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Experimental Investigations on Hair Fibers as Diffusion Bridges and Opiates as Solutes in Solution

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ABSTRACT: Diffusion experiments were performed using clipped hair fibers as diffusion bridges and aqueous solutions of morphine, codeine and dihydrocodeine. Natural as well as predamaged hair fibers were investigated. The test series were conducted at ambient temperature and at high humidity. After 312 or 372 hours the middle segments of the strands were clipped, washed and analyzed by GC/MS. Only when virgin hair samples were used the solutes passed along the fiber at full length resulting in a positive immunological finding at the end of the diffusion bridge. Most of the washing fluids were positive for opiates. All centerpieces had a high opiate content. The opiate concentration in damaged hair was significantly higher. Radial swelling of the hair fiber with radial diffusion was the first and main process to appear when hair was exposed to water. The diffusion process in hair could not be placed in a simple mathematical treatment.

KEYWORDS: forensic science, toxicology, hair analysis, opiates, radial diffusion, axial diffusion, external contamination

In recent years hair analysis for abused drugs has received considerable attention which is mainly due to potential advantages over current drug testing of body fluids such as urine and serum. First of all hair analysis provides a much larger window of detection. A decrease of drug levels with increasing distance of the hair segment from the scalp in hair samples of addicts was frequently observed although the degree of drug abuse had not been changed (1,2,3). Nakahara et al. found in an experimental study on the movement and stability of methoxyphenamine along the hair shaft that in cases of the same dosages the drug concentration in the root sections were higher than those in the tip sections (4). They concluded that some drugs may escape from hair by washing, especially from hair that was damaged by environmental influences. Further, drugs are also assumed to gradually decompose in the hair shaft with time.

This study was undertaken to examine if there are pathways in the hair fiber for small organic molecules like morphine, codeine and dihydrocodeine by which they leave or enter the fiber.

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Material and Methods

Hair Samples

The hair specimen used were natural Caucasian and Chinese hair, bleached Chinese and partly bleached Caucasian hair. Each test series was run twice with 300 fibers 8.5 cm in length. Additionally a sample blank was carried out for each hair species.

Experimental Design

The hair strands were subjected to water as diffusion bridges. Either end was put 1.5 cm in length into an Eppendorf tube (Fig. 1). One tube (A) was filled with an aqueous solution of morphine, codeine or dihydrocodeine, the other tube (B) was filled with tap water pH 7.8 (Fig. 1*a*). The experiments were performed in a humid chamber at ambient temperature. At given time intervals a 50 μL portion was taken out of tube B and replaced by fresh tap water. The samples taken were directly analyzed by immunoassay. Samples with test results above the highest calibrator value of 600 ng/mL were diluted with water in the ratio of one to ten.

Additional experiments were performed with natural hair strands after the middle segment (5 cm) of each hair fiber had been coated with nail polish prior to incubation (Fig. 1*b*). Both ends of the hair fiber were left untreated. The experiments were stopped after 312 or 372 hours. As for the coated fibers the test series was interrupted after 6 weeks.

The hair strands were removed and air dried. Then the middle segments were clipped and both end pieces were discarded. Only the centerpieces 4.5 cm in length were used for drug analysis by GC/MS.

Chemicals

All reagents were of analytical grade. Codeine and morphine were purchased from Merck (Darmstadt, Germany) and dihydrocodeine from Knoll (Ludwigshafen, Germany). Solutions were prepared with tap water pH 7.8 to yield concentrations of 1 mg/mL for morphine, codeine and dihydrocodeine. Pentafluoropropionic anhydride (PFPA) was provided by Fluka (Buchs, Switzerland).

Immunological testing was done with an Abuscreen Online® test kit for opiates from Hoffmann-La Roche (Grenzach-Wyhlen, Germany). The cross reactivity for the assay is approximately 134% for codeine and 95% for dihydrocodeine referred to a morphine concentration of 300 ng/mL. All values are given as morphine equivalents.

Instrumentation

The above mentioned immunoassay was performed on a Cobas Fara II autoanalyzer from Hoffmann-La Roche (Grenzach-Wyhlen,

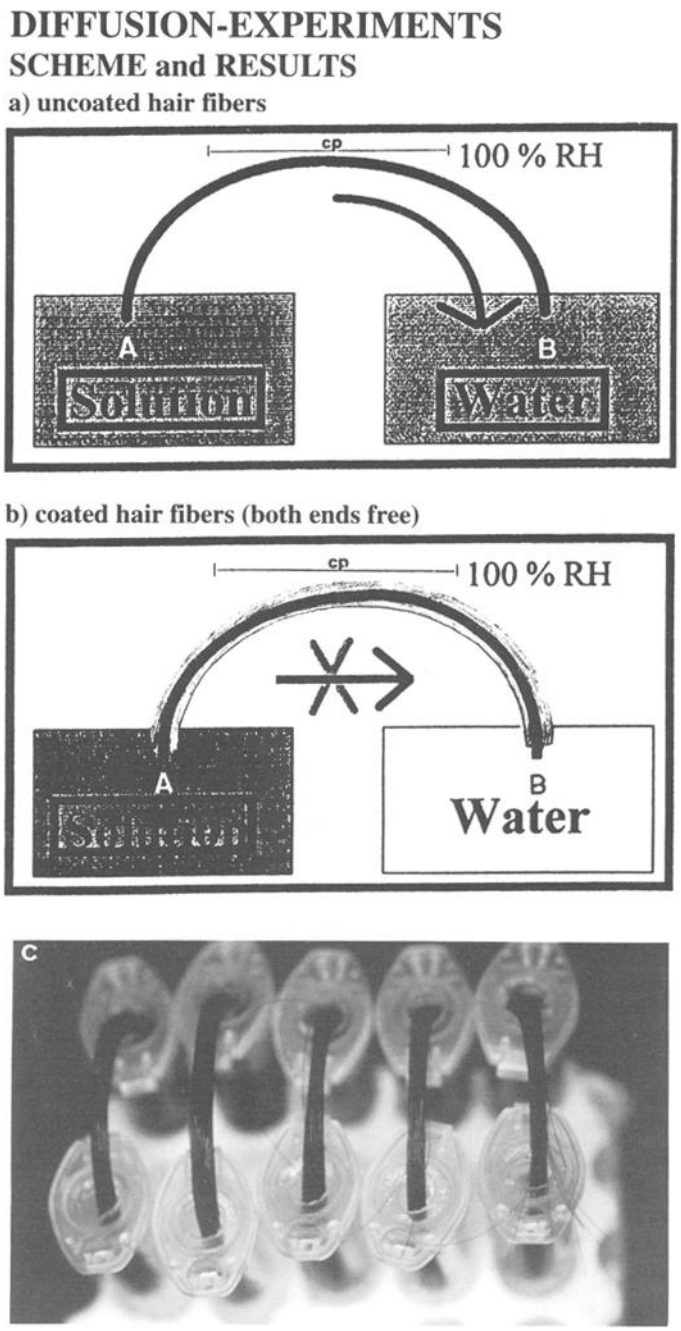

FIG. 1—*Experimental design, scheme and results of the diffusion experiments. Uncoated natural hair fibers used as diffusion bridges.* A: *solution containing opiate substances,* B: *tap water, cp: centerpiece used for GC/MS analysis. Coated hair fibers used as diffusion bridges. Incubation arrangement of the hair strands.*

Germany). The GC/MS system consisted of a HP 5890 gas chromatograph and a HP 5988 A mass spectrometer operated in EI mode with an electron energy of 70 eV (Hewlett Packard, Waldbronn, Germany). The gas chromatograph was equipped with a capillary CP-Sil 5 column (12 m, 0.25 mm i.d., 0.33 μm film thickness, Chrompack, Middelburg, the Netherlands). Samples were injected in the splitless mode. The injector and the detector were kept at 250°C. The gas chromatograph was temperature-programmed from 170°C (1 min hold) to 320°C (1 min hold) at 20°C/min.

Drug Analysis

The centerpieces of the hair strands were rinsed with 2 mL methanol and air dried again before each sample was pulverized in a ball mill. The methanol from the washing procedure was taken to dryness, the residue vortexed with 200 μL phosphate buffer (pH 7) and the supernatant tested with the above-mentioned immunoassay.

The pulverized hair samples were weighed, 2 mL of acetone and 500 ng/mg hair of deuterated morphine as internal standard were added. The samples were ultrasonicated for 4 hours and kept at 40°C overnight. After centrifugation 1.8 mL of the supernatant were evaporated to dryness under a stream of nitrogen. The residue was derivatized with 100 μL pentafluoropropionic anhydride for 30 min at 70°C. The derivatized residue was reconstituted in 200 μL of dichloromethane/isopropanol in a ratio of nine to one. 1 μL was injected into the GC/MS system. For quantitative determination m/z = 414 was used for morphine, 445 for codeine and 447 for dihydrocodeine respectively. Standard calibration curves were obtained using 1000, 500 and 100 ng of the corresponding opiate/mg hair. The linear correlation coefficients of the calibration curves were 0.995 for morphine, 0.989 for codeine and 0.988 for dihydrocodeine. Hair powder spiked with morphine, codeine and dihydrocodeine at 200 ng/mg was proceeded as described before. The recovery was 77% for morphine, 84% for codeine and 79% for dihydrocodeine. The limit of detection was 0.5 ng/mg hair according to a final volume of 200 μL. The precision of the determination was characterized by an intra-assay CV of 5.3% for a morphine level of 200 ng/mg, of 9.7% for a codeine level of 200 ng/mg and of 10% for a dihydrocodeine level of 200 ng/mg (n = 8). There was no interfering signal from the biomatrix.

Results

Immunological Drug Testing of Test Tube B

There was a positive immunological finding for the 50 μL samples taken out of the water containing test tube only when natural hair fibers were used. When natural Caucasian hair specimen were used as diffusion bridges there was a positive reaction (>300 ng/mL) for codeine 24 hours after the experiment had been started (Table 1). The corresponding time was 48 hours for morphine and 96 hours for dihydrocodeine. A kinetic curve for

TABLE 1—*Results (ng/mL) of the immunological screening of tube B for codeine, direction of hair fibers bridging tube A and B according to hair growth n.d.: not detectable, —: not measured.*

Time intervals hours	Natural Caucasian hair	Partly bleached Caucasian hair	Natural Asian hair	Bleached Asian hair
12	n.d.	n.d.	n.d.	n.d.
24	580	n.d.	n.d.	n.d.
48	965	n.d.	n.d.	n.d.
72	1575	n.d.	n.d.	n.d.
96	1922	n.d.	n.d.	n.d.
120	2170	n.d.	n.d.	n.d.
168	2645	n.d.	n.d.	n.d.
216	3500	n.d.	388	n.d.
264	3370	n.d.	—	n.d.
312	4740	n.d.	463	n.d.
372	—	—	512	—

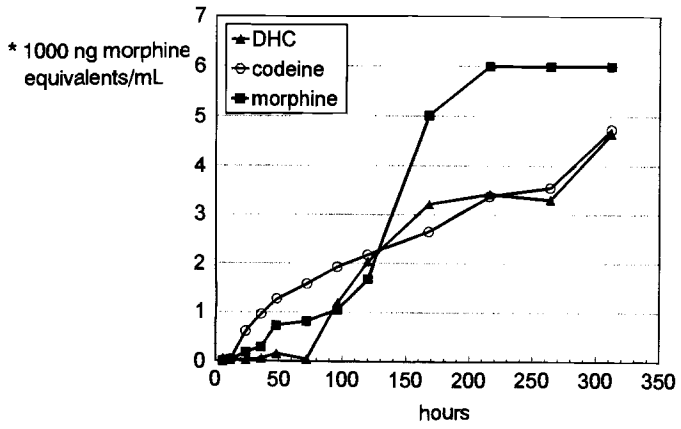

FIG. 2—*Monitoring of the opiate level in tube B at the end of the diffusion bridge (8.5 cm) for the natural Caucasian hair sample.*

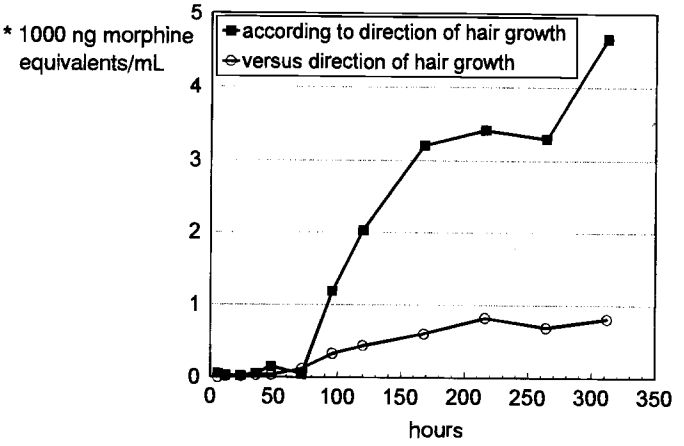

FIG. 3—*Monitoring of the dihydrocodeine level in tube B dependent on hair growth direction of the natural Caucasian hair sample at the end of the diffusion bridge (8.5 cm).*

the immunological findings for natural Caucasian hair is shown in Fig. 2. For the damaged and the predamaged hair samples drug testing of tube B remained negative. In the before-mentioned cases the diffusion front did not reach the end of the fibers resulting in a negative immunological finding throughout the experiment, although high opiate concentrations were found in these hair fibers (Table 2).

Influence of the Direction of Hair Growth on Drug Testing Tube B

Drug monitoring was completed by microscopical examination of the hair fibers used as diffusion bridges. The growth direction was established by the cuticula pattern. Accidentally the experiments revealed that the results obtained from immunological drug testing (tube B) were also influenced by the direction of the hair growth. When the root near end of a Caucasian natural hair strand was put into the opiate solution and the tip near end into water, an earlier positive immunological finding occurred compared to a strand that was bridging tube A and tube B against the direction of hair growth. The rate of diffusion and the dihydrocodeine concentration in the natural Caucasian hair specimen is shown in Fig. 3 (Table 3).

Drug Screening of the Washing Solution

For most of the samples the washing fluids were positive for opiates.

Drug Analysis of the Hair Centerpieces

In coated hair fibers opiates could not be detected (Fig. 1*b*). In uncoated hair fibers all centerpieces were found to be opiate positive (Fig. 1*a*). The opiate concentration in the middle segments ranged from 295 to 636 ng/mg hair for codeine, from 305 to 847 ng/mg for dihydrocodeine and from 141 to 608 ng/mg for morphine. The opiate concentrations were significantly higher in the Asian bleached hair sample and the partly bleached Caucasian hair sample (Table 2).

TABLE 2—*Opiate concentration of the hair centerpieces (ng/mg hair).*

	Natural Caucasian hair	Partly bleached Caucasian hair	Natural Asian hair	Bleached Asian hair
Morphine	280	608	141	452
Codeine	295	594	336	636
Dihydrocodeine	305	809	—	847

Discussion

The results showed that the solutes were absorbed from the solution by the hair fibers. Obviously the solution moved along the hair strands and penetration into the hair fibers occurred whenever water molecules were present. Only when the hair fibers were prevented from swelling by coating with nail polish diffusion effects were not observed.

As recently demonstrated by experiments using an aqueous rhodamine B solution and hair strands as diffusion bridges the processes that took place could be visualized (6). It could be shown that water or an aqueous solution is attracted by capillary forces between the single fibers. From the rhodamine B experiments it could be concluded that swelling of the hair with radial diffusion was the first and main process to appear when hair was exposed to water. As soon as the cross-sectional area of the hair fiber was saturated the capillary front moved forward.

This capillary action and movement along the fibers was found to be strongly influenced by the morphology of the hair specimen. When hair damage was present like in bleached hair samples the swelling and absorption capacity was increased resulting in a

TABLE 3—*Immunological screening of tube B for dihydrocodeine (ng/mL). Direction of the natural Caucasian hair strands bridging tube A and tube B according to and versus hair growth.*

Time intervals (hours)	According to hair growth	Against hair growth
48	n.d.	n.d.
72	n.d.	n.d.
96	1190	327
120	2030	437
168	3210	600
216	3420	820
264	3300	690
312	4660	810

negative drug testing of tube B. A relatively high drug concentration was found in these centerpieces.

The velocity of the movement of the solutes along the hair bundles was also influenced by the orientation of the scale edges.

As shown by drug analysis of the centerpieces the opiates had penetrated into the fiber from the fiber surface. These findings are in accordance with the results of the rhodamine B study (6). From other dyes like methylene blue and orange II it is known that they first enter the fiber between the cuticles along the endocuticula at the scale edges. Then diffusion occurs in the cortex, preferentially in the non-keratinous regions (5) within one hour. For opiates as solutes it is to be supposed that the process is the same.

The capacity of the hair fiber to accumulate exogenous organic molecules of small size was many times higher compared to findings in cases of drug abuse (7–10). Although the nature of drug binding to the hair fiber is not known the following conclusion can be drawn. All solutions were prepared with tap water of a pH value of 7.8. Under this condition the hair fiber is kept above its isoionic point. The isoionic point is a whole fiber property reflecting the equilibrium acid-base properties of the total fiber. According to the literature the isoionic point of a virgin hair fiber is from pH 5.6 to 6.2 (5). Therefore at any pH above the isoionic point the fiber bears a net negative charge and a ionic binding mechanism is favored.

The results gained up to now could not be placed in a simple mathematical treatment. Generally the diffusion process depends on temperature, reaction time, concentration, area of section and pH value. Most studies of diffusion into fibers employ equitations derived from Fick's law providing apparent instead of free diffusion coefficients. For example Crank (11) described an equitation from a solution of limited volume into a cylinder of infinite length. Assuming that the ratio of the amount of solute sorbed in time t to the maximum sorption capacity is linear to the square root of time the apparent diffusion coefficient may be calculated. But this assumption did not fit for our experiments. The hair fiber is not a homogenous cylinder of keratin. Its various structures represent a multicomponent system strongly influencing the diffusion process by its ultrastructure. Discontinous diffusion coefficients have to be discussed and the problem of a heterogenous moving boundary is obvious.

As already pointed out by Baumgartner (12) porous hair causes difficulties in the interpretation of the results of hair analysis. Recently Moeller (13) reported findings of low drug concentration in hair samples that had been frozen wet and stored at 4°C for some time. Kidwell and Blank (14) demonstrated that the external incorporated drugs cannot be removed by standard washing procedures. All these findings are in accordance with the results presented. The non-keratinous regions in human hair should provide channels for diffusion of small molecules in the presence of water. These pathways may be used in both directions: into and out of the fiber. The diffusion process can result in external contamination or loss of drug concentration dependent on molecular structure. As shown by the experiments the morphology and the physicochemical properties of the hair fiber are indespensable for a valid interpretation of the results of drug analysis in hair.

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