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**Cocaine detection in hair: Effect of retroviral infection, age,
morphine, and alcohol**

Poet, Torka Sue, M.S.

The University of Arizona, 1991

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**COCAINE DETECTION IN HAIR: Effect of
Retroviral Infection, Age, Morphine, and Alcohol**

by

Torka Sue Poet

A Thesis Submitted to the Faculty of the
DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
In Partial Fulfillment of the Requirements
For the Degree

MASTER OF SCIENCE
WITH A MAJOR IN TOXICOLOGY


In the Graduate College
THE UNIVERSITY OF ARIZONA

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STATEMENT BY AUTHOR


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This thesis is dedicated to my mother for trying to understand and because everyone dedicates their thesis to their mother.

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ABSTRACT

Factors affecting the deposition of cocaine into hair and the relationships between those concentrations, survey questionnaire results and natural killer cell activities were investigated. First, two and 18 month old mice were infected with LP-BM5, injected with cocaine, and hair cocaine content measured. There was no difference in cocaine concentration between the age groups. Retrovirally infected mice had greater amounts of cocaine in their hair. Next, a group of mice were fed an ethanol diet and injected with cocaine. There was a decrease in cocaine recovered in the hair of the ethanol-fed mice.

In a group of human drug users no correlation was found between survey questionnaire answers and hair cocaine concentrations. There was a correlation between natural killer cell activity and hair cocaine concentrations.

It was concluded that hair analysis for cocaine may have a place in clinical settings, but a predictive correlation between drug intake and hair concentration may not be possible.

CHAPTER 1: INTRODUCTION

The current methods commonly used to identify cocaine users have limited scope. Urinalysis is limited by the rate of elimination of cocaine and its metabolites. The major metabolite, benzoylecgonine, can be detected for up to 4 days (1). The only other common method used to identify cocaine users is self-reported drug history. Both of these methods have proven to be unreliable, many people will deny using drugs, and 4 to 5 days after last drug use urinalysis will be negative (2).

Several studies have shown that hair retains a variety of drugs and drug metabolites for extended periods of time (3). It is hypothesized that hair analysis may provide an indicator of past cocaine use which can be used in clinical situations to compare to biological effects of cocaine.

The main intent of the work reported in this thesis is to address the question of the effect of individual differences on deposition of cocaine in hair. In Chapter 1 the effect of a retrovirus infection, administration of a second drug (morphine) and age on the amounts of cocaine recovered in the hair of mice is investigated. In Chapter 2 the effect of concurrent ingestion of ethanol with cocaine

is investigated. The second goal of this work is to compare drug recovery in hair to verbal self reports of drug use by a sample population of admitted drug users. Also, a comparison of cocaine concentration in hair and one biological parameter shown to be affected by cocaine use (natural killer cell activity) (4,5) is discussed in Chapter 4.

Hair Analysis

Hair as a Matrix in Which to Detect Substances: More than 30 years ago it was suggested that hair could be used to identify the presence of trace metals being excreted from the body (6). At this time, the recovery of trace metals from hair caused a certain amount of excitement. It was heralded as a prime technique with which investigators could identify exposure to these substances. Some laboratories have found correlations between various trace elements in the diet and hair concentrations, for example, mercury (7,8), lead (9), cadmium (10,11), and arsenic (12). Similar correlations were not found in other studies of zinc (13,14,15), copper (16,17), and cadmium (18,19). Many other studies with hair and trace and heavy metals as well as micronutrients have been cited (20).

The use of hair to detect drugs was first reported in 1954 when Goldblum et al. measured barbiturate concentrations in the hair of guinea pigs (21). In 1974 Harrison et al. first suggested that hair had the potential to serve as a "sensitive indicator of past consumption by humans of amphetamines and related drugs" (22). In 1979 a process was described by which hair samples of opiate users could be screened for the presence of drug metabolites (23). A correlation was found between the time of opiate use and the distribution of a drug metabolite (morphine) along the length of the hair shaft. Subsequent studies indicated that a variety of other drugs including cocaine (24,25), phencyclidine (26), amphetamines (27,28), haloperidol (29), and nicotine (27,30) can also be detected by hair analysis. Although there have been reports of the detection of marijuana metabolites in hair (31,32), these remain to be validated (33).

Hair Growth and Drug Deposition: The hair shaft is a keratinized filament that is formed from the matrix of cells at the bottom of the hair follicle in a region of the epidermal epithelium that projects into the dermis. At the base of this filament is the area of formation and differentiation. The next region is the keratogenous zone and the region of the hair that exits the epidermis is the

permanent hair that results from these two processes (Fig. 1). The permanent hair fiber is a complex of special, highly cross-linked proteins that coalesce and condense into a permanent horny mass. The cross-section of the hair is divided into three general regions, the outer cuticle, the cortex and the central medulla (34).

Hair growth rate is highly variable at different body locations, between different individuals, at different times and ages, and even for different sexes and races (20,3). Hair is not continually being produced. Intra- and inter-individual variation in growth rate is remarkable in the head region alone (3,35). Different investigators have reported large differences in rates of growth (Table 1). There are three phases of hair growth which each individual follicle undergoes; anagen, catagen and telogen. Only during the actual growth phase, anagen, can substances become deposited in the hair (3). The normal cycling through the phases can be altered by disease and nutritional states (21).

It has never been shown exactly how trace elements are deposited in hair, but in 1977 Hopps proposed four possible sources (36): first, uptaken during histogenesis; second, deposition by sebum; third, absorption from eccrine sweat; and fourth, those deposition from the external environment or preparations applied to the scalp (e.g. cosmetics).

Various cleaning procedures have been used to differentiate between endogenous and exogenous sources of elements or drug metabolites (37). Assuming these methods to be effective, Hopps' suggestion of external sources of trace element deposition hair (and by extension drug metabolites) can be ruled out. During histogenesis substances can be deposited into the hair from either the circulating blood or from the sebum which is produced by the sebaceous gland at the base of the follicular epithelium and can flow into and impregnate the hair shaft (36,37). Baumgartner has proposed that drug metabolites are incorporated into the hair from the vast capillary beds that surround the growing hair follicle and that the metabolites are bound to sulfhydryl groups which are prevalent in hair (38).

Most methods of extraction of drug metabolites from hair are relatively mild techniques (39), with one enzymatic exception (40). This indicates that the drug or its metabolite are not covalently bound within the hair shaft. Hair also contains many carboxyl groups and these may also present possible non-covalent binding sites. Binding to amino groups has also been proposed (41).

Analysis: The plethora of variables already in existence makes standardization of the procedures involved with sample

collection, extraction, and analysis very important. Standardization has not been done, although suggestions for its achievement have been made. The International Atomic Energy Agency (IAEA) developed a protocol for the analysis of hair as an indicator of contamination by environmental trace elements in 1978, which they revised and made less complicated in 1984 (20,42). The Hair Analysis Standards Board also proposed a standardized procedure for the collection of samples (43). The only report specifically suggesting procedures for drug detection in hair was published in 1988 by Harkey and Henderson (3). All of these procedures discuss body location of sample collection in detail. The original IAEA procedure even suggested the collection of samples from 5 different specific sites on the scalp of all subjects. The second report suggests taking just one sample from the occipital region. Harkey and Henderson suggest always collecting samples from the vortex posterior region of the head. The collection of samples from other body areas has also been discussed, but the Hair Analysis Standards Board report disputes the "interpretability" of these data (43), and the suitability of samples collected from other areas is questioned by Harkey and Henderson (3). One report on marijuana in hair did compare pubic to scalp hair and the investigators concluded that pubic hair was actually a better source (32).

A year after this report, however all of their data was questioned (33).

There is no consistency in any reports on sample size or for the washing of the sample. It has not even been proven that any washing procedure removes all external contamination and at the same time only removes the external contamination (44). Extraction procedures are similar for similar drugs, but these also vary.

Hair versus Urine: Hair is touted as having many advantages over urine in which to detect the use of drugs (Table 2). In the early 1970s hair testing was also thought to be the key to defining toxic exposure to metals and for the diagnosis of nutritional deficiencies, however, it has since been shown that there is substantial variation among exposed and unexposed individuals (45,46,47) and Cone has gone so far as to suggest that hair testing for nutritional status is "quackery" (44). It seems that this labelling of hair testing for metals, some vitamins and essential elements has tainted the reputation of hair analysis and has caused its use to be questioned for any tests, including drugs.

The major advantage to hair analysis is the window of detection (37). Urine represents a "momentary look" at the individuals drug use (44). Drug use can be detected in hair for the length of time that that hair has been growing. If

an amount of drug is trapped in the keratin during hair cell growth it remains entrapped indefinitely after the cells die leaving behind the inert fiber. Thus hair should be able to give an historical pattern of drug exposure to the growing hair. The questions concerning drug entry and hair growth make quantification and validation of this procedure in the human population impossible to determine.

It has been proposed that hair be used as a "safety net" for urinalysis. False positives and negatives are said to occur more rarely (or never) in hair (48). Also, one positive urine test can be defended with a claim of external or inadvertent exposure, whereas a positive hair test can be sectioned and an individual, one exposure, area can be identified and compared to the rest of the length of hair. Alternatively, another, more-or-less equivalent, sample can be obtained.

Current Status of Hair Analysis for Drug Testing: A recent decision by the United States Food and Drug Administration (FDA) limits the use of hair analysis in mass screening procedures for drugs of abuse on the basis that quality control has not yet been established (49). The FDA issued a statement which expressed doubt as to the applicability of radioimmunoassay (the analytical method generally used) to prove the presence of a drug (50). In May of 1990 a panel

of judiciaries and scientists from the National Institute on Drug Abuse and the Society of Forensic Toxicology held a conference to evaluate hair analysis for drugs of abuse (51). This panel outlined a list of factors they believe must be attended to in order to evaluate any test results from hair analysis. Included in this list is the suggestion that hair analysis not stand alone in any diagnosis (i.e., should be confirmed by another test) and that standardizations should be developed. They also stressed the importance of compiling more information on certain aspects of drug deposition in hair and hair analysis; namely, mechanisms of drug incorporation, relationship between drug use and concentration in hair, minimum dose required for a positive result, time interval between use and appearance in hair, effect of various washing procedures, and how drug incorporation and retention is affected by individual differences.

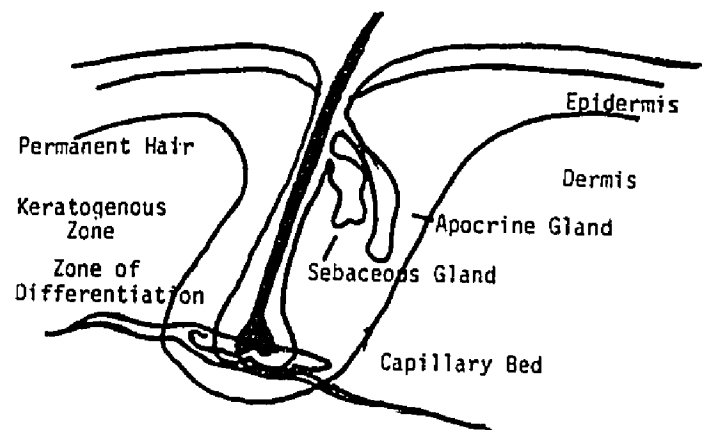


Figure 1. Diagram of hair follicle with the areas of growth and possible sources of deposition for various substances.

Scalp Hair Growth Rate	Reference
386 ± 64 μm/day	35
0.4 mm/day	52
0.2 - 0.5 mm/day	36
0.35 mm/day	53
0.75 - 1.12 mm/day	54
1.1 ± 0.2 mm/day	55
10 mm/day	56
7.1 - 12.0 mm/month	57
1.0 cm/month	58
0.9 - 1.2 cm/month	3
1.0 - 1.5 cm/month	7

Table 1. Some examples of different reported human scalp hair growth rates.

Hair Analysis	Urinalysis
Wide Window of Detection	Detects Short-Term Use (except Marijuana)
Sample Collection Without Embarrassment	Sample Collection Can Be Considered Demeaning
Difficult to Adulterate Sample - to affect analysis or to give false negative	Sample Can Be Diluted, Exchanged, or Adulterated With a Chemical Additive
Similar Sample Can Be Recollected in Case of Dispute	Equivalent Sample Cannot Be Recollected
Great Variability in Pocedures	Quality Control Pocedures Have Been Established
Legal Status Uncertain	Can Be Admissible as Evidence in Court

Table II. Comparison of Hair Analysis and Urinalysis.

**CHAPTER 2: EFFECT OF MURINE RETROVIRAL INFECTION ON
HAIR AND SERUM CONCENTRATIONS OF COCAINE AND MORPHINE**

Introduction: During the last few years the incidence of drug abuse in the United States has shown a dramatic increase. The National Institute on Drug Abuse reported at least a five-fold increase in the use of cocaine over the last decade (59). These data frequently come from self-reporting in survey questionnaires, with significant opportunity for respondent errors and hence inaccuracy of the numbers obtained (60). Testing for the presence of drugs of abuse and/or their metabolites in hair may provide a more reliable clinical measurement which also can be used to validate surveys (61).

While urine testing is the most common screening method for estimating drug use, the half-life of elimination of most narcotic drugs is generally only a few hours. With the exception of marijuana metabolites, which can be identified in urine for periods of seven to 30 days after exposure, results from body fluid analysis generally reveal only whether the individual has used drugs in the one to three days prior to sample collection (62). About 90% of the

administered dose of morphine, the active metabolite of heroin (diacetyl morphine), is excreted within 24 hours (60). Reports on the time limits for the detection in urine of benzoylecgonine, the major metabolite of cocaine, vary from 17 to 46 hours after administration (62). Detectable quantities of cocaine metabolites have been found in urine 120 hours after cessation in only a few cases following heavy, chronic drug use (63).

The ability to detect drugs in hair may provide an alternative to conventional urinalysis since, unlike biological fluids, hair provides a long term record of drug use. Drug metabolites may be detected in hair for several months after the last known use of the drug, in contrast to the few days during which they can be detected in urine (3,38). However, no information is available regarding the influence that age or viral infection may have relative to the amount of cocaine or morphine recovered in hair or serum.

The influence on drug disposition by viral infections may be important as studies have shown that many drug users have had hepatitis infections (64), and increased incidences of hepatitis B, cytomegalovirus and Epstein-Barr virus infections (65). In the United States, intravenous drug users constitute the second largest "at risk" group for developing AIDS (acquired immune deficiency syndrome) (66).

In the present study a mouse model was used to investigate the effects that age, retroviral infection, and drug injection have on concentrations of cocaine and morphine in serum and hair. The retrovirus used was the LP-BM5 murine leukemia virus, a mixture of ecotropic recombinant and defective murine leukemia viruses, which induces an acquired immunodeficiency syndrome in susceptible mice that has many features in common with human AIDS (67-69). We show here that retroviral infection influences the amount of drug incorporated into developing hair follicles following chronic administration of cocaine or morphine.

MATERIALS AND METHODS

Animals and Retroviral Infection: C57BL/6J female mice obtained from Charles River Labs (Willington, MA) (ages 2 months and 18 months) were housed in a ventilated animal room with a 12 hour light cycle. Food and water were supplied ad libitum. The mice were infected by intraperitoneal (i.p.) injection with 0.1 ml of the LP-BM5 murine leukemia virus inoculum which had an ecotropic titer (xc) of $4.5 \log_{10}$ plaque forming units per ml (70). The non-infected control group was injected i.p. with 0.1 ml of the saline vehicle.

Cocaine and Morphine Treatment: After one month of infection with the virus, the mice were injected i.p. with 0.1 ml of saline, cocaine hydrochloride, morphine sulfate, or both morphine and cocaine between 8:00 - 10:00 a.m. 5 days a week. Cocaine hydrochloride and morphine sulfate were kindly provided by the National Institute of Drug Abuse. The initial dosage of cocaine HCl was 20 mg/kg/day (first week), then 30 mg/kg/day (second week), and 50 mg/kg/day for the third week and thereafter. Morphine sulfate was given on the same schedule, beginning with 20 mg/kg/day, then 35 mg/kg/day and 55 mg/kg/day for the third week and thereafter. Mice that received both drugs were given half of the dosage of each in a single injection. At the same time, the saline control group received 0.1 ml of saline i.p. according to the same schedule. Treatment was continued for 6 weeks, after which time animals were sacrificed under ether, and blood was collected from an axillary vein, allowed to clot and centrifuged at 1500 rpm to separate the serum. The serum was stored at -70°C for later analysis.

Hair Sampling and Preparation: One day prior to beginning the drug schedule, a hair sample was obtained by shaving a small area (approximately 2 cm x 4 cm) just above the tail of each mouse using electric clippers (Ostar^R). This same

area was shaved again 28-30 hours after the last drug treatment. The clipper blades were swabbed with 70% alcohol between animals to avoid cross contamination. The hair was stored at -20°C in sealed plastic bags for later analysis.

Drug Extraction from Hair and Assay: The method of extraction of cocaine and morphine was modified from Balabanova et al (71). Each hair sample was thoroughly washed in 2-5 ml aliquots of a 10% soap solution (Tincture Green Soap, Medical Chemical Corp.) and rinsed 5 times with 5 ml aliquots of distilled water. Air-dried 25 mg hair samples were incubated at 37°C for 18 hr in 1.0 ml 0.1 M hydrochloric acid. Aliquots of 0.4 ml of the acid were neutralized with 40 μl of 1.0 M sodium hydroxide to approximately pH 7.5 and made up to 0.5 ml with phosphate-buffered saline. Fifty microliter samples were analyzed using a commercial radioimmunoassay kit purchased from Diagnostic Products Corporation (Los Angeles, CA) according to the manufacturer's recommended procedure. Twenty-five microliter serum samples were analyzed using the radioimmunoassay kit from Diagnostic Products Corporation according to the manufacturer's recommended procedure.

Statistics: Treatment and age groups were compared using analysis of variance. Any p value of 0.05 or less was considered to indicate a significant difference.

RESULTS

Drug Recovery From Hair: A standard curve was prepared from data obtained by dissolving the drugs to achieve concentrations of 0.0 to 10.0 mg/ml of cocaine hydrochloride or 0.0 to 1.0 mg/ml morphine sulfate in a 1:1 chloroform:methanol mixture. Aliquots (0.5 ml) were added to 25 mg samples of drug-free hair and the solvent evaporated under nitrogen gas at room temperature to achieve concentrations of 0.0, 250, 500, 1,000, and 5,000 ng/sample of cocaine, or 0.0, 2.5, 5.0, and 10.0 ng/sample of morphine. The test calibrators supplied with the radioimmunoassay (RIA) kit and the standard curve obtained from the hair prepared in this fashion were compared, and no appreciable difference was found (data not shown). Therefore, the standard curve solutions provided in the kit were used throughout the study. Data were reported as ng drug/mg of hair extracted.

Serum Cocaine and Morphine Concentrations: Serum samples were taken over a 30 hour period following the last i.p. injection. At 2 hr the mean cocaine concentration in the non-virus infected cocaine-treated mice was 4925 ng/ml serum, after 25 hr the concentration was 24 ng/ml, and no

cocaine was detected at 30 hr post injection. The cocaine serum concentrations from retrovirus infected mice were 5961 ng/ml at 2 hr and 74 ng/ml at 24 hr (Table 1).

The mean serum value of morphine in non-infected mice at 2 hr post injection was 682 ng/ml. The mean morphine concentrations 24 and 30 hr after injection were 53 ng/ml, and 16 ng/ml, respectively. In the LP-BM5 infected mice, the concentrations were 897, 69, and 25 ng/ml, respectively (Table 2). The difference in serum drug concentration between retrovirally infected and non-infected mice was significant after 2 hr for both drugs ($p < 0.01$). Cocaine and morphine were not detected in the serum of saline-treated mice.

The area under the plasma concentration versus time curve (AUC) for a drug is proportional to the total amount of that drug entering the blood stream and is inversely related to its clearance from the body. Thus, the AUC can be used as a measure of systemic availability (72). The AUC was determined using the trapezoidal rule. The AUC for cocaine in non-infected mice was 125.6 ($\mu\text{g} \times \text{hr}$)/ml and in the infected mice the AUC was 173.4 ($\mu\text{g} \times \text{hr}$)/ml. The AUC for morphine was 8.59 ($\mu\text{g} \times \text{hr}$)/ml in the non-infected while in the retrovirally infected mice the AUC was 10.37 ($\mu\text{g} \times \text{hr}$)/ml.

Cocaine and Morphine in Hair from Drug Injected Mice: All hair samples collected from mice injected with cocaine or morphine were found to contain measurable quantities of the drugs, while none was detected in the hair of saline-injected controls. Hair drug concentrations were in the range of 316 - 1609 ng/mg for cocaine and 6 - 22 ng/mg for morphine.

Retroviral Infection and Drug Levels: Hair samples from the retrovirally infected mice contained a significantly higher ($p < 0.001$) amount of cocaine and/or morphine than non-infected mice similarly treated. The hair of non-infected mice injected with cocaine contained a mean of 652 ng cocaine/mg hair, compared with 1609 ng/mg in infected mice (Fig 1). Retroviral infection was associated with a similar 2-to-3-fold increase in the amount of cocaine and morphine in the hair of mice injected with both cocaine and morphine compared with virus-noninfected animals. The mean morphine concentration obtained from the hair of non-infected mice was 11 ng/mg hair, whereas the amount found in LP-BM5 virus-infected mice was 20 ng/mg hair (Fig. 2). The concentrations of cocaine and morphine in mice injected with both of these drugs (at 50% of the dose of each drug injected individually) was about half that of mice injected with the single drug. Non-infected mice, which were treated

with both drugs, had a mean value for morphine of 7 ng/mg hair while infected mice from this group had an mean concentration of 10 ng/mg hair. No significant difference in the deposition of cocaine or morphine in the hair of the two different age groups was found (Fig. 1 and 2).

DISCUSSION

This study was initiated to investigate the possible influence of retroviral infection, a common ailment associated with users of street drugs (64,65), on the presence of drug metabolites in the systemic circulation. This situation was simulated by chronic administration of cocaine, morphine or a combination of both drugs to retrovirus-infected mice. The animals were of identical genetic background, thus reducing differences related to variations in individual pharmacokinetics. As all mice in a particular group were the same age and randomly assigned to experimental groups, differences in drug concentration should not be due to alterations in initial estrual cycle, weight, age, sex or genetics. Drugs are most likely incorporated into the hair shaft from the blood which supplies the growing hair follicle. The close affiliation between the arterial and venous blood supply and the active

hair follicle presumably permits the transfer of drugs from the circulation into the growing hair shaft (38). The shaft consists of a matrix of tightly coiled proteins that may bind and encase the drug molecules, permanently embedding them in the growing hair fiber. These entrapped substances cannot be washed out of hair by soap solutions, but can be released by chemical treatment. After hair is washed to remove external contamination, the released substances can be analyzed with conventional RIA technology.

Cocaine or morphine was detected in the hair of all treated mice. Neither drug was found in saline-treated animals. There were no differences due to age in hair concentration of either drug. In addition, a 50% reduction in the amount of cocaine or morphine injected yielded a similar reduction in hair levels. Thus there was no apparent effect of having cocaine present simultaneously with morphine on the level of either drug in hair.

There are conflicting reports as to the correlation found between self-reported drug use and concentrations subsequently found in samples of hair. Baumgartner et al. (38) reported a trend towards a relationship between drug levels in hair and self-reported intake for cocaine and heroin. In an earlier report, however, Baumgartner et al. (24) found significant differences between concentrations of cocaine recovered from the hair of individuals reporting the

same level of use. Puschel also found no correlation between concentration of opiates found in hair and self-reported heroin use (73). Our data show significant variation in the amount of drug incorporated into hair of similarly treated, genetically identical animals. The presence of drug could affect grooming, growth rate of hair, and food consumption with weight loss or gain (20,74), all of which could alter drug levels in hair. The animals treated with cocaine lost 4.4% of their original weight, whereas saline treated animals gained 6.2% of their original weight (Chen et al., submitted for publication).

Retrovirally infected mice progressing to AIDS had more than twice the amount of cocaine and almost twice the amount of morphine in their hair than non-infected mice. Retroviral infection also resulted in increased cocaine and morphine levels in serum, although in both cases the increase was less than two-fold. As determined by AUC, the overall serum concentration of cocaine was 1.4 times higher and that of morphine was 1.2 times higher in the retrovirally infected animals.

Variations in drug concentration could be due to hair growth rate, which varies from individual to individual and over different areas of the body (20,33), or differences in the pharmacokinetics of drug clearance. An individual that clears the drug more quickly would have less available drug

in the blood to deposit in the hair. Thus, the increased levels of both morphine and cocaine in the hair of retrovirally infected mice may reflect altered elimination. The higher drug levels in hair possibly suggest a longer exposure of other tissues during retroviral infection to injected cocaine and morphine. The higher, persistent level of serum cocaine and morphine during retroviral infection offers the opportunity for the same dose to produce greater physiological effects, including immunomodulation (75,76) which could be important in the progression of virally-infected individuals to AIDS. In another study, natural killer cell activity was shown to be increased proportionally to the levels of cocaine recovered in the hair of human subjects (Chapter 5). Other tissues also may be damaged by persistent cocaine and morphine levels during retroviral infection.

Serum was negative for cocaine 30 hr after the final treatment, and only small amounts of morphine were detected. As both drugs were found in the hair of all treated mice, but in none of the untreated animals, hair analysis seems to provide a better method of identification of previous drug use than biological fluids such as serum. The fact that the animals were treated daily over a long period of time means that cyclic variations between individual mice are unlikely to explain the variation in long term recovery in hair.

Our data support the use of hair testing to determine the level of intake for cocaine and morphine as a qualitative and semi-quantitative measure for these drugs in drug-abusing groups, although the suitability of analysis for these drugs in hair to quantitate individual past use needs further development. Data obtained from human subjects exhibit greater variability than that obtained from the animal studies, perhaps reflecting a variety of factors such as inaccurate recall, deliberate misrepresentation, differences in mode of administration, variations in rates of absorption or metabolism, and variable purity of drugs obtained on the street (Chapter 4).

The feature that distinguishes the hair radioimmunoassay from other tests to detect drugs of abuse is the unique potential of hair to provide a chronological record of drug use. Human hair grows at the rate of 1.2 ± 0.18 cm/month (Chapter 1), thus a history of drug exposure can be made by analyzing various sections of hair that represent different periods of growth. Such sectional analyses can be done using less than 10 mg of hair. The retrospective capacity of hair to record drug use has been replicated in several other laboratories (1,29,77). This type of data is not available from body fluid specimens typically used in drug screening (2,23,29,62,63). Studies in a variety of settings have shown that urinalysis tends to miss a rather large

number of drug users that are subsequently identified by hair analysis, mainly as a result of the wide window of detection available with the latter technique (38). The unique capacity of hair analysis to uncover chronic drug abuse patterns may make this technique an important adjunct to medical diagnosis, rehabilitation evaluation, and other applications where historical information is required.

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Supported by NIH grant DAO4827. The capable technical assistance of Bruce Smith and Guan-jie Chen in treating the animals is appreciated.

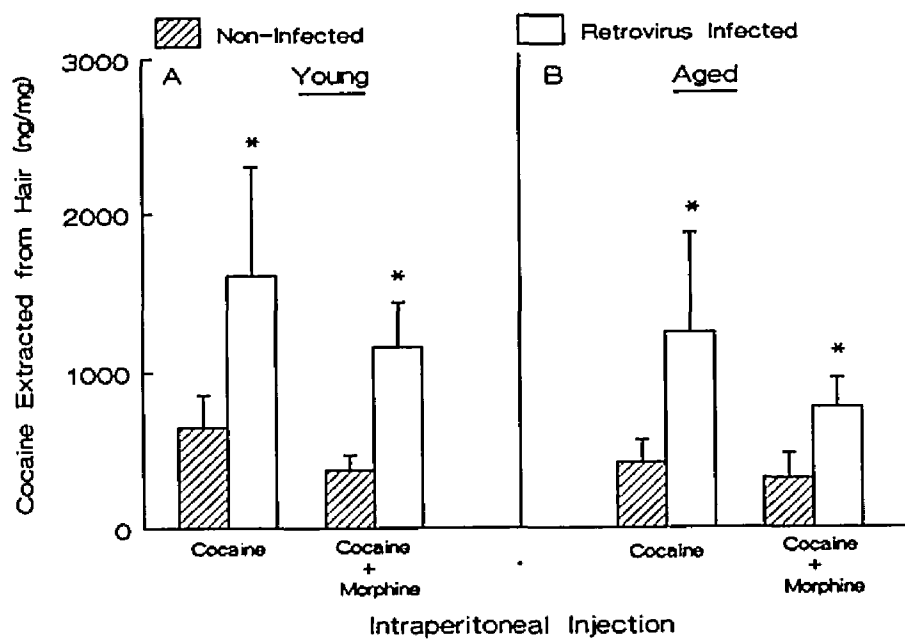


Figure 1. Cocaine concentrations in the hair of syngeneic C57/BL mice which were injected with 50 mg/kg cocaine or 25 mg/kg cocaine plus 25 mg/kg morphine.

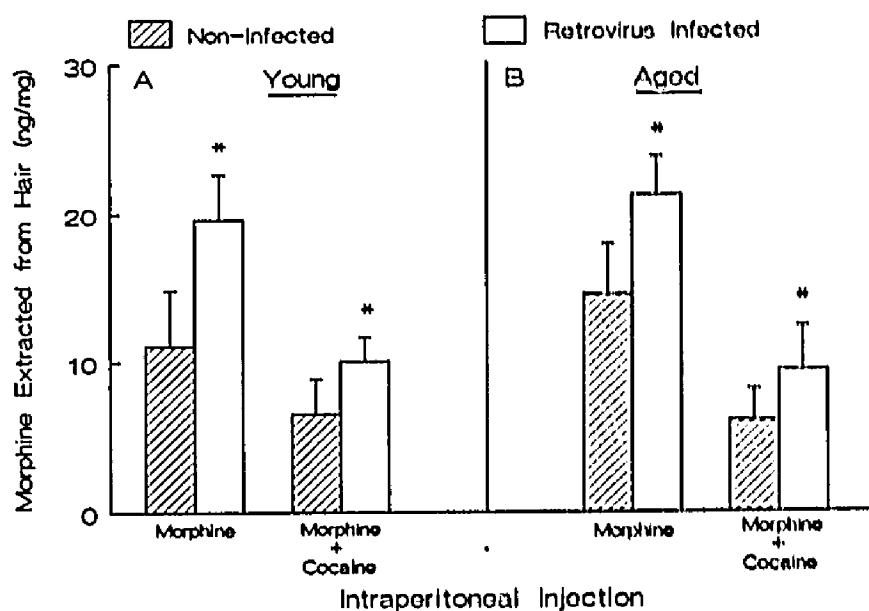


Figure 2. Morphine concentrations in the hair of syngeneic C57/BL mice infected with LP-BM5 or left uninfected and injected with 55 mg/kg morphine or 25 mg/kg morphine plus 25 mg/kg cocaine.

Time	Serum Cocaine ($\mu\text{g/ml}$)	
	Non-Infected	Retrovirally Infected
20 min	229.04 \pm 5.53	136.39 \pm 5.95
40 min	40.16 \pm 4.06	234.58 \pm 23.91
1 h	29.47 \pm 5.50	47.72 \pm 17.29
1.5 h	7.95 \pm 0.58	12.27 \pm 2.28
2 h	4.92 \pm 0.93	5.96 \pm 1.88
5 h	1.95 \pm 0.62	2.43 \pm 0.46
10 h	0.35 \pm 0.039	0.44 \pm 0.058
16 h	0.20 \pm 0.054	0.29 \pm 0.064
24 h	0.024 \pm 0.016	0.064 \pm 0.036
30 h	Not Detected	Trace

Table I. Serum cocaine concentrations. Syngeneic C57/Bl mice were infected with LP-BM5 or left non-infected before i.p. administration of 50 mg/ml of cocaine hydrochloride for 6 weeks. Mice were sacrificed at the times indicated and serum cocaine levels determined by RIA. Values are expressed as means \pm S.D. for 5 mice per time point.

Time	Serum Morphine (ng/ml)	
	Non-Infected	Retrovirally Infected
20 min	3,738 ± 996	3,419 ± 888
40 min	2,453 ± 309	3,556 ± 1,077
1 h	1,976 ± 217	2,264 ± 118
2 h	682 ± 43	897 ± 135
5 h	285 ± 4.0	346 ± 56
24 h	53 ± 19	69 ± 40
30 h	16 ± 14	25 ± 27

Table II. Serum morphine concentrations. Syngeneic female C57/B1 mice were infected with LP-BM5 or non-infected before i.p. administration of 55 mg/kg of morphine sulfate. Mice were sacrificed at the times indicated and serum morphine levels determined by RIA. Values are expressed as means ± S.D. for 5 mice per time point.

**CHAPTER 3: ETHANOL INDUCED CHANGES IN SERUM
AND HAIR COCAINE CONCENTRATIONS**

INTRODUCTION

A national survey on drug abuse showed the concurrent use of cocaine and alcohol to be common (78). Cocaine use has been shown to be associated with many toxicities, including, liver injury, rhabdomyolysis, cerebrovascular accidents, pulmonary edema, ischemia, and immune abnormalities (75,79-81). Hepatotoxicity of cocaine in mice has been shown to be strain and sex dependent (82-84), and only two case reports of hepatotoxicity in man have been reported (85,86), although liver dysfunction has been reported in a cross-sections of cocaine using patients (79). Ethanol has been shown to potentiate cocaine-induced liver injury in mice (87,88). The hepatotoxicity of ethanol, on the other hand, is well documented in humans and in animal models (89,90).

Although cocaine is metabolized to several products, only norcocaine seems to have biological activity (91). The largest group of cocaine metabolites in man are the products

of hydrolysis, ecgonine, benzoylecgonine, and ecgonine methylester. These comprise about 90% of the excreted metabolites, with only about 5-10% being excreted as the parent compound (92,93). N-hydroxynorcocaine and norcocaine nitroxide are more minor metabolites which are produced by N-demethylation, mainly in the liver, by the P-450 system (92). N-hydroxynorcocaine appears to be responsible for the toxicity seen in some cases through its oxidation to a nitrosonium ion which can ultimately lead to lipid peroxidation (94).

It is probable that the potentiation of cocaine hepatotoxicity by ethanol is due to an interaction with the P-450 system which may result in an increase in N-hydroxynorcocaine produced. It has been shown that ethanol is metabolized by the P-450 system as well as by acetaldehyde dehydrogenase (95). Ethanol also induces some P-450 isozymes, namely P-450IIE1 (89). A general proliferation of endoplasmic reticulum has been noted as well (96).

A dose-response curve for the elevation of serum glutamate-oxaloacetate transaminase in response to cocaine has been demonstrated (94). Ethanol could change this dose-response curve or alters systemic availability of cocaine. Therefore, the effect of ethanol on cocaine concentrations in hair and serum of male C57/Bl mice was investigated.

MATERIALS AND METHODS

Animals: Three-to-five week old male C57/BL6 mice (Charles River Labs, Wilmington, MA), weighing between 19-22g, were randomly housed 5 to a cage in an approved animal care facility with a 12 hr light cycle. Animals were assigned to one of four treatment groups: group 1 (control), which was fed a dextrin-maltose (isocalorically substituting for ethanol) Lieber-Decarli diet (Dyets; Lieber/Decarli Formula; Dyets, Inc. Bethlehem, PA) and injected with saline (0.1 ml); group 2, fed a Lieber-Decarli ethanol diet (5.05% v/v, representing 27% of the total calories) and injected with saline; group 3, fed the Lieber-Decarli control diet and injected with cocaine (40mg/kg/day); and group 4, fed the Lieber-Decarli ethanol diet and injected with cocaine (40mg/kg/day) (Table I).

Ethanol was introduced into the diet for one week at half the final concentration used in the experiment to allow the mice to become accustomed to it. The diet was prepared according to the manufacturer's recommended procedure (Dyets, Inc., Bethlehem, PA). The animals were maintained on this diet for either 5 or 9 weeks.

Cocaine hydrochloride was obtained from the National Institute for Drug Abuse (Rockville, MD). Cocaine

administration was by i.p. injection. Prior to the first week of treatment with the full concentration of alcohol the mice injected with cocaine were given half the final concentration (20 mg/kg) to reduce cocaine and ethanol induced mortality. Following that week, mice were injected with 40 mg/kg cocaine hydrochloride 7 days a week over the 5 or 9 week time periods. The animals were sacrificed under ether at the end of either the fifth or ninth weeks.

Serum Samples: For the first four weeks of treatment, blood was collected once a week from the retrosinus orbital of 4 mice per group 2 hr after cocaine injection. Blood was also collected from the axillary vein at the time of termination of the experiment, 24 hr after the final injection of cocaine. Serum was obtained by centrifuging the blood at 1500 rpm after the blood was allowed to clot. Twenty-five microliter serum samples were analyzed for cocaine concentration using the radioimmunoassay (RIA) kit from Diagnostic Products Corporation (Los Angeles, CA) according to the manufacturer's recommended procedure.

Serum ethanol concentrations were determined by gas chromatography. One microliter serum samples were injected onto a glass wool plug on top of a Porpack Q Column (Alltech Assoc., Deerfield, IL) maintained at 180°C (97). Peak area for individual samples were compared to those obtained by

injection of standard solutions of known amounts of ethanol onto the column.

Hair Samples: At the end of either the 5 or 9 week treatment periods, an area just above the tail of each mouse was shaved close to the skin using electric clippers (Ostar^R). The hair was stored at -20°C in sealed plastic bags for later analysis. The analysis of these hair samples for cocaine was performed as in chapter 1.

Statistics: All data were reported as mean \pm standard deviation (S.D.). Values were analyzed by one-way analysis of variance to determine if there was a difference between groups. A p value of less than 0.05 was considered significantly different.

RESULTS

Serum Cocaine Concentrations: Two hours following injection of cocaine (at approximately 11:00 am) the serum cocaine concentrations were consistently higher in the mice fed the control diet and injected with cocaine (group 3) over the ethanol-fed, cocaine injected mice (group 4). In the first week, the cocaine concentration in serum was highest overall

in the control-fed animals, and the difference between groups 3 and 4 was the greatest, $4.8 \pm 0.9 \mu\text{g/ml}$ compared to $1.5 \pm 0.7 \mu\text{g/ml}$, respectively ($p < 0.001$). Over the next three weeks the serum cocaine concentration in group 2 mice declined to a minimum of $2.4 \pm 0.5 \mu\text{g/ml}$ ($p < 0.005$); whereas there was no significant decline in the concentration of cocaine recovered in the serum of the group 4 mice during this time period (Fig. 1).

At termination, the serum cocaine concentrations were still detectable 24 hr after the final injection. Again, group 3 (control fed) mice had serum cocaine concentrations which were higher at 5 and 9 weeks than in the group 4 mice. The cocaine concentrations within either group were not different after 5 or 9 weeks (Fig. 2). No cocaine was detected in the serum of saline injected animals.

Hair Cocaine Concentrations: The hair cocaine concentrations followed the same pattern as the serum concentrations. In the cocaine-injected, maltose-dextrin fed animals (group 3) the hair cocaine levels were higher after both treatment lengths. In group 3 animals, the concentrations were 35.2 ± 6.1 after week 5 and $53.8 \pm 12.2 \mu\text{g/mg}$ hair after the 9th week ($p < 0.01$). In group 4 animals the hair cocaine concentrations were $22.9 \pm 3.8 \mu\text{g/mg}$ hair after week 5 and $41.5 \pm 6.8 \mu\text{g/mg}$ hair after week 9 ($p < 0.001$). At the end

of the experiment, the hair cocaine concentration was significantly lower in the ethanol-fed animals ($p < 0.005$). No cocaine was detected in the hair of the saline injected animals.

Serum Ethanol Concentrations: The serum ethanol concentrations varied greatly both within and between groups. In the first two weeks no ethanol was recovered in the serum of the non-cocaine injected animals (group 2). Approximately 58 mg ethanol/100 ml serum were recovered from the serum of the cocaine-injected mice (group 4), with standard deviations encompassing almost 90% of the mean value. The ethanol concentrations for the group 4 animals did not change significantly over the 4 week period. In weeks 3 and 4 ethanol was detected in the ethanol-fed saline-injected animals, again with standard deviations approximately 90% of the mean values (Fig. 4).

CONCLUSIONS

It is believed that drug and/or drug metabolite(s) are deposited in the growing hair by diffusing from the capillary bed which supplies the bulb of the hair follicle with nutrients (38). A measure of cocaine in hair should correlate with blood cocaine concentration over the length

of time the hair is growing (Chapter 2). The present data demonstrate that a diet containing ethanol decreases the systemic availability of cocaine in mice as estimated by hair cocaine level.

The RIA used does show a cross-reactivity with cocaine metabolites, but not with a variety of other substances tested (Diagnostic Products Corp.). A test of benzoylecgonine, ecgonine methyl ester and ecgonine added to drug free urine and measured using this procedure indicated a 98.8% antibody specificity for benzoylecgonine with approximately a 10% cross-reactivity for ecgonine methyl ester and approximately a 0.8% cross-reactivity with the ecgonine (Chapter 4). Another report found similar cross reactivities for these metabolites and a cross reactivity for norcocaine to be 63.5%, while cocaine was shown to cross react 70 times greater than benzoylecgonine (97). Therefore, the decrease in hair and serum cocaine concentrations may simply be due to a different drug-drug metabolite profile.

The mechanism of cocaine-induced hepatotoxicity is unknown, but it has been proposed to be mediated through lipid peroxidation (98), possibly with the depletion of hepatic reduced glutathione as a secondary consequence (99). Norcocaine produced through cytochrome P-450 oxidation may take part in a futile cycle which uses up the glutathione

(94). However, an increased hepatic glutathione concentration has been observed with chronic cocaine administration with hepatic toxicity, possibly representing a compensatory mechanism (99).

A great deal of strain, sex and developmental differences have been noted for the development of cocaine-induced hepatotoxicity in mice (83). This has led to the suggestion that the dissimilarity in hepatotoxicity seen is due to a difference in one or more of the enzymes involved (100). Although cytochrome P-450 enzyme induction by ethanol has been demonstrated, it has been reported that induction of P-450IIE1 by ethanol does not result in an increased binding of cocaine in microsomal systems (101).

Only cocaine and norcocaine have been shown to have clinical activity (91,94,102). These data suggest that a change in the systemic profile of these compounds may be responsible for the altered toxicity of cocaine with ethanol. The ethanol-mediated cocaine toxicity may be due to an increase in the metabolism to norcocaine (through cytochrome P-450 induction) or through a decreased hydrolysis leading to a greater availability of the parent compound.

Treatment Group	Diet	Injection
1	Dextrin-Maltose	Saline
2	Ethanol	Saline
3	Dextrin-Maltose	Cocaine
4	Ethanol	Cocaine

Table I. Treatment groups, mice were fed Lieber-DeCarli Dextrin-maltose (control), or Ethanol Diets and injected with either cocaine or saline.

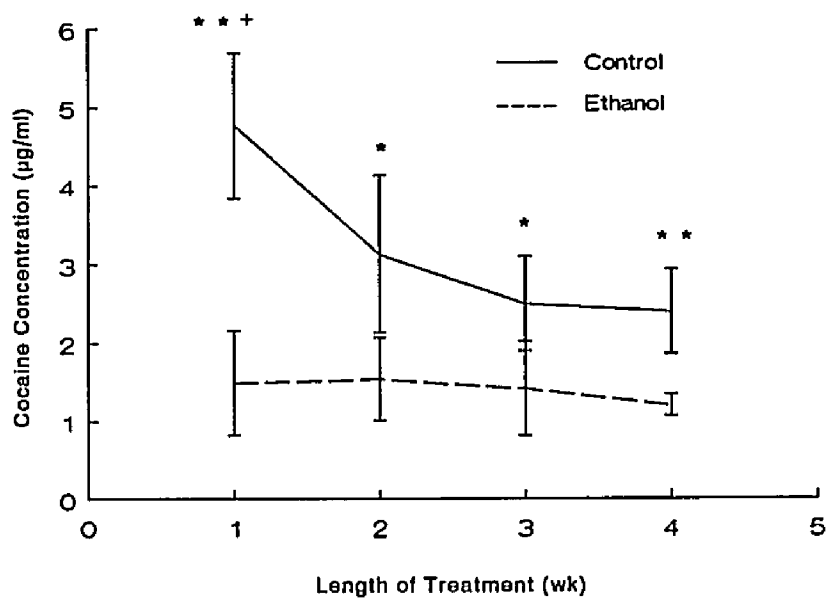


Figure 1. Weekly serum cocaine concentrations. * significant difference between treatment groups, $p < 0.05$, ** $P < 0.001$. + significant difference between time points in the same group, $p < 0.005$.

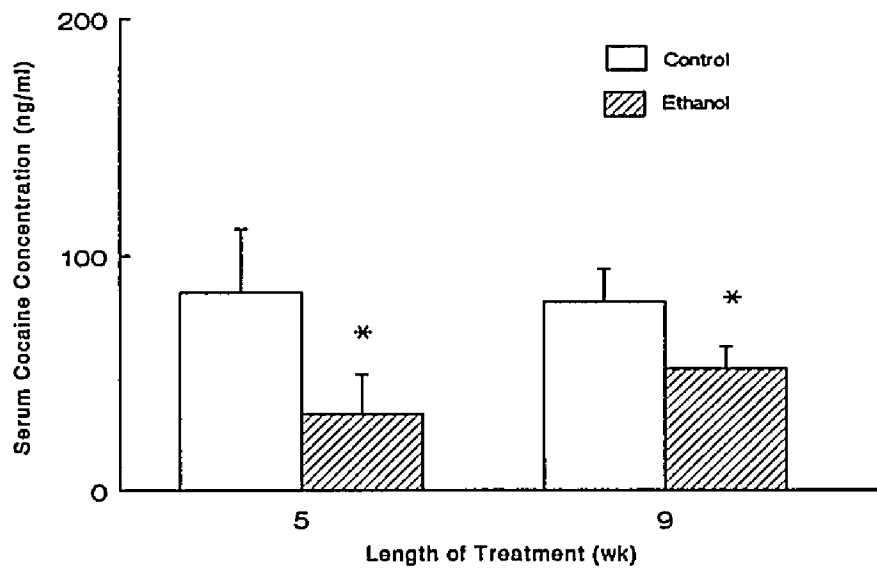


Figure 2. Serum cocaine concentrations 24 hr after final treatment. * significant differences between treatment groups, $p < 0.005$.

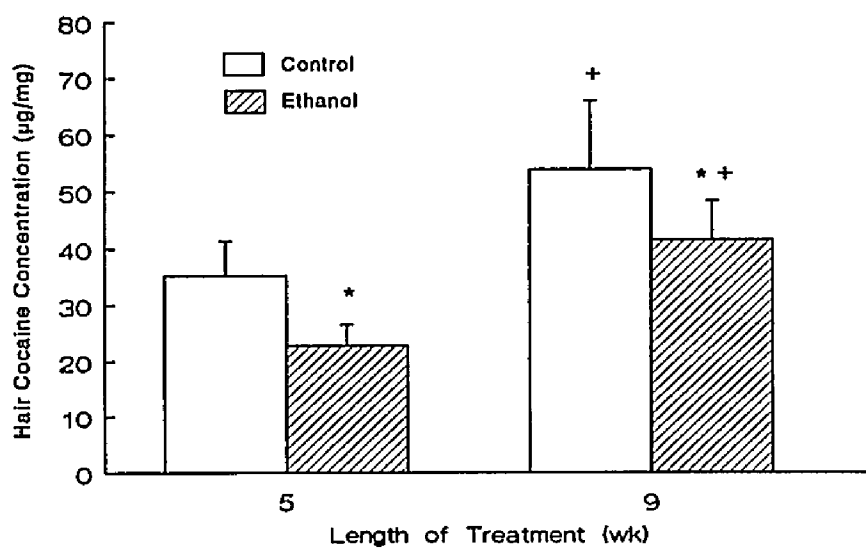


Figure 3. Hair cocaine concentrations after final treatment.
* significant difference between treatment groups, $p < 0.005$. + significant difference between time points, $p < 0.005$.

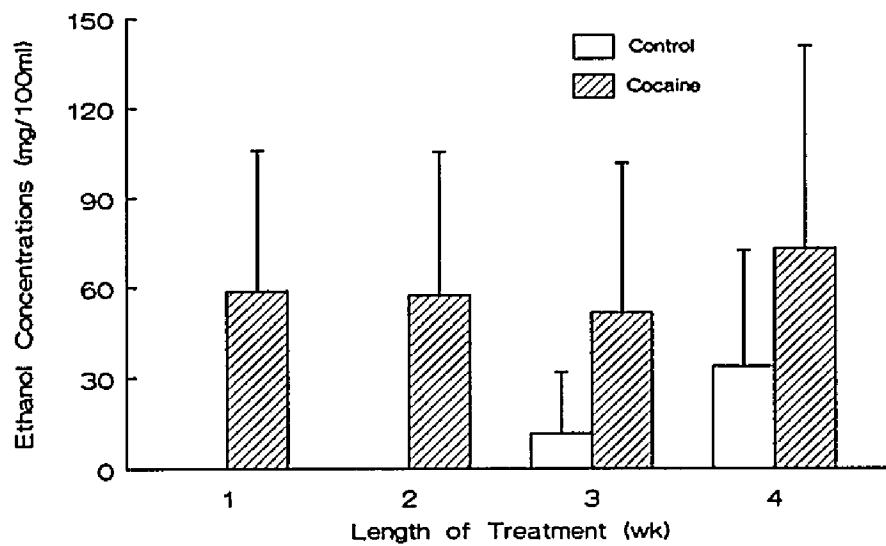


Figure 4. Serum ethanol concentrations, mean \pm S.D., over the first 4 weeks of treatment.

CHAPTER 4: COCAINE METABOLITE IN
HAIR AND URINE OF DRUG USERS

INTRODUCTION

According to the 8th National Household Survey on Drug Abuse (1985) the number of U.S. citizens who were current users of marijuana, cocaine or heroin was estimated at 18 million, 6 million and 160,000 respectively (104). Drug testing by urinalysis has long been the standard method by which illegal drug use is identified since it provides a rapid and inexpensive evaluation of an individual's drug status. However, the technique is limited not only by the relative ease with which samples may be adulterated or exchanged but also by the short half-life of elimination of some drug metabolites. For opiate and cocaine metabolites, detection limits in urine are usually reached between 48 and 72 h following administration of the parent drug (60). These restrictions have led to the development of techniques designed to provide greater information on the pattern and duration of drug abuse in habitual users.

While some quality control parameters remain to be established (3,49), the method of hair analysis may prove useful for clinical studies which require a comparison

between the amount of drug in biological samples, and the effects of drugs of abuse on biological processes. We considered it of interest, therefore, to determine the correlation that might be established between two different methods used to identify drug use, namely hair and urine analysis. We report here that the status of a suspected drug-using individual is not always adequately described by self-report, and that hair analysis does appear to provide a wider window of drug identification than the more commonly used method of urinalysis.

METHODS

Subjects: Hair and urine samples, and verbal data were collected from 47 men attending the Community Outreach Program for AIDS in Southern Arizona (COPASA) in Nogales, Arizona (age range 14-41 years, mean 25.9 years). The 47 subjects were recruited from this pool. The study was approved by the Human Subjects Committee which also approved advertisement for intravenous drug users and non-users. COPASA clients are primarily individuals with a drug-using life style. The overall incidence of HIV seropositivity in 800 subjects screened in the COPASA program has been found to be less than 3% (Erickson & Estrada, unpublished data) and none of the participants in our study reported either

infection with HIV or symptoms of AIDS. Interviewers were told to take any male who volunteered and to inform subjects that participation did not depend on drug-use history. All subjects taking part in the study completed a detailed questionnaire relating to past and present drug use. Informed consent was obtained from all subjects, using forms approved by the Human Subjects Committee of the University of Arizona. A control group consisted of 10 male laboratory personnel (age range 18-49, mean 33.5 years).

Extraction of Drugs from Hair: Scalp hair was cut close to the skin at the back of the head of each individual, placed into a zip-lock plastic bag and stored at -20°C . Scissors were cleaned by swabbing with 75% ethanol on gauze after each collection to prevent cross-contamination of hair samples. Urine samples were obtained at the time of participant presentation and frozen at -70°C until analysis. Each hair sample was placed into a clean Buchner flask, washed once with soap solution (Tincture Green Soap; 10% in distilled water), rinsed four times with distilled water and left to air-dry on absorbent paper. A portion of hair (100 mg) was taken from the proximal (cut) end of the original sample and was approximately 3.5 cm in length, representing the past 2-3 months of growth. To extract drug from hair, each hair sample was incubated with hydrochloric acid (0.1

M, 1.0 ml) for 18 h at 37°C. The acid extracts were neutralized with sodium hydroxide (1.0 M, 100 μ l) and phosphate-buffered saline (0.15 M, pH 7.4) was added to give a final volume of 2.0 ml. Fifty microliters of each sample was analyzed in duplicate by radioimmunoassay (RIA) on the same day. The amount of drug per 2.0 ml of extract was assumed to be equivalent to the amount per 100 mg of hair.

Radioimmunoassay: Prior to analysis, urine samples were centrifuged (300 x g, 10 min) to sediment particulate material. The pH at 25° C of each supernatant fraction was found to be in the range 5.5-7.5 and was not adjusted. Aliquots (50 μ l) of hair extracts and urine samples were analyzed for cocaine metabolite, benzoylecgonine (BE) (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA) (competitive binding ligand 125 I-labelled BE) and urine samples additionally assayed for cannabinoids (including Δ -9-tetrahydrocannabinol, THC) by a double-antibody 125 I solid phase RIA (Coat-a-Count) according to the manufacturer's recommended procedure. Briefly, 200 μ l of 125 I-labeled metabolite is added to the sample, then 200 μ l of the metabolite anti-sera is added. After the samples are incubated for 1 hr, 1.0 ml of precipitating solution (donkey anti-sheep gamma globulin and polyethylene glycol) is added and the samples are incubated for another 15 min. The

sample is then centrifuged at 3000xg for 15 min, the supernatant decanted, and the pellet counted on a gamma counter. The amount of radioactivity per sample was determined using an LKB Automatic Gamma Counter interfaced with an IBM microcomputer. Calibration curves were generated using processed human urine standards for BE and cannabinoids as supplied by the manufacturer. The line of best fit between duplicate of 7 different concentrations samples was generated by microcomputer and correlation coefficients were consistently 0.997 or higher. Drug levels in urine were expressed as nanograms of drug per milligram creatinine (creatinine levels for all samples determined by MedTox Laboratories., St. Paul, MN).

Adsorption of Drug to Drug-Free Hair: To determine whether the manufacturer's standard curves for benzoylecgonine (generated from urine-based calibrators) were applicable to the measurement of these compounds in extracts of hair samples, "test" standard curves were generated by overnight incubation of washed, drug-free hair from the laboratory controls with cocaine or BE hydrochloride (obtained from the National Institute on Drug Abuse (NIDA), Rockville, MD) in a methanol-chloroform (1:1) mixture. The solvent was removed by nitrogen evaporation. Following acid hydrolysis and neutralization, extracts were analyzed by RIA and the

standard curve generated compared with that obtained by assay of the manufacturer's urine-based calibrators.

RESULTS

According to the manufacturer, the lower limits of detection for BE and cannabinoids, the values approximately 2 standard deviations below the counts of maximum binding, were 3 and 2 ng/ml, respectively. We established our own cut-off levels by adding known amounts of drug to drug-free, processed human urine (DPC) or (in the case of BE) to drug-free normal hair and performing serial dilutions to give final concentrations in the ng/ml range. Lines of best fit were calculated using the SlideWrite Plus computer software program (Sunnyvale, CA) and cut-off levels for positivity taken at the point at which the standard curve lost linearity. For THC we used a cannabinoids standard previously quantified by gas chromatography/mass spectrophotometry (GC/MS) (ConDoa, DPC). BE was used in the form BE hydrochloride, dissolved initially in distilled water and finally in drug-free processed human urine.

The standard curve derived in this manner for cannabinoids in urine was linear to approximately 20 ng/ml (Fig. 1). This value was twice as high as that obtained as

the mean + 2 S.D. of cpm values (9.3 ng/ml) of urine samples from 10 laboratory controls presumed not to be using illegal drugs, and thus was used to represent the cut-off level for THC metabolite in urine. Similarly we obtained a standard curve for BE which was linear to approximately 50 ng/ml BE for BE in urine (Fig. 2) and to 60 ng BE added to hair (Fig. 3). Although it is as yet impossible to obtain a "gold" standard for drug in hair (i.e. a known quantity of drug embedded in the hair matrix as opposed to surface adsorbed) we showed that the standard curve of L-cocaine-spiked hair samples (100 mg hair) deviated only slightly and not significantly from that generated from the manufacturer's calibrators and was linear to a concentration of added cocaine of 25 ng (Fig. 4). The BE antibody used in this RIA exhibits cross-reactivity of 104% with BE and 7259% with L-cocaine (98), and based on this information and on the data obtained from serial dilution procedures outlined above we chose cut-off points for positivity of test urine samples of 50 ng/ml, and for hair samples of 25 ng/sample (100 mg hair). Again, because the anti-BE antibody exhibits a high degree of cross-reactivity with cocaine, results were expressed as cocaine and BE combined (cocaine/BE), rather than as BE alone.

Crossreactivity with other Metabolites: BE added to drug-free urine at 6000, 2000, 667, 222 and 74 ng/ml was determined by RIA as 5672, 2226, 700, 206 and 67 ng/ml, indicating 98.8% antibody specificity. Ecgonine methyl ester (NIDA) added at 5000 and 1000 ng/ml was determined by RIA as 557 and 135 ng/ml respectively, indicating 10% crossreactivity, and ecgonine HCl (NIDA) added at 5000 and 1000 ng/ml was determined as 40.5 ng/ml and undetectable, suggesting crossreactivity of approximately 0.8%.

GC/MS Confirmation: Cannabinoid-containing urine-based solutions determined by GC/MS (ConDoa, DPC) to contain 117 ng/ml and 17 ng/ml cannabinoids corresponded with similar results determined by RIA (116 ng/ml and 14 ng/ml cannabinoids, respectively) (91% concordance). Drug-free human urine samples spiked with BE were kindly assayed by Dr. Pooran Chand (EHRT, Birmingham, AL) by GC/MS. Samples containing 2000, 667, 222 and 74 ng/ml BE were determined by GC/MS to contain 1754, 623, 221 and 78 ng/ml respectively (96% concordance). Of test subject hair samples, four (100 mg portions) were found to contain 45.5, 36.1, 22.9 and 16.1 ng cocaine/BE per mg of hair by RIA; 10 mg portions of the same samples analyzed by GC/MS (Drs. W. Baumgartner, C. Berka & T. Donahue, Psychomedics Corp., Santa Monica, CA) contained 31.8, 8.3, 28.6 and 5.70 ng cocaine/BE per mg

hair. The difference between the two methods in terms of quantity of cocaine/BE is presumably due to differences in the part of the hair sample analyzed: we had already removed 3 cm portions from the end of each sample for analysis by RIA whereas the Psychomedics Corp. determined quantity of cocaine/BE in 10-100 uncut strands of the same samples. Nevertheless, the four hair samples determined by RIA to contain the highest levels of cocaine/BE were also found to be the highest drug-containing samples by GC/MS. In addition, hair samples determined by RIA in this laboratory to be drug-negative were also negative by GC/MS, suggesting a reasonable degree of correlation between the two methods of analysis.

Cocaine/BE in Urine and Hair Samples of Subjects: Based on the cut-off levels stated above, only 4.3% of urine samples were determined to be cocaine/BE-positive (113 and 161 ng/mg creatinine). Neither of the individuals donating these samples claimed use within the previous 96 hr. Both, on the other hand, were positive for cocaine/BE in hair. Five subjects reporting use between 36 and 72 hr previously, tested negative for cocaine/BE in urine, suggesting either a discrepancy between verbal report and actual assay data or that metabolite elimination had taken place during this time period.

In contrast to the low incidence of positive urine samples, approximately 55% of subjects tested positive for cocaine/BE in hair. The majority of these samples (42%) contained between 25-100 ng cocaine/BE, the remainder being distributed approximately equally between those containing 100-250 ng, and more than 250 ng (Fig. 5). This indicates that hair analysis can readily identify drug use which is not detectable by urinalysis. Although the majority of cocaine/BE positive hair samples were obtained from subjects that reported using cocaine between 10 days and two months prior to sample collection, we found there to be no relationship between the stated frequency of cocaine use (as provided by verbal questionnaire) and levels of cocaine/BE found in hair.

Marijuana: Seventy-four per cent of subjects were positive for cannabinoids as determined by RIA. The majority of urine samples (57.6%) contained cannabinoids in the range 100-1000 ng/mg (creatinine), whereas only one sample contained more than 5,000 ng/mg (Table I). The pattern of excretion of the THC metabolites with respect to verbal report of last use was approximately normal with the peak rate of elimination in the time period 13-24 hr following last (reported) use. However, metabolites were also detected in samples obtained

from several individuals who claimed abstinence of drug over at least the previous three days (Fig. 6).

DISCUSSION

The main purpose of the present study was to further establish methods by which drug histories could be evaluated in order to determine the effects of regular use on biological parameters. Analyses of urine samples indicated that most of the subjects investigated in this study were regular marijuana users, whereas urinalysis identified only 4.3% (two subjects) as recent users of cocaine. The data indicate that analysis of urine samples appears to be adequate only for the identification of recent and frequent drug use. Measurement of urine levels of cocaine/BE in the absence of a simultaneous analysis of hair extracts would have prevented the identification of almost 93% of cocaine users. On the other hand, claims of recent use of marijuana were always supported by the detection of cannabinoids in urine. Marijuana has an apparent half-life of excretion in the range 1-10 days (105) while the half-lives of both cocaine and its two major metabolites, ecgonine methyl ester and benzoylecgonine, are comparatively short (0.5-1.5, 4 and 6 h respectively) (106). Furthermore, chronic use of marijuana can result in cannabinoid-positive urine samples

up to 36 days after the last reported use (107) and particularly heavy use may give positive results for more than 60 days (108). In concordance with these reports we found that intermediate and high concentrations of cannabinoids were still detectable in several urine samples from individuals claiming a 4-6 day abstinence from the drug. Conversely, the detection time of BE in urine following a single administration of cocaine is generally less than 72 h (60,109) although it was shown recently that BE may be excreted for as long as 120 h after cocaine use (63). These findings were, however, attributed to chronic, extremely heavy use of cocaine. Our subjects did not (according to hair assay data) fit into this particular category but appeared instead to be low-level, infrequent users of cocaine. This postulate was confirmed by the finding that the content of BE found in hair samples of subjects was generally low (less than 250 ng).

Although little is known about drug uptake into hair, the process may involve interaction of the metabolite with cystine residues of the hair protein fiber network (3). Incorporation into the hair matrix is thought to occur in proportion to drug plasma concentration, which in turn depends on the amount of drug administered and rates of clearance. In the present study we observed several instances in which admission of use of cocaine conflicted

with a cocaine/BE-negative hair sample, and vice versa. Secondly, when in a subsequent study the levels of heroin metabolite (morphine) in urine and hair were examined, the number of samples actually testing positive (no urine samples, two hair samples) bore little relationship to the 51% of subjects that claimed use. Our experiments with genetically-identical mice injected with cocaine or morphine have indicated that the amount of drug extracted from hair is related to dose (Chapter 1). In these mice the co-injection of cocaine and morphine did not affect the hair levels of either BE or morphine, and there were no false positive or false negative hair samples. Hence the conflicting data obtained with human subjects regarding frequency of self-reported use of drug and actual content of metabolite detected in hair samples could be due to differences in individual pharmacokinetics, poor recall or ignorance of the purity of the drug administered (61). Our results suggest the need to use analytical procedures to support and show validity of data provided by verbal reports, particularly if payment for participation is made. The subjects in our study were Hispanic men rather than a random sample of the drug-using population in the U.S., and thus, the data (as with any small sample) are not appropriate for extrapolation to that of other populations. Nevertheless, the pattern of drug use observed with these

individuals appears similar to data obtained by survey analysis, i.e., a high incidence of cocaine and marijuana abuse, with a lower number of cases of heroin use. Our study illustrates that surveys which depend only on verbal data for evidence of drug use may suffer from a lack of recall or veracity of reporting on the part of the subject. Thus it is important to establish a reliable method whereby past and present drug use may be properly evaluated. Hair analysis as a method for evaluating the drug status of an individual has several obvious advantages over urinalysis. First, long-term drug use may be evaluated, depending on hair length; second, samples are not easily adulterated and can be recollected later if results are questioned; and last, the method of collection does not cause embarrassment to the subject. On the other hand, a recent dictum by the U.S. Food and Drug Administration limits use of the technique in mass screening procedures (e.g. pre-employment) on the basis that quality control procedures have not yet been established (49). Further, sample preparation can be more labor-intensive than urinalysis, particularly if a large number of subjects is involved. However, we believe that, for studies using small numbers of subjects for measurement of biological changes, hair analysis shows particular merit for confirmation of drug use.

Acknowledgements

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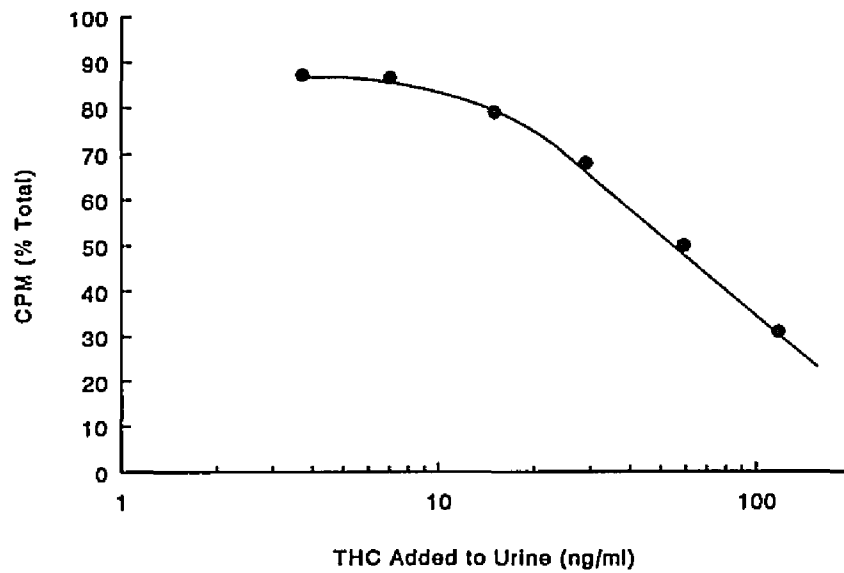


Figure 1. Detection limit of cannabinoids in urine. The cut-off level for positivity was taken at the point at which the dilution curve lost linearity. Values are expressed as percent cpm of reference counts (duplicate samples).

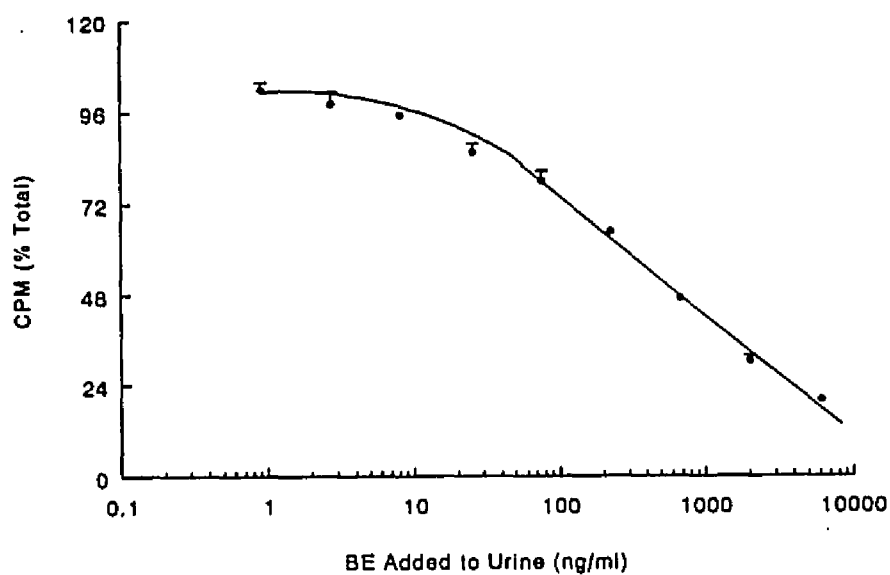


Figure 2. Detection limit of BE in urine. Values are means \pm S.D. of three separate experiments and are expressed as percent cpm of reference counts.

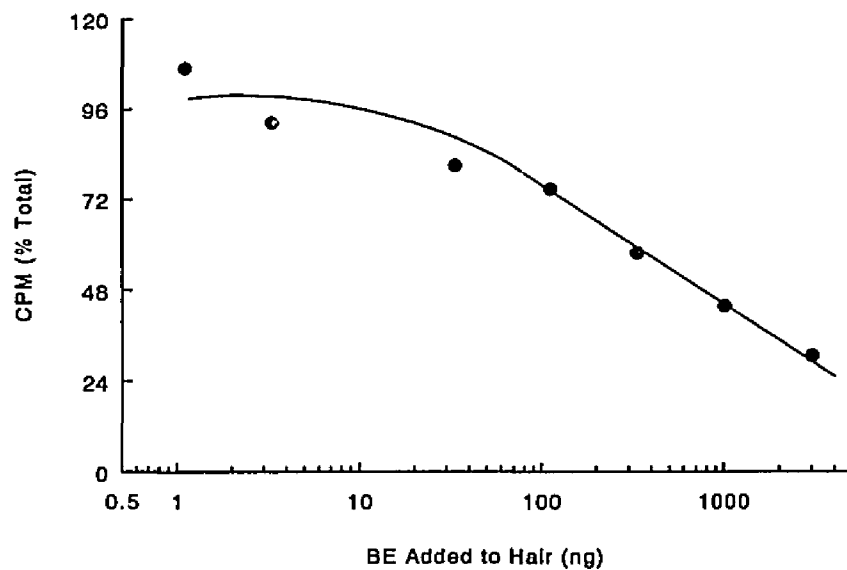


Figure 3. Detection limit of BE in hair. BE concentrations in extracts were determined by RIA to obtain a cut-off level for positivity. Values are expressed as percent cpm of reference counts (duplicate samples).

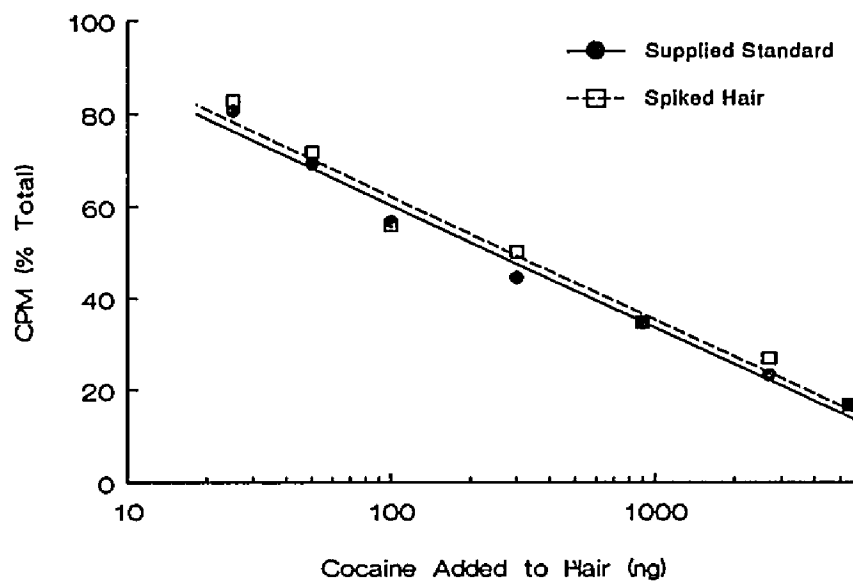


Figure 4. Cocaine hydrochloride was added to drug-free hair and extracts analyzed by RIA. There were no appreciable differences between spiked hair extracts and urine-based calibrators. Values are expressed as percent cpm of reference counts (duplicate samples).

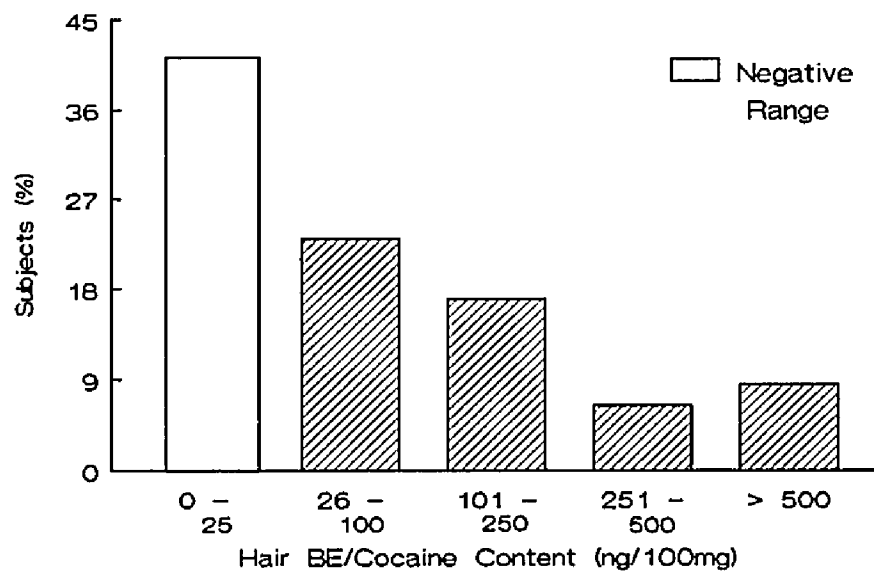


Figure 5. Cocaine/BE content in hair samples of drug users.

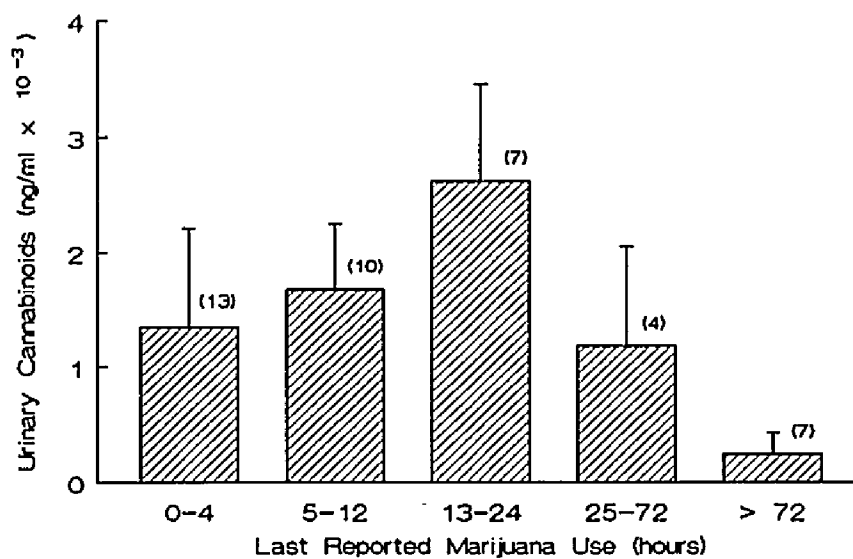


Figure 6. Comparison of reported last use of marijuana with the detection of cannabinoids in urine. Values are mean \pm S.E. of the number of subjects in each group - indicated in parentheses.

Urinary Cannabinoids (ng/ml creatinine)	Positive Samples (% total)
negative	27
20-100	7
101-1000	42
1001-5000	22
> 5000	2

Table I. Measurement of urinary cannabinoids. Values are expressed as per mg of urinary creatinine.

CHAPTER 5: COCAINE ASSOCIATED ABNORMALITIES IN
NATURAL KILLER CELL FUNCTION IN DRUG ABUSERS

INTRODUCTION

Cocaine (benzoylecgonine) is an alkaloid obtained from the leaves of Erythroxylon coca, a plant found predominantly in Western South America. Considered the "champagne of drugs", increasing use of cocaine as a recreational drug in recent years has prompted questions regarding the public health risks of abusing this drug and reports document the toxic effects of cocaine (110,111). Alterations of the immune system have been noted following cocaine intake (75,112,113), although much remains to be elucidated about the actual immunotoxicological mechanisms involved. Cocaine has been proposed to be a co-factor in the development of the Acquired Immunodeficiency Syndrome (AIDS) (114).

Natural Killer (NK) cells appear to play a role in a variety of diseases. Compromised or absent NK cell activity has been linked to development and progression of cancer as well as chronic and acute viral infections including AIDS (115-117). Studies also show that NK cells are involved in

multiple effector, regulatory and developmental activities of the immune system (118-120).

The present study was undertaken as part of a detailed investigation into the immunotoxicology of cocaine and other drugs of abuse. Few reports are available regarding the effects of cocaine on the immune system which may include both suppressive and stimulatory actions (75). An important consideration in such studies involving drug addicts is an accurate assessment of drug use. Most studies take into account the history of drug use given by the subject. However this can be misleading and inaccurate, varying with the reasons or incentives facing the subject coming in for the study. Having faced such inconsistencies, we screened all our subjects for the presence of cocaine in urine and hair. Analysis for cocaine in hair samples was done taking into account its reliability (3) and the short duration of time the drug and its metabolites remain in serum and urine. On the other hand, it has been proposed that cocaine is deposited at a consistent rate over time in hair and can be used to correlate drug use with immunological functions (38).

MATERIALS AND METHODS

Drug Users: A non random sample of 47 Hispanic male drug users were recruited in Nogales, Arizona. All subjects were part of the National Institute of Drug Abuse (NIDA) funded Community Outreach Project on AIDS in Southern Arizona (COPASA). The details of the study were explained to all subjects and informed consent was obtained as per the regulations of the Human Subjects Committee of the University of Arizona. Using a standardized questionnaire administered by a trained bilingual interviewer, each subject was asked his history of substance abuse including cocaine, heroin, marijuana, alcohol and tobacco. Hair, urine and blood samples were taken by trained personnel at the time of the interview. None of the subjects reported infection with Human Immunodeficiency Virus (HIV). The overall incidence of seropositivity in the 800 subjects screened in the COPASA program was less than 2% (Erickson & Estrada, unpublished data).

Controls: A convenience sample of 15 disease-free healthy males was recruited from laboratory and hospital personnel. None of the controls had a history of substance abuse of any

kind, and this was confirmed by hair and urine analysis for cocaine, morphine and marijuana.

Screening for cocaine use: To determine which of the subjects actually used cocaine or other drugs over the recent past, an analysis of urine and hair was done. Extraction of the drug and its derivatives from hair was done as described by Balabanova et al. (71) and modified by us (Chapter 3), using an RIA kit (Diagnostic Products Corporation, Los Angeles, CA). Urine samples were analyzed for cocaine, morphine and for marijuana derivatives (tetrahydrocannabinols).

Blood samples: Blood was collected by venipuncture into Leucoprep tubes (Becton & Dickinson, Lincoln Park, NJ). These sterile heparinized tubes, containing lymphocyte separation medium, can directly be processed for mononuclear cell separation, avoiding blood dilutions and keeping personnel contact with blood to the minimum. The tubes were centrifuged at 1500 g for 20 minutes. Mononuclear cells were isolated, washed and depleted of adherent cells by plastic adherence as described before (121).

Assay For NK Cell Activity: NK cell activity was measured using the direct ⁵¹Cr release assay with the erythroleukemia

cell line K562 as targets (122). Briefly, lymphocytes and labelled target K562 cells were incubated together at different effector:target concentrations (50:1, 25:1 and 12.5:1) in U bottom micotiter plates (Falcon, Becton & Dickinson, NJ). After centrifugation at 40 g for 5 minutes the plates were incubated at 37° C in a humidified atmosphere of 5% carbon dioxide in air for 4 hours. Following incubation the plates were centrifuged at 400 g for 10 minutes and 100 µl of supernatant was removed from each well and counted in a gamma counter. Total release of ⁵¹Cr was determined in triplicate wells by lysis of labelled K562 cells with 1% NP 40 solution. Spontaneous release was determined by incubation of effector cells with media alone. Percentage cytotoxicity was calculated by using the mean of triplicate values as follows:

$$\% \text{ Cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100\%$$

Determination of Circulating NK Cells: The number of circulating NK cells were determined by flow cytometry of peripheral blood using the monoclonal antibody against the CD 16 antigen found predominantly on NK cells (Leu 11c).

Data Analysis: Characteristics of the sample including demographics and substance abuse patterns were analyzed

using descriptive statistics. Distribution of data for NK cells, NK cell activity and drug levels were examined using histograms and scatterplots.

Due to the non-normal distribution of drug and immune parameters, comparisons among groups of subjects were made using nonparametric techniques. Subjects were grouped by level of cocaine measured in their hair. Groups were compared using the Kruskal-Wallis 1-way ANOVA. When a significant difference was demonstrated among the groups, the Mann-Whitney U test examined differences between groups. Bivariate relationships between cocaine levels in hair and NK cell activity were estimated by Spearman rank order correlation coefficients. Differences for all analyses were considered statistically significant at $p \leq 0.05$.

RESULTS

Sample Characteristics: 47 Hispanic males participated in the study. Average age of the sample was 25.9 years (S.D. = 6.3) with a range of 14-41 years. On a scale of non use to heavy use of cocaine, the majority (n=27; 58%) reported heavy current use; 11 reporting moderate use, 1 with occasional use, 2 with infrequent use and 6 with non use. Thirty six subjects said they used tobacco with the majority

(n= 23) smoking less than one pack a day. In addition, 38 subjects used alcohol, with 5 reporting daily use, 27 with weekly use and 6 with monthly use. On a scale of 1 = poor to 4 = excellent, 26 reported fair health, 15 reported good health and 2 reported poor health. None of the subjects reported infection with HIV, hepatitis, gonorrhea or syphilis. One user reported being diagnosed with tuberculosis and two with genital herpes in the last month. Controls included 15 males of mixed ethnicity with an age range of 18-49 years in good health with no infections (Table I).

Screening for Cocaine: The median cocaine level in the hair of the 47 test subjects was 33.7 ng/100 mg hair (range: 1.4 to 4552.1 ng/100 mg hair). Four subjects tested positive for cocaine in the urine. Two subjects tested mildly positive for morphine in the hair and 38 of the 47 subjects tested positive for marijuana derivatives in urine. To study the relation between levels of cocaine in the hair and NK cell activity, the sample of drug users were categorized into 3 groups. Group 1 had cocaine levels less than 18.7 ng/100 mg hair and were classified as the low cocaine group (n =15). Some of these individuals had hair cocaine levels which were comparable to the hospital staff controls who were known to be not using the drug. This could be due to a low frequency

of use or that insufficient time or insufficient amounts had been taken for a detectable amount of cocaine metabolite to be recovered in the hair. Two times the standard deviations added to the mean value of the controls (18.7 ng/100 mg hair) was used as a cut-off point. Group 2 had levels greater than 18.7 ng/100 mg hair but less than 100 ng/ 100 mg hair were classified as the moderate cocaine group (n =16). Subjects with cocaine levels greater than 100 ng/100 mg hair were placed in the high cocaine group (n =16).

Circulating NK Cells Among Cocaine Users: The percent NK cells ranged from 1-39% (median = 11%) for the low cocaine group, 4-44% (median = 10%) for the moderate cocaine group, and 5-40% (median = 14%) for the high cocaine group. Absolute NK cell counts were in the range 90.2-1310.4 (median =327.6) for the low group, 25.7-1584.0 (median = 228.3) for the moderate group and 118.9-706.8 (median = 445.2) for the high group. No statistically significant differences were evident between the three groups for percent or number of NK cells. Differences among the three cocaine groups and the controls for circulating NK cells were not statistically significant using the Kruskal- Wallis 1 way ANOVA (Table II).

NK Cell Activity Among Drug Users : Comparison of NK cell activities at the three Effector:Target (E:T) ratio levels for the low, moderate and high cocaine groups were performed. Results from all three ratios showed a consistent pattern, and hence data for the 50:1 ratio is shown (Table III). Significant differences were demonstrated among the three groups of cocaine users (Kruskal-Wallis 1-way ANOVA). Mann-Whitney U tests were used to determine which 2 groups were different for each of the three cytotoxicity parameters. There were significant differences between the low and moderate, and between the low and high cocaine groups. All significant differences revealed higher levels of activity for moderate and high cocaine groups than for the low cocaine group. No difference was demonstrated between the moderate and high groups (Table II).

Scatterplots of ranked data for NK cell activity at all three E:T ratios and cocaine levels in the hair demonstrated significant positive relationships (Spearman's rho). At the 50:1 ratio, the rho was 0.54 (Figure 1). NK cell activity was therefore seen to increase with higher levels of cocaine in the 47 drug users. Differences among the three groups of drug users and controls were statistically significant. Mann-Whitney U tests were used to determine which of the four groups were different (Table II). Controls were compared with all cocaine groups and showed higher NK cell

activity than the low and moderate cocaine groups, but showed no difference from the high cocaine group.

DISCUSSION

Concerns about immunotoxicological effects of drugs of abuse are on the increase, particularly since the AIDS epidemic. If drugs of abuse significantly compromise the immune system, their use may well be a predisposing factor in the development or enhancement of AIDS. All subjects were selectively screened for cocaine abuse. They were analyzed on the basis of differential levels of drug residue in hair indicating extent of drug abuse in the recent past (Chapter 4).

Findings in this study reveal aberrations in NK cell activity in cocaine users, many of whom also used marijuana, alcohol and tobacco. The various groups used similar amounts of these substances (as confirmed by self admission and verified by biochemical tests) but they were significantly different in their use of cocaine. The interesting finding of this study is the positive correlation seen between levels of cocaine in the hair and NK cell function. The observed changes in NK activity could not have been due to changes in the counts of lymphoid cells with NK markers,

since no group showed any significant changes in the levels of circulating NK cells. Thus it appears that the lifestyle of the drug users lowered NK cell functions but not numbers of NK cells. In addition, the use of larger amounts of cocaine caused a relative rise in NK cell activity. This finding might be interpreted in a different way. It is possible that cocaine can be initially immunosuppressive, explaining the low NK cell activity in subjects with low concentrations of cocaine in their hair. On prolonged or chronic use, the abuser becomes tolerant. This view is supported by the fact that subjects with the highest levels of cocaine in their hair and therefore the longest users, had NK cell function values comparable to normal controls.

Associations between cocaine and NK cell activity have been reported before. In a controlled study, known amounts of either cocaine hydrochloride or benzoylecgonine tetrahydrate (a major metabolite of cocaine) were administered to eight subjects (4). An increase in NK cell activity was demonstrated within 5 minutes following the administration of the cocaine. No such increase was found following the administration of benzoylecgonine. Also, there was an increase in CD16 positive lymphocytes which are believed to have NK activity. When added directly to lymphocytes *in vitro*, cocaine had no influence on NK activity (4).

In the present study, it is not probable that the increased NK cell activity among the drug abusers with high residues of cocaine in their hair was due to such a rapid effect of cocaine. Almost none of our subjects had detectable levels of cocaine metabolites in their urine which indicates absence of use during the past day. Therefore, the effects on NK cell function observed in this study may not be an immediate or direct one.

Cocaine may increase NK cell function through a neuro-endocrine mediated process (75). *In vivo*, cocaine binds to specific sites on brain membranes (123), resulting in multiple neurochemical actions including altered metabolism of neurotransmitters such as serotonin, dopamine, acetylcholine and norepinephrine (124,125). Cocaine causes inhibition of the reuptake of these neurotransmitters by presynaptic nerve endings, causing a prolongation of their actions. In addition, cocaine also stimulates the release of beta endorphin (126). Beta endorphin and norepinephrine are potent stimulants of NK cell activity (127-130). Whether this increased activity is due to an increase in circulating NK cells or due to an increased activation of existing cells is not clear. We could not detect any increases in NK cell numbers in the cocaine users based on surface phenotyping.

Another factor to be considered is the role of nutrition. It is known that malnutrition can cause immune

derangement (131) and that drug users are usually found to have nutritional deficiency (132). NK cells have been recognized primarily for their cytotoxic reactivity against malignant cells, microorganisms and viral infected cells. However, it is now evident that these cells also have powerful immunoregulatory roles in bone marrow cell maturation and regulation of antibody production (117-120). A recent study also demonstrated that NK cells could directly suppress activation and proliferation of B lymphocytes (133). The antibody producing mechanism is one of the front line defense systems against pathogenic insult. Any inhibition of this mechanism could have serious repercussions for the host. In addition, NK cells also may act as suppressor cells in some immune responses (115).

It therefore appears that the immunotoxicology of cocaine in drug users, may involve complex metabolic interactions. Moreover the stress resulting from pathological, disruptive and nutritional consequences of cocaine use may further contribute to the immunological abnormalities. Since drug users are very high risk groups for AIDS, it is possible that cocaine-induced defects in NK cell function, whether suppressive or abnormally stimulatory, can influence HIV infection (114). Our preliminary studies on cocaine and NK cell function in retrovirally infected mice seem to agree with this (5). A

comprehensive and multiparameter approach is therefore required to elucidate this seemingly complex problem.

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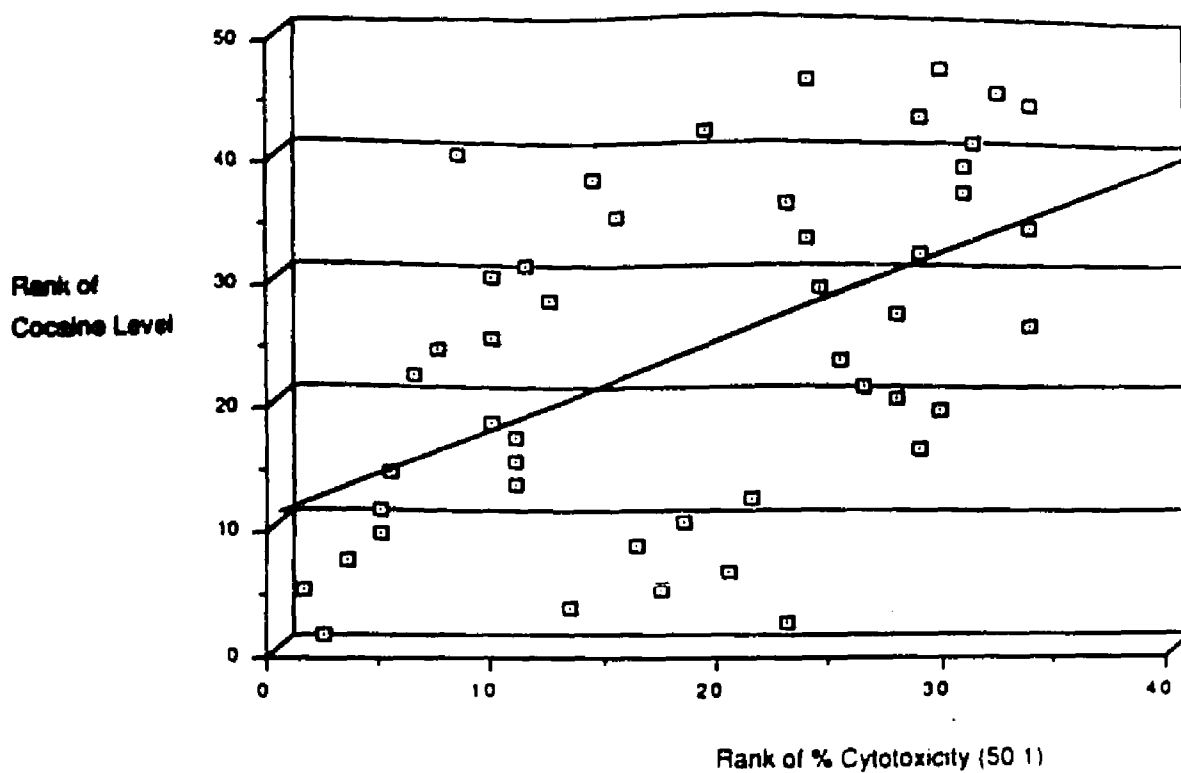


Fig. 1. Scatterplot and Spearman's correlation between ranked cocaine levels in hair and percent cytotoxicity. (E:T = 50:1) $\rho = 0.54$, $p < 0.0001$.

Cocaine	subjects (%)	Health	Subjects (%)	Tobacco	Subjects (%)	Alcohol	Subjects (%)
Heavy	27 (58)	Excellent	4 (9)	Heavy	13 (28)	Daily	5 (11)
Moderate	11 (23)	Good	15 (32)	Moderate	23 (49)	Weekly	27 (57)
Occasional	1 (2)	Fair	26 (55)	Non-Use	11 (23)	Monthly	6 (13)
Infrequent	2 (4)	Poor	2 (4)			Non-Use	9 (19)
Non-Use	6 (13)						

Table I. Self-reported characteristics of sample. The subjects' questionnaire answers as to; cocaine use, health state, tobacco and alcohol use.

Group	Percent NK Cells	Absolute NK Cells/mm ³
Controls (n = 15)	15 (18 - 36)	420.4 (110.6 - 1264.3)
Low Cocaine (n = 15)	11 (1 - 39)	327.6 (90.2 - 1310.4)
Moderate Cocaine (n = 16)	10 (4 - 44)	228.3 (25.7 - 1584.0)
High Cocaine (n = 16)	14 (5 - 40)	445.2 (118.9 - 706.8)

Table II. Circulating NK cells in cocaine users and normal controls. No significant differences were found as analyzed by Kruskal-wallis one-way ANOVA. Values are expressed as median with range in parentheses.

Group	Percent Cytotoxicity
Controls (n = 15)	42.1 (24.0 - 50.4)
Low Cocaine	29.4 (20.4 - 36.4)
Moderate Cocaine	36.8 (26.9 - 46.4)
High Cocaine	39.7 (28.1 - 46.8)

Mann Whitney Analysis

Significant differences:

Low < Controls (p = 0.0003)

Low < Moderate (p = 0.0222)

Low < High (p = 0.0003)

Moderate < Control (p = 0.0032)

Table III. NK cell activity for cocaine users and normal controls (E:T = 50:1). Values are expressed as median with range in parentheses.

CHAPTER 6: CONCLUDING DISCUSSION

The dramatic increase in cocaine use in the United States over the last 10 years has prompted much interest in its toxicity. There is no doubt that cocaine has a variety of toxic effects in animals and in humans (80,81). A dose-response relationship in humans has been difficult to ascertain due to the problems involved in measuring the extent of cocaine use (Chapter 4). We sought to establish a method by which drug use might be accurately determined. An animal model was used to develop a method to analyze cocaine content in hair, and the procedure that was developed to extract and measure cocaine from hair was then applied to humans self-reporting drug use.

Few reports are available regarding the effect of cocaine on the immune system or on the hepatotoxicity of cocaine in humans. In humans it has been impossible to confirm that any toxicity observed has not been due to other factors associated with the lifestyle of the drug user. In animals, these effects do not appear to be mediated directly by cocaine, the immune changes observed are possibly neuroendocrine-mediated and the hepatotoxicity seen is most likely due to a metabolite of cocaine (75,94). In either case, *in vitro* studies are

difficult to relate to *in vivo* situations. Also, the animal models that have been used have shown a great deal of variability in the effects of cocaine in different strains and species (75,84). On the other hand, cocaine has been listed as a Schedule II drug (i.e. high abuse potential with limited medical application), and the only accepted uses of cocaine are for topical administration for mucous membrane anesthesia and vasoconstriction (81).

Studies on drug addicts have shown a variety of toxicological effects (79,111), but it has not been possible to define the cause of these effects (79,134). Hair analysis may provide the information needed to determine the level and quantity of drugs used (Chapter 1), and, thus, give a value by which correlations between use and toxicity can be drawn.

The data given in these experiments do not, however, validate the use of hair analysis to quantitate drug use. The findings that retroviral infection and ethanol intake both alter the recovery of cocaine in hair promote further speculation as to the effects of the multiplicity of variables found in the human population on drug recovery from the hair of any one individual.

The fact that serum cocaine concentrations were also altered by ethanol intake and by retroviral infection is

interesting, and lends credence to the results from the hair analysis. This suggests that hair drug levels do indeed represent exposure to that drug.

The question to be answered in the clinical situation is the level of tissue exposure to cocaine. Hair may be a very good way to assess that exposure. There are several pathways for cocaine metabolism and the pattern by which the bulk of the cocaine (parent compound) is transformed may be influenced by strain and species variability which could lead to the differences seen in cocaine toxicity (92,133). In a comparison of cocaine metabolism in 2 human subjects it was found that the subject with the higher serum cholinesterase activity showed less N-demethylation activity (92). In this case hair cocaine levels would presumably reflect systemic cocaine concentrations rather than the amount of cocaine administered, regardless of the route of metabolism.

In studies on human subjects the ranked level of cocaine recovered from the hair of drug users correlated with NK cell activity as well as with interleukin 2 receptor levels (Chen et al., submitted for publication), but not with self-reported cocaine intake. The differences to self-reported cocaine intake may be due to many factors.

The use of hair analysis for drugs of abuse is not currently allowed in criminal proceedings because sufficient quality control and standardized procedures are not available. The difference between the amount of cocaine and morphine recovered in the hair of mice retrovirally infected or fed ethanol and the amount recovered in non-infected or sucrose-fed mice injected with equivalent amounts of cocaine may indicate this technique may be of limited use for the quantification of cocaine or morphine used, but hair analysis may still be valid in clinical applications.

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