. The increased mass of pentadeuterated cocaine (molecular weight = 308) is sufficient to ensure its distinction from unlabeled cocaine (molecular weight = 303) by mass spectrometry. The chemical formulae and molecular weights for cocaine, d5-cocaine, BE, and d5-BE are shown in Figure 2.

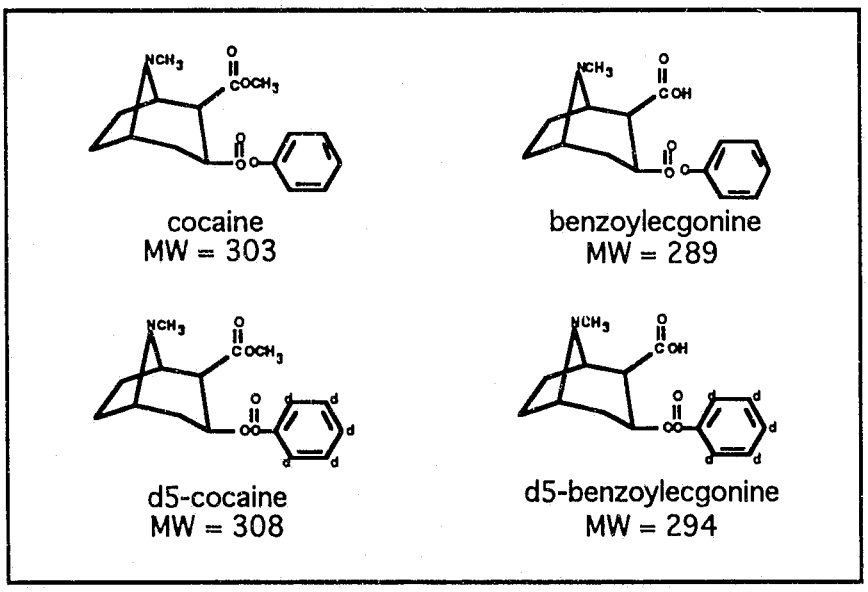

Figure 2. Chemical formulae and molecular weights of cocaine, benzoylecgonine, d5-cocaine, and d5-benzoylecgonine.

By administering deuterated cocaine and then measuring deuterated cocaine and metabolites in hair, any cocaine administered during the studies could be distinguished from any residual cocaine in the subjects' tissues or any cocaine taken surreptitiously during the study.

The appropriateness of using d5-cocaine was confirmed by determining that its metabolic disposition was identical to unlabeled cocaine. The reason this is necessary is that the replacement of hydrogen with deuterium can under some conditions alter the rate of drug metabolism, i.e., an isotope effect. Significant isotope effects on kinetics are rare unless a carbon-hydrogen (or carbon-deuterium) bond is broken in a major metabolic pathway. The positions of the deuterium label on the benzoyl group of cocaine were chosen because they are not involved in the major routes of cocaine metabolism. Consequently, an isotope effect is unlikely. Nevertheless, this was confirmed by the following experiment.

Seven subjects were tested for the isotope effect evaluation. A 50:50 mixture of labeled (5 mg/ml) and unlabeled cocaine (5 mg/ml) was administered as a 0.6 mg/kg total dose (i.e., 0.3 mg/kg labeled and 0.3 mg/kg unlabeled cocaine) given intravenously over a 1 min injection time. Plasma and urine samples were assayed using GC-MS procedures. Relevant

pharmacokinetic parameters, half-life, clearance, volume of distribution, and amount of metabolites (benzoylecgonine and ecgonine methyl ester) were determined from the measured concentrations of drug and metabolite in the biofluids. It was determined from the subjects studied that deuterated cocaine produces no isotope effect.

Isotope Effect of d5-Cocaine

Data from the experiments in which we compared the pharmacokinetics of deuterated and non-deuterated cocaine are shown in Figure 3. In this figure, the mean concentrations of non-deuterated and deuterated cocaine in plasma of the seven test subjects are plotted versus the time the sample was collected. At all sampling times the concentrations of non-deuterated and d5-cocaine are not significantly different; thus, there appears to be no isotope effect.

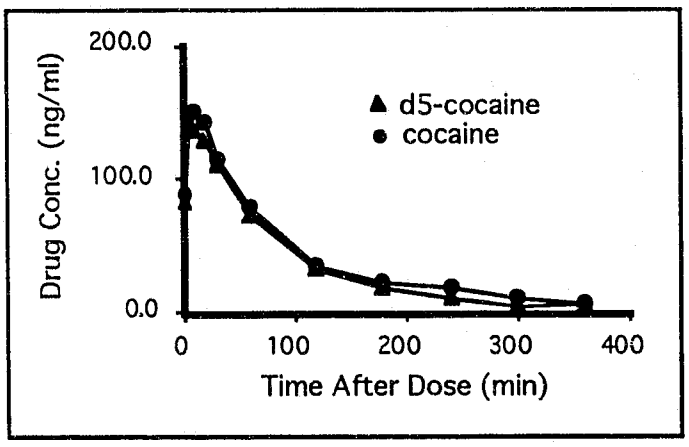

Figure 3. Plasma decay curves for d5-cocaine (deuterated) and non-deuterated cocaine following intravenous administration of 0.3 mg/kg. Values shown are means for seven subjects.

Figure 4 shows the plasma decay curves for deuterated and non-deuterated benzoylecgonine, the primary metabolite of cocaine, in three subjects. Although there is more variability than with cocaine, there was no significant isotope effect evident with this metabolite.

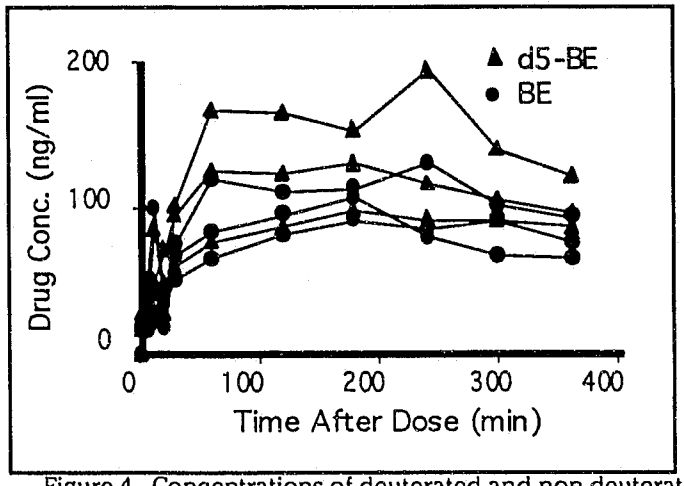

Figure 4. Concentrations of deuterated and non-deuterated benzoylecgonine in three subjects after administration of of a mixture of deuterated and non-deuterated cocaine.

Similarly, Table 2 shows half-life and clearance values for the test subjects. The mean values for plasma half-life and clearance of non-deuterated and deuterated cocaine are not significantly different and correlate with an r^2 of about 0.8 which indicates that the pharmacokinetics of non-deuterated and deuterated cocaine are similar.

Table 2.
Plasma half-life and clearance values for non-deuterated and deuterated cocaine in seven subjects.

Subject	Plasma Half Lives (min)		Plasma Clearance (ml/min/kg)	
	Cocaine	d5-Cocaine	Cocaine	d5-Cocaine
90255	83.6	81.1	21.5	19.3
90256	61.7	61.4	32.8	28.9
90272	81.1	102.4	30.8	22.4
90286	37.8	41.6	23.0	21.5
90292	83.9	76.9	15.5	15.1
90303	44.5	46.7	24.5	26.5
90317	78.3	68.9	17.6	15.2
Mean	67.3	68.4	23.7	21.3

Drug Administration and Formulation

IV Administration

Cocaine for intravenous administration was prepared from synthesized deuterium-labeled cocaine hydrochloride. A stock solution was prepared under sterile conditions as a Millipore-filtered 10 to 20% stock solution in sterile 0.9% sodium chloride. The administered dose was diluted to a volume of 3 to 7 ml for intravenous injection by syringe pump. Stock solutions were refrigerated and were used within one month of preparation. As a quality control procedure, samples from each dose administered were assayed to verify concentration and stability.

Intranasal Administration

For intranasal doses, cocaine was administered as a 20% solution of the hydrochloride salt in saline delivered in a fine mist to the nasopharynx. Although snuffing of the crystalline material is the preferred route by cocaine users and some researchers, in our lab we find that the spray delivers a more reproducible dose than does crystalline cocaine and we find little difference in kinetics or effect when the two dose forms are compared. The advantage of using cocaine solution introduced by insufflation is better control of the dose administered. The use of the

nasal insufflator also reduces idiosyncrasies of dose delivery due to subject variability in their method of dosing. We used a Macintosh Oxford sprayer modified so that the reservoir held approximately 1 ml of solution. It delivered a 150 mg dose in about 4 squeezes. Assay of the residual material in the spray system allowed precise determination of the actual dose delivered to the nasopharynx.

Doses Used

Three dose ranges were used in studies in which d5-cocaine was administered either intravenously or intranasally as a single dose (Table 3). To administer the largest dose of cocaine safely, multiple doses of drug were administered intranasally every 3 - 5 days. Under this dosing scheme, over three-quarters of a gram of cocaine (825 mg) were administered in less than three weeks. With this dosing regimen we simulated a single use of cocaine as well as a pattern of repeated, chronic use. Obviously, many chronic cocaine users use much higher doses and for longer periods of time, but we were limited by safety considerations of the research subjects.

Table 3.
Dosing regimen for single dose and multiple dose studies.

Route	Dose Range	Amount of d5-Cocaine (mg)	Dose of d5-Cocaine (mg/kg)	Number of Subjects
IV	Low	12 - 23	0.3	4
IV	Medium	39 - 55	0.6	9
IV	High	90 - 151	1.2	6
Nasal*	Low	60 - 180	0.4 - 3*	4
Nasal*	High	300 - 750	4 - 11*	5

* Nasal doses listed were corrected for bioavailability, estimated to be 30%.

Procedures for Collecting Biologic Samples

Blood Collection

For the pharmacokinetic studies, blood was collected from the dominant arm via an indwelling venous catheter that was removed at the end of the first study day. On days 2 through 4, blood was obtained by venipuncture. Blood samples, usually 8–10 ml, were obtained before and at 15, 30, 60, 180, and 360 min, as well as 24, 48, and 72 hr after drug administration. Blood samples were placed in test tubes containing 5 µl of a saturated solution of sodium fluoride to prevent degradation of cocaine by blood esterases. The tubes were mixed gently to ensure thorough mixing and iced immediately after filling. Plasma was separated and frozen at -20°C within 1 hr.

Urine Collection

Urine samples were obtained before dose administration and at 6, 23, 49, and 73 hr postdose. For each sample the pH and volume were recorded, a 20 ml aliquot removed, acidified with HCl to a pH of less than 4, then frozen and stored at –20°C until assayed. Each sample was labeled with study name, date, time of collection, volunteer number and sample number. Spot urines were obtained at pre, 23, 49, and 73 hr postdose, and sent for a toxicological screen.

Sweat Collection and Processing

Arm and hand sweat were collected using polyethylene shoulder length gloves (0.00125" gauge x 34 inch, 3-mil thick). Particular care was taken to avoid inadvertent environmental cocaine contamination. A shoulder length plastic glove was placed over the arm and hand then secured with an elastic band. Over this glove another shorter plastic hand glove was used to protect the longer glove from tears. After each exercise period the 2 to 5 ml of accumulated sweat was removed, pH and total volume measured, and then acidified to a pH of less than 4 using 6 M HCl. The acidified sweat sample was centrifuged at 2400 rpm for 10 min, then pipetted and placed into a 15-ml tube and stored at -20° C until analyzed.

Hair Collection

Hair samples (at least 100 mg per subject) were collected from the posterior vertex region of the scalp before each drug administration and at monthly intervals. At each sampling time, approximately 100 hair strands were collected by cutting the hair as close to the scalp as possible (within a millimeter). Care was taken to orient hair so that the proximal ends matched and could be identified for precise segmental studies. Hair sampling continued at monthly intervals for at least one month past the time when drug was no longer measurable in hair.

Physiological Measures

Physiologic data were obtained before and after each cocaine administration and sweat induction period. Measurements of heart rate, blood pressure and skin temperature were recorded on a PhysioControl device with subjects always recumbent for at least 5 min before each determination. Core body temperature was measured with tympanic membrane thermocouple probes placed in the external ear canal. Tympanic temperature is highly correlated with core body temperature. Subjective high ratings (verbal rating on a 0 to 100 scale) and stimulant drug symptom check lists (20 items, 0 to 5 intensity rating administered by hand held computer) were given often enough to track peak symptom intensity and duration of major symptoms. Room temperature and percentage relative humidity were also recorded during the sessions when sweat was collected.

Analytical Procedures

Quantitation of d5-Cocaine and Metabolites in Human Hair

A sensitive and specific method for the simultaneous detection and quantification of cocaine and its two principle metabolites was developed during this research program and published in the Journal of Analytical Toxicology (Harkey, Henderson et al., 1991b).

Chemicals and Reagents

Cocaine and BE standard solutions (1 mg/ml in methanol) were obtained from Sigma Chemical Company, ecgoninemethyl ester (EME) was obtained from Alltech-Applied Science Company, and diflurococaine obtained from El Sohly Laboratories. Tris (hydroxymethyl) aminoethane hydrochloride (Tris HCl), Tris (hydroxymethyl)aminomethane (Tris base), sodium dodecyl sulfate (SDS), dithiothreitol (DTT), and Proteinase K (protease type XXVIII, 13.6 units/mg) were obtained from Sigma Chemical Company. N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) was obtained from Pierce Chemical Company; Bond Elut Certify™ extraction columns and Vac-Elut SPS 24™ vacuum manifold from Analytichem International; Isobutane (99.99%, reagent grade) from AGA Gas, Inc.; Helium (99.999%) from Liquid Carbonic Company; methanol (HPLC and Pesticide grades) from Fisher Scientific; isopropanol (High Purity grade) from Baxter Healthcare; and methylene chloride (Nanograde®) from Mallinckrodt Chemical Works. All other reagent grade chemicals were obtained from either Fisher Scientific or Mallinckrodt Chemical Works. All drug solutions were prepared in Pesticide grade methanol and HPLC grade solvents were used in all extraction procedures.

Standards and Solutions

A stock solution containing d5-cocaine, cocaine, d5-BE, BE, and EME, each at 1 mg/ml in methanol, was prepared and stored at - 5° C. For calibration standards and daily quality control samples, working solutions of d5-cocaine, cocaine, d5-BE, BE, and EME were prepared in methanol at 0.1, 0.5, 1, 5, and 10 ng/µL. Daily calibration plots were obtained by analyzing hair samples fortified with each drug at 0.1, 0.5, 1, 5, and 10 ng/mg hair. Both positive and negative quality control hair samples were analyzed daily. Two positive controls were prepared by fortifying drug-free hair samples with d5-cocaine, d5-BE, and EME, all at either 0.1 or 1 ng/mg hair. To dissolve the hair samples prior to extraction, the following stock and working solutions were prepared: 1 M Tris buffer, pH 7.5; 10% SDS; 0.4 M DTT in 10 mM sodium acetate, pH 5.2; Proteinase K, 10 mg/ml; and digest buffer, which contained 1 ml of 1 M Tris buffer, 20 ml of 10% SDS, and 79 ml deionized water. For solid phase extraction procedures, the following solutions were prepared: 0.1 M potassium phosphate buffer, pH 6.0; 1 M potassium hydroxide; 0.1 M hydrochloric acid; methylene chloride:isopropyl alcohol (80:20) with 2% ammonium hydroxide.

Sample Preparation and Derivatization

Before digestion, all hair samples were washed to remove any potentially interfering substances from the external surface of hair. Before washing, the root ends of the hairs were aligned, the samples (weighing approximately 10 mg) cut into a 1 cm length and placed into a 100 ml beaker containing 25 ml 1% SDS. The hair samples were stirred on a magnetic stirrer for 5 min, then poured though filter paper to retain the hair for further rinsing. Samples were rinsed with deionized water (50 ml X 10), then methanol (30 ml X 3). Hair samples were allowed to drain completely between each rinse and were dried under a hood overnight before weighing. After drying, the samples were cut into very small sections (≤ 1 mm) before weighing a 10 mg aliquot for analysis. The procedure for digesting the hair samples is similar to that reported by Gill *et al.* (Gill 1985), but was modified to improve the recovery of cocaine and to reduce chemical background. Approximately 10 mg of hair was placed in a screw-capped silanized glass centrifuge tube (10 mm x 100 mm) with 2.6 ml digest buffer and 0.4 ml of 0.4 M DTT in 10 mM sodium acetate buffer, then vortexed and incubated for 2 hr at 40° C.

After the first incubation, 55 µL of Proteinase K solution was added, the sample vortexed again, and incubated overnight at 40° C.

d5-Cocaine, cocaine, d5-BE, BE, and EME were extracted from the digested hair samples using Bond Elut Certify™ columns and Vac-Elut SPS 24™ vacuum manifold. After conditioning the column with 2 ml methanol and 2 ml 0.1 M phosphate buffer, the digested hair samples were added to the extraction columns, the columns rinsed with 3 ml deionized water, 3 ml 0.1 M hydrochloric acid, and 8 ml methanol. Drugs were eluted with 2 additions of 2 ml methylene chloride:isopropyl alcohol (80:20) with 2% ammonium hydroxide. The extracts were evaporated under nitrogen at 40° C, reconstituted in 0.25 µL methylene chloride, and vortexed. Each extracted sample was then transferred to a 1-ml screw-capped injection vial, evaporated under nitrogen at 40° C, 10µL of MTBSTFA added, and the vial tightly closed. The samples were vortexed, incubated at 40° C for 10 min, vortexed again, then 1 µL injected into the GC/MS.

Gas Chromatography-Mass Spectrometry

Chemical ionization mass spectrometric analysis of hair extracts was performed on a Finnigan ITS-40 ion trap mass spectrometer interfaced with a Varian 3400 gas chromatograph equipped a Varian 1075 injector and a J & W Scientific DB-5 capillary column (15 M x 0.25 mM i.d., 0.1 µM film thickness). Helium was used as the carrier gas with a head pressure of 5 psi and a linear velocity of 55 cm/sec at 100° C. The injector was held in the splitless mode from 0.01 to 0.6 min after the injection of a 1 µL sample. The column temperature was held initially at 100° C for 1 min, increased to 300° C at 25°/min, then held at 300° C for 2 min. The injector and transfer line were maintained 260° C, and the manifold at 240° C. The filament emission current was 10 µA and the electron multiplier voltage was 1600 V. Isobutane was used as the reagent gas with the following ionization parameters: maximum ionization time, 2000 µsec; maximum reaction time, 80 msec; ionization level, 20 amu; and reaction level, 40 amu. Full scan GC/CIMS analysis of mass range 80 to 440 was performed at 1.0 s/scan. Using the gas chromatographic conditions described above, retention times were 4.13 min for EME-TBDMS, 5.62 min for internal standard, 5.85 min for d5-cocaine, and 6.90 for d5-BE-TBDMS.

Quantitation

A five-point calibration plot was prepared daily by analyzing 10 mg drug free hair samples fortified with d5-cocaine, cocaine, d5-BE, BE, and EME at concentrations of 0.1, 0.5, 1, 5, and 10 ng/mg hair. The ITS-40 Auto Calibration program was used to generate calibration plots, and the Auto Quantitation program was used to determine the concentration of d5-cocaine, cocaine, d5-BE, BE, and EME in hair samples. A single ion (MH^+) was used for quantitation based on the peak area ratios of cocaine, the *tert*-butyldimethylsilyl derivative of EME (EME-TBDMS), and the *tert*-butyldimethylsilyl derivative of BE (BE-TBDMS) to the internal standard, difluorococaine. Masses 308, 408, 314, and 340 were used for d5-cocaine, d5-BE-TBDMS, EME-TBDMS, and internal standard, respectively. Masses 304, 404, 314, and 340 were used for cocaine, BE-TBDMS, EME-TBDMS, and internal standard, respectively. Correlation coefficients for calibration plots were calculated by linear regression analysis using the ITS-40 Auto Calibration program. Calibration plots were obtained daily. During validation studies, correlation coefficients for five calibration plots of 0.1 to 10 ng/mg hair were 0.992 ± 0.009 for d5-cocaine, 0.991 ± 0.008 for d5-BE-TBDMS, and 0.979 ± 0.01 for EME. The detection limit, developed using drug-free hair samples spiked with d5-cocaine, d5-BE, and EME at 10 ng/mg hair, was set at 0.1 ng/mg hair for d5-cocaine and d5-BE, and 0.5 ng/mg for EME, based on a

signal to noise ratio ≥ 3. Although EME could be detected in some samples at ≤ 0.1 ng/mg hair, the cutoff was set at 0.5 ng/mg hair due to a small co-eluting peak that was observed in some negative control hair samples. We found that the detection limit for the cocaine analytes varied depending on the type of hair of hair used to produce the calibration curves. To improve the ruggedness of our assays, we used coarse black Asian hair to validate the method and to prepare all standards and controls because this hair type is more difficult to digest and produced a higher chemical background than Caucasian hair.

Precision

The precision of this method was determined by analyzing quality control samples that contained cocaine, BE, and EME at known concentrations. For hair samples spiked with each drug at 0.1 ng/mg hair, the observed mean ± standard deviation and coefficient of variation (% CV) were 0.11 ± 0.02 ng/mg (18.2% CV) for cocaine; 0.09 ± 0.02 ng/mg (22.2% CV) for BE-TBDMS; and 0.14 ± 0.04 ng/mg (28.6% CV) for EME,-TBDMS. At 1 ng/mg hair, the observed mean ± standard deviation and % CV were 1.07 ± 0.11 ng/mg (10.3% CV), 1.10 ± 0.18 ng/mg (16.3% CV), and 0.94 ± 0.26 ng/mg (27.7% CV), for cocaine, BE-TBDMS, and EME-TBDMS, respectively.

Recovery

The percentage recovery for cocaine and BE was typically in the range of 90.4 to 115.4%. For samples spiked at 0.1, 0.5, and 1 ng/mg hair, the mean recovery (± standard deviation) was found to be: at 0.1 ng/mg hair, 97.9% (± 17.4) and 115.4% (± 10.8), for cocaine and BE, respectively; at 0.5 ng/mg hair, 90.4% (± 6.8) and 93.3% (± 4.5); and at 1 ng/mg hair, 90.5% (± 9.3) and 94.0% (± 6.2), for cocaine and BE, respectively. Using this method to calculate absolute recovery, the values for EME were consistently higher (e.g., 109.6% (±18.7) at 0.1 ng/mg hair, 126.8% (± 29.5) at 0.5 ng/mg hair, and 124.97% (± 26.8) at 1 ng/mg hair). This difference may be due to a small peak found in blank hair with m/z 314 and a retention time similar to EME.

Linearity

Linearity of our method was determined by analyzing drug free hair samples spiked with cocaine, BE, and EME at 1, 5, 10, 50, and 100 ng/mg hair. Although the method appeared to be linear to 100 ng/mg hair, the 5-point calibration plot from 1 to 100 ng/mg hair was used only as an initial estimate of concentration. If the concentration was less than 1 ng/mg hair, precision was much better if a 3-point calibration plot of 0.1, 0.5, and 1.0 ng/mg hair was used, as described previously. Similarly, for concentrations in the range of 1 to 10 ng/mg hair, a 3-point calibration plot of 1, 5, and 10 ng/mg hair was used. At concentrations above 10 ng/mg hair, the initial 5-point calibration curve at 1 to 100 ng/mg hair was acceptable. Precision in this range was verified by analyzing hair samples fortified with cocaine, BE, and EME, each at a concentration of 50 ng/mg hair. The mean ± standard deviation (and % CV) for these samples were 55.3 ± 3.29 (5.95%), 59.7 ± 3.20 (5.36%), and 54.0 ± 11.34 (20.99%), for cocaine, BE, and EME, respectively.

Quantitation of d5-Cocaine and Metabolites in Blood, Urine and Sweat

Cocaine and Benzoylecgonine

Cocaine and benzoylecgonine were measured by capillary gas chromatography with nitrogen–phosphorus detection using the method developed previously by Jacob *et al.* (Jacob et al. 1987). The method utilizes structural analogs of both analytes, m–toluylecgonine and m–toluylecgonine methyl ester, as internal standards, and can be used to determine concentrations as low as 10 ng/ml in 1 ml samples. This method was developed originally for plasma and urine assays, but has been successfully applied to various biologic fluids including whole blood, saliva, sweat and CSF. The assays were carried out using automated gas chromatographs with a microcomputer data system to facilitate studies involving large numbers of samples.

Deuterium–Labeled Cocaine and Deuterium–Labeled Benzoylecgonine

The stable isotope–labeled cocaine and benzoylecgonine were determined by GC–MS. The extraction procedure and derivatization of benzoylecgonine are identical to the method for unlabeled cocaine and benzoylecgonine described above (Jacob et al. 1987) with some minor modifications for sweat. GC–MS analyses were carried out using a 0.2 mm x 25 m methyl silicone capillary column interfaced to a desk–top quadrupole mass spectrometer (Hewlett Packard mass selective detector). Quantitation was achieved by selected ion monitoring of the molecular ions produced by electron ionization of the analytes and internal standards, m/z 303 for cocaine, m/z 308 for d5-cocaine, m/z 317 for the internal standard m–toluylecgonine methyl ester, m/z 345 for the butyl ester derivative of benzoylecgonine, 350 for the butyl ester derivative of d5-benzoylecgonine, and 359 for the butyl ester derivative of the internal standard, m–toluylecgonine. Sensitivity of the GC–MS method was 10 ng/ml.

Calculations and Presentation of Data

As yet there is no universally accepted way of expressing hair analysis data; however, in the scientific literature, hair analysis data are often presented in units of concentration (e.g., ng drug/mg hair). In our studies this was not a useful unit because we sampled hair for up to 10 months and the length of hair varied considerably with the subject and with time. Thus, two hair samples could have the same amount of drug, but quite different concentrations simply because they differed in length.

Two major objectives of our study were to ascertain : 1) whether hair analysis could be used to correlate the amount of drug administered with the amount of drug incorporated into hair and 2) to ascertain how accurately segmental analysis is in recording a history of drug use. Thus, we were interested in correlating the dose (amount of drug administered) with the amount of drug in the entire hair sample and with the amount of drug in various hair segments. This is complicated by the fact that the length of the hair samples varied considerably from subject to subject and, for each subject, the length of the hair sample varied with time.

Other investigators attempt to correct for differing hair lengths by using a standardized hair sample (e.g., the first 3- 6 cm of hair from the root) which theoretically will contain only drug ingested during the past few months. However, we found that cocaine was not always incorporated into discrete areas adjacent to the root but was often found distributed over many segments far from the root.

Therefore, to prevent any possible experimental bias we are presenting our data in a variety of formats.

Results Expressed as Concentration of Drug in Hair

In this report, results are expressed in concentration terms (ng drug per mg hair tested) only when the hair samples tested were too small to perform segmental analysis.

In the scientific literature hair analysis results have usually been presented as concentration of drug in the entire hair sample tested or in individual hair segments (e.g., amount of drug present per amount of hair, ng/mg hair). However, in our studies we were attempting to correlate the amount of drug administered with the amount of drug incorporated into hair and expressing data in terms of concentration could be misleading. We obtained samples from some subjects for up to 10 months and the length of their hair varied considerably throughout the study period. Thus, to correct for the differing lengths of the various hair samples collected and any diffusion of the drug over the hair fibers, we expressed our results as amount of drug in a "standard hair sample". This "standard hair sample" consisted of a bundle of hair fibers composed of successive 1 cm segments with each segment weighing 10 mg. The length of this bundle varied from subject to subject and with time.

Results Expressed as Amount of Drug in the Hair Sample

Except in a few cases all hair samples were analyzed by segmental analysis. A typical hair sample from a subject would consist of a bundle of hair about the thickness of a pencil. This bundle was carefully cut into 1-cm segments starting at the root. Each segment was further divided into 10-mg portions, analyzed, and a mean value for that segment determined. The amount of drug in the hair sample was calculated by summing the amount of drug present in all individual hair segments. Thus, our results are expressed as amount of drug in a hypothetical hair sample composed of a varying number of 1-cm segments with each segment containing 10 mg of hair.

However, for any individual the total amount of drug present in his or her hair changed with time. Typically, the total amount of drug in hair reached a maximum at one to two months after drug administration and then decreased with time. Therefore, the "amount of drug in hair" was then expressed in three ways: maximum amount of drug (in any of the samples), mean amount of drug (in all samples), and area under the curve (AUC).

"Maximum Amount of Drug (in any of the samples)"

The maximum amount of drug is the highest value observed in any hair sample obtained at any sampling time. This usually occurred between one and three months after drug administration. This value is derived from the data shown in Table 5 of the Results section.

"Mean Amount of Drug (in all samples)"

The mean amount of drug present was calculated by determining the mean value of all positive hair samples obtained from an individual. Expressing data this way could prevent the results from being biased by one or two unusually high samples.

"Area Under the Curve (AUC)"

Expressing the amount of drug present as area under the curve (AUC), measures the area under the amount versus time curve (i.e., the amount of drug present in a hair sample versus the time since drug administration). Although this term is not used typically in expressing the results of hair analysis, it is commonly used in pharmacokinetic calculations to define the total amount of drug incorporated into the body over time and is useful in measuring total drug "bioavailability". AUC is calculated according to the "trapezoidal rule" in which the areas of the trapezoids that comprise the amount-time curve are summed. This is illustrated in Figure 5.

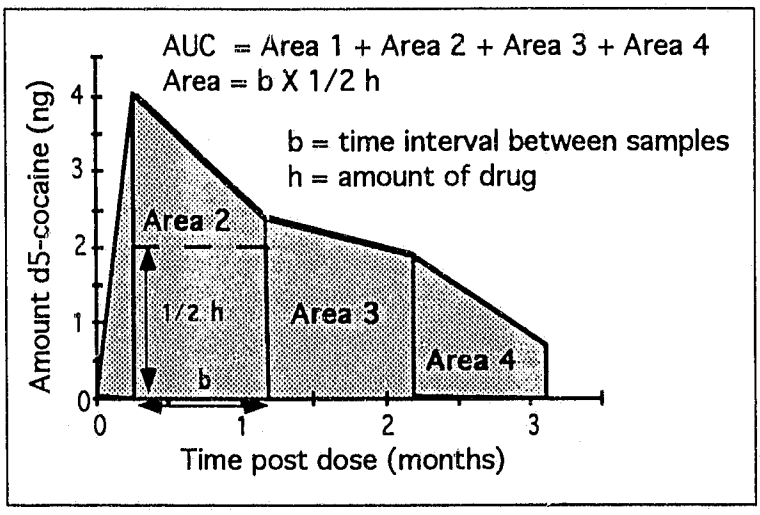

Figure 5. Calculation of the area under the curve (AUC) by the trapezoidal rule.

Examples of the various ways hair analysis data are expressed in this report are shown in Table 4.

Hair samples positive for d5-cocaine were obtained at 0.26, 1.17, 2.2 and 3.13 months. The "maximum amount" of drug found in any hair sample was 4.01 ng (the sample obtained at 0.26 months) and the "mean amount" of drug was 2.25 ng (the mean of values for all four samples). The amount expressed as "AUC" (Area Under the Curve) was 6.84 ng• months when calculated by the trapezoidal rule.

Table 4.
Segmental analysis results for subject number 88173 used to demonstrate the calculation of the area under the curve (AUC) by the trapezoidal rule.

		Amount d5-Cocaine in Segment (ng)				
Hair Collected (mo post dose)	Amount d5-Cocaine in Sample (ng)	Seg 1	Seg 2	Seg 3	Seg 4	Seg 5
0.26	4.01	1.20	2.82			
1.17	2.40	1.26	0.54	0.38	0.22	
2.20	1.89	0.83	0.38	0.25	0.19	0.24
3.13	0.68	0.11	0.23	0.34		

Maximum amount of d5-cocaine = 4.01 ng
Mean amount of d5-cocaine = 2.25 ng
AUC d5-Cocaine = 6.84 ng

RESULTS

Measurement of d5-Cocaine and Metabolites in Hair

Tables 5 and 6 are master tables showing the amount of d5-cocaine and d5-BE respectively found in all positive segments of all hair samples of subjects who received controlled doses of d5-cocaine. For clarity only positive samples are shown. The metabolite ecgonine methyl ester (EME) was detected very infrequently, usually at the limit of detection, therefore data for this metabolite is not shown. Subsequent illustrations and graphs presented in the report use data extracted from these tables.

Analyte Profile

As can been seen from the data in Tables 5 and 6, cocaine, not the metabolites BE or EME, was the primary analyte found in hair. This was true for all doses and following all routes of administration. Even when multiple doses were given, e.g., an IV dose followed by successive intranasal doses, cocaine was still the primary analyte. BE was detected in only 10 of the 25 subjects (Table 6) and in only a few of the multiple hair samples obtained from these individuals. Usually it was found in subjects who received the higher doses of cocaine. The mean ratio ± SD of d5-cocaine to d5-BE was 5.5 ± 3.3.

Table 5
Master table showing amounts of d5-cocaine in segments of hair samples obtained from subjects who received a controlled dose of d5-cocaine.

Number	Subject ID	Dose (mg)	Route	Dose (mg/kg)*	Hair Collected (mo post dose)	Total Amount d5-C in Hair (ng)	Amount d5-Cocaine in Segment**														
							1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	88173	149.1	N	1.2	0.26	4.01	1.20	2.82													
		153.6	N	1.2	1.17	2.40	1.26	0.54	0.38	0.22											
					2.20	1.89	0.83	0.38	0.25	0.19	0.24										
					3.13	0.68	0.11	0.23	0.34												
2	90308	35.2	IV	0.6	1.13	0.37	0.19	0.18													
					2.25	0.52		0.17	0.22	0.13											
3	90339	39	IV	0.6	1.00	0.19	0.19														
					2.00	0.26	0.13	0.13													
					3.00	0.10			0.10												
	90339	120	N	0.60	2.00	0.23			0.23												
					4.00	0.12					0.12										
					5.00	0.24					0.11	0.13									
					8.00	0.20									0.10	0.10					
4	90340	38.7	IV	0.60	1.50	0.10			0.10												
5	90350	40.6	IV	0.6	4.75	0.38	0.15				0.10	0.13									
		270	N	1.2	5.63	0.10	0.10														
					6.63	0.10		0.10													
					7.53	0.59		0.10	0.11	0.10			0.18		0.10						
6	90354	42	IV	0.6	1.27	0.33	0.33														

Table 5
Master table showing amounts of d5-cocaine in segments of hair samples obtained from subjects who received a controlled dose of d5-cocaine.

Number	Subject ID	Dose (mg)	Route	Dose (mg/kg)*	Hair Collected (mo post dose)	Total Amount d5-C in Hair (ng)	Amount d5-Cocaine in Segment**														
							1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
7	90356	45.8	IV	0.6	1.23	0.32	0.15	0.17													
					2.23	0.19			0.19												
8	90358	45.5	IV	0.6	2.50	0.10			0.10												
9	90361	44.5	IV	0.6	2.00	0.18	0.18														
10	90363	39.5	IV	0.6	0.75	0.14		0.14													
					1.75	0.10				0.10											
11	90364	39.5	IV	0.6	0.23	0.12	0.12														
					1.23	0.31	0.17	0.14													
					2.20	0.16			0.16												
	90364	140	N	0.60	0.03	0.12	0.12														
					0.10	0.18	0.18														
					1.00	0.72	0.21	0.19		0.10		0.10			0.12						
					1.90	0.27		0.10	0.17												
					3.00	0.16				0.16											
					4.00	0.12					0.12										
12	90375	39.5	IV	0.6	2.00	1.09		0.38	0.59	0.12											
					5.30	0.27							0.27								
					7.23	0.33									0.33						
					8.00	0.22									0.22						

Table 5
Master table showing amounts of d5-cocaine in segments of hair samples obtained from subjects who received a controlled dose of d5-cocaine.

Number	Subject ID	Dose (mg)	Route	Dose (mg/kg)*	Hair Collected (mo post dose)	Total Amount d5-C in Hair (ng)	Amount d5-Cocaine in Segment**														
							1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
13	90376	72.8	IV	1.2	1.20	0.42	0.25	0.17													
					2.20	0.28		0.16	0.12												
					4.70	0.34					0.16	0.18									
					5.73	0.18								0.18							
					6.77	0.14								0.14							
					7.90	0.14									0.14						
14	90377	64.9	IV	1.2	0.03	0.19	0.19														
					1.07	0.46	0.23	0.23													
					2.07	0.45		0.24	0.20												
					8.30	0.36															
15	90378	63.8	IV	1.2	2.00	0.87	0.76	0.11													
					4.50	0.58		0.35	0.23												
					5.50	0.27					0.10	0.17									
					6.53	0.36					0.14	0.10	0.12								
16	90381	72.5	IV	1.2	0.80	0.60	0.48	0.12													
					2.00	0.34		0.18	0.17												
17	91001	749.5	N	3	2.70	0.88		0.18	0.54	0.16											
					3.50	1.19			0.17	0.92	0.11										
					4.73	0.52					0.34	0.18									
					5.70	0.43						0.22	0.22								
					6.73	0.70						0.25	0.44								
18	91003	95.5	IV	1.2	2.77	2.24	0.10	0.44	1.07	0.62											
					5.27	3.89		0.11	0.65	2.53	0.53										
					7.40	3.89					0.14	0.59	1.07	1.29							

Table 5
Master table showing amounts of d5-cocaine in segments of hair samples obtained from subjects who received a controlled dose of d5-cocaine.

Number	Subject ID	Dose (mg)	Route	Dose (mg/kg)*	Hair Collected (mo post dose)	Total Amount d5-C in Hair (ng)	Amount d5-Cocaine in Segment**														
							1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
19	91013	108	IV	1.2	1.20	1.81	0.82	0.47	0.35	0.17											
					2.27	2.51	0.61	1.05	0.63	0.22											
					3.60	4.62	0.42	0.70	2.47	0.83	0.10	0.10									
					4.53	2.67		0.13	1.64	0.91											
					5.63	4.27	0.25	0.15	0.23	0.74	2.08	0.83									
					6.67	4.39				0.65	1.79	1.52	0.43								
	91013	170	N	0.60	7.03	5.05					0.30	0.46	2.82	1.47							
					7.10	4.26					0.37	1.38	1.94	0.56							
					7.90	3.26					0.31	0.88	0.81	1.25							
					8.27	4.04		0.98	0.14	0.10	0.30	0.95	1.02	0.56							
					9.40	0.75							0.17	0.28	0.15		0.16				
					10.53	0.20									0.10	0.10					
20	91014	90.5	IV	1.2	0.50	0.15	0.15														
		764.2	N	3	1.00	0.34	0.34														
					2.10	1.14	0.44	0.53	0.18												
					3.00	0.55		0.21	0.18	0.17											
21	91015	65.5	IV	1.2	3.10	0.30		0.30													
					4.10	0.36			0.21	0.15											

Table 5
Master table showing amounts of d5-cocaine in segments of hair samples obtained from subjects who received a controlled dose of d5-cocaine.

Number	Subject ID	Dose (mg)	Route	Dose (mg/kg)*	Hair Collected (mo post dose)	Total Amount d5-C in Hair (ng)	Amount d5-Cocaine in Segment**														
							1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
22	91021	120	N	0.6	0.03	0.20		0.10	0.10												
					0.10	0.28		0.16	0.12												
					0.90	0.14		0.14													
					2.13	0.37			0.21	0.16											
					3.00	0.11				0.11											
					4.00	0.14					0.14										
					5.00	0.11							0.11								
					8.00	0.10						0.10									
23	91030	120	N	0.60	0.03	1.70				0.16	0.11	0.12	0.19	0.14	0.10	0.12	0.13	0.29	0.36		
					0.10	1.75		0.17	0.18	0.16	0.15	0.12	0.10	0.16		0.13	0.16	0.19	0.23		
					1.00	0.74		0.12	0.15	0.17	0.10	0.10							0.10		
					1.90	1.33			0.09	0.16	0.14	0.12	0.11	0.10	0.13	0.11		0.11	0.13	0.13	
					3.00	0.71							0.12	0.10				0.20	0.13		0.16
					4.00	0.37													0.13	0.12	0.12
24	91031	150	N	0.60	0.03	0.57									0.23	0.34					
					0.10	0.67			0.10	0.10		0.12		0.18	0.17						
					1.00	0.19		0.19													
					1.90	0.19			0.19												
					3.00	0.15				0.15											
					4.00	0.10					0.10										
					7.00	0.11								0.11							

Table 5
Master table showing amounts of d5-cocaine in segments of hair samples obtained from subjects who received a controlled dose of d5-cocaine.

Number	Subject ID	Dose (mg)	Route	Dose (mg/kg)*	Hair Collected (mo post dose)	Total Amount d5-C in Hair (ng)	Amount d5-Cocaine in Segment**														
							1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
25	91033	140	N	0.6	2.00	0.21			0.11	0.11											
					3.00	0.17				0.17											
					4.00	0.22						0.22									
					5.00	0.21							0.11	0.10							
					5.83	0.10								0.10							
					7.00	0.11										0.11					
					8.00	0.20											0.10	0.10			

* Nasal doses corrected for bioavailability estimated to be 30%.

** Each segment is 1 cm measured from hair root.

Table 6
Master table showing the amounts of d5-benzoylecognine (d5-BE) in all segments of hair samples obtained from subjects who received a controlled dose of d5-cocaine.

Subject ID	Dose (mg)	Route	Dose (mg/kg)*	Time Hair Collected (mo post dose)	Total Amount d5-C in Hair (ng)	Amount d5-Cocaine in Segment**								
						1	2	3	4	5	6	7	8	9
88173	149.1	N	1.20	0.26	0.12	0.47								
	153.6	N	1.20	1.00	0.11	0.17	0.19	0.13						
				2.20	0.17	0.16		0.10		0.16				
				3.13			0.22							
90354	42.0	IV	0.60	1.27	0.26									
90356	45.8	IV	0.60	1.23		0.10								
90375	39.5	IV	0.60	2.00			0.11							
				7.23									0.18	0.11
90381	72.5	IV	1.20	0.80	0.11									
				2.00		0.12	0.13							
91001	148.4	N	2.00	2.70			0.11							
	148.4	N	2.00	3.50				0.20						
				5.70						0.11	0.15			
91003	95.5	IV	1.20	2.77			0.10							
				5.27				0.41						
				7.40						0.11	0.21	0.28		

* Nasal doses corrected for bioavailability estimated to be 30%.
** Each segment is 1 cm measured from root.

Table 6
Master table showing the amounts of d5-benzoylecognine (d5-BE) in all segments of hair samples obtained from subjects who received a controlled dose of d5-cocaine.

Subject ID	Dose (mg)	Route	Dose (mg/kg)*	Time Hair Collected (mo post dose)	Total Amount d5-C in Hair (ng)	Amount d5-Cocaine in Segment**								
						1	2	3	4	5	6	7	8	9
91013	108.0	IV	1.20	1.20	0.15									
				2.27	0.10	0.13								
				3.60			0.32	0.12						
				4.53				0.10						
				5.63					0.18					
				6.67					0.19	0.15				
91014	143.6	N	2.00	1.00		0.15								
91013	170.0	N	2.00	7.00						0.19	0.16			
				7.00					0.12	0.14				

* Nasal doses corrected for bioavailability estimated to be 30%.
** Each segment is 1 cm measured from root.

Amounts and Ratios of Analytes in Hair

The amounts of d5-cocaine and d5-BE in the hair of the experimental subjects were typically in the high picogram to low nanogram range. Following intravenous administration of 0.3 mg/kg d5-cocaine, the resulting d5-cocaine concentrations in hair were below the limit of quantitation for our GC/MS method. d5-cocaine was detected in all hair samples obtained after a dose of 0.6 mg/kg or greater. The amounts in each hair sample were in the high picogram to low nanogram range. The metabolite BE was present in approximately 1/6 these amounts. The finding that cocaine is present in hair in much higher amounts than BE or EME has been confirmed by all other researchers who have used sensitive and specific analytical methods capable of distinguishing cocaine from BE and EME. The analyte ratios we found in the present study agree with the ratios we obtained in another experiment (not funded by NIJ or NIDA) in which hair samples were obtained from individuals who self report ingesting cocaine daily.

Table 7 shows the concentrations of cocaine, BE and EME in hair samples obtained from six South American Indians who chew cocaine daily. These hair samples were obtained from native peoples living in the Sierra Nevada region of Columbia or in the Amazon Basin region of Colombia, Brazil and Ecuador. The samples were sent to our laboratory by Dr. Guozano Cediel, an archaeologist working with the Colombia Institute of Agrarian Reform. Little information was available about these individuals except the name of their tribe, sex, region where they live and they fact that they self-report chewing coca leaves daily. These data have been published in the Journal of Analytical Toxicology, 1992 (Henderson *et al.*, 1992).

Table 7.
Concentrations of cocaine and metabolites
in the hair of South American coca chewers.

Subject	Cocaine (ng/mg)	BE (ng/mg)	EME (ng/mg
1	18.6	3.4	1.2
2	28.9	4.4	4.4
3	19.8	2.3	2.0
4	7.6	3.7	0.5
5	1.0	0.3	0
Mean ±SD	15.2 ± 11.0	2.8 ± 1.6	1.6 ± 1.7

The concentration of cocaine and metabolites in the South American samples (before and after washing) are shown in Table 8. Because samples from most subjects contained very small amounts of hair, it was possible to make this comparison in only two samples. In sample 2, washing reduced the amount of cocaine, BE and EME detected by 10%, 14%, and 5%, respectively, and in sample 3, washing reduced the amount of cocaine, BE and EME by 24%, 26% and 53%, respectively.

Table 8.
Concentrations of cocaine and metabolites in the hair of South American coca chewers before and after washing [a].

	Cocaine (ng/mg hair)		BE (ng/mg hair)		EME (ng/mg hair)	
Subject	Before Washing	After Washing[a]	Before Washing	After Washing[a]	Before Washing	After Washing[a]
2	32.6	28.9	5.1	4.4	4.7	4.4
3	25.8	19.8	3.2	2.3	4.2	2.0

[a] Samples were washed with 1% SDS, deionized water and methanol before digestion and GC/MS analysis.

Threshold Dose

Our GC/MS procedure could detect d5-cocaine in hair after a single dose of drug was administered. No drug could be detected in the hair of subjects who received the lower doses of cocaine (11.8 mg - 22 mg), even when two small doses were given one week apart. However, cocaine could be found in the hair of all the subjects who received doses greater than 35.2 mg (approximately 0.5 mg/kg). Thus, the minimum detectable dose for our analytical methods appears to be between 22 and 35 mg, an amount somewhat less than that found a single "line" of street cocaine (e.g., 50 - 100 mg).

Effect of Dose on Amount of Cocaine Incorporated into Hair

The relationship between the dose of d5-cocaine administered and the amount of cocaine incorporated into hair is illustrated in figures 6 through 11. In these figures the amount of drug is expressed as either maximum amount of drug, mean amount of drug, or area under the curve (AUC). The amount of drug incorporated into hair for any given dose varied considerably between subjects and there was no clear increase in the amount of drug with an increase in dose. This held true over the nearly four-fold range of doses administered (from 0.6 to 4.2 mg/kg). Statistical analysis (linear regression by the least squares method) of the data confirmed this observation. Regression lines and correlation coefficients are shown in Figures 6-11.

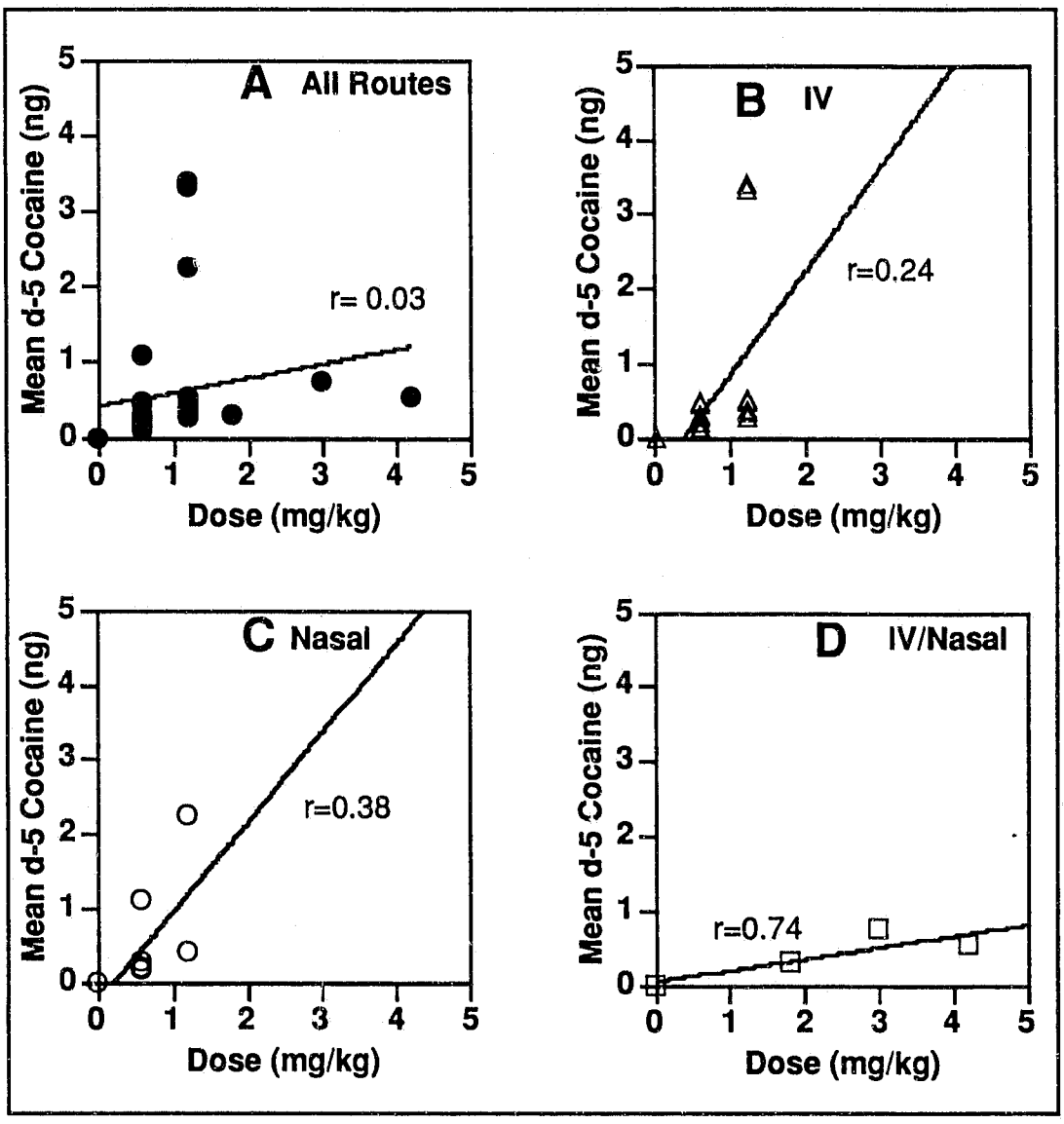

Figure 6. Correlation between dose of d5-cocaine and the mean amount of drug found in hair after IV, nasal, or IV followed by nasal administration.

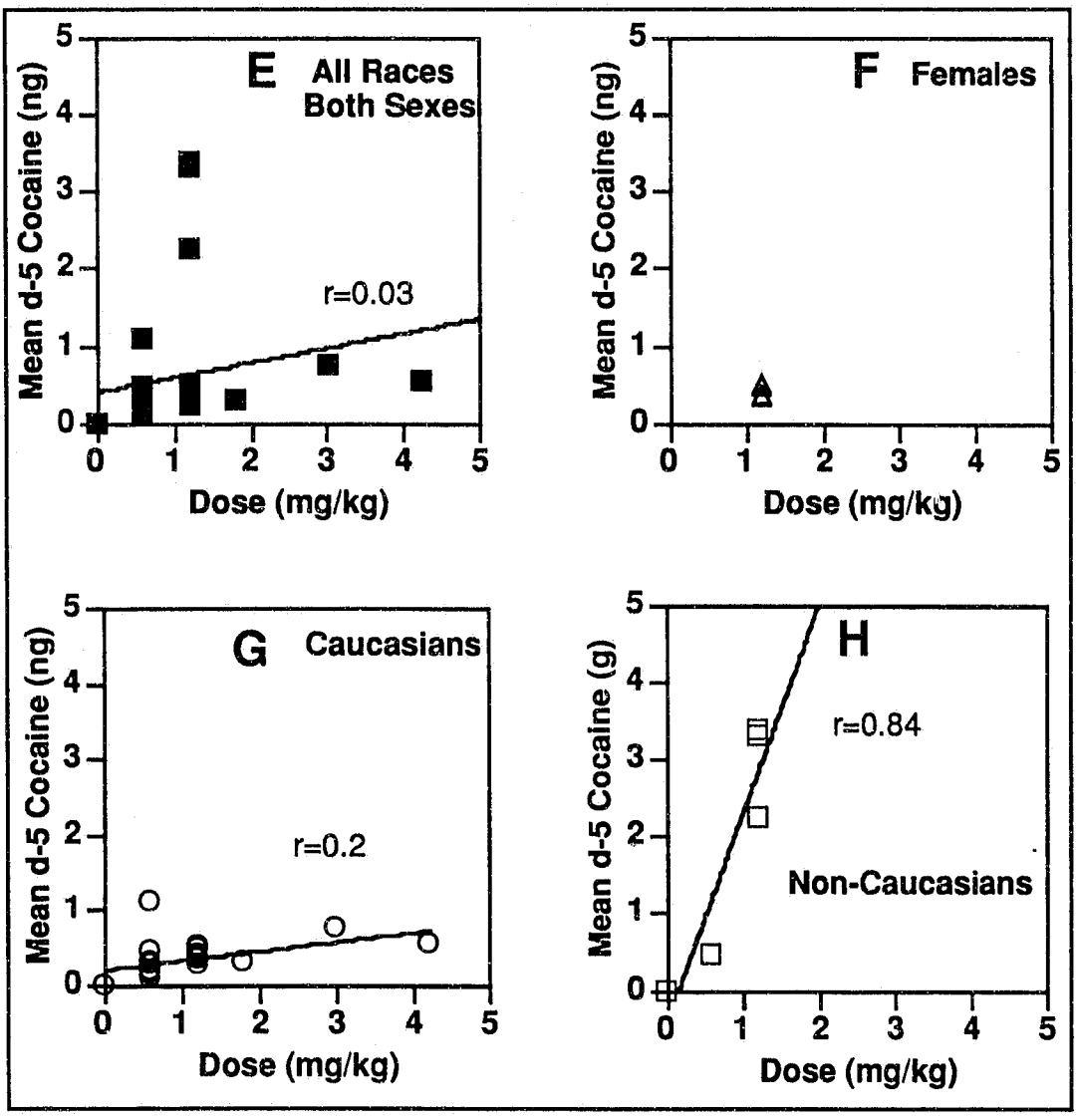

Figure 7. Correlation between dose of d5-cocaine and the mean amount of drug found in hair of all subjects, females (Caucasian only), all Caucasian, and non-Caucasian subjects.

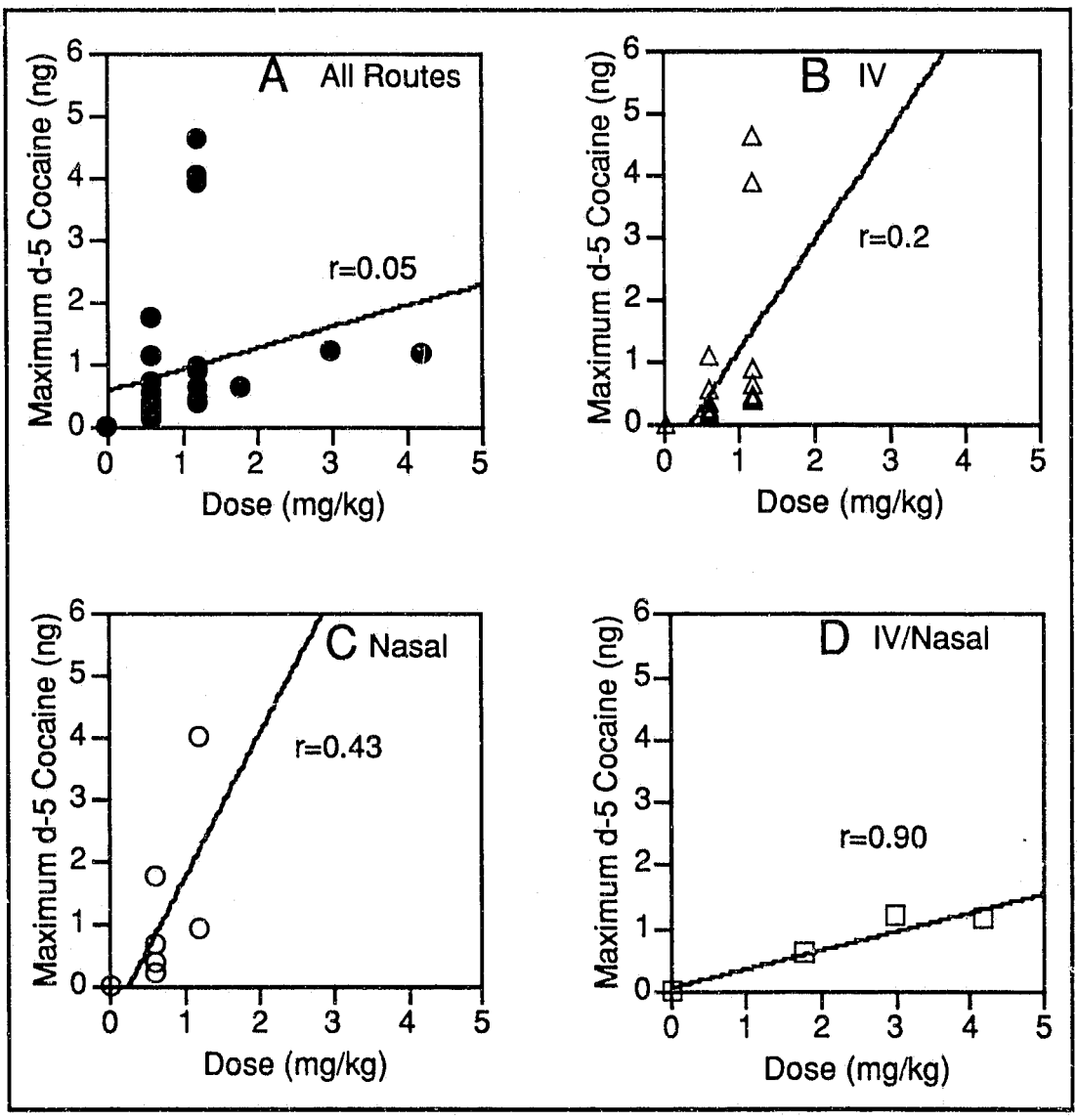

Figure 8. Correlation between dose of d5-cocaine and the maximum amount of drug found in hair after IV, nasal, or IV followed by nasal administration.

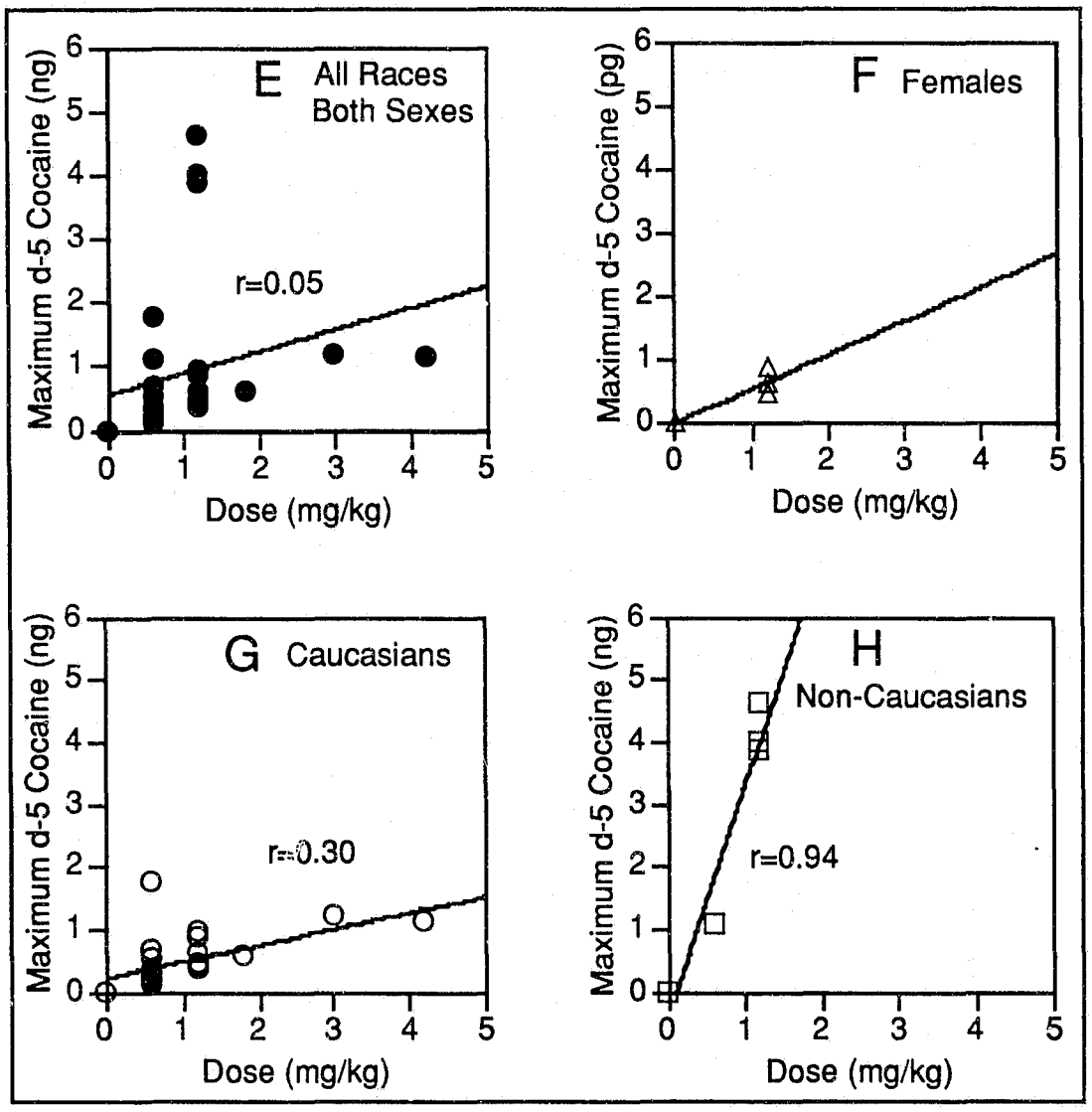

Figure 9. Correlation between dose of d5-cocaine and the maximum amount of drug found in hair of all subjects, females (Caucasian only), all Caucasian, and non-Caucasian subjects.

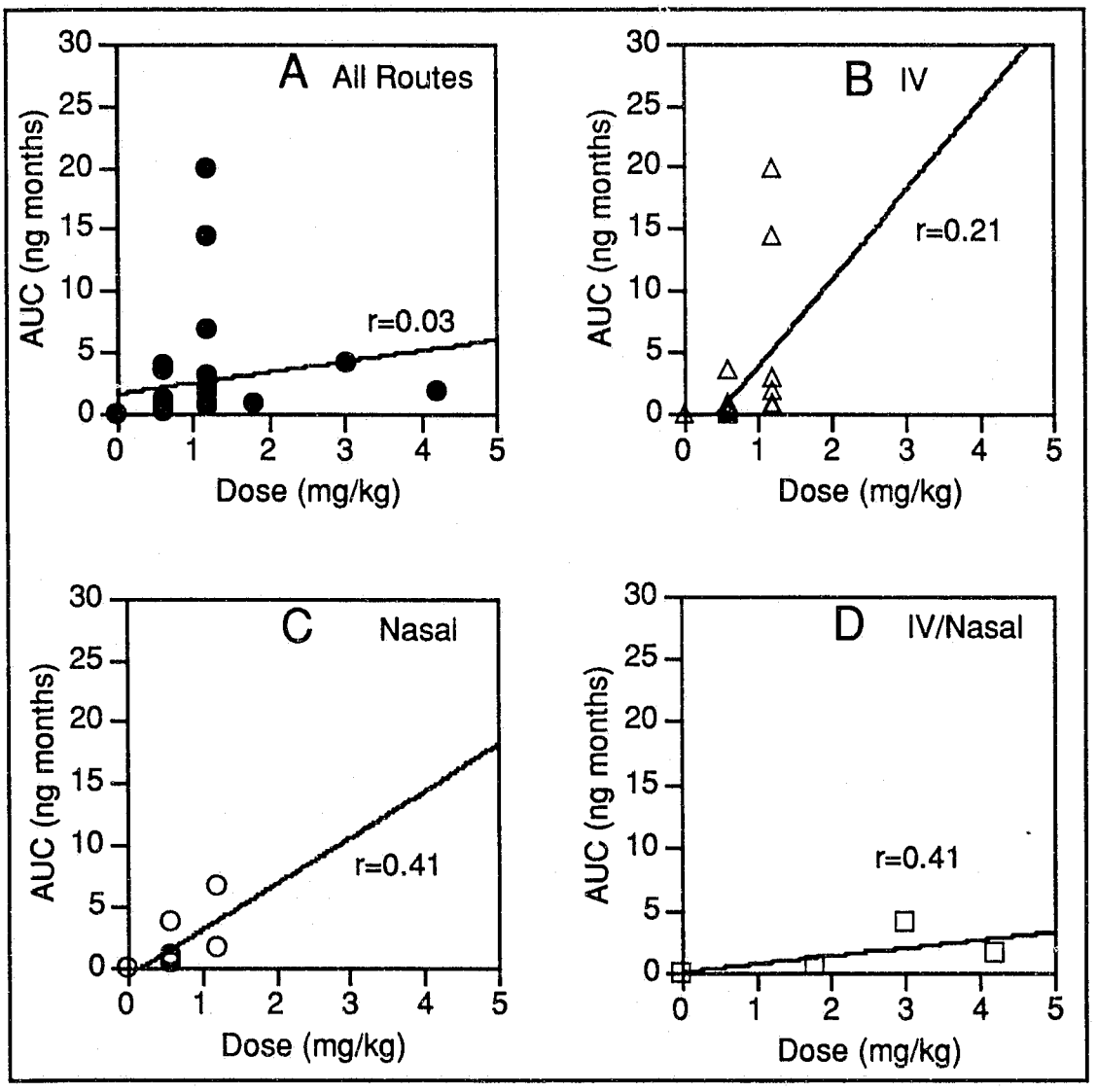

Figure 10. Correlation between dose of d5-cocaine and the amount of drug (expressed as area under the curve) found in hair after IV, nasal, or IV followed by nasal administration.

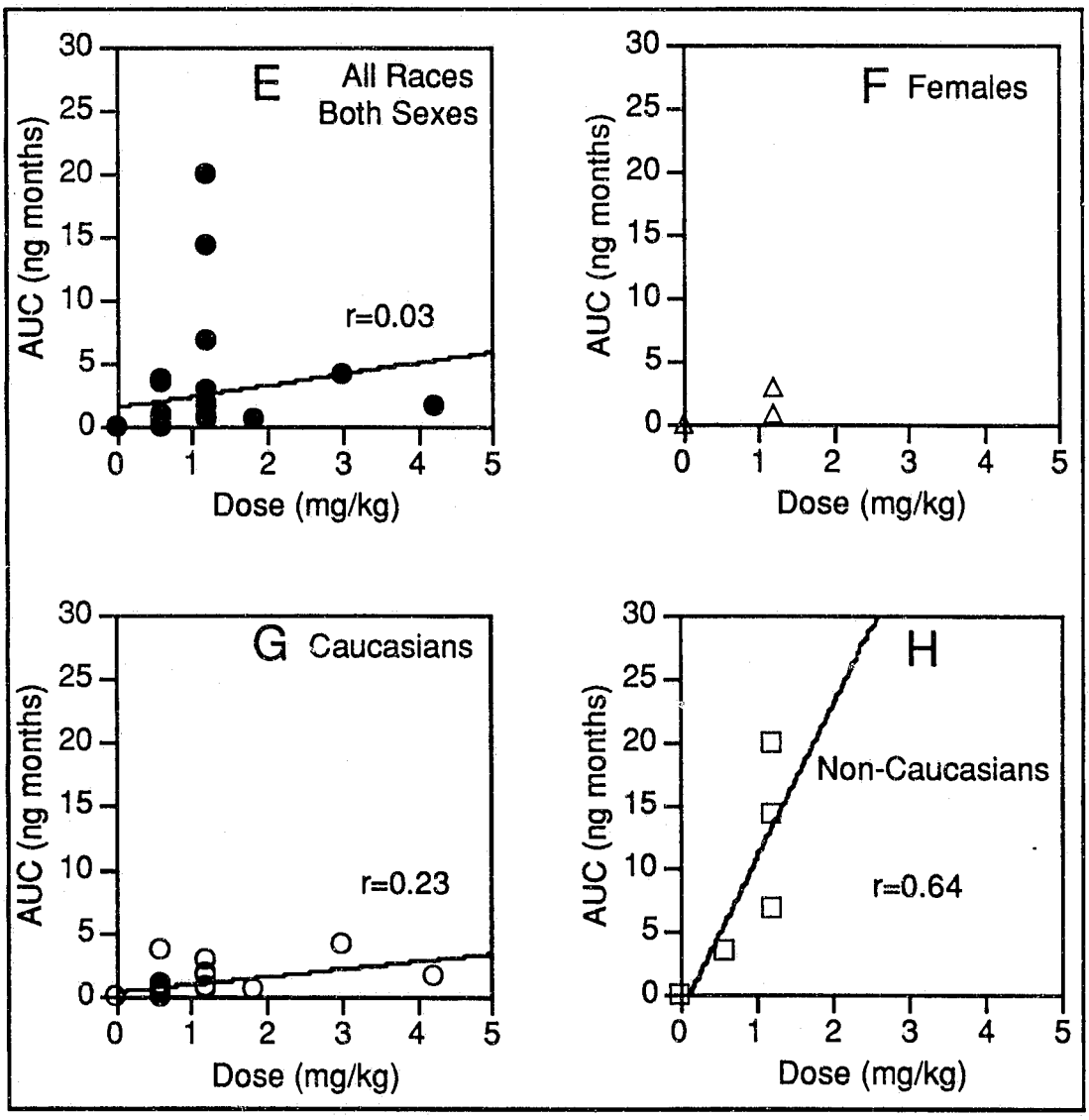

Figure 11. Correlation between dose of d5-cocaine and the amount of drug (expressed as area under the curve) found in hair of all subjects, females (Caucasian only), all Caucasian, and non-Caucasian subjects.

Effect of Route of Administration on Amount of Cocaine Incorporated into Hair

None of the routes of administration showed a predicable relationship between the dose and the amount of drug incorporated into hair. Theoretically, IV administration should yield the best dose - response relationship because it eliminates absorption as a variable. However, even with this route there was considerable variability among the subjects. The correlation was best for the group that received cocaine by IV followed by intranasal administration, but there were too few subjects in the group (all non-Caucasians) to make a meaningful determination. Also, the slope of the correlation line was very shallow. If this graph does represent the actual relationship between dose and amount in hair for non-Caucasians, then hair analysis would be of little use in determining the dose of drug ingested. Large increases in dose result in very small changes in the amount of cocaine incorporated into hair.

Effect of Subjects' Gender on Amount of Cocaine Incorporated into Hair

All female subjects received the same dose so we are unable to infer anything about their dose-response relationship. There was, however, little variability between the subjects and all four female subjects had very similar amount of d5-cocaine in their hair.

Effect of Subjects' Race on Amount of Cocaine Incorporated into Hair

The most significant variable affecting the incorporation of cocaine into hair was race. All four non-Caucasians had significantly more cocaine incorporated into their hair than did their Caucasian counterparts (Figures 7, 9, and 11). The four non-Caucasians were clearly outliers whether the amount of cocaine was expressed as mean amount, maximum amount, or AUC. Non -Caucasians had between 2 and 12 times (depending upon how the amount of drug was expressed) as much drug in their hair as did Caucasians (Table 9).

Table 9.
d5-Cocaine uptake into the hair of Caucasian versus Non-Caucasian subjects.

Race	Dose Regimen	N	Mean Amount ± SD (ng)	Maximum Amount ± SD (ng)	AUC ± SD (ng months)
Caucasian	0.6 mg/kg IV	9	0.21 ± 0.12	0.25 ± 0.14	0.40 ± 0.29
Non-Caucasian	0.6 mg/kg IV	1	0.48	1.09	3.57
Caucasian	1.2 mg/kg IV	5	0.40 ± 0.12	0.54 ± 0.20	1.44 ± 1.03
Non-Caucasian	1.2 mg/kg IV	2	3.36 ± 0.05	4.26 ± 0.52	17.15 ± 3.89
Caucasian	1.2 mg/kg Nasal	1	0.4	0.95	1.69
Non-Caucasian	1.2 mg/kg Nasal	1	2.25	4.01	6.84

Segmental Analysis

By inspection of the data in Table 5 it can be seen that the position of cocaine along the hair shaft correlated only to a limited degree to the time since drug administration. Generally, once cocaine appeared in hair it was confined to a few segments and these drug-containing segments did move down the hair shaft toward the tip with time. However, there was considerable variability in the number of segments in which the drug was confined and in the rate at which the band of drug traveled from root to the tip.

Time Until Drug Is First Detected in Hair

Although determining the minimum time for drug to appear in hair was not an original objective of our studies and this was not studied systematically, we did observe that there was considerable variability in the time it took for drug to first appear in hair. During our studies we inadvertently discovered that cocaine could be detected within eight hours of drug administration. We detected d5-cocaine in a "control" (i.e., pre-dose) hair sample from subject 90377. After checking with the staff we discovered that the sample was collected on "Day 0" (the dosing day); however, it was actually obtained 8 hr after drug administration rather than before drug administration. A control sample that had been obtained from this subject three weeks prior was negative. We subsequently obtained hair samples from four other subjects (91021, 91030, 91031, and 91033) on days 1 and 3 following drug administration. Three of the four subjects (91021, 91030, and 91031) had positive hair samples the day after the drug was administered. These results are limited, however they do suggest that cocaine can be detected in hair very soon (within hours) after drug administration.

Relationship Between Location of Drug in Hair Shaft and Time of Drug Administration

There was also considerable variability in the distribution of drug throughout the hair once it appeared in hair. In five subjects, cocaine was found in more than three adjacent segments even though a single dose was administered. In one subject (91030) who received a single 0.6 mg/kg intranasal dose, drug was found in 10 segments on the day following drug administration. This subject participated in the sweat study and exercised on the day the drug was administered. On the other hand, one subject (91014) received six doses of cocaine over a one month period (simulating chronic use), but had drug distributed over three segments only. Results from segmental analysis of these two subjects' hair are compared in Figure 12.

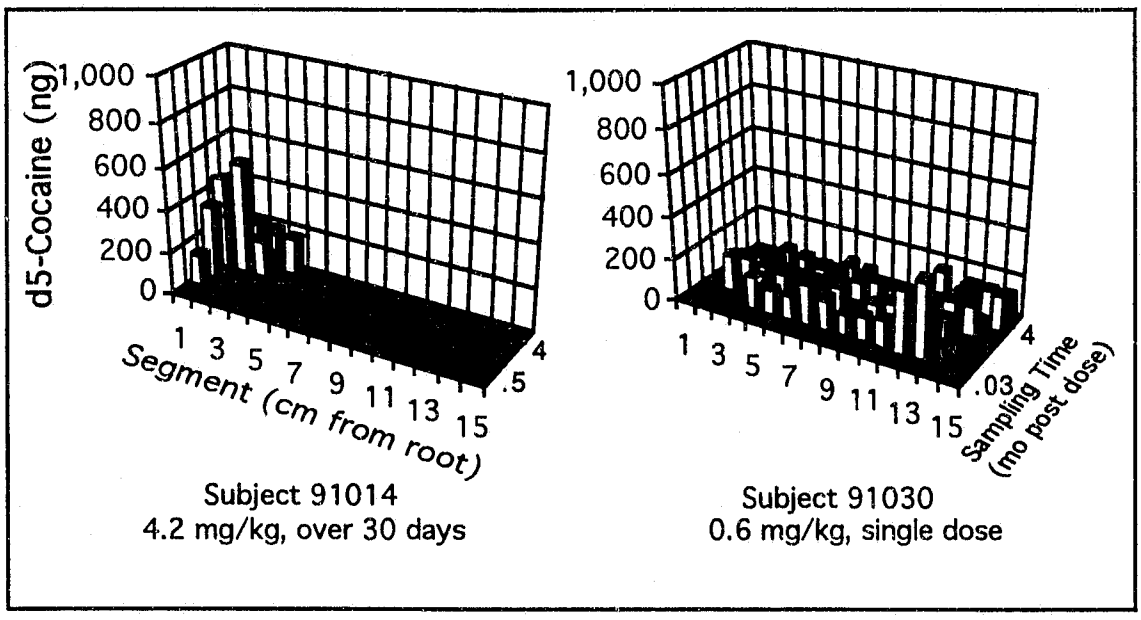

Figure 12. A comparison of segmental analysis results of hair samples collected from subject 91014 (who received 4.2 mg/kg by the intravenous and intranasal routes over a 30 day period) and subject 91030 (who received a single intravenous dose of 0.6 mg/kg). Results are shown in a 3D representation in which the amounts of d5-cocaine (X axis) found in the individual hair segments (Y axis) are plotted against the time the sample was collected (Z axis).

Detection Window

By inspection of the data in Table 5 it can be seen that the time cocaine can be detected in hair following a single dose varied remarkably between subjects. The amount of drug in hair decreased with time; however, it would be difficult to predict with any accuracy how long a single dose of cocaine could be detected. Some subjects had hair samples positive for d5-cocaine for only for 2 months while others who received a single dose had positive hair samples for up to 8 months. Theoretically, the factor that should affect the detection window most would be the amount of drug incorporated into hair immediately after drug administration. That is, the greater the amount of drug found in hair after one month, the longer the drug should be detected. This was not always the case. Subject 88173 had relatively large amounts of d5-cocaine in his hair (4 ng) detected seven days after drug administration, yet the drug disappeared rapidly and could not be detected after the third month. On the other hand, subject 91021 had much less drug in hair (0.2 ng on day 1 and 0.14 ng on day 28), but the drug could still be detected in hair 8 months after administration.

For 14 of the 25 subjects the amount of drug in hair appeared to decrease at an approximate exponential rate after drug administration. We calculated the half-life for d5-cocaine in the hair of these subjects based on the assumption that loss of drug from hair occurred by a first-order process. The means for four groups of subjects are listed in Table 10.

Table 10.
Apparent half-life values for the disappearance of d5-cocaine from hair.

	Half-life for d5-cocaine in hair (mo)			
	All Subjects	Caucasians	Non-Caucasians	Females
Mean	1.9	2.1	1.6	2.3
SD	1.3	1.4	1.1	0.8
N	14	11	3	3

A half-life of 1.9 months for cocaine disappearance from hair would suggest that a reasonable detection window would be approximately 6 months. By that time (approximately 4 half-lives) more than 80% of the drug would have disappeared from hair. Non-Caucasians appear to have a shorter half-life, but the number of subjects and data points are so few that this is not likely to be significant.

Measurement of Cocaine and Metabolites in Blood

Plasma Pharmacokinetics of d5-Cocaine in the Test Subjects

To test whether the intersubject variability in the amount of drug incorporated into hair resulted from differences in their pharmacokinetics, d5-cocaine was administered then sequential blood samples were collected and analyzed. Figures 13 and 14 show the plasma concentrations of d5-cocaine and d5-BE in seven subjects after an intravenous administration of 0.6 mg/kg of deuterated cocaine.

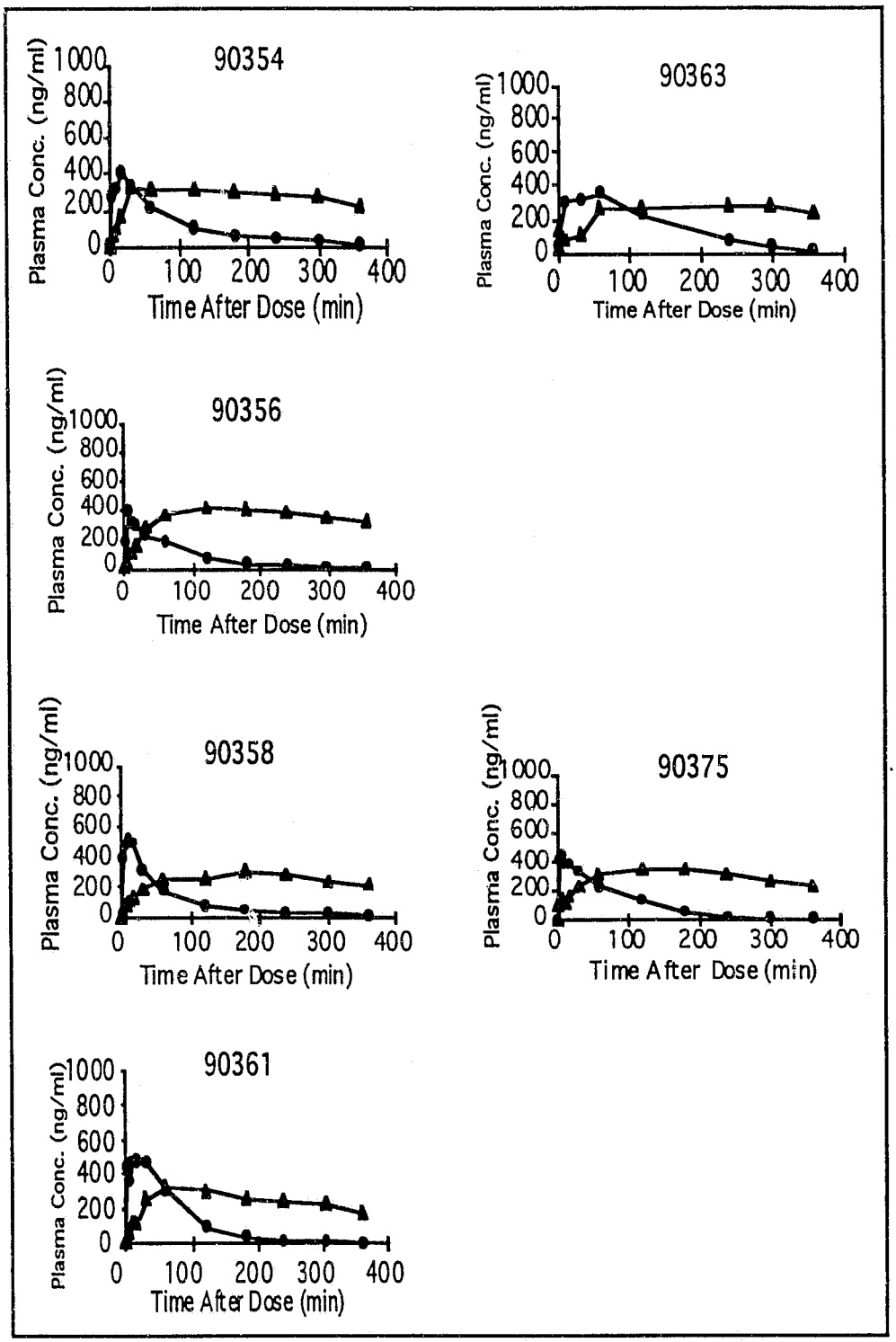

Figure 13 . Plasma concentrations of d5-cocaine (solid circles) and d5-BE (solid triangles) in seven subjects after an intravenous administration of 0.6 mg/kg d5-cocaine.

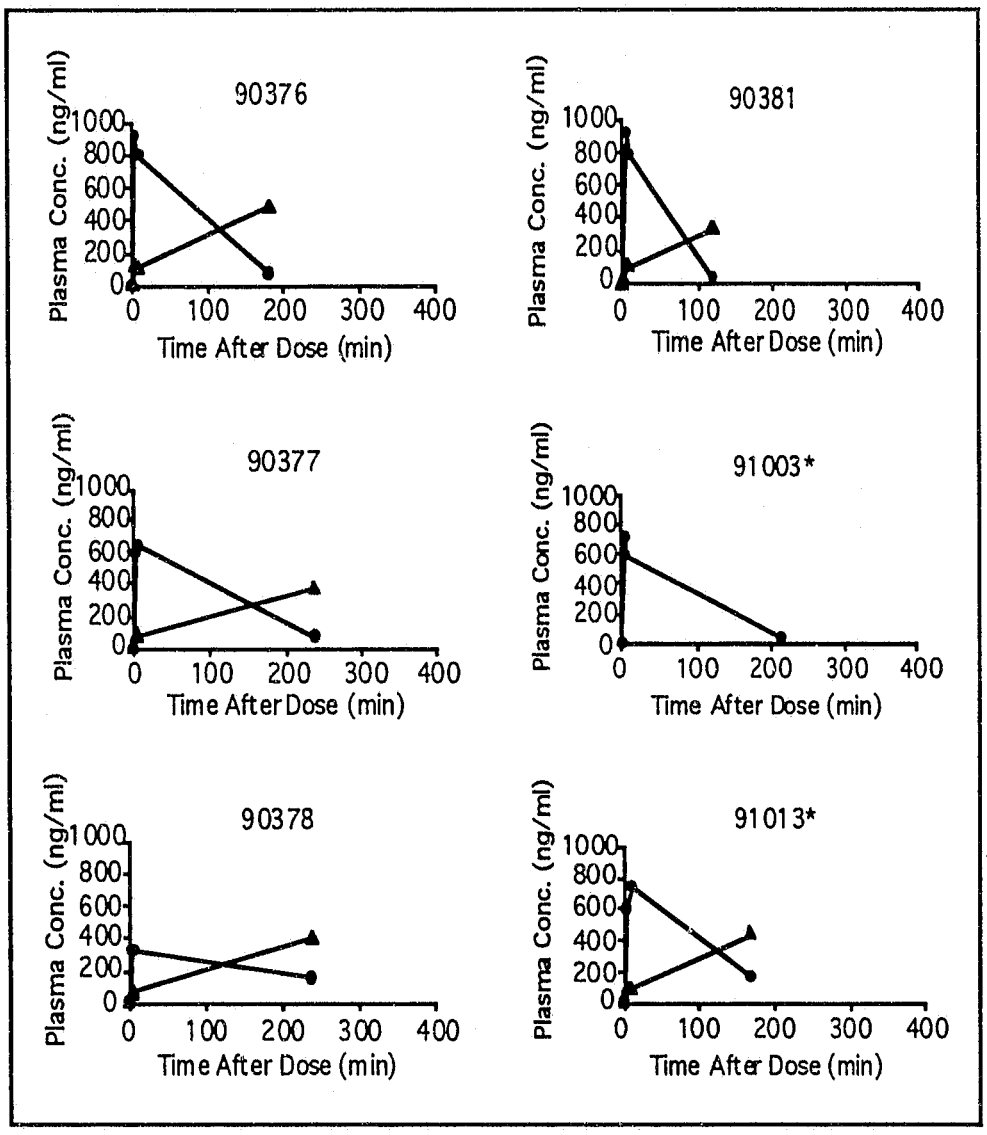

Figure 14 . Plasma concentrations of d5-cocaine (solid circles) and d5-BE (solid triangles)in six subjects after an intravenous administration of 1.2 mg/kg d5-cocaine. Asterisk denotes non-Caucasian subject.

Plasma decay curves of d5-cocaine for the subjects within each test group were quite similar. For the 0.6 mg/kg dose group, peak d5-cocaine concentrations of about 400-500 ng/ml occurred in the first few minutes and then declined rapidly over the next few hours. Conversely, d5-BE concentrations increased over time to a plateau value of about 400 - 500 ng/ml. For the 1.2 mg/kg dose group, peak d5-cocaine concentrations of about 800 ng/ml occurred in the first few minutes and then declined rapidly over the next few hours. Conversely, d5-BE concentrations increased over time to a plateau value of about 400 ng/ml. The mean half-life of d5-cocaine in these subjects was 66 $\pm$ 14 min.

There was some variability among the subjects in their plasma pharmacokinetics, which is typical, but much less than the variability in the amount of cocaine incorporated into their hair. More importantly, the plasma pharmacokinetics for the non-Caucasian subjects, numbers 90375, 91003, and 91013, were unremarkable yet these subjects incorporated more cocaine into his hair than anyone in their respective group.

Measurement of Cocaine and Metabolites in Sweat

The concentrations of d5-cocaine and d5-BE in the sweat of subjects at various times after an intranasal dose of 0.6 mg/kg d5-cocaine are shown in Figure 15. Sweating was induced by exercising on a stationary bicycle. Sweat samples were collected from the arm and hand using a cotton glove.

Sweat Induction

Sweating was induced by exercising on a bicycle ergometer for 15 min during which time heart rate was monitored and work load regulated to ensure a heart rate between 120 to 150 beats per minute. Core temperature was also monitored. Exercise periods were repeated at 45 min, 2 hr 45 min, and 5 hr 45 min post dose. Between exercise periods, subjects rinsed with distilled water and dried their arm and hand to remove residual sweat that may have accumulated. Subjects returned to the laboratory at 24, 48 and 72 hr postdose, showered, and after donning their sweat collection gloves, underwent another sweat facilitation exercise session identical to the one on the day before.

Sweat Collection

Arm and hand sweat was collected using polyethylene shoulder length gloves (0.00125" gauge x 34 inch, 3-mil thick). Particular care was taken to avoid inadvertent environmental drug contamination. A shoulder length plastic glove was placed over the arm and hand then secured with an elastic band. Over this glove another shorter plastic hand glove was used to protect the longer glove from tears. After each exercise period the 2 to 5 ml of accumulated sweat was removed, pH and total volume measured, and then acidified to a pH of less than 4 using 6 M HCl. The acidified sweat sample was centrifuged at 2400 rpm for 10 min and pipetted and placed into a 15-ml tube and stored at -20° C until analyzed.

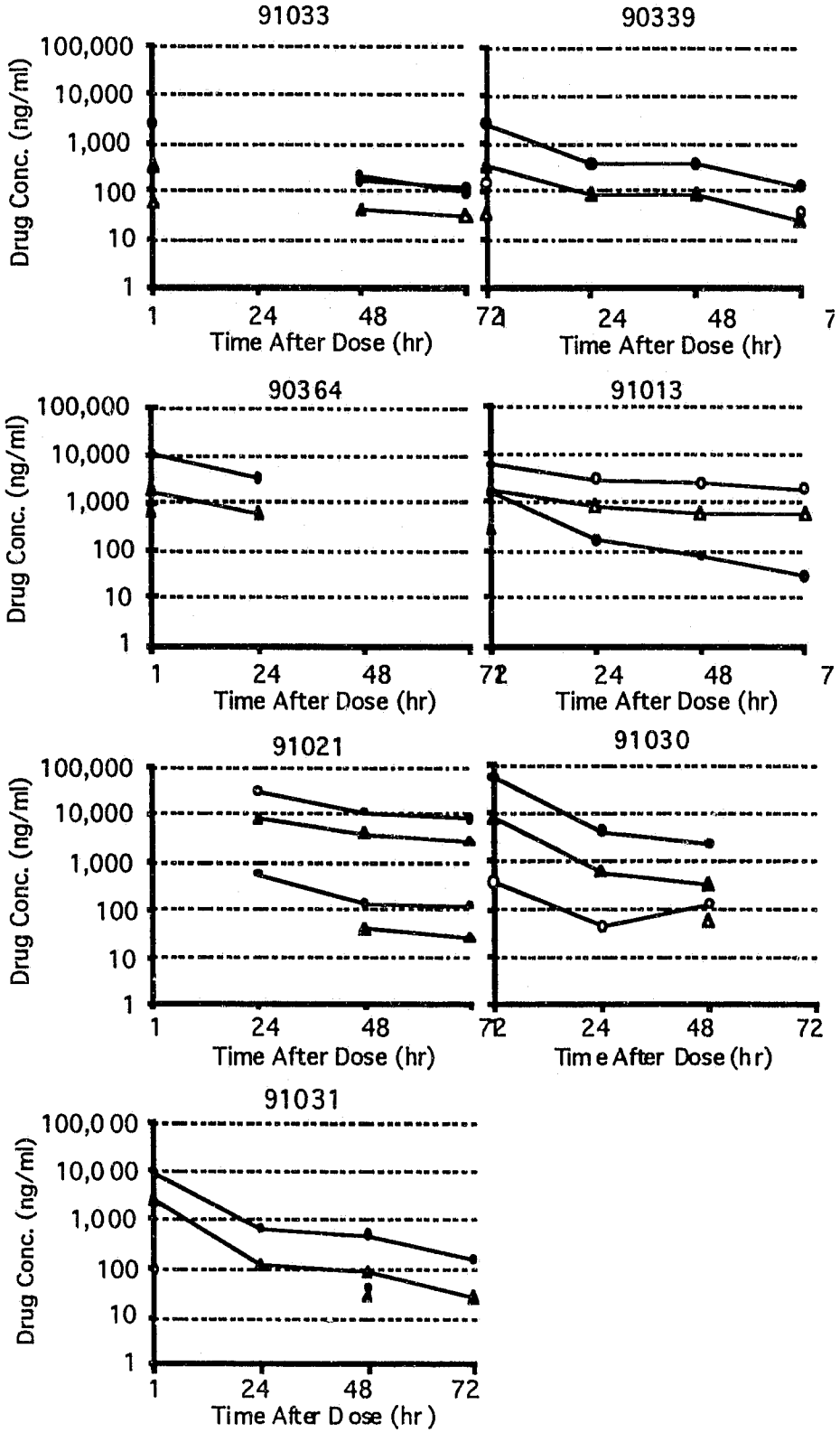

Figure 15. Sweat concentrations of d5-cocaine (closed circles), d5-BE (closed triangles), cocaine (open circles), BE (open triangles) in seven subjects after receiving a 0.6 mg/kg intranasal dose of d5-cocaine. Note - Scale for these graphs is logarithmic.

The analysis of the sweat samples yielded three striking results. First, the concentrations of cocaine and BE in sweat were very high – approximately 10-times higher than are found in plasma. Secondly, cocaine is generally present in higher concentrations than BE and in a ratio similar to that found in hair. And thirdly, cocaine and metabolites persist in sweat longer than they do in plasma or urine. The concentration of these two drugs in sweat was more variabile than in plasma, but the drug concentrations in sweat did not correlate well with the amounts of cocaine found in hair. For example, one subject, 91030 had extremely high levels of d5-cocaine in his sweat, but did not have unusually high amounts of drug in his hair.

Although there were relatively few data points, the d5-cocaine concentrations in sweat appeared to decrease at an exponential rate and individual half lives were calculated from these semi-logarithmic plots. The mean half of d5-cocaine (± standard deviation) in the sweat of 13 subjects was found to be 14.9 ± 3.7 hr. The half-lives for d5-cocaine in the three biological samples analyzed – plasma, sweat, and hair – are compared in Table 11.

Table 11.
Half-life of d5-cocaine in plasma, sweat and hair of human volunteers.

	Half-life of d5-cocaine		
	Plasma (min)	Sweat (hr)	Hair (mo)
Mean	66.4	14.9	1.9
SD	14.1	3.7	1.3
N	12	13	14

DISCUSSION

The results reported herein suggest that analysis of hair samples by a sensitive and specific method such as GC/MS can be used to detect cocaine use. However, the considerable variability observed between the subjects tested in this study under controlled dose conditions is a cause of some concern.

There are no simple apparent explanations for the high levels of drug found in the hair of non-Caucasians. Also, it should be noted that the non-Caucasians were not all Afro-Americans. Two of the four were of mixed Hispanic, Asian, and East Indian decent. Thus, such differences may be seen in a number of non-Northern European populations. It is tempting to speculate that sweat is one medium by which cocaine is transferred from the body to hair and that the interindividual differences in sweating might account for the differences in cocaine incorporation into their hair.

A number of drugs of abuse have been detected in sweat including alcohol (Brown, 1985), cocaine (Smith and Liu, 1986a,b) and the amphetamines (Suzuki, Inoue *et al.*, 1989; Vree, Muskens *et al.*, 1972). However, the non-Caucasians did not appear to have unusually high sweating rates or unusually high concentrations of drug in their sweat. One subject did have high concentrations of 5-cocaine in his sweat, but he was Caucasian and did not have unusually high levels of cocaine into his hair. However, in this study sweat was collected from

the hands which, unlike the scalp, do not have large numbers of sebaceous glands associated with their sweat glands. For very lipophilic drugs like cocaine the lipid secretions of the sebaceous glands may be a more efficient vehicle for its transfer to hair than the aqueous secretions of the sweat glands. More studies will be needed to resolve the relative importance of sweat and/or sebaceous secretions as vehicles for transferring drugs to hair. At the very least, sweat may be an important medium for the external contamination of hair. Excretion of d5-cocaine in scalp sweat may explain why the drug could be detected in hair as early as 8 hours after dosing and why the drug was distributed over a large area of the hair shaft even though a single dose was administered. In a series of *in vitro* studies shown in the next section it was demonstrated that sweat can be a vehicle for external contamination of hair.

IN VITRO STUDIES

The objectives of the *in vitro* studies were to:

Determine the extent to which externally applied cocaine (in aqueous solution and as a vapor, simulating "crack" smoke) was retained or incorporated into hair.

Determine how efficient various washing procedures are in removing the externally applied drug.

Determine whether cosmetic treatments of hair such as bleaching and perming affect the detection of cocaine in hair.

Determine whether sweat can be a source of external contamination.

EXTERNAL CONTAMINATION OF HAIR BY COCAINE IN AQUEOUS SOLUTION

EXPERIMENTAL METHODS

Exposure of Hair Samples

Brown Caucasian hair was obtained from barber shops, washed with detergent and methanol to remove any hair care products or natural oils, then air dried. Approximately 800 mg of hair was cut into 2 mm pieces and incubated for 48 hr in 5 ml of 0.9 % saline (simulating sweat) containing radiolabeled cocaine (4-^{3}H-cocaine, specific activity of 14.77 mCi/mg). Two drug concentrations were used in the incubations: 15 ng/ml (which contained only radiolabeled cocaine, approximately 229,000 cpm/ml) and 1 mg/ml (which contained approximately 229,000 cpm/ml radiolabeled cocaine and 1 mg/ml unlabeled cocaine). At the end of the incubation period, the 15 ng/ml and 1 mg/ml solutions yielded hair containing approximately 7 pg cocaine/mg hair and 4 µg cocaine/mg hair, respectively.

Washing the Hair Samples

Hair samples (10 mg) were placed in a vacuum filtration device and washed with successive 1 ml aliquots of wash solution. The hair was allowed to stand in contact with the wash solution for 5 min and the reservoir was agitated gently at one minute intervals. The stopcock was opened and vacuum applied until all the wash solution was recovered (approximately 2 min). Each 1 ml wash solution was collected in a scintillation vial, 8 ml of scintillation fluid added, and the radioactivity determined by scintillation spectrometry. Each hair sample was washed with 12 successive volumes of solution, after which the hair was dried, digested in base and the residual radioactivity determined. Two 10 mg hair samples were used to evaluate each wash solution and five wash solutions were tested: distilled water, methanol, acetone, chloroform, and 1% sodium dodecyl sulfate.

RESULTS

The amount of radiolabeled cocaine remaining in hair after washing with four solutions (water, methanol, acetone and chloroform) is shown below.

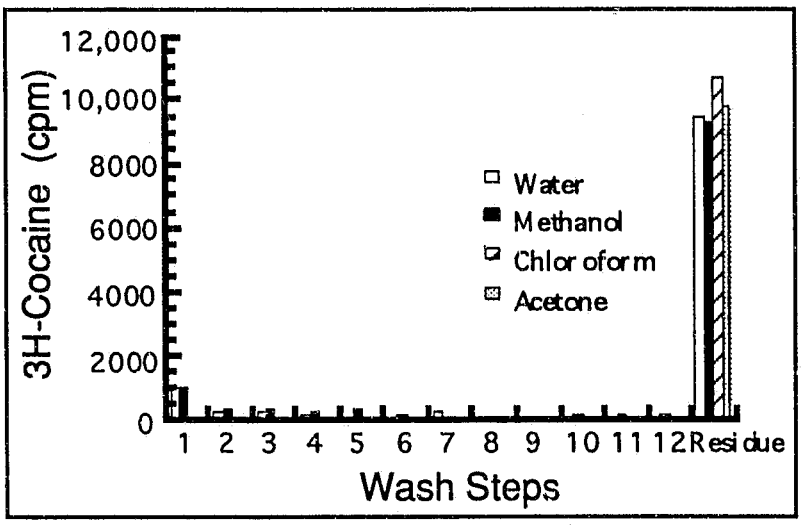

Figure 16. Radiolabeled cocaine remaining in hair after washing with various solutions. Hair (brown, Caucasian) was soaked in an aqueous solution of 3H-cocaine (1 mg/ml), dried, and washed as described in Experimental Methods. Values shown are the mean of two determinations.

A similar pattern was observed when hair was soaked in more dilute solutions of radiolabeled cocaine. Figure 17 shows the efficiency of five wash solutions (water, methanol, acetone, chloroform, and 1% dodecyl sulfate) in removing radiolabeled cocaine from hair exposed to a solution of 15 ng/ml cocaine.

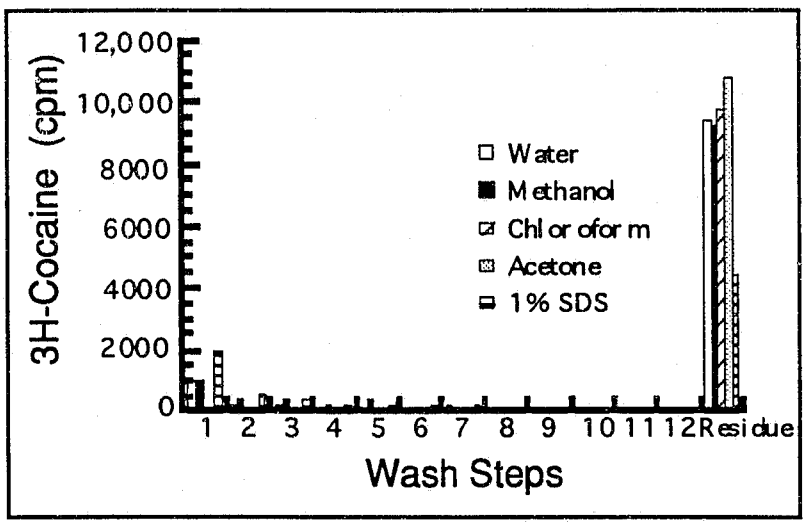

Figure 17. Radiolabeled cocaine remaining in hair after washing with various solutions. Hair (brown, Caucasian) was soaked in an aqueous solution of 3H-cocaine (15 ng/ml), dried, and washed as described in Experimental Methods. Values shown are the mean of two determinations.

The efficiency of the various washing solutions in removing externally applied radiolabeled cocaine from hair is summarized in Tables 12 and 13.

Table 12.
Efficiency of wash solutions in removing cocaine from hair soaked in a 1 mg/ml solution.

	Wash Solution			
	Distilled Water	Methanol	Acetone	Chloroform
% Drug Removed	40	36	7	7
% Drug Remaining	60	64	93	93

Table 13.
Efficiency of wash solutions in removing cocaine from hair soaked in a 15 ng/ml solution.

	Wash Solution				
	Distilled Water	Methanol	Acetone	Chloroform	1% SDS
% Drug Removed	21	25	6	7	43
% Drug Remaining	79	75	94	93	57

DISCUSSION

The results of these two *in vitro* studies suggest that the wash solutions were not completely effective in removing externally applied cocaine. None of the wash solutions removed more than 50% of the externally applied cocaine, even after 12 successive washings. Most of the drug that was removed was extracted in the first wash step and successive washings were substantially less effective. Although the solution from the last wash step contained little or no drug, analysis of the digested hair revealed that from 57% to 94% of the absorbed drug remained in the hair.

1% Sodium dodecyl sulfate was the most effective washing solution in removing cocaine from the hair. Surprisingly, chloroform, an organic solvent in which cocaine is very soluble, was not as effective as distilled water in removing cocaine. Similarly, acetone, another organic solvent in which cocaine is highly soluble, was not effective in removing the drug from hair. It is likely that more vigorous washing techniques may remove more of the externally applied drug. However, these studies do suggest that human hair will absorb significant amounts cocaine from aqueous solutions. Further, this absorbed drug cannot be removed completely by commonly used washing solutions such as water, methanol, acetone, chloroform and sodium dodecyl sulfate despite successive washing attempts. Even chloroform, which is the preferred solvent for extracting cocaine from biological fluids and tissues, removed very little of the externally applied drug from hair. Further, the observation that aqueous solutions (which hydrate the hair) remove more drug than organic solvents suggests that externally applied drug penetrates the cuticle and becomes deposited in the cortex. This seems intuitively possible since hair hydrates very easily when exposed to an aqueous solution.

Thus, the problem of external contamination may be greater than anticipated and it may not be possible to distinguish externally applied drug from that which is incorporated into the growing hair shaft.

KINETICS OF COCAINE UPTAKE AND WASHOUT IN HAIR

A second series of experiments were conducted to study, in more detail, the kinetics of cocaine uptake and washout in hair.

EXPERIMENTAL METHODS

Human hair samples were obtained from barber shops and washed with detergent and methanol briefly to remove any hair care products or natural oils then air dried. Approximately 800 mg of hair was cut into 2-mm pieces and incubated for 72 hr in 5 ml of 0.9 % saline (simulating sweat) containing radiolabeled cocaine (4-^{3}H-cocaine, specific activity of 14.77 mCi/mg). Two drug concentrations were used in the incubations: 15 ng/ml (which contained only radiolabeled cocaine, approximately 229,000 cpm/ml) and 1 mg/ml (which contained approximately 229,000 cpm/ml radiolabeled cocaine). Duplicate ten mg hair samples were removed from the bathing medium at various time periods, dried, weighed, digested, and the radioactivity determined by scintillation spectrometry. At the end of 72 hr, the dried remaining hair was placed in vial containing distilled water and incubated with gentle shaking for 25 hr. Duplicate 10 mg hair samples were removed at 0.33, 0.66, 1, 1.5, 2,3, and 25 hr, dried, digested, and the radioactivity determined by scintillation spectrometry. The rinse solution was changed after each sampling time.

RESULTS

Figure 18 shows the uptake and washout of radiolabeled cocaine in brown Caucasian hair.

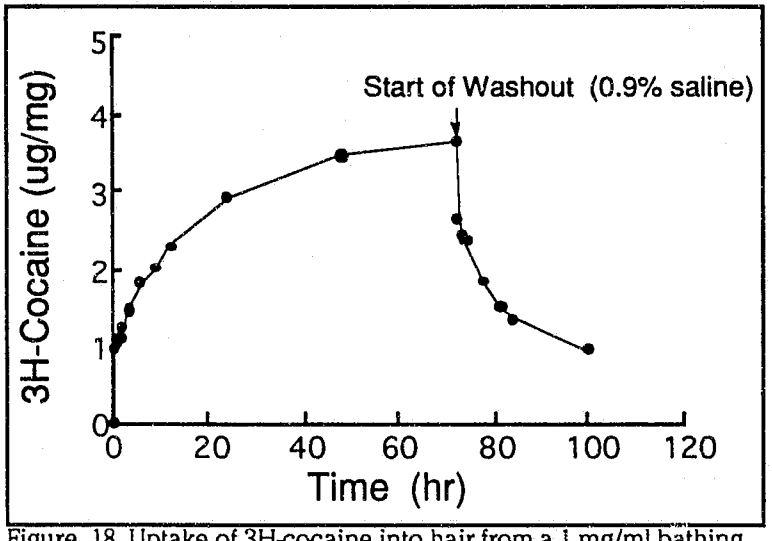

Figure 18. Uptake of 3H-cocaine into hair from a 1 mg/ml bathing solution followed by washout from a 0.9% saline solution. Each data point is the mean value of duplicate 10 mg hair samples.

Significant amounts of radiolabeled cocaine were taken up by hair throughout the 72 hour incubation period and there appears to be at least two distinct phases of drug uptake. During the first phase (which occurs within minutes of exposure), radiolabeled cocaine was taken up quite rapidly and by 20 min (the first sampling time) more than 20% of the total accumulated cocaine had been absorbed. Thereafter, radiolabeled cocaine accumulated at a slower but

persistent rate and by 6 hr one-half of the total drug had been incorporated . During the washout phase the opposite occurred, approximately 25% of incorporated drug was removed by 30 min (the first sampling time). Thereafter, the radiolabeled cocaine was removed at a slower but continuous rate.

DISCUSSION

The time course for accumulation of radiolabeled cocaine into human hair is quite similar to that described for the incorporation of dyes into hair or wool (Robbins, 1988) which suggests that the physical processes are similar. During the dyeing process there is a phase of rapid accumulation of dye (thought to be due to the attraction and binding of dye to the cuticle), followed by a slower phase (thought to be due to the subsequent penetration and diffusion of the chemical into cortex). When dyed hair or wool is washed, the opposite occurs. In the first few washings, dye bound to the cuticle is removed rapidly, while dye incorporated into the cortex is removed at a much slower rate with successive washings.

EXTERNAL CONTAMINATION OF HAIR BY VAPORIZED COCAINE

("Crack Smoke")

Studies on the uptake and washout kinetics of externally applied cocaine were extended to include exposure from cocaine vapor ("Crack" smoke). Four types of hair were used: Caucasian brown; African-American black; Caucasian blonde; and Caucasian brown that was bleached with 30% H_2O_2.

EXPERIMENTAL METHODS

Exposure Chamber

As shown in Figure 19, a Plexiglas chamber, measuring approximately 1 ft x 2 ft x 2 ft, was placed over an electric hot plate. Hair samples were placed in wire mesh trays approximately 1 ft above the hot plate and offset from the center of the hot plate by approximately 6 inches.

Exposure Conditions

After the hot plate was heated to 200° C, a small metal cup containing a 10 mg sample of cocaine free base was carefully lowered onto the plate. The cocaine vaporized immediately and the white fumes slowly dispersed throughout the chamber. Hair samples were exposed for 5 min, then removed from the chamber. Triplicate samples were taken from each of the four types of hair then analyzed by GC/MS. This experiment was repeated three times. For each experiment the position of each hair type was changed to ensure there was no experimental bias due to the position of the sample in the exposure chamber.

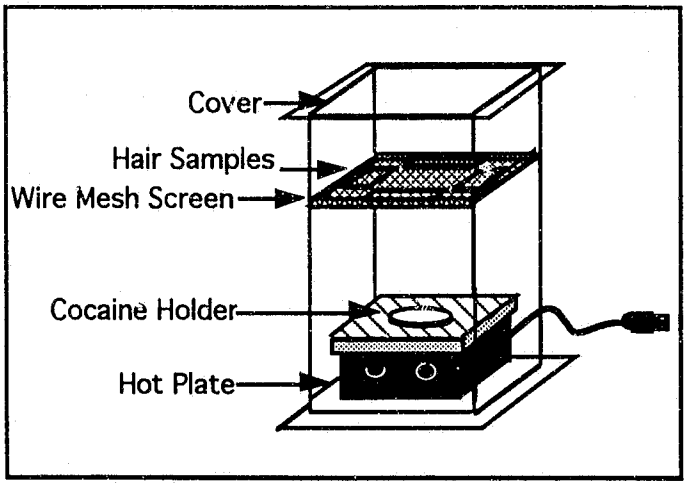

Figure 19. Plexiglas chamber for exposing hair samples to cocaine vapors.

RESULTS

The amount of cocaine present on hair following exposure to cocaine vapor is shown in the table below.

Table 14.
Cocaine in hair exposed to cocaine in vapor form ("Crack" smoke).

	Cocaine Concentration in Hair (ng/mg)[a]				
	Blonde[b]	Brown[c]	Bleached[c]	Asian	African - American
Before Washing	163.0 ± 22.2	131.9 ± 21.5	276.6 ± 11.9	133.1 ± 23.3	168.8 ± 19.0
After Washing	14.9 ± 2.1	22.7 ± 3.9	6.3 ± 1.0	52.6 ± 3.0	25.9 ± 2.6
% Remaining	9.1	17.2	2.3	39.5	15.3

[a] Values represent the mean ± SD of three to six samples.
[b] Caucasian natural blonde
[c] Caucasian

Table 15.
Cocaine in hair after exposure to cocaine in vapor form ("Crack" smoke) followed by treatment with various cosmetic preparations.

	Cocaine Concentration in Hair (ng/mg)[a]			
	Blonde[b]	Brown[c]	Asian	African - American
Before Washing or Treatment	163.0 ± 22.2	131.9 ± 21.5	133.1 ± 23.3	168.8 ± 19.0
After Washing	14.9 ± 2.1	22.7 ± 3.9	52.6 ± 3.0	25.9 ± 2.6
After Lightening[d]	20.8 ± 1.2	21.7 ± 1.6	39.0 ± 3.8	45.9 ± 1.0
After Perming[d]	0.8 ± 0.1	2.2 ± 0.3	8.2 ± 0.7	-
After Straightening[d]	-	-	-	10.0 ± 2.2

[a] Values represent the mean ± SD of three to six samples.
[b] Caucasian natural blonde
[c] Caucasian
[d] After exposure to cocaine vapor and treatment, but without vigorous washing.

Table 16.
Benzoylecgonine concentrations in hair exposed to "Crack" smoke followed by treatment with various cosmetic preparations.

	Benzoylecgonine Concentration in Hair (ng/mg)[a]			
	Blonde[b]	Brown[c]	Asian	African - American
Before Washing or Treatment	0.0	0.0	0.0	0.0
After Washing	0.0	0.0	0.0	0.0
After Lightening[d]	1.4 ± 0.4	1.1 ± 0.2	3.4 ± 1.3	3.9 ± 0.9
After Perking[d]	0.3 ± 0.1	0.0	0.4 ± 0.02	
After Straightening[d]				0.9 ± 0.1

[a] Values represent the mean ± SD of three to six samples.
[b] Caucasian natural blonde
[c] Caucasian
[d] After exposure to cocaine vapor and treatment, but without vigorous washing.

DISCUSSION

These data suggest that external contamination of hair from cocaine present in vapor form is possible. From 9 - 39% of the "crack" smoke remained on the hair samples even after they were washed. These washed samples would be considered positive if they were tested by routine hair analysis procedures.

There may be some differences in the amount of cocaine absorbed by the different hair types, as well as differences in the efficiency of the wash solutions in removing cocaine from the different hair types. In general, the greatest amount of cocaine was found in the unwashed coarse black hair samples, and cocaine was most easily removed from bleached brown hair. However, the number of samples in this study is too small to be conclusive.

Data in Tables 15 and 16 show that cosmetic treatment of hair may decrease the detection window for cocaine. Bleaching or lightening the hair seemed to produce the greatest decrease in the amount of cocaine present, followed by straightening (for African-American hair).

EXTERNAL CONTAMINATION OF HAIR BY COCAINE IN SWEAT

EXPERIMENTAL METHODS

Seven subjects were given a pharmacologically active dose of 2 mg/kg of deuterium-labeled cocaine as a saline solution containing 200 mg/ml of cocaine HCl. Administration was by a device producing a very fine and evenly distributed spray with the total dose delivered by 3 to 5 puffs of spray from the device inserted into the nose. Care was taken to avoid contamination of the subjects' hands with the spray. Assaying the sprayer residue allowed for correction of dose due to drug lost in the system. This was usually less than 1%.

Sweat Induction

Sweating was induced by exercising on a bicycle ergometer for 15 min during which time heart rate was monitored and work load regulated to ensure a heart rate between 120 to 150 beats per minute. Core temperature was also monitored. Exercise periods were repeated at 45 min, 2 hr 45 min, and 5 hr 45 min postdose. Between exercise periods, subjects rinsed with distilled water and dried their arm and hand to remove residual sweat that may have accumulated.

Exposure of Control Hair Samples

At 2 hr postdose (1 hour after the exercise period), each subject was given a 200 mg sample of blank, cocaine free hair and held this sample in their dominant hand for 30 min. The blank hair samples were from a larger pool of hair that had been tested previously and found to be free of cocaine. As a control against any inadvertent contamination of the samples in the laboratory environment, samples from this pool of drug free hair were sent to the clinical site in which the experiments were performed and then returned to the analytical laboratory. All control samples were negative.

Collection of Subsequent Hair Samples

Hair samples were collected from the subjects in this study at 2 and 4 days post dose, and on a monthly basis thereafter. These hair samples were collected and analyzed as described previously and the results are shown in Tables 5 and 6.

RESULTS

Table 17 shows the concentration of cocaine detected in the control (drug-free) hair that had been held in the subjects' hand 2 hr after receiving d5-cocaine.

Table 17. Concentration of d5-cocaine in control hair samples held in the hand of subjects who received 0.2 mg/kg d5-cocaine intranasally.

	d5-Cocaine in Hair (ng/mg)[a]	
Subject ID	Before Washing	After Washing
90339	3.04 ± 0.1	0.9 ± 0.0
90364	1.22 ± 0.25	n.d.*
91013	2.02 ± 0.18	0.22 ± 0.01
91021	2.72 ± 0.32	0.29 ± 0.03
91030	47.8 ± 9.29	10.90 ± 0.78
91031	0.31 ± 0.03	n.d.
91033	0.74 ± 0.07	n.d.

[a] Values shown are the mean ± SD of three samples.
* n.d. = None detected at a cutoff of 0.1 ng/mg hair.

DISCUSSION

The results in Table 17 demonstrate that external contamination of hair can occur via sweat. All samples were positive for d5-cocaine, and most were positive even after washing. In most cases the amount of cocaine found the the control hand-held sample was greater than the amount found in the hair of subjects who had received cocaine in our studies. The amount of cocaine in the control hair held by subject 91030 is remarkably high and it is very likely that had this sample been sent to a hair analysis laboratory it would have resulted in an evidentiary "false positive" test, i.e., the sample is positive, but not from cocaine ingestion. Even after washing, the amount of cocaine in this externally contaminated hair sample was higher than that found in his hair when it was tested over the next few months (See Table 6).

It is difficult to foresee all the possible circumstances in which cocaine might be transferred to hair via sweat and result in a false positive. The circumstances in this experiment were rather extreme – sweating was induced in these subjects and the hair was held in the hand for total of 30 min. This does not simulate brief, casual contact. However, the test subjects received only

one, rather modest intranasal dose and it is likely that an even greater amount of drug could be transferred by someone who used cocaine regularly and had a high body burden of the drug. At the very least, our results suggest that extreme care must be taken by all personnel engaged in collecting or processing the hair samples to avoid physically touching the hair sample.

Although our data suggest that sweating may be an additional route by which cocaine is transferred from the body to hair, this still may not explain fully the large intersubject differences in cocaine accumulation in hair. Subjects who had the greatest amount of d5-cocaine in their hair did not always have the highest concentration of drug in their sweat. However, the sweat was collected from the arms and hands and would not contain the substantial sebaceous secretions that would be expected in sweat from the scalp. However, cocaine excretion in sweat may explain why the drug was sometimes found over a wide area of the hair shaft even though the subject received only a single dose. For example, subject 91030 transferred significant amounts of cocaine to the control hair he held in his hand. In addition, he had d5-cocaine distributed over 10 segments of his hair when it was tested two days after drug administration.

This experiment, along with the other external contamination experiments described previously, demonstrates that external contamination of hair with cocaine can occur rather easily whether cocaine is in aqueous or vapor form. Washing the hair sample before testing will reduce much of the external contamination, but not all the externally applied cocaine will be removed. More exhaustive or efficient washing procedures might be developed for removing externally contaminated drug from hair; however, our studies suggest that externally applied drug resides not only on the outside of the hair shaft, but penetrates past the cuticle into the cortex. More vigorous washing may remove more externally applied drug, but it will also remove internally incorporated drug as well.

It has been suggested that the ratio of drug found in the rinse solution to that found in hair after digestion can be used as a criterion for distinguishing externally bound from internally incorporated drug. For example, if little or no drug is found in the last rinse solution, but large amounts are found in hair after digestion then it is assumed that all externally bound drug has been removed by washing and only internally incorporated drug is liberated during digestion. Our data show that this is not always the case. Most externally applied drug is removed in the first wash step. Therefore, any sample with large amounts of drug in the wash solutions and much smaller amounts after digestion would be suspected of external contamination. However, consider the case of a subject who was exposed to drug through external contamination, but who washed his hair (as part of normal daily hygiene)before it was collected and sent to the laboratory. His sample would have a low concentration of drug in the wash solution (it was removed in the prior wash), but it could still have a significant amount of drug remaining in the hair after digestion. If the "wash ratio" was used as the sole criterion for detecting external contamination, it would lead to the wrong conclusion.

The presence of cocaine as the primary analyte in hair also complicates the external contamination issue. In urine it is the metabolite BE which is the major analyte, so external contamination is rarely used to challenge the results of urinalysis. However, in hair, cocaine would be the principle analyte after either external contamination or cocaine ingestion. It might be possible to identify unique metabolites, such as cocaethylene, which form only through biochemical processes in the body and not through chemical process in the environment, which could be used to rule our external contamination in some samples (Cone, Yousefnejad, *et. al.*, 1991). However, at the present time it is not possible to categorically rule

out external contamination as a cause of a positive hair sample by washing techniques alone. In fact, soaking hair samples in drug solutions (containing the appropriate mixture of parent drug and metabolite) might be a convenient way of preparing highly standardized hair samples for use in quality control and proficiency testing. It is likely that samples prepared this way could not be distinguished from actual field samples hair testing laboratories.

CONCLUSIONS

What is the relationship between amount (i.e., the dose) of the drug used and the amount of drug found in hair? (Objective 1 of the grant)

There does not appear to be a predictable relationship between the dose of cocaine administered and the amount of drug eventually incorporated into hair. The administration of even a single intravenous or intranasal dose of cocaine can be detected by hair analysis. The minimum detectable dose for our ion trap mass spectrometric method is about 25- 35 mg, an amount less than that typically found a single "line" of street cocaine (e.g., 50 - 100 mg). However, within the range of doses we tested (0.6 - 4.2 mg/kg) the amount of cocaine incorporated into hair varied so much between subjects, that it is impossible to determine the dose of cocaine a subject received based upon the amount of cocaine in his or her hair.

In our studies, the individual (# 91014) who received the largest total dose of cocaine of all the subjects, a total of 855 mg (91 mg IV plus 764 mg intranasal) administered over a period of 31 days, had approximately the same amount of drug incorporated into his hair as did one subject (90375) who received only 40 mg d5-cocaine intravenously. When these doses are corrected for bioavailability and body weight, subject 91014 received seven times as much drug as subject 90375, but had essentially the same amount of drug incorporated into his hair. This large interindividual variability could not be explained by differences in the subjects' plasma pharmacokinetics.

Our studies also suggest that for cocaine segmental analysis cannot determine with any accuracy the time of drug ingestion. In none of the 25 test subjects tested did segmental analysis show the distribution of drug along the hair shaft predicted by the model in which drug is incorporated into the hair shaft at the time of ingestion and moves down the shaft at the rate of 1 cm per month (the presumed growth rate of human hair). There was considerable intersubject variability in: 1) the time when d5-cocaine first appeared in hair, 2) the extent the drug was distributed along the hair shaft, and 3) the time d5-cocaine remained in hair. In only two subjects was a single dose of d5-cocaine confined to a single 1-cm segment. More typically, the dose was distributed over at least two segments. This is not unexpected considering the variability that can occur in aligning and cutting the hair segments even when considerable care is taken in this procedure. In four subjects, however, a single dose was distributed over four segments and in one subject, a single dose was distributed over 10 segments.

Our studies suggest that the simple biological model proposed in which drugs are incorporated into hair by simple diffusion of drug from blood into the growing hair cells may not be correct, at least for cocaine. This model predicts that the amount of drug incorporated into hair is proportional to the concentration of drug in the blood and thus proportional to the dose. This model would predict also that BE and EME (the metabolites of cocaine that are present in higher concentrations than cocaine in blood) would also be the major analytes found in hair.

This is not the case. Even when cocaine is ingested regularly and the concentrations of BE and EME in blood are many-fold higher than those of cocaine, the levels of these metabolites are quite low in hair. Thus, it seems more likely that cocaine enters hair via multiple mechanisms and possibly at multiple times during the hair growth cycle

Can the drug administered during the experimental procedures be distinguished from any drug taken surreptitiously during the course of the study or any drug retained in the body from previous use? (Objective 2)

Yes. In our studies we administered deuterium-labeled cocaine (d5-cocaine) to the human volunteers. d5-Cocaine is pharmacologically and pharmacokinetically identical to non-deuterated cocaine, but produces a unique signal or response in a mass spectrometer. Thus, in the blood, sweat or hair samples we were always able to distinguish the drug we administered from any residual cocaine in the subject's body or any drug surreptitiously during the course study. We detected, but did not quantify, non-deuterated cocaine in many of our samples. This must have come from the ingestion of cocaine by the subjects either before the study or during the study. This is not too surprising considering some of our subjects were in the study for up to 15 months and they all had a prior history of cocaine use. It would have been impossible to conduct the precise kind of experiments for as long as we did without the use of this specially tagged cocaine.

Is Sweat an Important Mechanism For the Incorporation of Drugs into Hair? (Objective 3)

Possibly. Sweat may be one of many mechanisms by which cocaine is incorporated into hair. We have found that cocaine is excreted in sweat in very high concentrations for many days after a single administration of drug. Excretion of cocaine in sweat may explain why the drug can be detected in hair within hours after drug ingestion and why the drug is distributed over multiple hair segments following a single dose. It is not clear from our data whether the large intersubject variability seen in our dose-response studies can be explained by differences in the excretion of cocaine in sweat in these subjects. Sweat may also be a possible vehicle for external contamination. When subjects, who had received a single dose of cocaine previously, held control ("drug free") hair in their hands for a short time, significant amounts of cocaine were transferred to the blank hair. Even after washing, the amounts of cocaine were great enough to produce an evidentiary false positive.

Is External Contamination an Important Variable Which Must Be Considered in Hair Analysis? (Objective 4)

Our studies show that external contamination is potentially an important variable and should always be considered when interpreting hair analysis results. First, unlike other biological samples such as blood and urine, hair is always exposed to the environment. Secondly, the amounts of drug found in hair are quite small so contact of the hair sample with even traces of cocaine during any stage of the testing process (from sample collection to final analysis) could result in a false positive. Thirdly, the major analyte found in hair is cocaine, not its metabolites, which is exactly what you would find if the hair sample were contaminated from an external source. Finally, hair readily absorbs cocaine whether it is present in an aqueous solution or in vapor form ("crack smoke"). The amount absorbed depends upon the concentration and

duration of exposure. In fact, hair can be contaminated by simply being handled by someone who has ingested cocaine. In the strictest sense some of the cocaine incorporated into hair after drug ingestion may get there via "external contamination"; that is, from sweat. Preventing external contamination of the sample must be a prime objective in all phases of the hair testing process and external contamination must always be ruled out when interpreting hair analysis results.

Can washing procedures and/or evaluations of relative ratios of drug to metabolite and handling procedures prevent evidentiary challenges based on claims of environmental contamination? (Objective 5)

Our studies suggest that the washing procedures typically used in hair analysis will remove most, but not all externally applied drug. Our studies show that most externally applied drug is removed in the first few washes, while smaller amounts are removed thereafter. This suggests that the drug is not simply adhering to the surface but is likely embedded into the hair fiber. Thus, washing alone is not sufficient to guarantee removal of externally applied drug. It has been proposed that comparing the amount of drug removed in the wash steps with the amount of drug found in hair after digestion will serve as an indicator of external contamination, i.e., a large amount of drug in the washes but a small amount of drug in hair suggests external contamination. This might be a useful (although rather expensive for routine use) procedure for identifying samples recently contaminated. However, this would not identify a sample that has been contaminated then washed before the sample is acquired. If an individual's hair is contaminated somehow via an external source and then washed as part of the normal daily hygiene, most of the drug will be washed off but some of the drug will be absorbed into the hair shaft. If a hair sample is then taken from this individual and tested, the washings will show very little cocaine (most was removed in the previous washing), but cocaine will be found in the hair.

Using relative ratios of cocaine to BE and/or EME may be more useful in identifying externally contaminated samples, but this will require the identification and quantitation of additional analytes and thus make the test more expensive. Further, these metabolites cannot be used as unambiguous indicators of cocaine ingestion because they can be formed by non-biochemical means. Our studies showed that BE can be formed in samples exposed to cocaine vapor then treated with cosmetics such as bleaching, perming, or straightening solutions. Looking for highly unique metabolites such as cocaethylene, which forms in the body when alcohol and cocaine are used concurrently, might be useful in ruling our external contamination; however, this metabolite is present in relatively low levels in hair and occurs only when cocaine use is combined with alcohol use.

After more information has been obtained concerning the levels of cocaine likely to be present following external contamination, threshold levels, similar to those used in urine drug testing, might be developed for hair analysis for cocaine. As is done in urine testing for marijuana, threshold levels should be set high enough to preempt any reasonable challenge of external contamination as well as insure analytical accuracy.

Can individuals intentionally reduce the drug content of their hair through extensive washing or other cosmetic treatments? Objective 6

Our studies were too limited in this area to answer this with any certainty. Cosmetic treatments such as bleaching were found to be somewhat more effective than washing in removing externally applied cocaine. Thus, individuals might reduce the drug content in their hair

somewhat through extensive washing (especially with very alkaline dandruff shampoos) or cosmetic treatments, but whether this would be effective enough to avoid a positive hair test result would depend upon how much cocaine was there initially and the cutoff or threshold level for the hair test. Extensive washing and cosmetic treatments may not be effective enough to avoid a positive result with hair testing, but they may result in differences in drug content in hair from individuals with the same drug history and thus may complicate the interpretation of hair analysis results.

Can hair analysis test results vary with age, race, or gender? Objective 7

Race may be the most important variable in determining the amount of drug incorporated into hair. In fact within the range of doses used in our studies, race was more important than dose in determining the level of d5-cocaine in hair. Although there were only four non-Caucasian subjects in our studies, all four were "outliers", that is, they had statistically higher levels of drug in their hair than did their Caucasian counterparts. Conversely, all the outliers in the study were non-Caucasians. Although the total number of non-Caucasians was small (4 of the 25 test subjects) they did represent 16% of the study group. Further, this does not appear to be an analytical artifact or due to any error in drug administration or hair sampling. Non-Caucasians typically had higher amounts of cocaine in all hair segments of all positive samples collected from them. It is important to stress that these non-Caucasians were not only Afro-Americans, but included individuals of Hispanic, East Indian, and Eurasian decent. At this time we have no explanation for this seeming anomaly.

The four females in the study group were all Caucasian and did not have unusually high or low amounts of drug in their hair. Based on the results of our small sample group, we conclude that gender does not appear to be an important variable affecting cocaine uptake into hair. Similarly, age did not appear to be a significant variable as the ages of the four outliers were very close to the mean of the study group – 30 years. The range of ages in the study group was relatively narrow (21 - 39 years), but is representative of the cocaine using population.

ADDITIONAL WORK PRODUCTS RESULTING FROM THESE STUDIES

As called for in the contract, progress reports were submitted to the National Institute on Justice. In addition, a number of papers and abstracts were published in the scientific literature and a number of presentations were made, usually by invitation, at various law enforcement, toxicology, and health care symposia and meetings. These are listed below.

PAPERS PUBLISHED

1. "Simultaneous Determination of Cocaine, Benzoylecognine and Ecgonine Methyl Ester in Human Hair by Gas Chromatography - Mass Spectrometry", M. R. Harkey, G. L. Henderson and C. Zhou. *Journal of Analytical Toxicology*, 15:260-265, 1991.

2. "Cocaine and Metabolite Concentrations in the Hair of South American Coca Chewers", G.L. Henderson, M.R. Harkey, and C. Zhou, *Journal of Analytical Toxicology*, 16:199-201, 1992.

3. "Biology and Physiology of Hair" , M.R. Harkey, Journal of International Toxicology, in press.

4. "Mechanisms of Drug Incorporation into Hair" , G.L. Henderson, Journal of International Toxicology, in press.

ABSTRACTS PUBLISHED

1. "Effect of External Contamination on the Analysis of Hair for Cocaine", G.L. Henderson, M.R. Harkey, R.T. Jones, and C. Zhou, Joint Meeting of Society of Forensic Toxicologists and the Canadian Society of Forensic Scientists, Montréal, Québec, September 23 - 27, 1991.

2. "Quantitation of d5-Cocaine and Its Metabolites in Human Hair", M.R. Harkey, G.L. Henderson, R.T. Jones, and C. Zhou, Joint Meeting of Society of Forensic Toxicologists and the Canadian Society of Forensic Scientists, Montréal, Québec, September 23 - 27, 1991.

3. "Quantitation of d5-Cocaine and Its Metabolites in Human Hair", G.L. Henderson, M.R. Harkey, R.T. Jones, and C. Zhou, American Society of Criminologists Annual Meeting, San Francisco, CA, November 20 - 23, 1991.

PRESENTATIONS AT SCIENTIFIC MEETINGS

1. "Overview of Hair Analysis for Drugs of Abuse", M.R. Harkey, Society of Forensic Toxicologists/National Institute on Drug Abuse Joint Conference on Hair Analysis for Drugs of Abuse, Washington, DC, May 27-79, 1990.

2. "Update on Hair Analysis for Drugs of Abuse", M.R. Harkey, California Association of Toxicologists, Yosemite, CA, November 2-3, 1990.

3. "Hair Analysis to Determine *In-Utero* Exposure to Cocaine", G.L. Henderson, Presented at the California Birth Defects Program, Berkeley, CA, May 3, 1991.

4. "Hair Analysis for Drugs of Abuse: Potential Uses and Limitations", M.R. Harkey, SyvaFocus '91 Toxicology Conference, Milwaukee, WI, May 29, 1991.

5. "Hair Drug Testing Workshop", M.R. Harkey, Administrative Law Judges Association Forum, Monterey, CA, May 31, 1991.

6. "Effect of External Contamination on the Analysis of Hair for Cocaine", G.L. Henderson, M.R. Harkey, R.T. Jones, and C. Zhou, Joint Meeting of Society of Forensic Toxicologists and the Canadian Society of Forensic Scientists, Montréal, Québec, September 23 - 27, 1991.

7. "Quantitation of d5-Cocaine and Its Metabolites in Human Hair", M.R. Harkey, G.L. Henderson, R.T. Jones, and C. Zhou, Joint Meeting of Society of Forensic Toxicologists and the Canadian Society of Forensic Scientists, Montréal, Québec, September 23 - 27, 1991.

8. "Effect of External Contamination on the Analysis of Hair for Cocaine", G.L. Henderson, M.R. Harkey, R.T. Jones, and C. Zhou, Quarterly Meeting of the California Association of Toxicologists, San Diego, CA, November 2, 1991.

9. "Quantitation of d5-Cocaine and Its Metabolites in Human Hair", M.R. Harkey, G.L. Henderson, R.T. Jones, and C. Zhou, Quarterly Meeting of the California Association of Toxicologists, San Diego, CA, November 2, 1991.

10. "The Use of Hair Analysis in Forensic Investigations", G.L. Henderson, 11th Annual Symposium, Institute of Forensic Sciences, Oakland, CA, May 15, 1992.

11. "Biology and Physiology of Hair" , M.R. Harkey, First International Symposium on the Use of Hair Analysis in Forensic Investigations", Genoa, Italy, December 10,11, 1992.

12. "Mechanisms of Drug Incorporation into Hair" , G.L. Henderson, First International Symposium on the Use of Hair Analysis in Forensic Investigations", Genoa, Italy, December 10-11, 1992.

References

1. Arnold, W. and K. Puschel. (1981). "Experimental Studies on Hair as an Indicator of Past or Present Drug Use." J. Forensic Sci. Soc. 21: 83.

2. Baumgartner, A. M., P. F. Jones, W. A. Baumgartner and C. T. Black. (1979). "Radioimmunoasay of Hair for Determining Opiate-Abuse Histories." J. Nucl. Med. 20: 748-752.

3. Baumgartner, A. M., P. F. Jones and C. T. Black. (1981). "Detection of Phencyclidine in Hair." J. Forensic Sci. 26(3): 576-681.

4. Baumgartner, W. A. (1986). Detection of Drug (Cocaine) Use by Hair Analysis., Final Report, U.S. Navy Rehabilitation Center Study.

5. Baumgartner, W. A., C. T. Black, P. F. Jones and W. H. Blahd. (1982). "Radioimmunoassay of Cocaine in Hair: Concise Communication." J. Nucl. Med. 23(9): 790-792.

6. Bell, M. R. and S. Archer. (1960). "L(+)-2-tropanone." J. Amer. Chem. Soc. 82: 4642.

7. Brown, D. J. (1985). "The Pharmacokinetics of Alcohol Excretion in Human Perspiration." Meth. and Find. Exptl. Clin. Pharmacol. 7(10): 539-544.

8. Chatt, A., C. A. Secord, B. Tiefenbach and R. E. Jervis. (1980). "Scalp Hair as a Monitor of Community Exposure to Environmental Pollutants". Hair, Trace Elements, and Human Illness. New York, Praeger Publishers.

9. Cone, E.J. (1990). "Testing Human Hair for Drugs of Abuse. I. Individual Dose and Time Profiles of Morphine and Codeine in plasma, Saliva, Urine, and Beard Compared to Drug-Induced Effects on Pupils and Behavior. J. Anal. Tox. 14: 1-7.

10. Cone, E.J., D. Yousefnejad, W.D. Darwin, and T. Maguire. (1991), "Testing human hair for drugs of abuse. II. Identification of unique cocaine metabolites in hair of drug abusers and evaluation of decontamination procedures. J. Anal. Tox. 15: 250-55.

11. Gill., P., A.J. Jefferies, and D.J. Werrett, (1985). "Forensic Application of DNA Fingerprints." Nature 318: 577-579:

12. Haley, N. J. and D. Hoffman. (1985). "Analysis for Nicotine and Cotinine in Hair to Determine Cigarette Smoker Status." Clin. Chem. 31(10): 1598-1600.

13. Harkey, M. R. and G. L. Henderson. (1989). Hair Analysis for Drugs of Abuse. Advances in Analytical Toxicology. Chicago, Year Book Medical Publishers, Inc.

14. Harkey, M. R., G. L. Henderson, R. J. Jones and C. Zhou. (1991a). Quantitation of d5-Cocaine and its Metabolites in Human Hair. Presented at the Joint Meeting of the Society of Forensic Toxicologists and the Canadian Society of Forensic Scientists, Montreal, Quebec, September 23-27.

15. Harkey, M. R., G. L. Henderson, C. Zhou and R. T. Jones. (1991b). "Simultaneous Determination of Cocaine, Benzoylecognine and Ecgonine Methyl Ester in Human Hair by Gas Chromatography - Mass Spectrometry." J. Anal. Tox. 15: 260-265.

16. Harrison, W. H., R. M. Gray and L. M. Solomon. (1974a). "Incorporation of D-amphetamine into Pigmented Guinea Pig Hair." Brit. J. Dermatol. 91: 415-418.

17. Harrison, W. H., R. M. Gray and L. M. Solomon. (1974b). "Incorporation of L-DOPA, L-alpha-Methyldopa and DL-Isoproterenol into Guinea Pig Hair." Acta Dermatol. 54: 249-253.

18. Henderson, G. L., M. R. Harkey, R. J. Jones and C. Zhou. (1991). Effect of External Contamination on the Analysis of Hair for Cocaine. Presented at the Joint Meeting of Forensic Toxicologists and the Canadian Society of Forensic Scientists., Montreal, Quebec, September 23-27.

19. Henderson, G. L., M. R. Harkey, C. Zhou and R. T. Jones. (1992). "Cocaine and Metabolite Concentrations in the Hair of South American Coca Chewers." J. Anal. Tox. 16: 199-201.

20. Ishiyama, I., T. Nagai, T. Nagai, E. Komoru, T. Momose and N. Akimori. (1979). "The Significance of Drug Analysis of Sweat in Respect to Rapid Screening for Drug Abuse." Z. Rechtsmedizin. 82: 251-256.

21. Ishiyama, I., T. Nagai and S. Toshida. (1983). "Detection of Basic Drugs (Methamphetamine, Antidepressants, and Nicotine) from Human Hair." J. Forensic Sci. 28: 380-385.

22. Jacob, P.III, B.A. Elias-Baker, R.T. Jones, and N.L. Benowitz .(1987). "Determination of Benzoylecognine and Cocaine in Biological Fluids by Gas Chromatography." J. Chromatog. 417: 277-286.

23. Kidwell, D. A. (1989). Analysis of Drugs of Abuse in Hair by Tandem Mass-Spectrometry. Presented at the 36th American Society of Mass-Spectrometry Conference on Mass Spectrometry and Allied Topics., San Francisco.

24. Klug, E. (1980). "Zur Morphinbestimmung in Kopfhaaren." Z. Rechtsmed. 84: 189-193.

25. Michalodimitrakis, M. (1987). "Detection of Cocaine in Rats from Analysis of Hair." Med. Sci. Law. 27(1): 13-15.

26. Niwaguchi, T., S. Suzuki and T. Inoue. (1983). "Determination of Methamphetamine in Hair after Single and Repeated Administration to Rat." Arch. Toxicol. 52: 157-164.

27. Parton, L., D. Warburton, V. Hill and W. Baumgartner. (1986). "Quantitation of Fetal Cocaine Exposure by Radioimmunoassay of Hair." Ped. Res. 21(4): 372A.

28. Robbins, C.R. (1988). "Interactions of Shampoo and Creme Rinse Ingredients with Human Hair." In *Chemical and Physical Behavioral of Human Hair*, Springer-Verlag, New York, Chap. 5, pp. 122 - 170.

29. Smith, F. P. and R. H. Liu. (1986a). "Detection of Cocaine Metabolite in Perspiration Stain, Menstrual Bloodstain, and Hair." J. Forensic Sci. 31(4): 1269-1273.

30. Smith, F. P. and R. H. Liu. (1986b). "Detection of Cocaine Metabolite in Perspiration Stain, Menstrual Bloodstain, and Hair." J. Forensic Sci. 31(4): 1269-1273.

31. Sramek, J. J., W. A. Baumgartner, J. A. Tallos, T. N. Ahrens, J. F. Heiser and W. H. Blahd. (1985). "Hair Analysis for Detection of Phencyclidine in Newly Admitted Psychiatric Patients." Am. J. Psychiatry. 142(8): 950-953.

32. Suzuki, O., H. Hattori and M. Asano. (1984). "Detection of Methamphetamine and Amphetamine in a Single Human Hair by Gas Chromatography/Chemical Ionization Mass Spectrometry." J. Forensic Sci. 29(2): 611-617.

33. Suzuki, S., T. Inoue, H. Hori and S. Inayama. (1989). "Analysis of Methamphetamine in Hair, Nail, Sweat, and Saliva by Mass Fragmentography." J. Anal. Toxicol. 13: 176-178.

34. Valente, D., M. Cassini, M. Pigliapochi and G. Vansetti. (1981). "Hair as the Sample in Assessing Morphine and Cocaine Addiction." Clin. Chem. 27(11): 1952-1953.

35. Van Dyke, C., A. Stesin and R. Jones. (1986). "Cocaine Increases Natural Killer Cell Activity." J. Clin. Invest. 77: 1387-1390.

36. Vree, T. B., A. T. J. M. Muskens and J. M. Van Rossum. (1972). "Excretion of Amphetamines in Human Sweat." Arch. Int. Pharmacodyn. 199: 311-317.