

Radioimmunoassay of Hair for Determining Opiate-Abuse Histories

Annette M. Baumgartner, Peter F. Jones, Werner A. Baumgartner, and Charles T. Black

*The Aerospace Corporation, El Segundo, California, and the Veterans Administration Wadsworth
Medical Center, Los Angeles, California*

Heroin and morphine metabolites can be detected in hair with the use of commercially available radioimmunoassay reagents and with minor sample preparation. Hair samples obtained from morphine-treated mice and heroin users contained nanogram levels of the drug per milligram of hair (single human hair). The results of the hair analyses for all subjects admitting the use of heroin were positive, whereas the results of only 30% of thin-layer chromatographic urinalyses of these same subjects were positive. In addition, differences in drug concentration for sections of hair near the scalp and near the distal end correlated with the length of time the drug had been used. These results exemplify the potential advantages of the use of hair analysis over urine and serum analyses in terms of accessibility, sample stability, and long-term retention of information.

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Drug levels in body fluids can be determined only when the drugs have been administered within 1 or 2 days before sample collection. Although body tissues are known to retain or concentrate traces of drugs, the urine and serum levels are not necessarily accurate reflections of these tissue levels. Nevertheless, the body fluids are generally preferred as samples for diagnostic and detection purposes, mainly because of the accessibility of these fluids. A complementary approach that could provide a history of drug use—and be used in toxicology, law enforcement, and personnel screening—would be invaluable.

In 1954 it was reported (1) that barbiturate residues had been found in the hair of guinea pigs 1 mo after the administration of the drug. However, the

methods of extraction and analysis, and the large sample required, indicated that this approach would not be practical for routine drug analysis. In a later study (2), drugs were again found to be present in the hair of guinea pigs, as shown by the presence of carbon-14 in the hair after the administration of C-14 labeled amphetamines and dopamines. From these early studies it was surmised that hair might serve as an indicator of past or present drug use if more sensitive and specific analytic procedures were developed.

We chose to investigate hair analysis to determine opiate-use histories because of the widespread opiate abuse. Commercially available radioimmunoassay kits for opiates provided the required sensitivity and specificity.

MATERIALS AND METHODS

Animal studies. Twelve mice (B16-C57) were injected, i.p., with 0.15 mg of morphine in a saline solution three times per week for 2 wk.

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For reprints contact: Annette Baumgartner, The Aerospace Corp., P.O. Box 92957, Los Angeles, CA 90009.

A small area on the back of each mouse was plucked free of hair before the morphine treatment. This hair was used as a control sample throughout the study. Hair grown during and after the treatment was obtained from this previously plucked area.

Hair was cut from another part of the body of the mice in order to evaluate the sample preparation procedure. Each hair was cut in half. The distal ends represented hair growth before the drug treatment; the bases (the hair closest to the scalp) represented growth during drug treatment. After careful washing, no evidence of the drug was found in distal ends, whereas measurable amounts were found in the bases.

In order to remove any external contamination, each 10-mg hair sample was washed three times in a 1-ml detergent solution and rinsed three times in 1 ml of distilled water. The effect of successive washings in the removal of any loosely bound drug from mouse hair (presumed to result from external contamination, especially urine) is shown in Fig. 1.

The hair was dried and pulverized with a mortar and pestle. Maximum extraction was achieved when the 10-mg samples were heated in 5 ml of methanol for 2 hr (Fig. 2). The hair was separated from the methanol by centrifugation, the methanol was evaporated, and the residue was redissolved in 1 ml of a phosphate buffer (pH 7.4).

Human studies. Human hair samples were collected from admitted heroin users at the Long Beach Alcohol and Drug Rehabilitation Center. The users' urine was tested by means of thin-layer chromatography at an independent laboratory (3). The drug histories of these users were recorded at the time of collection of the hair sample.

The first phase of the human study involved the use of the whole length of the hair. In the second phase, 2.5-cm sections of the hair closest to the scalp were used to represent recent growth; 2.5-cm sections cut at 15 cm away from the scalp represented earlier growth. In no case was the hair root used in the analyses.

Radioimmunoassay for morphine and heroin. The commercial radioimmunoassay kit* for these opiates was used according to the manufacturer's recommended procedures (4). Extracts of the control hair samples, to which morphine had been added, were used to establish calibration curves (Fig. 3). It was found that these curves compared closely with those obtained from the control urines in the kit. However, the molarity and the pH of the buffer did affect the slope and shape of the curve. A 0.1 M phosphate buffer, at pH 7.4, yielded the curve shown in Fig. 3.

The sample, 0.1 ml of the extract (approximately

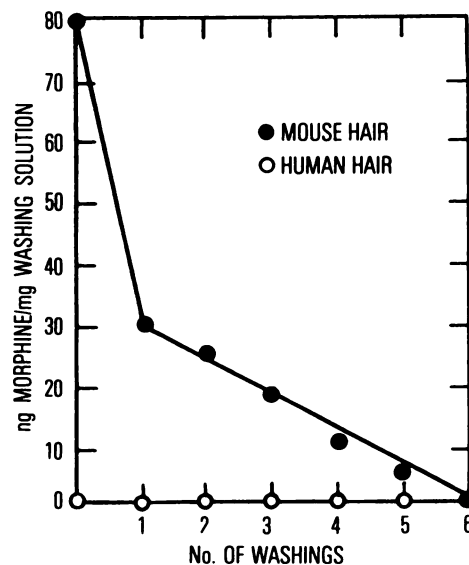


FIG. 1. Morphine washed from hair in successive washings; average obtained from six individual mice.

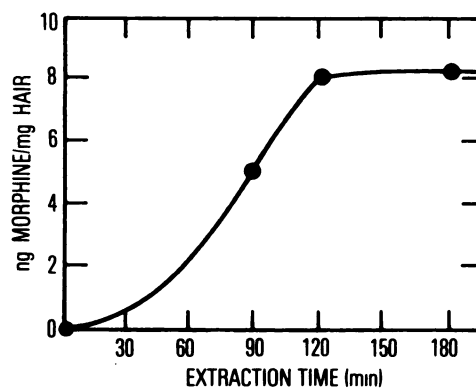


FIG. 2. Rate of morphine extraction; average obtained from six individual mice.

equivalent to one human hair), is added to a test tube containing morphine antibodies and I-125-labeled morphine. After 20 minutes of incubation, the bound antigen is precipitated with ammonium sulfate, and the supernatant containing the free antigen is transferred to a gamma counter.

Sample preparation. All human hair samples were washed according to the procedure used for the animal hair. No measurable drug levels were found in the wash or rinse water, which indicates that there was no external contamination or no extraction with water (Fig. 1).

The extraction procedure established for the animal hair was equally effective for the human hair.

RESULTS

Animal studies. When the samples were first ana-

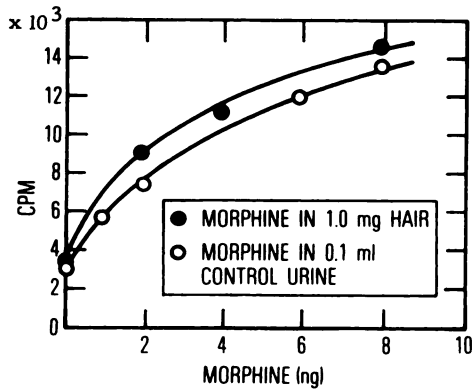


FIG. 3. Radioimmunoassay calibration curves: comparison of urine controls from manufacturer and buffer-reconstituted hair extracts to which morphine was added.

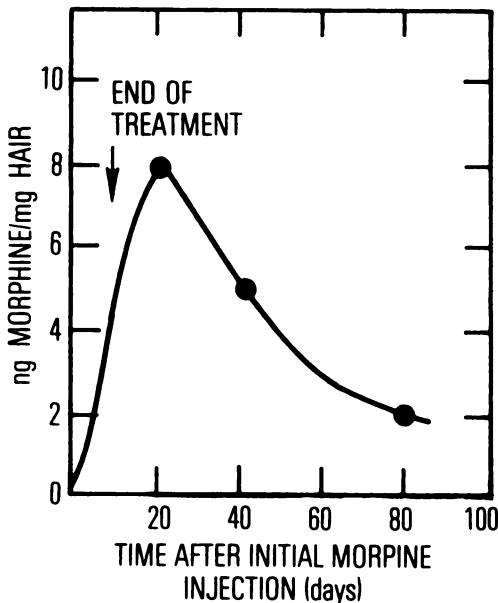


FIG. 4. Morphine content of whole hair plotted against time of drug treatment; averages obtained from 10 mice; maximum variation from animal to animal was 12%.

lyzed (1 wk after the last injection) approximately 8 ng of morphine per milligram of the whole length of hair were found (Fig. 4). Whole hair samples removed at later times showed a decline in the drug level, and 2 mo after the last injection approximately 2 ng per milligram of whole hair were found. By sectioning hair collected 2 mo after the last injection, we could distinguish those areas primarily grown during the drug treatment from those grown after treatment. Hair from the section grown during the treatment contained 6–8 ng of morphine per milligram of hair, whereas the hair grown later contained no measurable levels of morphine.

Human studies. For the initial analyses, whole lengths of hair, without the roots, were used to

determine the presence of any morphine. Although the results of only 30% of the urinalyses were positive (by thin-layer chromatography), the results of hair analyses for all 60 subjects admitting opiate use were positive. Even in the 52 subjects whose last dose had been within 24 hr of the sample collection, only 44% of the urinalyses were positive. Data obtained from the first 25 users—with the use grouped as long, medium, or short, according to the duration of the drug use—are given in Fig. 5. Long indicates use for 8 mo or longer; medium, 3–8 mo; short, 2 wk–3 mo. There is a definite correlation between the total drug content in whole lengths of hair and the length of drug use. In order to clarify the reason for the two exceptions in Fig. 5 (both of which involved short hair), the second phase of our study involved sectioning the hair.

The most recently grown section of the hair (Fig. 6) consistently contained high drug levels for all users, whereas only long-term users had high drug levels in the earlier hair growth (hair at least 15 cm from the scalp). Thus, the sectional analyses made it possible to distinguish among the three groups of users and to explain the apparent exceptions (Fig. 5).

DISCUSSION AND CONCLUSION

The animal studies were carried out in order to develop the necessary sample-preparation methods under controlled conditions of drug administration. Although care was taken to avoid drug contamination of the hair during the injections by housing the mice in metabolic cages, contamination, particularly from urine, had to be taken into account. By cutting the mouse hair into sections grown before and during the drug treatment, we demonstrated that external contamination of the hair could be removed by careful washing. Only hair actually grown during the drug treatment retained evidence of morphine after washing.

Figure 1 suggests that successive washing removes loosely bound morphine from mouse hair (morphine presumably present from external contamination). After three washes and rinses, no further morphine was detected in the wash water, leaving only the drug incorporated in the hair, the removal of which requires vigorous extraction procedures.

The decrease in the drug concentration in the animal hair with time after the last injection (Fig. 4) was expected because of the continued growth of the hair when no new drug is administered. In view of the rapid growth and loss of hair in mice, it is indeed satisfying that measurable amounts of the drug were still found after 3 mo. After 2 mo of growth, the hair was sectioned. The recently grown

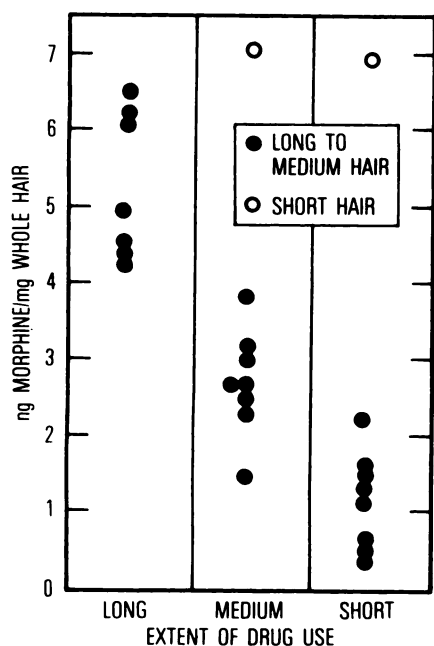


FIG. 5. Correlation of heroin use with human hair analysis. Long drug use = more than 8 mo; medium = 3-8 mo; short = less than 3 mo.

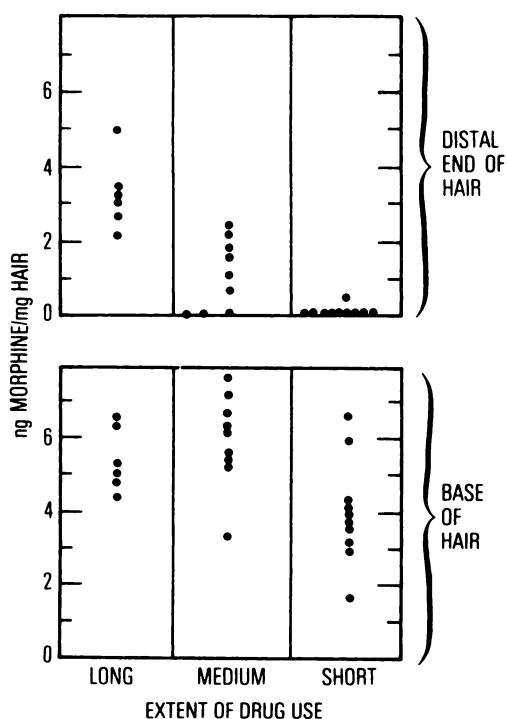


FIG. 6. Sectional analysis of human hair.

hair samples contained no detectable morphine, and the tips, grown earlier, had the higher drug concentrations.

The human studies clearly demonstrated the advantage of radioimmunoassay in hair over the thin-

layer chromatographic analysis of urine samples. Radioimmunoassay is known to be more sensitive than the thin-layer chromatography, and permits the detection of concentrations in the 1- to 10-ng/mg range. Hair retains the drug evidence much longer than urine or serum, apparently throughout its lifetime, and this permits the detection of past drug abuse. Blood or urine analysis indicates use only within a few days. By sectioning the hair, the approximate time of drug use can be determined, since the drug is apparently detectable only in the hair grown during, or possibly immediately after, drug use.

For additional reasons the use of hair samples is significantly more advantageous than the use of urine or serum samples: hair samples are readily accessible, stable, and retain the drug for long periods of time. Although the hair sample is not likely to replace urine and serum, it is expected that hair analysis will serve as a valuable complement to the current approach in that it is a practical method for determining drug histories.

Because of the widespread concern over the abuse of opiates, the focus was on them. Future work will include the analysis of other drugs in hair by the use of radioimmunoassay techniques. Other methods of analysis—e.g., gas chromatography/mass spectrometry—may provide for the simultaneous detection of several drugs in hair. Confirmation of a history of drug abuse is of great interest to law-enforcement agencies, e.g., for monitoring drug use among prisoners and parolees; to drug clinics in evaluating applicants for methadone treatment; and to employers and the Armed Forces for personnel screening.

The analysis of human hair by means of a sensitive and convenient method such as radioimmunoassay may be used in other applications. The information obtained from hair may aid in the diagnosis of the chronic toxic effects of drugs or pollutants, and in the monitoring of workers exposed to dangerous chemicals and of astronauts confined to artificial environments.

FOOTNOTE

* Abuscreen for morphine, I-125, Roche-Diagnostics, Nutley, N.J.

ACKNOWLEDGMENTS

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