Werner A. Baumgartner,[1] *Ph.D.; Virginia A. Hill,*[2] *B.S.; and William H. Blahd,*[3] *M.D.*

Hair Analysis for Drugs of Abuse

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ABSTRACT: Hair analysis for drugs of abuse by radioimmunoassay and gas chromatography/mass spectrometry (GC/MS) is an effective means for identifying drug abusers. Hair can be collected under close supervision without embarrassment and is not subject to evasive maneuvers (false negatives) such as temporary abstention, excessive fluid intake, and substitution or adulteration of specimens. Hair analysis has a wide window of detection ranging from months to years and provides information concerning the severity and pattern of an individual's drug use. Hair analysis is also not subject to evidential false positives, such as those caused by poppy seed ingestion, spiking of drinks or food, and mix-up or contamination of specimens. In part, these problems can be avoided because hair analysis can always be repeated with a newly collected specimen. The results of animal experiments and of various clinical, forensic, and criminal justice applications are described.

KEYWORDS: criminalistics, workshop, hair, radioimmunoassay, chemical analysis, cocaine, PCP, heroin/morphine, marijuana, criminal justice

Background

The detection of heroin/morphine by radioimmunoassay (RIA) techniques in the hair of human subjects was first reported in 1978 by our Nuclear Medicine group at the West Los Angeles Veterans Administration Medical Center, in collaboration with Annette Baumgartner and Peter Jones of The Aerospace Corporation's Chemistry & Physics Laboratory [*1*]. We subsequently extended radioimmunoassay of hair (RIAH) to phencyclidine (PCP) [*2*], cocaine [*3*], marijuana, digoxin, methadone, and benzodiazepines [*4*].

These studies showed that hair analysis and urinalysis were complementary rather than competing tests [*5*]. Urinalysis provides short-term information of an individual's drug use (and is thus the test of choice for post-accident testing), whereas long-term histories

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[1]Director, RIA Laboratory, Nuclear Medicine and Ultrasound Service, West Los Angeles Veterans Administration Medical Center, Wadsworth Division, adjunct assistant professor, University of Southern California School of Pharmacy, Los Angeles, CA, and scientific director, Psychemedics Corp., Santa Monica, CA.

[2]Research scientist, Nuclear Medicine and Ultrasound Service, West Los Angeles Veterans Administration Medical Center, Wadsworth Division, Los Angeles, CA.

[3]Chief, Nuclear Medicine and Ultrasound Service, West Los Angeles Veterans Administration Medical Center, Wadsworth Division, and professor of medicine, University of California, School of Medicine, Los Angeles, CA.

(from months to years) are accessible through hair analysis. Moreover, in contrast to qualitative information from urinalysis, hair analysis provides quantitative information on the severity and pattern of an individual's drug use. Furthermore, many of the problems associated with urinalysis can be resolved by hair analysis [*6*]. These unique aspects of hair analysis will be detailed below following a presentation of its development and technological status.

At the outset, it is important to distinguish hair analysis for drugs of abuse from nutritional trace metal analysis, where lack of agreement on normal ranges and conflicting interpretations concerning the medical significance of the trace metal content of hair have created considerable controversy [*7,8*]. Rather, in its interpretational aspects, hair analysis for drugs resembles heavy metal analysis of hair (for example, mercury, lead), which has been an established tool of forensic and environmental toxicology and clinical pathology in view of the relatively straightforward interpretation of the presence of heavy metals in hair [*9–11*].

Our early RIAH studies [*1–3*] were performed with cumbersome solvent extraction techniques. These were subsequently replaced by more efficient and cost-effective procedures (patent pending) to facilitate the introduction of hair analysis for mass screening purposes: clinical testing, high-security employee screening, and forensic science and criminal justice applications. This new method of hair analysis was extensively field-tested in over 1000 human subjects in various clinical and forensic science settings described in the present report.

The accumulation of drugs in the hair of drug users has been confirmed by several independent laboratories in the United States, Europe, and Japan [*12–32*]. These researchers also extended testing to amphetamine, antipsychotic agents, and nicotine. In addition to its obvious utility in the drug abuse area, hair analysis also appears promising for monitoring compliance of medication intake, for establishing toxic chemical exposure, and even for monitoring control of diabetes by measuring the glycosylation of hair proteins [*33*]. Solid-probe tandem mass-spectrometry (MS) confirmation techniques of drugs in hair performed by the FBI and Naval Research Laboratories [*34,35*], in addition to specimen matching with deoxyribonucleic acid (DNA) fingerprinting [*36*], appear to offer additional advantages for hair analysis.

The first court case involving hair analysis for abused drugs was reported by Dr. Frederick Smith from the University of Alabama [*16*]. Dr. Ronald Siegel, the University of California at Los Angeles, in collaboration with our laboratory, has evaluated the utility of hair analysis for forensic psychopharmacological purposes [*37*].

Methodological Considerations

The accumulation of drugs in hair has been studied in human subjects and in animal models. The latter offer the advantage of providing carefully controlled conditions unavailable for human subjects, and animal hair is also much easier to analyze. One reason for this is that the ratio of hair weight to body weight is much larger for animals than for humans. Consequently, the fraction of ingested drug which becomes entrapped in animal hair is proportionally greater. Furthermore, with human subjects, cosmetic considerations dictate that only a small fraction of the total head hair be taken for analysis (for example, 10 mg). In contrast, one can readily obtain a 50-mg hair sample from a 30-g mouse. For these reasons, as well as in screening for multiple drug use, it became necessary to develop hair testing procedures which require only between 0.5 to 1.0 mg of hair per drug analysis.

Beyond their advantages, animal studies also pose unique problems, for example, the contamination of animal fur by drug-containing urine or feces. We addressed these problems in our studies by housing animals in individual cages on wire mesh to minimize

contamination from excretion-impregnated bedding material, and by collecting hair only from the back of animals, that is, from a location less likely to have come in contact with the wire cage. Before analysis the hair must be carefully cleaned from such contamination. We used specially designed wash procedures, developed with hair which had been soaked in drug-containing urine for various periods of time. Finally, to simulate the steady growth rate of human hair, animal hair was removed from the back by plucking before intraperitoneal (i.p.) injection with known amounts of drug. Only the regrown hair was assayed for drugs. This approach avoids the problem of dealing with a population of hair where a significant fraction is in the nongrowing, that is, nondrug-entrapping phase.

Decontamination Procedures

The decontaminating procedures that we have found effective for human hair involve washing the specimen (approximately 5 or 10 mg) in 1 mL of ethanol for a period of 15 min at 37°C, followed by three 30-min washes in phosphate buffer (pH 7) at 37°C. Our experience has shown that most hair samples do not exhibit any external contamination, since such contamination is removed by washing with shampoo. Generally, if external contamination is found, the washout kinetics of repeated washing show that contamination is rapidly removed, that is, it is present in the first and second washes but not in the third or fourth wash. Continued washing of hair in such cases does not remove any further material deposited in the deeper layers from the bloodstream during the synthesis of the hair fiber. However, in some instances, exceptions to this general rule have been found—we have seen cases where more extensive washing was required. To identify such cases, we assay the drug content of the last wash and compare this to the drug content of the final hair extract. If the extract-to-wash ratio is not at least ten (and in most cases it is much greater) we repeat the analysis on another sample of the same specimen after the wash kinetics on this sample show that a plateau has been reached. Thus, by using the above criteria and washout kinetics in conjunction with gas chromatography/mass spectrometry (GC/MS) confirmation (of the metabolite, if possible), we can establish with reasonable certainty whether a drug found in hair is derived from the bloodstream or from external contacts.

Extraction Procedures

Our initial publications used solvent-based extraction procedures, applied at elevated temperatures (boiling point of solvent) and for extended times, to produce plateau extraction kinetics similar to those described for the wash procedures. Other investigators have used a similar approach with different solvents or have applied acid/base extraction techniques or alkaline digestion of the hair sample [*12–32*]. Although such procedures are cost-effective in forensic science cases, this is not so when medical or employee screening is involved. This is particularly true for multiple drug screens where each drug may require its own time-consuming extraction procedure.

In view of these difficulties, we sought an analytical procedure in which the hair is dissolved and where the antibody subsequently used in RIAH acts as a specific analyte-extracting agent. Such an approach requires the rapid destruction of the organic protein matrix of hair under sufficiently mild conditions that will not damage the entrapped analyte or the protein antibodies subsequently added for the RIA assay. Matrix effects due to variations in the protein or lipid composition or both of hair specimens should also be absent. Our proprietary extraction and assay procedures fulfill all of these conditions. Recovery experiments with analyte spikes added to intact hair before the hair dissolution process yield recoveries near 100% at analyte concentrations close to the detection limit of the assay.

Unfortunately, most investigators in the field of hair analysis for drugs of abuse have not evaluated the efficiency of their decontamination and extraction procedures, nor have they provided recovery data. Consequently, many studies can only be compared on a qualitative basis. These differences in experimental rigor undoubtedly contribute to the conflicting results cited in a recent critical review of hair testing for drugs [*9*].

Effects of Hair Treatment

We have investigated the effects of cosmetic treatment on the drug content of hair. Although these studies with drug-containing hair from different donors are still in progress, a general picture has begun to emerge. We have focused specifically on the effects of repeated shampooing, perming, relaxing, and dyeing of hair. Repeated shampooing was found to have no significant effect on the drug content of hair. Drug levels, however, have been found to be affected under some conditions by the other kinds of treatment. The extent to which this occurs depends on the type of hair (fine, coarse, or ethnic origin), severity and type of treatment, and type of drug. However, to date, we have not been able to apply treatments which reduce drug levels to a point at which a drug user would have escaped detection. This is true even for hair that had been damaged by treatment to cosmetically unacceptable levels.

Most importantly, one's ability to detect drug use under a variety of hair treatment conditions depends critically on the efficiency of the extraction process. Specifically, the method must be able to extract drugs from the deeper regions of the hair fiber which were inaccessible to the treatment agents. Ideally, therefore, the analysis of treated hair should be undertaken with methods showing extraction efficiencies close to 100%.

Hair damage as a result of cosmetic treatments can be readily identified by staining of hair with methylene blue solution (20-mg/100-mL water). However, for the convenience of mass screening, we developed a second method for identifying treated hair. This procedure has been integrated into the extraction process (patent pending).

The degree of methylene blue uptake by hair, as determined by microspectrophotometry, can be correlated with the extent of hair damage due to treatment and this, in turn, with decreases in the drug content of the hair. Correction factors are being developed from these measurements, thereby enabling us to maintain our semiquantitative correlations between extent of drug use and drug levels in hair (see below) even under the unfavorable conditions of hair treatments.

GC/MS Confirmation

Mass spectrometer confirmation methods have been developed in collaboration with Dr. J. Peterson and G. Hisayasu of the Pacific Toxicology Laboratories. A sample size as low as 5 mg of hair is sufficient for this purpose. Procedures used for serum and urine analyses were adapted for this purpose [*38–40*]. With our present technology, GC/MS cut-off levels (expressed per 10 mg of hair) are as follows: cocaine, 5 ng; morphine, 5 ng; PCP, 1 ng.

RIA Cutoff Levels

Cutoff levels for RIA were determined by experiments with hair specimens from proven nonusers of drugs. In determining these levels, it was necessary to establish whether or not matrix effects caused by differences in lipid or protein composition or both contribute to the observed statistical scatter of the response variable (counts per minute at the zero drug concentration mark). In other words, it is not sufficient to do repeated response variable determinations with a small number of guaranteed negatives for the determi-

nation of these levels, or even less, to determine the statistical scatter of the zero-value response variable with only aqueous samples.

Our proven drug-free population consisted of personnel from the U.S. Navy Rehabilitation Center in San Diego, where individuals were under close visual supervision and urine was tested three times per week. The statistical distribution of the zero-concentration response variable was determined with a number (N) of different hair specimens. From these data, the mean value and the values for 2, 3, and 5 standard deviations were calculated. From the RIA calibration curve (passing through the mean value of the zero-concentration response variable), we determined the drug concentration cutoff values corresponding to 2, 3, and 5 standard deviations (Table 1). For forensic science and employee screening purposes, the MS confirmation cutoff levels are used. For research and clinical purposes, where GC/MS confirmation is not required because of absence of punitive consequences or legal precedence, we use the 3 and 5 Standard Deviation RIA cutoff levels, respectively. Potential problems due to cross reactivity can be identified by the RIA dilution test. So far we have not observed instances of cross-reactivity in hair, probably because analyte and cross-reacting substances are present in hair at similar concentrations. The cutoff at two standard deviations is customarily defined as the RIA Detection Limit.

An interesting feature which emerged from the RIA experiments was the virtual absence of matrix effects for our RIA procedures over a sample range of 50 to 200 μL at a concentration of 10-mg hair per mL of hair extract; that is, RIA results under these conditions were identical to those obtained with water. In this respect, hair analysis differs significantly from urinalysis, since the latter exhibited major matrix effects with different specimens. This, in turn, resulted in a substantial statistical scatter in the zero-concentration response variable. Thus, the same RIA kit was found to be less sensitive for urinalysis than for hair analysis.

Sample Collection and Growth Rate of Hair

In the case of trace metal analysis of hair, considerable differences have been observed in the concentrations of these substances in hair collected from different regions of the head [9]. These data and our own preliminary findings suggest that this may also be the case for drugs of abuse. It is therefore desirable to collect hair specimens from a well-defined site on the head, for example, from the posterior vertex. The latter is a region where hair growth is less influenced by age and sex, and where a more constant number of hair follicles are in the growing phase (85%) [9]. The vertex area is easily located by imagining a line from the top of the ears running over the top of the head. The vertex region is the area 1 to 2 in. (2½ to 5 cm) back from the center of that line.

On rare occasions, it may be necessary to establish an accurate growth rate of hair to define precisely the time of a particular drug exposure. In such cases, the actual growth rate of an individual's hair should be measured. One simple, but time-consuming, method for doing this is to bleach or dye an individual's hair right up to the scalp and to measure the extent of hair growth after a suitable period of time (for example, one month). A quicker, but more complicated, procedure has been described by Masaji Saitoh et al. [*41*]. In this method, a glass capillary tube, graduated every 0.2 mm, is fitted around a growing hair. Growth rates can be measured within three to four days with a Zeiss Dermascope with greater than 1% accuracy between 0 to 40°C and 0 to 100% humidity.

Quantitative Aspects of Hair Analysis

If all urine specimens collected over a three-day period after a one-time cocaine drug use were analyzed for drug content, one would obtain the sharply rising and descending

TABLE 1—*Cutoff values determined from drug-free hair samples.*

Substance	*N*	2 S.D.		3 S.D.		5 S.D.	
		B/B_o, %	Ng/10-mg Hair	B/B_o, %	Ng/10-mg Hair	B/B_o, %	Ng/10-mg Hair
Marijuana (low-sensitive antibody)	20	91.0	0.17	86.5	0.28	77.5	0.48
Marijuana (high-sensitive antibody)	99	87.3	0.08	81.0	0.12	...	...
Cocaine	145	92.1	0.85	88.1	1.4	80.3	3.8
Heroine/ morphine	40	89.4	0.52	84.1	0.78	73.5	1.7
PCP	42	93.4	0.43	90.1	0.64	83.5	1.1
Methadone	15	92.5	0.65	88.8	1.1	81.3	2.0

curve shown in Fig. 1. The area under the curve would represent the total amount of drug excreted in the urine and this would be correlated to the amount of ingested drug. Obviously, this procedure is not practical for drug screening. Here, only one specimen is collected at a particular point in time; consequently, we are not certain whether the collected sample falls on the ascending or descending limb of the curve. In other words, a low urine drug level could mean that an individual took a small amount very recently or a larger amount at a more distant point in the past.

With hair we have a different situation. Essentially, one can view hair as sequestering drugs which are circulating in the bloodstream (Fig. 2), doing so in direct proportion to

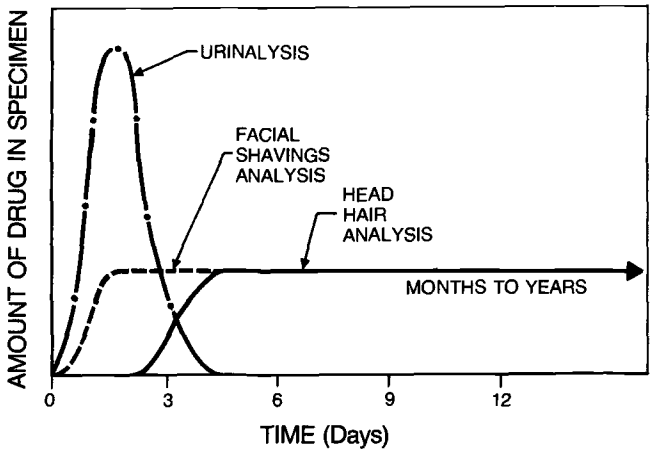

FIG. 1—*Accumulation of drugs in urine and in hair as function of time.*

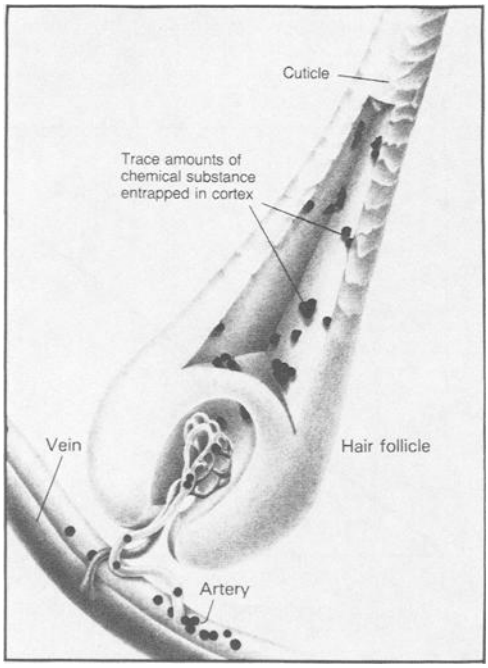

FIG. 2—*Transfer of drug from the circulatory system to hair follicle and its subsequent encapsulation in keratin fibers of hair shaft.*

their concentration. In this respect, hair acts as an "integrating monitor." The concentration circulating in the bloodstream depends, of course, on the amount of drug taken and its mode of administration, as well as on the speed of its metabolism, excretion, and distribution to other body compartments. But, as in the case of alcohol, the concentration circulating in the bloodstream should be correlatable to the concentration entering the primary target organ, the brain.

We tested the first component of our hypothesis, that is, the "integrating monitor" function of hair by injecting syngeneic C57BL6 mice three times per week with different amounts of drug for a period of three weeks. This was done for heroin, cocaine, PCP, and methaqualone (Figs. 3–6). Each point on the curve represents the data from two

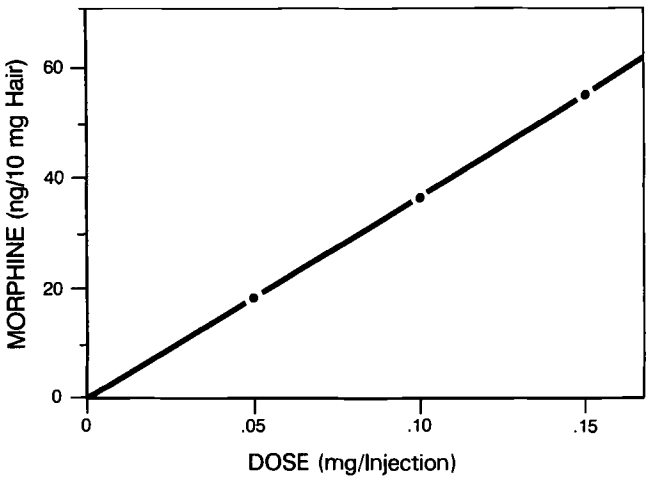

FIG. 3—*Correlation between injected heroin dose and heroin content of mouse hair 24 days after first injection into C57BL6 syngeneic mice. Injection schedule: 3 times per week for 3 weeks.*

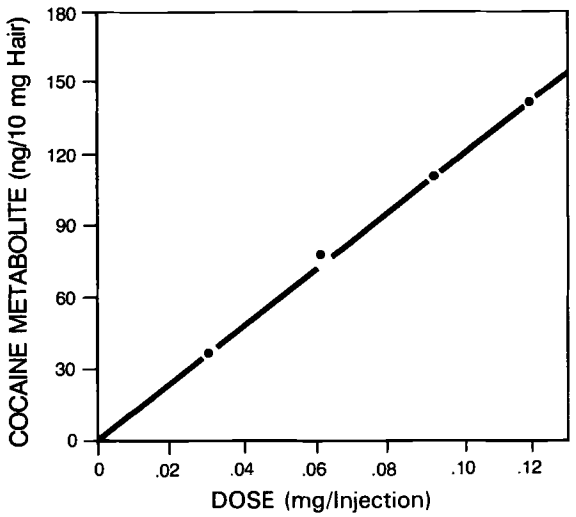

FIG. 4—*Correlation between injected cocaine dose and cocaine content of mouse hair 24 days after first injection into C57BL6 syngeneic mice. Injection schedule: 3 times per week for 3 weeks.*

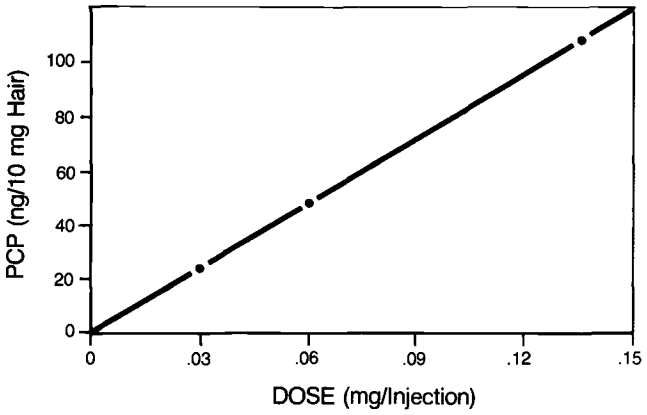

FIG. 5—*Correlation between injected PCP dose and PCP content of mouse hair 24 days after first injection into C57BL6 syngeneic mice. Injection schedule: 3 times per week for 3 weeks.*

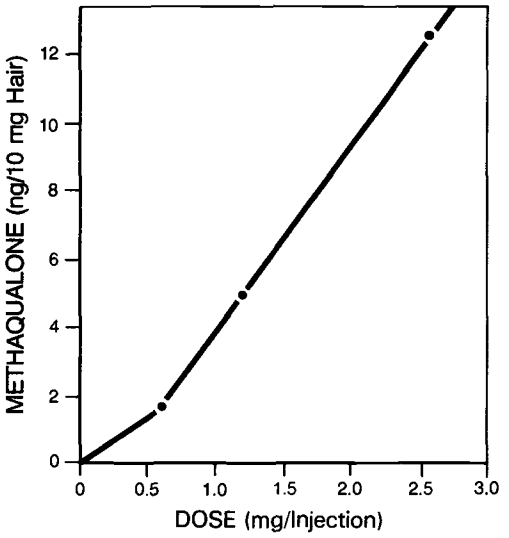

FIG. 6—*Correlation between injected methaqualone dose and methaqualone content of mouse hair 24 days after first injection into C57BL6 syngeneic mice. Injection schedule: 3 times per week for 3 weeks.*

animals. The results show an excellent linear correlation between the drug content of mouse hair and increasing dose, all the way to the LD_{50} dose.

Next, we attempted to obtain evidence for this hypothesis with human subjects. One approach was to use model compounds such as digoxin, a pharmaceutical agent administered in quantities of 0.1 to 0.5 mg per day. The main clinical objective is to adjust the daily intake of digoxin in such a way as to attain a serum digoxin level of between 0.8 and 1.8 ng/mL. This therapeutic level is characterized by the attainment of approximately steady state kinetics 6 h post digoxin ingestion, that is, after the initial absorption-peak kinetics in serum have subsided. These pharmacokinetics of digoxin absorption lead to

considerable fluctuations in serum digoxin levels under constant dose conditions; for the same patient these frequently range between 1.0 to 1.6 ng/mL. Practical considerations precluded us from obtaining sufficient serum readings for the statistical evaluation of each data point chosen in Fig. 7; instead, most points represent only the mean of three to four serum readings.

Consistent with our hypothesis, we see that serum digoxin levels between 0.5 and 2.0 ng/mL result in a tight cluster of digoxin levels in hair (2 to 6 pg of digoxin/10-mg hair) with a distinct trend towards an increase with higher serum digoxin levels. Similar experiments with methadone are currently in progress with Charles Charuvastra, M.D., at the West Los Angeles Veterans Administration Medical Center [*4*]. We anticipate that this study will allow us to investigate a greater serum concentration range.

Another approach to testing our hypothesis was to investigate the correlation between the amount of self-reported drug use and the drug content found in the hair. Admittedly, self-reporting has its deficiencies: inaccuracies of recall, veracity of reporting, unknown drug purity, and varying efficiencies of drug administration. However, one has very little choice in this matter, given that the controlled conditions available for studying low drug use (that is, administration of illicit drugs under medical supervision) cannot be applied in intermediate and high drug use. In spite of these difficulties, we obtained reasonably good correlations between self-reports and the drug contents of hair for cocaine, PCP, and heroin (Figs. 8–10). The poor correlation obtained with marijuana (Fig. 11) is attributed, in part, to technical difficulties with the assay, that is, to the degree with which the antibody reacts with the numerous pyrolysis products of cannabinoids which are formed during smoking. Highly specific monoclonal antibodies are currently being tested to minimize this problem.

It is useful to compare the correlation graph data (Figs. 8–11) to the cutoff levels listed in Table 1. From these two sets of data, one can calculate that as little as one to two lines (approximately 100 mg) of cocaine use per week should be detectable by hair analysis. It would be possible to detect even lower drug use if the size of the hair sample per analysis were increased to more than 1 mg. Similarly, one can calculate the lowest detectable use levels for the other drugs: heroin, 3.5 bags/month; PCP, 0.30 cigarette/month; marijuana, 1.3 joints/month.

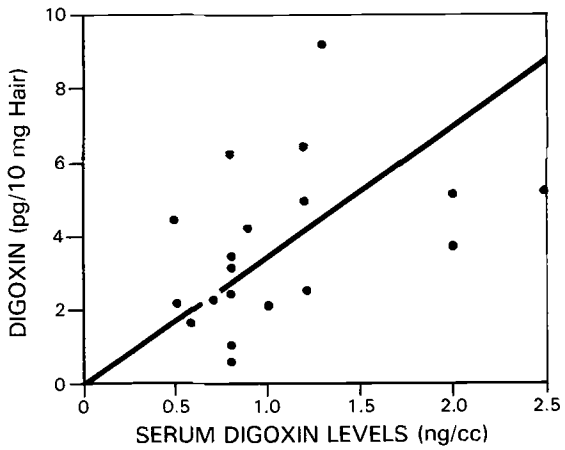

FIG. 7—*Correlation between serum digoxin levels and hair digoxin levels in patients receiving daily digoxin doses of 0.1 to 0.5 mg.*

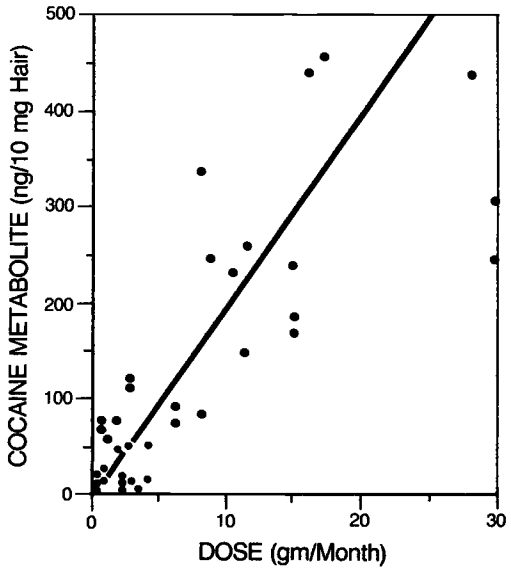

FIG. 8—*Correlation between self-reported amounts of cocaine use and cocaine levels in hair, based on confidential self-reports from patients, probationers, and parolees.*

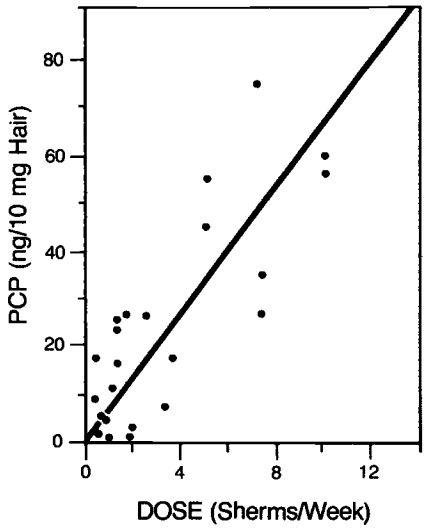

FIG. 9—*Correlation between self-reported amounts of PCP use and PCP levels in hair, based on confidential self-reports from patients, probationers, and parolees.*

Wide Window of Detection

Because head hair grows at approximately 1.3 ± 0.2 (ISD) cm per month [*41*], it is readily seen that the window of detection for hair analysis can range over many months to years, thereby affording unique opportunities for a retrospective immunological analysis of hair (RIAH). Furthermore, if the window of detection is compartmentalized by

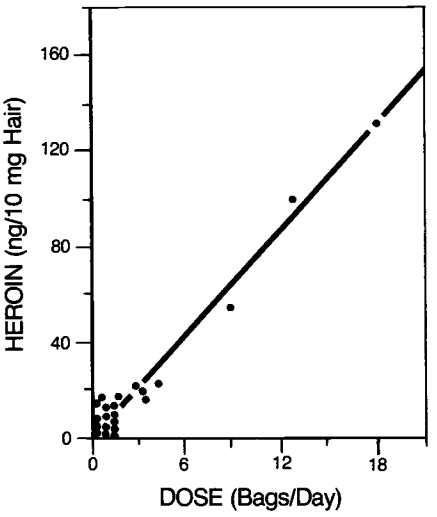

FIG. 10—*Correlation between self-reported amounts of heroin use and heroin levels in hair, based on confidential self reports from patients, probationers, and parolees.*

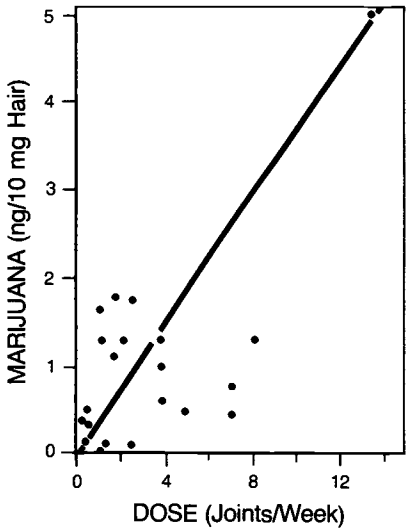

FIG. 11—*Correlation between self-reported amounts of marijuana use and marijuana levels in hair, based on confidential self-reports from patients, probationers, and parolees.*

cutting strands of hair into sections (for example, one-month intervals), one is able to obtain information on the pattern of an individual's drug use, that is, whether use is decreasing, constant or increasing. Thus, relative changes in an individual's drug use can be established with a high degree of certainty, even though absolute estimates may be subject to the vagaries of biochemical individuality.

This relative measure of drug use through hair analysis appears particularly promising for monitoring compliance of medication intake. For example, a measure of compliance

may be obtained by comparing the drug levels in hair corresponding to the time a patient received medication under the controlled conditions of a hospital setting to those noted after discharge. In these circumstances, the patient has become his/her own control.

Typical examples of sectional analysis are shown below. Figure 12 illustrates the utility of hair analysis in a rehabilitation setting. The individual in question is seen to reduce her heroin habit from 14 bags per day to 2 to 3 bags per day over a 15-month period. In this case, the advantages of sectional hair analysis over urinalysis are obvious from the fact that urinalysis would have yielded at best consistently positive results without indicating that an improvement was actually taking place. Figure 13 illustrates a case of relatively constant heroin use, whereas the patient in Fig. 14 shows an escalating use

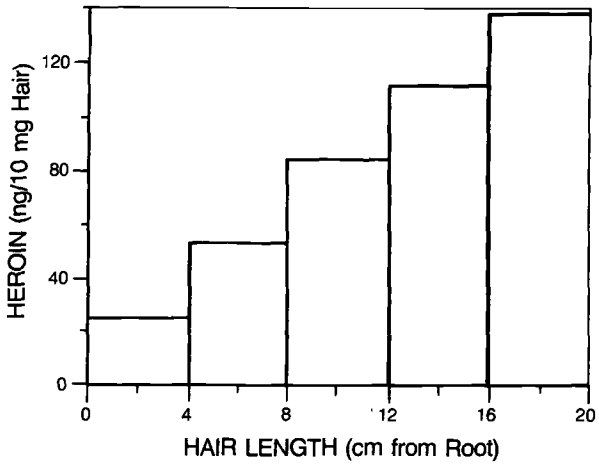

FIG. 12—*Individual's drug use pattern by the analysis of hair strands cut into small sections corresponding to different periods of time; hair growth rate: 1.3 cm/month. Hair analysis corroborates self-reported decrease in heroin use from 14 bags/day to 2 bags/day over 15-month period.*

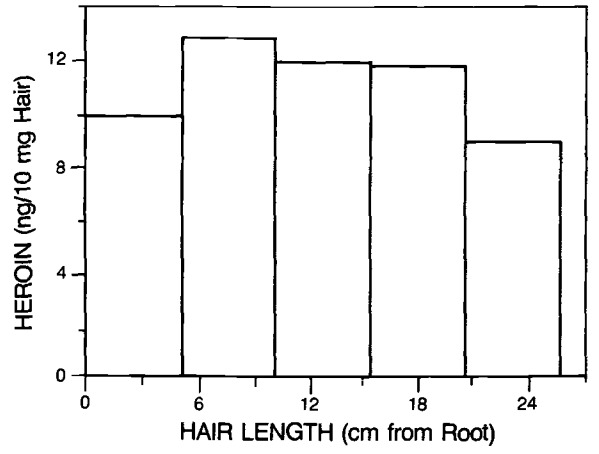

FIG. 13—*Individual's drug use pattern by analysis of hair strands cut into small sections corresponding to different periods of time; hair growth rate: 1.3 cm/month. Hair analysis corroborates self-reported constant heroin use of approximately 1 to 2 bags/day over a 9-month period.*

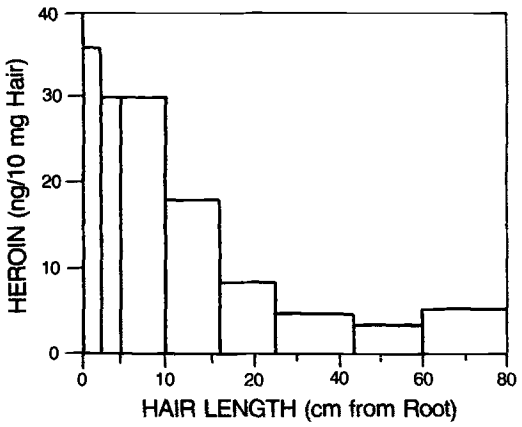

FIG. 14—*Individual's drug use pattern by analysis of hair strands cut into small sections corresponding to different periods of time; hair growth rate: 1.3 cm/month. Hair analysis corroborates self-reported escalation of heroin use over 60-month period.*

over a period of 60 months. A typical case of decreasing marijuana use is shown in Fig. 15.

A particularly interesting case is the one shown in Fig. 16, our first example of prenatal drug exposure. Work on this pressing societal problem has been greatly expanded in recent years [*42*]. The mother in this situation had taken small quantities of PCP during and after her pregnancy. Hair specimens were obtained from the mother and her baby approximately one year after delivery when family members became concerned about the baby's abnormal development. From the hair analysis it is evident that the PCP had been transferred to the developing fetus as seen by the positive regions in the baby's hair. These values abruptly dropped to zero after birth.

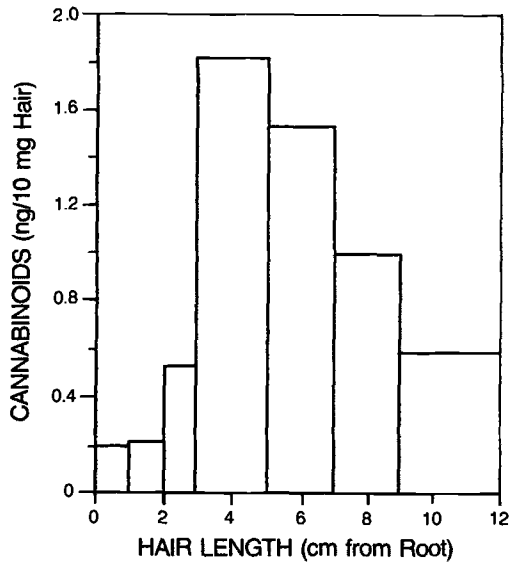

FIG. 15—*Individual's drug use pattern by analysis of hair strands cut into small sections corresponding to different periods of time; hair growth rate: 1.3 cm/month. Hair analysis corroborates self-reported decrease of marijuana use from one to three joints/week to no use during last month.*

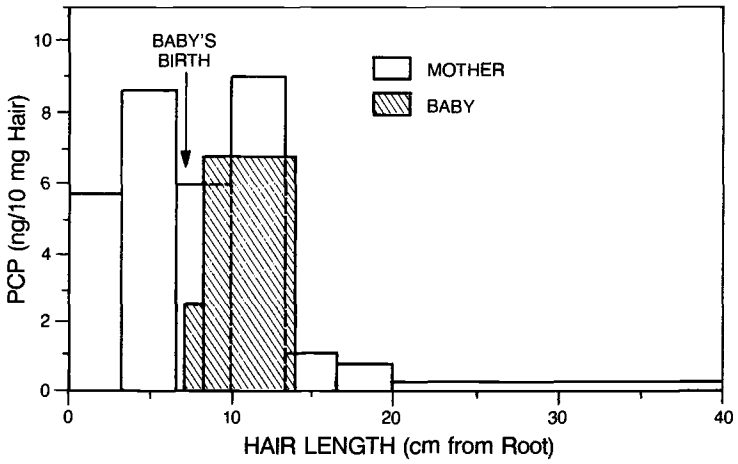

FIG. 16—*Drug use pattern during and after pregnancy by analysis of hair strands from mother and baby cut into small sections corresponding to different periods of time; hair growth rate: 1.3 cm/month. Hair analysis corroborates PCP use during pregnancy and demonstrates significant fetal exposure.*

The results of our first postmortem case, performed in collaboration with Dr. Ronald Siegel, are shown in Fig. 17. Hair samples were obtained from the remains of a murder victim one and one-half years after death. The well-preserved hair sample was cut into sections as indicated and analyzed for cocaine content. The results show that the deceased had used cocaine at a fairly constant rate up to the last month, at which time a precipitous decrease in drug use was indicated. These results corroborated eyewitness accounts that the deceased had been unable to support his former habit during the last month because of financial difficulties and that he was killed while trying to maintain his former level of drug use without the necessary financial resources.

In another case with Dr. Siegel, we obtained the authenticated hair (five hairs, 3 in.

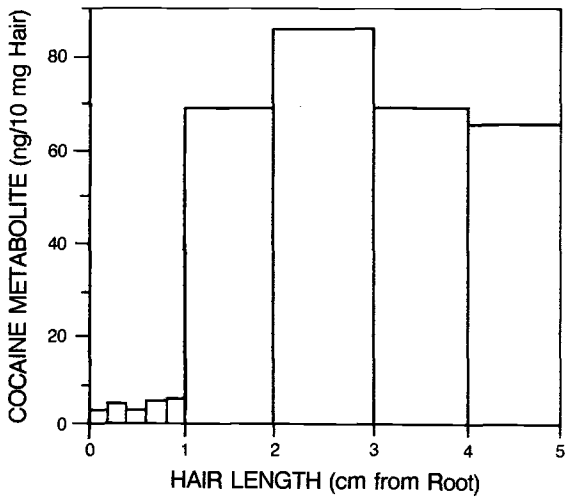

FIG. 17—*Drug use pattern of deceased individual established from remains one and one-half years after death. Analysis of hair strands cut into small sections corresponding to different periods of time corroborate eyewitness accounts of an abrupt decrease in cocaine use during last month of life.*

[7.6 cm] in length) from the Victorian poet (pharmaceutical chemist and physician) John Keats. The hair is believed to be from a period of the poet's life during which he took laudanum to control the pain of terminal tuberculosis. Our analysis revealed the presence of opiates 167 years after their deposition in the hair, thereby attesting to the fact that hair can provide a stable matrix for storage of entrapped material even in the absence of refrigeration.

Resolving the Problems of Urinalysis Drug Testing

There are essentially three types of problems with urinalysis drug testing: false positives, personal degradation of observed urine collection, and opportunities for evasive maneuvers (false negatives). These problems can be greatly mitigated or eliminated through hair analysis.

Concerning the problem of false-positive urinalysis results, one must distinguish between technical false positives and evidential false positives. Technical false positives can essentially be avoided if positive urines from drug screens are confirmed by GC/MS procedures. The prevention of evidential false positives is quite another matter.

Examples of evidential false positives are contamination or mix-up of urine specimens at the collection site or in the laboratory. Such specimens obviously will test positive by both the initial screen and by the GC/MS confirmation technique, even though the individual may not be a drug user.

Hair analysis does not suffer from such contamination problems, since hair is thoroughly cleansed before analysis and, of course, we can always obtain a fresh identical hair sample if there is any claim of a specimen mix-up or breach in the chain of custody. This makes hair analysis essentially fail-safe, in contrast to urinalysis, since an identical urine specimen cannot be obtained at a later date. Clearly, hair analysis can thus function as a "safety net" for urinalysis.

Another potential problem is the possible inadvertent contamination of urine specimens or hair extracts in the laboratory by a drug-using chemist through the handling of certain types of equipment used in drug testing (for example, pipet tips, and so forth). This possibility is further enhanced by the paradoxical fact that most drug-testing laboratories do not test their personnel for drug use. And, even if laboratories did such testing, it would probably be done too infrequently and by the readily evaded, false-negative-prone urine test. By the time a drug-using chemist is identified by urinalysis, many evidential false positives could have been generated. Thus, the testing of the testers by essentially evasion-proof hair analysis should be a prime concern in any drug-testing program.

Another possible source of evidential false positives is through the inadvertent ingestion of drinks or food which had been laced with drugs. Whether there is an actual occurrence of this kind or not, such a scenario can be a powerful legal defense, known as the "brownie defense."

While GC/MS analysis of urine specimens cannot distinguish between chronic use or inadvertent exposures, hair analysis can make this distinction. This is achieved simply by looking for evidence of drug use in different sections of hair corresponding to different periods of time. A one-time exposure, if the drug was taken in sufficient quantity, would only show up in a particular segment of the hair strand; chronic use would manifest itself throughout the length of the hair corresponding to the period of chronic use. A related problem is a false evidential positive resulting from the opiates present in poppy seeds. An amount equaling those in three bagels appears to be sufficient for the creation of a positive urine result. In contrast to this, we have shown that the ingestion of up to 30 g of poppy seeds per week did not result in a positive hair result. The same source of poppy seed, however, produced positive urines.

The collection of hair is neither a degrading nor an embarrassing experience as is

closely supervised collection of a urine specimen. But even supervised urine collection cannot prevent evasion, particularly in a preemployment setting, since the forewarned individual merely has to abstain from drug use for a few days before the test or engage in excessive fluid intake (flushing). Other methods for evading urinalysis are switching of samples or adulteration of the specimen with interfering substances. None of these evasive actions can confound hair analysis. Hair analysis is, therefore, particularly useful for the screening of personnel in highly sensitive positions at regularly scheduled annual physicals, thereby avoiding Fourth Amendment problems encountered by random urine testing.

Hair Analysis: An Effective Means for Identifying Drug Users

Several field studies have shown that drug users can be identified more effectively by hair analysis than by urinalysis. The higher identification efficiency of hair analysis stems from its wide window of detection, its resistance to evasive maneuvers, and the highly effective drug-trapping mechanism of hair which allows the identification even of low-level drug users [*43*].

The superior overall performance of hair analysis has been demonstrated in a number of field studies. In our clinical studies with cocaine [*3*], heroin [*1*], and PCP [*2*] users, we compared confidential self-reports with the results of hair analysis and urinalysis. Hair analysis identified drug users in these populations with essentially 100% efficiencies; urinalysis, on the other hand, yielded detection efficiencies of only 0, 12, and 30%, respectively. In another study, hair analysis surpassed the ability of urine or plasma analysis to identify PCP ingestion as a cause of psychotic episodes [*44*]. In a sample of 47 newly hospitalized psychiatric patients, 11 patients who had used PCP were identified by hair analysis; blood and urine (analyzed by GC with a cutoff level of 1 ng/mL) failed to identify any of these cases.

In a current two-year study funded by a National Institute of Justice (NIJ) grant and in collaboration with James Baer of the U.S. Probation and Parole Department, hair analysis is being used to evaluate the relationship between the extent of prior drug use and parole/probation infringements in a population of parolees and probationers [*45*]. Client volunteers provide a hair specimen at intake and at three-month intervals for the duration of the one-year program. The results have been compared with client treatment records, confidential self-reports of drug use, supervision records (including number of arrests), and urinalysis data. Each client is required to provide approximately six urine samples per month at randomly scheduled intervals. Urines are analyzed by the methods in current use by the probation/parole department. Marijuana is screened with enzyme multiplied immunoassay (EMIT®) and confirmed by high-performance, thin-layer chromatography, with a cutoff of 100 ng/mL of Δ-9 carboxy-tetrahydrocannabinol (THC). PCP and cocaine are screened using thin-layer chromatography, and confirmed by GC with cutoffs, respectively, at 2 and 0.5 μg/mL. Opiates are screened using EMIT and thin-layer chromatography and confirmed by GC with cutoffs at 0.5 μg/mL.

Table 2 summarizes the hair and urinalysis data and self-reports collected to date in the NIJ study. The urine and hair data are classified into two categories based on self-report of either use or non-use. The hair and urine results for corroborating the self-report are indicated in each of the four columns: A U+ signifies at least one positive urine obtained within the three-month sampling period. H+ is used to denote a level of drug detection in hair above the three standard deviation cutoff levels (as established in the previous study conducted with U.S. Navy personnel, Table 1).

The data clearly show that RIAH surpasses even frequently applied random urinalysis for the detection of drug use. The superior performance of hair analysis is actually greater than indicated by the data in Table 2. For example, if urinalysis was positive at least

TABLE 2—*Comparison of urinalysis and hair analysis in a parolee/probationer population.*[a]

Client Status	Drug	Positive Self-Report					Negative Self-Report				
		N	U+ H+	U+ H−	U− H+	U− H−	*N*	U+ H+	U+ H−	U− H+	U− H−
At intake to drug program	cocaine	65	14	0	46	5	109	3	0	21	85
	PCP	8	3	0	5	0	181	0	0	3	178
	morphine/ heroin	29	6	1	17	5	151	1	2	2	146
3 months after intake	cocaine	10	3	0	4	3	87	2	0	12	73
	PCP	1	0	0	1	0	79	0	0	1	78
	morphine/ heroin	6	2	0	4	0	84	4	1	2	77
6 months after intake	cocaine	6	0	0	3	3	44	0	0	5	39
	PCP	3	1	0	0	2	44	0	0	2	42
	morphine/ heroin	2	0	0	0	2	47	1	1	1	44

[a] U+, positive urine; U−, negative urine; H+, positive hair; H−, negative hair.

once for the 18 random urine samples collected over the three-month "detection window," this was scored as a U+; but if the self-report in this example shows continuous drug use during this time, these urine results should be scored as 1 U+ and 17 U−, giving a 5.5% detection efficiency. A more refined analysis, such as this, will be applied to these data in the final report of the study.

The results of the Navy Study (Table 1) and our excellent correlation between the results of the RIAH screen and GC/MS confirmation suggest that the conflict between positive hair analysis results and negative self-reports is attributable to false self-reports and not to false-positive RIAH screen results.

In summary, it appears that the value of hair analysis for the identification of drug users is steadily gaining recognition. This can be seen from its growing use in preemployment screening and forensic science and criminal justice applications. In the medical arena, we see hair analysis increasingly applied in the identification of drug use, particularly for diagnosing toxic psychosis and problems relating to prenatal drug exposure. As to the ultimate objective of our research endeavors, we anticipate that hair analysis will also prove to be a valuable tool for guiding the recovery process of people suffering from addictive disorders.

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Address requests for reprints or additional information to
W. A. Baumgartner
Psychemedics Corp.
1807 Wilshire Blvd., Suite B-2
Santa Monica, CA 90403