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Detection of Basic Drugs (Methamphetamine, Antidepressants, and Nicotine) from Human Hair

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ABSTRACT: Human hair contains methamphetamine, amitriptyline, imipramine, nicotine, and their metabolites in some amount, which can be detected by routine toxicological methods. Sometimes, the level of drugs reaches over 100 μg/g. Animal experiments indicate that these drugs are found solely in sections of hair grown after administration of the drugs. The negative stage after the administration of drugs means that the hair section containing drugs has not come out of the hair follicle. Toxicological examination of the hairs may give some clue helping to identify the chronology of the intoxication.

KEYWORDS: toxicology, hair, methamphetamine, tricyclic antidepressants, nicotine, intoxication chronology

Information pointing to a history of drug abuse (especially methamphetamine) from biological specimens other than urine and blood is of great interest not only to toxicologists, but also to law enforcement agencies in Japan. Cases in which urine was collected from addicts using urinary catheterization—they usually refuse to give the urine voluntarily—have sometimes been rejected in the courts because of invasion of the individual's right in collecting the evidence. Although this difficulty could partly be overcome by using their perspiration [*1*], some addicts claim contamination of the drugs from a third person.

Meanwhile, several investigators have reported that human hairs are useful in screening drug abuses [*2-5*]. Based on these reports, we have investigated the detection of methamphetamine from hair. In addition, we have investigated whether basic drugs, such as amitriptyline, nortriptyline, imipramine, and nicotine are contained in human hair.

Materials and Methods

Hairs (weighing from 6 to 220 mg) from methamphetamine addicts were supplied by the Japanese Ministry of Welfare. Because of the individual's rights, no precise history of each patient was given, except in one case.

Human hair from patients who have undergone long-term treatment with antidepressants with a daily dose of 25 to 50 mg was obtained from a psychiatric hospital. The weight of the hair was approximately 15 to 20 mg.

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Hair of smokers and nonsmokers were collected from laboratory personnel.

Animal experiments were performed to confirm our results. Male mice of different strains were administered intraperitoneally with a daily dose of methamphetamine 100 μg/0.1 mL. After 2, 5, 8, 14, and 24 days of successive administration, the animal was killed and the hairs were collected by using a hair clipper for mice.

In order to remove any external contamination of drugs from perspiration and other biological fluids, the hairs were washed with a kind of detergent (5% Extran MA 02 neutral, E. Merck, Darmstadt West Germany) thoroughly, then with 0.1% sodium dodecyl sulfate SDS). They were then rinsed with distilled water thoroughly. After this, the samples were washed several times with amount of 0.01*N* hydrochloric acid. After the last washing, the samples were incubated in 1 mL of 0.01*N* hydrochloric acid at 37°C for 1 h. This hydrochloric acid aliquot contained no trace of drugs in gas chromatograph (GC) analysis. The cleaned samples were washed with acetone and dried. It was then incubated in 1.5*N* sodium hydroxide at room temperature overnight. In this condition, no remarkable change of the hair structure was observed. When 1.5*N* hydrochloric acid was added to pH 1 to 2, the hair was dissolved almost homogenously. After this, the aliquot was brought to pH 10 to 12 by adding 1*N* sodium hydroxide. Methamphetamine and nicotine were extracted according to Jain's method [*6*]. In the case of the tricyclic antidepressants, Burch's extraction was used [*7*].

Gas chromatographic (GC) and gas chromatographic/mass spectrometric (GC/MS) analyses were performed by using Hitachi and Shimazu GC and GC/MS apparatuses. The precise conditions are described in each of experimental results. Following internal standards were added in the quantitative assay of the drug: *N*-ethyl-benzylamine in the case of methamphetamine; dextromehorphan, chlorimipramine, and demethylchlorimipramine in the case of tricyclic antidepressants; and methamphetamine in the case of nicotine.

Experimental Results

Occurence of Methamphetamine in Various Biological Stains and Hairs

Figure 1 represents the results of experiments concerned with the detection of methamphetamine in various biological samples in the dried stains and hairs in one criminal case. This was a female of 48 years of age who had abused methamphetamine during the period from 20 June to 19 Sept. 1981. She admitted during police inquiry that she was administered the drug intravenously by her husband, presumably twice a day (each dose approximately 10 mg). All biological samples were taken within four days after the arrest; dried stains were prepared with the usual gauze. No precise description was given as for their original volumes. They were sealed in plastic bags and delivered to our laboratory. The experiments were performed one month after the samples were taken.

Dried stains on gauze of 5 by 10 cm were extracted with 30 mL of warm distilled water (approximately 50°C) for 30 min and the methamphetamine content was assayed. Total amount of the methamphetamine in the samples was as follows: urine 56.8, perspiration 11.4, and saliva 0.48 μg. As indicated in this figure, the extract of the hairs (18 mg) shows a distinct peak suggesting the presence of methamphetamine estimated at 0.69 μg. In calculating the concentration level, it reaches to 38.3 μg/g. The confirmation of the presence of methamphetamine (trifluoroacetyl [TFA] derivative) in the hair was performed by GC/MS analysis in the following conditions. A Shimazu 6020 gas chromatograph/mass spectrometer was equipped with a glass column of 1-m by 3-mm inner diameter packed with 3% OV-17 on Gas Chrom Q (80–100 mesh). The injection temperature was 250°C, ion source temperature was 200°C, ion accelerating voltage was 3.5 kV, ionizing energy was 70 eV, and the carrier gas (helium) flow rate was 30 mL/min. Distinct ionic responses at 154, 118, 110, and 91 *m/z* were detected.

For the confirmation of the presence of the metabolite, amphetamine, the extract was treated with TFA and analyzed using OV-17. As described previously [*8*], a distinct response

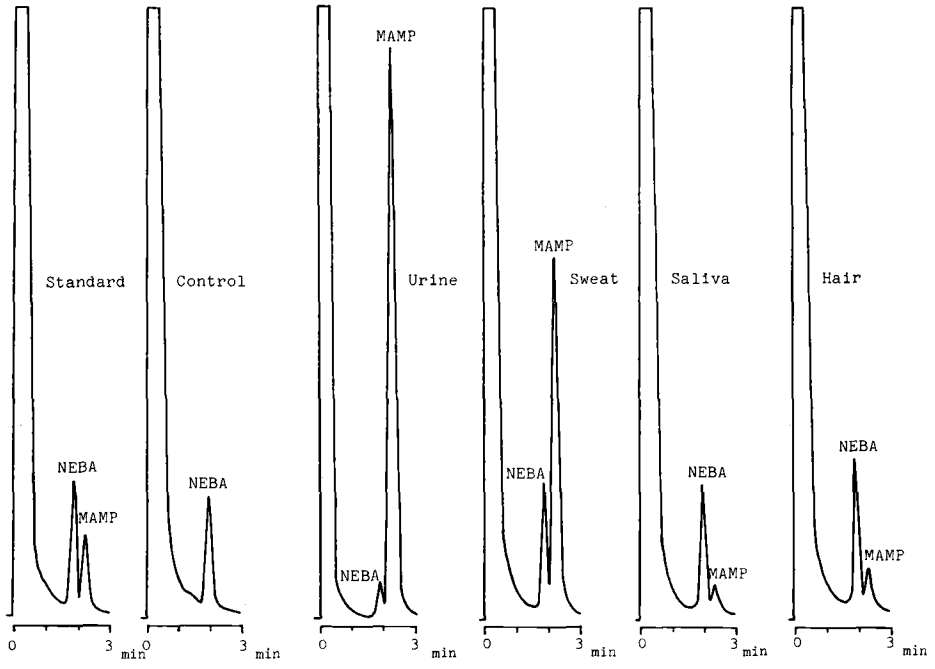

FIG. 1—*Gas chromatographic analysis of various samples from a methamphetamine addict where the ordinate is gas chromatographic response, the abscissa is the retention time. NEBA* = N-*ethyl-benzylamine (internal standard), and MAMP = methamphetamine. A Hitachi 073, flame ionization detector gas chromatograph was equipped with a glass column (1-m by 3-mm inner diameter) packed with 5% polyethylene glycol-6000 + 5% potassium hydroxide on Chromosorb G (80-100 mesh) treated with acid washing and dimethyldichlorosilane. The column temperature was 140°C, the injection temperature was 190°C, and the carrier gas (nitrogen) flowed at a rate of 30 mL/min. The injection volume was 2 μL (50 ng of NEBA and MAMP are contained in the standard test). The sensitivity was ×1, the attenuation was ×32 in the case of urine, and ×8 in other specimens.*

corresponding to amphetamine-TFA was found in the cases of stains from urine and perspiration. In the cases of saliva and hairs, some indication suggesting the presence of amphetamine-TFA was obtained.

Examinations of the hairs from other two patients showed following results; 1.1 μg in 227 mg (4.8 μg/g) and 27.2 μg in 216 mg (125.9 μg/g) of hair. Figure 2 represents an example of analysis using TFA derivative of the extract and OV-17 as the GC column. Here, a distinct peak corresponding to the amphetamine-TFA was discerned. The amount of methamphetamine and amphetamine was 27 and 3.7 μg, respectively. Another case was examined using 5.9 mg of hair. No distinct peak of methamphetamine was detected, but its presence was discerned by examining it with mass fragmentography at 154, 118, and 110 *m/z*.

Animal Experiment

The results of the animal experiment are shown in Fig. 3. It is reasonable that the amount of drugs in the mouse hair increases along with the length of the term of administration. It is also evident that the hairs collected during the first three to four days after methamphetamine administration were completely free of the drug. This suggests that the negative stage was the term of hair growth until the section of hair containing the drug became observable. Some

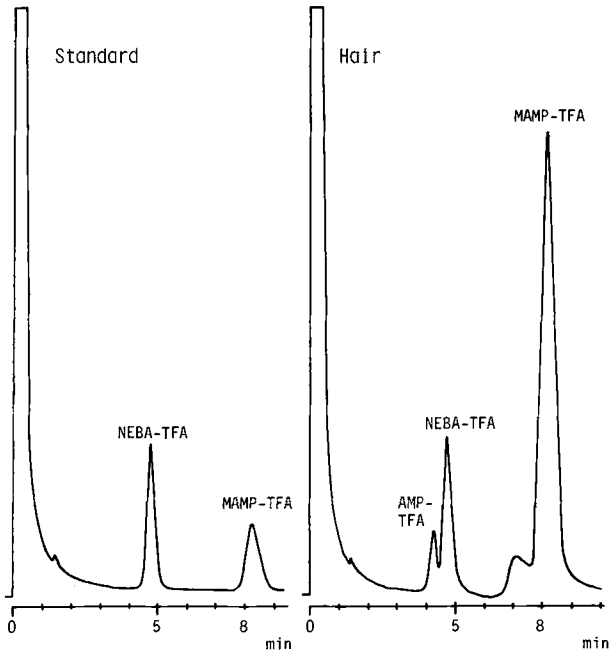

FIG. 2—*A gas chromatographic analysis of extract from the hair of a methamphetamine addict where the ordinate is gas chromatographic response, the abscissa is retention time, and NEBA and MAMP are the same as in Fig. 1; each material was pretreated with TFA. The gas chromatograph was a Hitachi 073 flame ionization detector equipped with a glass column (1-m by 3-mm inner diameter) packed with 3% OV-17 on Chromosorb W (80-100 mesh) treated with acid washing and dimethyldichlorosilane. The column temperature was 130°C and the injection temperature was 180°C. The carrier gas (nitrogen) flowed at a rate of 30 mL/min. The sensitivity was ×1 and the attenuation was ×8. The injection volume was 2 μL (80 ng of NEBA and MAMP are contained in the standard test).*

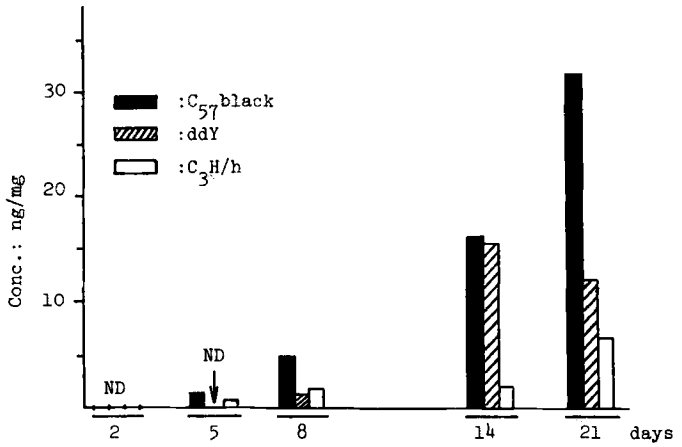

FIG. 3—*Effect of time on the amount of methamphetamine in the hair of various mice where the ordinate is the days of administration of methamphetamine-hydrochloric acid into intraperitoneal cavity (100 μg/day) and the abscissa is the concentration of methamphetamine in the hair (ng/mg).*

indications suggest that the deposition of the methamphetamine is dependent on the animal species.

Detection in Human Hairs of Drugs Other Than Methamphetamine

As shown in the experiments using perspiration [*1, 9*], it may be possible that many drugs, such as amitriptyline, nortriptyline, imipramine, nicotine, and so forth are also found in human hair. These investigations may be applied, not only in the field of toxicology, but also in the identification of individuals.

Hairs from the smokers and nonsmokers contain nicotine of 18 to 177.2 μg/g. Although there is a tendency to indicate that the hairs of smokers contain a larger amount of nicotine, no clear cutoff level can be attained by examining the nicotine content in the hair. In the examinations to estimate the tricyclic antidepressants in the hairs, following results were obtained.

Case 1—Administration of amitriptyline and imipramine with 12.8 μg/g of amitriptyline and 11.2 μg/g of its metabolite nortriptyline and 16.5 μg/g of imipramine and 16.5 μg/g of its metabolite demethylimipramine.

Case 2—Same drug combination with 23.9 μg/g of amitriptyline, 68.4 μg/g of nortriptyline, 46.9 μg/g of imipramine, and 20.4 μg/g of demethylimipramine.

Case 3—Administration of nortriptyline and imipramine with 118.5 μg/g of nortriptyline, 69.2 μg/g of imipramine, and 22.6 μg/g of demethylimipramine. This experiment indicates that the toxicological investigation of only a few milligrams of hair (a few pieces) determine definitely whether the patients are under the long-term treatment of medicines.

Discussion

Amphetamines (methamphetamine and amphetamine), tricyclic antidepressants, and nicotine are also found in the hair, as in the cases of heroin and its morphine metabolites [*4*], morphine [*3*], phenobarbital [*5*], and phencyclidine [*4*]. The amount reaches levels that are caught easily by usual GC and radioimmunoassay (RIA) assays. In previous forensic toxicology, much attention has been given to the field of intoxication because of inorganic substances found through hair analysis. Since organic substances are also contained in hairs, they should be regarded as an important source of the screening of drug abuses. Some more important aspects of hair analysis may be mentioned. At present, there is no available method to confirm addicts (chronic intoxications) by toxicological examinations alone. When a methamphetamine addict is prevented from taking the drug, its excretion into the urine decreases suddenly, so that it cannot be found after a few weeks by routine techniques. No difference between acute and chronic intoxication can be discerned at this stage. Although some tissues, like sweat glands, retain the methamphetamine longer [*1*], an examination of the sweat gives no clearcut evidence to indicate chronic intoxication. A quantitative analysis of the hair gives presumably the most important information on the dose during long-term administration; the localization pattern of the amphetamines in a single hair, as indicated by Baumgartner et al [*2,4*] will give the toxic state of the addict more distinctly.

The determination of the drugs from the biological stains is considered an important approach for the trace evidence examination [*5*]. Although the amount of methamphetamine in various stains in our experiment could not be evaluated on the scientific basis, because of the lack of precise description of the original volume, some indication may be attained. Usually, the excretion of the amphetamine into the saliva is a scanty one, which is hardly possible to be detected in analyzing 1 mL of saliva using the routine GC method [*10*]. Detection of the methamphetamine in the amount of 0.43 μg surely could be due to the concentration of a few millilitres of saliva.

The level of the accumulation of methamphetamine, nicotine, and tricyclic antidepressants

in the hair tissue is very high—sometimes over 100 μg/g—in comparing that to the concentration in the other tissues for acute intoxication with the high dose [*11*]. Harrison et al [*12*] suggested that the amphetamines might be incorporated into the melanin pigment. This cannot be supported by our experiment, because the hair of ddY mice, which lacks this pigment, also contains this substance. The simple bindings of drugs with hair proteins may be a more reliable mechanism to explain the accumulation of various kinds of drugs in the hair.

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