Paper Chromatography Study of *In Vitro* and *In Vivo* Hydrolysis of Heroin in Blood

By GEORGE R. NAKAMURA* and TYUNOSIN UKITA†

A study was conducted using paper chromatography to elucidate the pathway of heroin hydrolysis in human blood *in vitro* and in dog's blood *in vivo*. Results indicated that heroin deacetylated rapidly to monoacetylmorphine which remained relatively stable in blood and left the bloodstream as such. Only a trace amount of morphine was detected *in vitro* and *in vivo*. Both compounds became undetectable after 30 min. in the *in vivo* experiment.

THIS LABORATORY has yet to report a positive finding of morphine or morphine derivatives in whole blood specimens. The problem seems to be in the limited sensitivity of the methods (1, 2) used or/and in the timing of blood collection. While the urine remains a preferred body fluid specimen for narcotic analysis since it acts as a reservoir of metabolic waste, the examination of blood specimens may yield information as to the origin of morphine eliminated in the urine.

For forensic consideration, it seems more convincing to inform the court that the subject had taken heroin, especially when he has had heroin in his possession. A finding of monoacetylmorphine in blood would strongly imply that heroin was taken.

Therefore, some experiments were performed to determine the nature of heroin hydrolysis in human blood and the length of time heroin and its hydrolysis products remain in the blood circulation, *in vivo*.

PROCEDURE

Preparations of diacetylmorphine, 6-monoacetylmorphine, and 3-monoacetylmorphine were the same as those described in a previous paper (3).

Ten milligrams of diacetylmorphine hydrochloride were dissolved in 20 ml. of fresh human serum in a 50-ml. conical flask and to this mixture was added 1 mg. of oxytetracycline HCl to suppress bacterial growth. The flask was fixed in a water bath at 37.5°. At various time intervals, a 1-ml. aliquot was removed and introduced into a small separator containing 0.5 ml. of saturated $NaHCO_3$. The alkaline mixture was immediately shaken with 20 ml. of 9:1 chloroform–ethanol mixture. Then, the extract was filtered through a pledget of cotton into a small beaker. The clear extract was evaporated to near dryness and the entire residue was applied onto a Whatman No. 1 chromatography paper. The chromatographic procedure was the same as that described by Genest and Farmilo (4) and was used as a standard method in this study.

To determine if nonenzymic hydrolysis, *i.e.*, autohydrolysis of heroin and 6-monoacetylmorphine could occur in human blood, serum was this time heated to 60° for 1 hr. and the substrate diacetylmorphine was introduced into the deactivated serum. The resulting mixture was incubated at 37°, extracted, and chromatographed under standard conditions. For further confirmation of possible autohydrolysis of heroin, the substrate was introduced into $^1/_{15}$ *M* phosphate buffer, pH 7.3, and the same standard procedure was followed for the hydrolysis study.

Three dogs, each weighing about 20 lb., were selected for the *in vivo* study of heroin hydrolysis. Each received an intramuscular injection of 80 mg. of pure diacetylmorphine hydrochloride. Twenty milliliters of blood was collected from the jugular vein at 10-min. intervals as shown in Fig. 3. The specimens were treated with heparin and chilled in an ice water bath. Plasma was separated from these specimens using a refrigerated centrifuge, adjusted to pH 8.0 with saturated $NaHCO_3$, and then shaken with 9:1 chloroform–ethanol mixture.

RESULTS AND DISCUSSION

The chromatogram in Fig. 1 showed that the *in vitro* hydrolysis of heroin in human serum proceeded rapidly and it disappeared within 1 hr. Furthermore, the hydrolysis proceeded by pathway of 6-monoacetylmorphine. 3-Monoacetylmorphine was not a product of hydrolysis indicating the more rapid cleavage of the phenolic acetyl group. The amount of morphine liberated at the end of 7 hr. was comparatively small. 6-Monoacetylmorphine was still present in the serum in a large proportion after 7 hr. of incubation, indicating its stability and its possible role as an important mediator for the pharmacological activity of heroin (5). Also this stability in blood offered a possibility for its forensic detection in human blood.

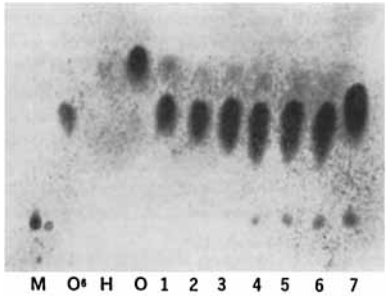

Fig. 1.—*In vitro* hydrolysis of heroin in human serum at 37.5°. Aliquots from reaction mixture examined at 1-hr. intervals. Key: M, morphine; 0^6, 6-monoacetylmorphine; and H, heroin.

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In the research reported here, the "Principles of Laboratory Animal Care," as established by the National Society for Medical Research, were adhered to.

* Present address: Alcohol and Tobacco Tax Laboratory, Internal Revenue Service, U. S. Treasury Department, San Francisco, Calif. 94102.

† Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan.

Control experiments using enzyme-deactivated human serum (Fig. 2) indicated that a rapid hydrolysis of the phenol acetyl group occurred without enzymic catalysis. The same chromatographic pattern was obtained by using phosphate buffer instead of deactivated serum. This observation further indicated that heroin could be degraded mostly to 6-monoacetylmorphine in the human body by autohydrolysis. Autohydrolysis seems to hold true for further degradation of 6-monoacetylmorphine to morphine since the amount of morphine liberated from both the heat-treated and untreated serum was approximately the same. Ellis (6) demonstrated by a manometric method of analysis that human blood was devoid of 6-monoacetylmorphine esterase activity. However, in the authors' study, the rate of morphine liberation was slightly faster in nontreated serum, therefore, the possible occurrence of enzyme catalysis could not be entirely precluded.

Cochin *et al.* (7) has shown that the plasma level of morphine in dogs after a subcutaneous injection attained a peak level at 45 min. The authors' paper chromatographic study of heroin metabolism in dogs showed that 6-monoacetylmorphine was the principal breakdown product of heroin in blood and that it persisted on the chromatogram for a 30-min. period. The chromatogram in Fig. 3 showed strong spots for 6-monoacetylmorphine and weaker spots for morphine for a 30-min. period, indicating again the relatively stable monoacetylmorphine in blood even under *in vivo* conditions. Only a faint spot for morphine could be detected after 30 min. suggesting that most of the degraded products of heroin had left the blood stream by that time. 6-Monoacetylmorphine and morphine were characterized by ultraviolet absorption spectra examination and color tests described by the authors previously (3).

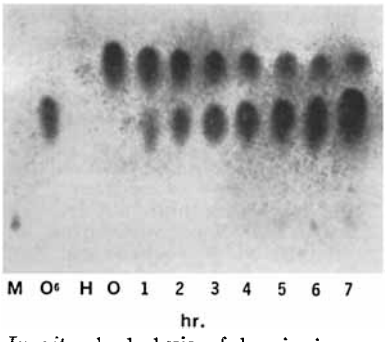

Fig. 2.—*In vitro* hydrolysis of heroin in enzyme-deactivated serum at 37.5°. Same key and conditions as those in Fig. 1.

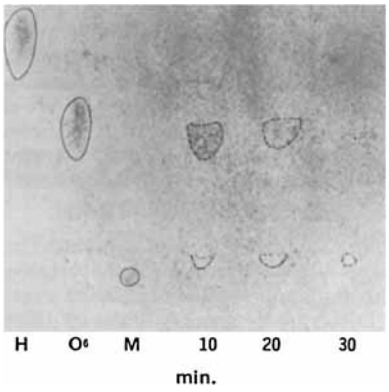

Fig. 3.—*In vivo* hydrolysis of heroin in dog, administered intramuscularly 80 mg. of diacetylmorphine hydrochloride; 20 ml. of blood collected at 10-min. intervals and examined for metabolites. Key same as in Fig. 1.

SUMMARY

In vitro and *in vivo* study of heroin in blood revealed that monoacetylmorphine was the principal product and it was relatively stable *in vitro* for at least 7 hr. in blood at 37°. The study further indicated that heroin entered the blood stream and was rapidly deacetylated to monoacetylmorphine, leaving the circulation virtually in this form. The detection of monoacetylmorphine was, therefore, indicated for forensic purpose. However, the study showed that blood must be collected within a 30-min. period after administration.

Morphine was detected on the chromatogram upon *in vitro* and *in vivo* hydrolysis at a much lower level.

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