Urinary Excretion of Hydromorphone and Metabolites in Humans, Rats, Dogs, Guinea Pigs, and Rabbits

E. J. CONE ˣ, B. A. PHELPS, and C. W. GORODETZKY

Abstract □ Hydromorphone was administered as a single dose to humans, rats, dogs, guinea pigs, and rabbits, and timed urinary collections were made. GLC–mass spectrometric and GLC analyses of the samples revealed the presence of the parent compound and both 6-hydroxy epimers as metabolites in the urine of all species. Free or conjugated parent drug predominated, while levels of free or conjugated 6β-hydroxy metabolite were higher than or equal to those of the 6α-form. The time courses of excretion of drug and metabolites were similar for all species, with the major portion being excreted in the first 24 hr. Generally, free and conjugated drug were undetectable in human urine after 8 and 48 hr, respectively.

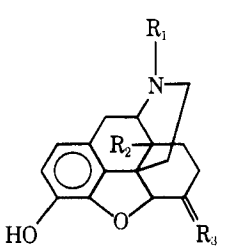
Keyphrases □ Hydromorphone and metabolites—urinary excretion in humans, rats, dogs, guinea pigs, and rabbits □ Excretion, urinary—hydromorphone and metabolites, humans, rats, dogs, guinea pigs, and rabbits □ Metabolites—hydromorphone, urinary excretion in humans, rats, dogs, guinea pigs, and rabbits □ Narcotic analgesics—hydromorphone and metabolites, urinary excretion in humans, rats, dogs, guinea pigs, and rabbits

Hydromorphone (I) (dihydromorphinone) is a potent narcotic analgesic widely used for pain relief orally and parenterally. Its metabolism in rabbits is by conjugation and reduction to dihydromorphine (1). However, little is known regarding its metabolism and excretion in humans and other mammalian species.

Since hydromorphone is structurally related to the narcotic antagonists naltrexone (II) and naloxone (III) whose metabolic profiles include C-6 reduction to both α- and/or β-hydroxy metabolites, depending on species, it was of interest to determine if analogous pathways existed for hydromorphone. This report describes the determination of free and conjugated hydromorphone and the 6-hydroxy metabolites dihydromorphine (Iα) and dihydroisomorphine (Iβ) in the urine of humans, rats, dogs, guinea pigs, and rabbits given single doses of hydromorphone.

EXPERIMENTAL

Reagents and Chemicals—Reagent grade chloroform, 2-propanol, and methanol were used as received. Ethyl acetate was dried over calcium hydride. Compounds I[1], Iα[2], and Iβ[2] were used as received. The drug purity was determined prior to the study by TLC, GLC, and GLC–mass spectrometry (2).



I: $R_1 = CH_3$, $R_2 = H$, $R_3 = O$

II: $R_1 = CH_2$—cyclopropyl, $R_2 = OH$, $R_3 = O$

III: $R_1 = CH_2CH{=}CH_2$, $R_2 = OH$, $R_3 = O$

Iα: $R_1 = CH_3$, $R_2 = H$, $R_3 = \alpha$-OH

Iβ: $R_1 = CH_3$, $R_2 = H$, $R_3 = \beta$-OH

Table I—Protocol for Animal Experiments

Species	*n*	Average Weight	Dose of I Hydrochloride, mg
Guinea pig	6	998 g	5
Rat	5	462 g	5
Dog	2	7.75 kg	10
Rabbit	4	4.15 kg	5

GLC—A gas chromatograph[3], equipped with a flame-ionization detector, was used with a 1.83-m × 2-mm glass column packed with 3% OV-225 on 100–120-mesh Gas Chrom Q. The injector, detector, and column oven were maintained at 250, 260, and 215°, respectively. The air, hydrogen, and nitrogen carrier gas flows were 300, 30, and 26 ml/min, respectively. The retention times (in minutes) of the trifluoro derivatives were 2.60 for I, 2.94 for Iα, 2.13 for Iβ, and 3.56 for the internal standard, morphine.

GLC–Mass Spectrometry—Chemical-ionization mass spectral data were obtained on a quadrupole gas chromatograph–mass spectrometer[4] equipped with a glass column (1.52 m × 2 mm) packed with 3% OV-225 on 100–120-mesh Gas Chrom Q. The gas chromatograph was coupled to the mass spectrometer by a 0.31-cm glass-lined stainless steel tube and a venting valve. The electron energy was 80 ev. Methane, with a flow rate giving an ion source pressure of 1000 μm, was used as the carrier and reagent gas.

The temperatures of the injector, column, and ion source were 230, 215, and 100°, respectively. After sample injection, the venting valve was opened for 20 sec, allowing solvent and highly volatile substances to escape without entering the ion source. Selected ion recording was performed while focusing on *m/e* 480, 478, and 366 using a 1.8-kv multiplier setting.

TLC—Compounds I, Iα, and Iβ were applied to glass fiber sheets[5] and eluted in a saturation-type chamber containing chloroform saturated with aqueous ammonia. Conditions in the chamber were allowed to equilibrate for approximately 10 min prior to elution. The R_f values for I, Iα, and Iβ were 0.26, 0.08, and 0.04, respectively.

Sample Collections—The animals were housed individually in stainless steel metabolic cages equipped with urine collectors. Predrug control urine was collected the day before drug administration. A single dose of I hydrochloride was administered subcutaneously, and the urine was collected in 24-hr aliquots for 2 days. At the end of each collection period, the cages were rinsed with water, which was then added to the urine collection. Feces remained separated from the urine during collection and were discarded. The samples were centrifuged, decanted, and frozen until analyzed. The animal species, number in each experiment (*n*), average weight, and dose used are summarized in Table I.

The human subjects were healthy, adult male, federal prisoners from whom informed consent was obtained. Their ages ranged from 25 to 46 years. All were former narcotic addicts incarcerated at the National Institute on Drug Abuse Addiction Research Center. The subjects were drug free. Control urine from five subjects was collected for 24 hr before drug administration. Compound I hydrochloride (4 mg po) was administered, and all urine was collected for 144 hr, with collection periods ending at 2, 4, 8, 12, 24, 48, 72, 96, 120, and 144 hr after drug administration.

All samples for each individual were pooled for each collection period.

[1] Knoll Pharmaceutical Co., Whippany, N.Y.
[2] Drug Addiction Laboratory, University of Virginia.
[3] Varian model 2700.
[4] Finnigan model 3300 equipped with a Finnigan model 6000 interactive data system.
[5] Gelman I.T.L.C. type SG, Gelman Instrument Co., Ann Arbor, Mich.

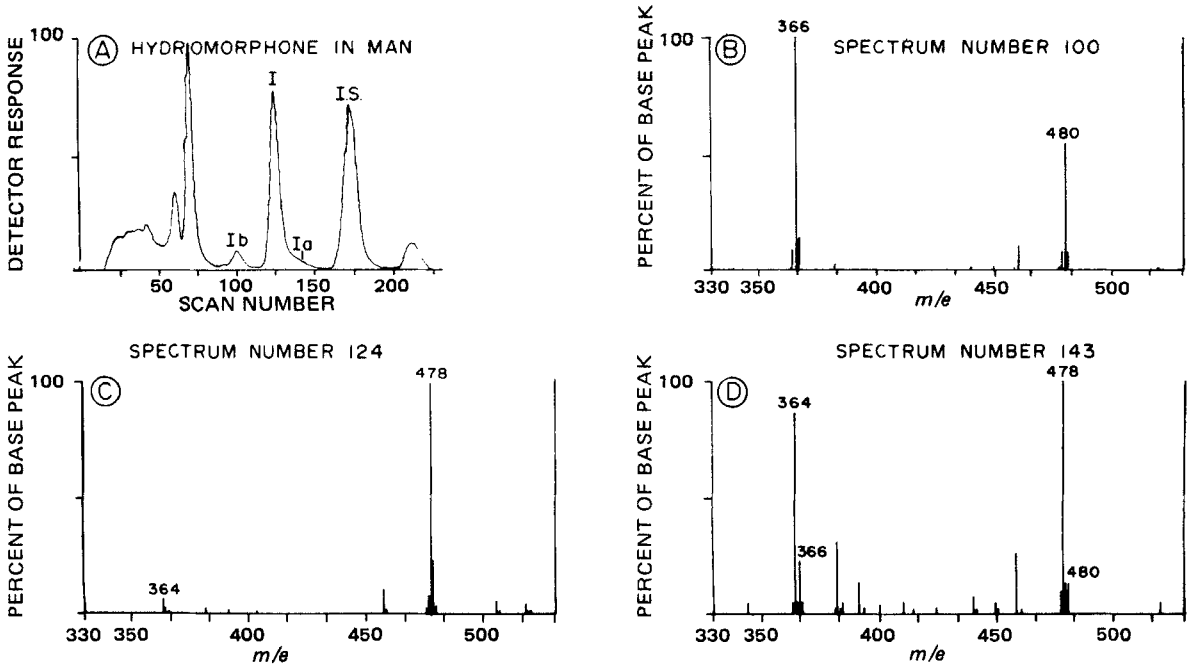


Figure 1—*GLC–chemical-ionization mass spectral (methane) analysis of drug in human urine. Key: A, integrated total ion current chromatogram of urine extract derivatized with trifluoroacetic anhydride; IS, internal standard (morphine); B, spectrum 100 identified as the 6β-hydroxy metabolite of I; C, spectrum 124 identified as I; and D, spectrum 143 identified as mixture of I and Ia.*

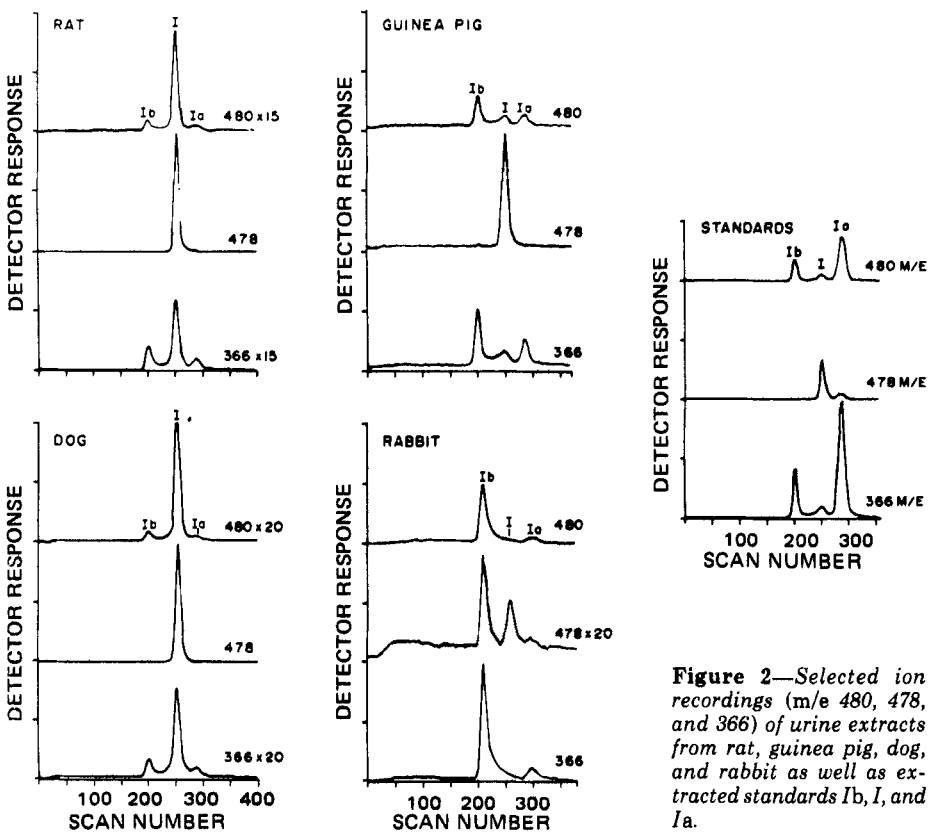


Figure 2—*Selected ion recordings (m/e 480, 478, and 366) of urine extracts from rat, guinea pig, dog, and rabbit as well as extracted standards Ib, I, and Ia.*

During the first two collection intervals (postdrug), the subjects were requested to drink at least 240 ml of liquid to ensure adequate urine volume. No special food or water intake conditions were maintained thereafter. The urine volume was measured, and the samples were frozen until assayed.

Extraction of Samples—Urine samples were acid hydrolyzed for 30 min in 10% HCl in an autoclave at 1.27 kg/cm^2 in a manner similar to that reported (3) for the acid hydrolysis of morphine. The free and acid-hydrolyzed samples were extracted as previously described for the analysis of II (4), with chloroform–2-propanol (9:1) substituted for the extracting solvent. The sample pH during extraction was maintained at 10.0 ± 0.02.

The extract was evaporated to dryness in an acylation tube[6], and 100 μl of trifluoroacetic anhydride[7] was added. The tube was sealed and

[6] Regis Chemical Co., Morton Grove, Ill.
[7] Aldrich Chemical Co., Milwaukee, Wis.

Table II—Recovery of Drug and 6-Hydroxy Metabolites from Urine of Animals Given Hydromorphone[a]

	I				Ib				Ia					
	Free		Conjugated		Free		Conjugated		Free		Conjugated		Total	
Species (n)	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Guinea pig (6)	33.2	0.8	10.7	0.6	18.7	0.4	0	0.2	8.1	0.2	0	0	70.7	2.2
Rat (5)	8.4	0.7	17.0	2.4	0.6	0.1	0.8	0.3	0.7	0.4	0.2	0.3	27.7	4.2
Dog (2)	10.5	0.7	46.6	3.9	0.9	0.2	1.0	0	0.5	0.1	1.0	0	60.5	4.9
Rabbit (4)	6.0	1.6	9.1	3.9	2.0	0.2	3.8	0.9	0.2	0.1	0.7	0.1	21.8	6.8
Human (5)	5.6	0	29.9	6.9	—[b]	—[b]	1.0[c]	0[c]	0	0	0.1[d]	0	36.6	6.9

[a] Values represent the means of triplicate determinations and are expressed as the percent of the administered dose. Levels of conjugated drug and metabolites were determined by subtraction of free from total concentrations after acid hydrolysis. [b] Not determined. [c] Value is based on one subject. [d] Estimated by GLC–mass spectral determination.

heated in an oil bath at 90–95° for 1–2 hr. After heating, the tube was cooled under tap water, and the mixture was evaporated to dryness under dry nitrogen at room temperature. Ethyl acetate (50 μl), dried over calcium hydride, was added; then the tube was resealed, shaken, and analyzed by GLC or GLC–mass spectrometry immediately. Each sample was worked up just prior to analysis to prevent sample hydrolysis.

pH Study—The effect of pH in the 8–12 range on the extraction of I, Ia, and Ib was determined by extracting triplicate samples of each drug with chloroform and adding the internal standard in the final step prior to analysis. A pH range of 9.5–10.5 was optimum.

Extraction Efficiency Study—The efficiency of chloroform *versus* chloroform–2 propanol (9:1) for extraction of I, Ia, and Ib at pH 10.0 was determined in a manner similar to that for the pH study. Peak height ratios of the extracted sample were compared to those of the unextracted substances. Percent recoveries ± SE (n = 6) for chloroform and chloroform–2-propanol were 64.1 ± 20.7 and 49.2 ± 6.8 for I, 39.3 ± 11.8 and 64.0 ±10.7 for Ia, and 25.8 ± 8.2 and 66.8 ± 8.1 for Ib, respectively.

GLC Quantitation—Standard curves for I, Ia, and Ib were prepared by adding known amounts (0–50 μg of I and Ib and 0–12.5 μg of Ia as the free base) to predrug control urine containing morphine (40 μg) added as the internal standard. The samples were extracted with or without acid hydrolysis, derivatized, and analyzed by GLC. Linear relationships of peak height ratios of I, Ia, and Ib were observed for this concentration range. The standard curves for I, Ia, and Ib can be described by $R = 0.0374C - 0.0420$, $R = 0.0399C - 0.0032$, and $R = 0.0541C + 0.0017$, respectively, where R is the peak height ratio of drug to internal standard and C is the concentration.

Prediction limits were constructed from these data as generally described (5). Day-to-day variability of the analysis procedure was monitored by inclusion of standard control samples. Analyses in which the standard control samples fell outside the 95% prediction intervals were rejected. The samples from the untreated and acid-hydrolyzed urine were analyzed on the same curve since the standard controls taken through the hydrolysis and extraction procedures were within the prediction intervals. The reported values represent the mean of at least three determinations.

RESULTS AND DISCUSSION

Qualitative identification of hydromorphone (I) and the 6-hydroxy-metabolites in the urine of humans and animals was made by a series of GLC–mass spectral studies. Figure 1 contains a typical GLC–chemical-ionization mass spectral (methane) chromatogram and associated spectra obtained for a human urine extract. Compounds I (spectrum 124) and Ib (spectrum 100) were identified by comparison of their retention times and chemical-ionization spectra with analytical standards. Small amounts of Ia (spectrum 143) also could be detected.

Table III—Order of Urinary Excretion of Hydromorphone and Metabolites within Species

Species	Relative Amount in Urine
Guinea pig	I > Ib > conjugated I > Ia ≫ conjugated Ib
Rat	Conjugated I > I ≫ conjugated Ib = Ia > Ib > conjugated Ia
Dog	Conjugated I ≫ I ≫ Ib ≥ conjugated Ib = conjugated Ia > Ia
Rabbit	Conjugated I > I > conjugated Ib > Ib > conjugated Ia > Ia
Human	Conjugated I ≫ I ≫ conjugated Ib > conjugated Ia

Screening of the animal urines (Fig. 2) for these metabolites was achieved by selected ion recordings of m/e 480 (M + 1 ion of Ia and Ib), 478 (M + 1 ion of I), and 366 (M + 1 ion − CF_3COOH of Ia and Ib). A detector response at these settings at scan numbers 200 (3.40 min), 250 (4.23 min), and 285 (4.84 min) was characteristic of Ib, I, and Ia, respectively. Both Ib and Ia as well as I were present in the urine of all species.

Quantitative studies on the urinary excretion of these compounds were performed using GLC. Figure 3 shows a sample chromatogram for a guinea pig urine extract, demonstrating the separation of I and the epimeric 6-hydroxy metabolites (Ia and Ib). By using the GLC method described, near baseline resolution was achieved for each component on the relatively polar OV-225 liquid phase. This method was used for the quantitative determination of these substances in urine as free and acid-hydrolyzable conjugates of I, Ia, and Ib in several animal species.

Resolution problems were encountered only in human samples, where caffeine presented serious interference in the determination of the small amounts of Ib present. For these samples, prior elimination of the interfering peak was necessary and was accomplished by elution of the sample extract on glass fiber TLC with chloroform saturated with

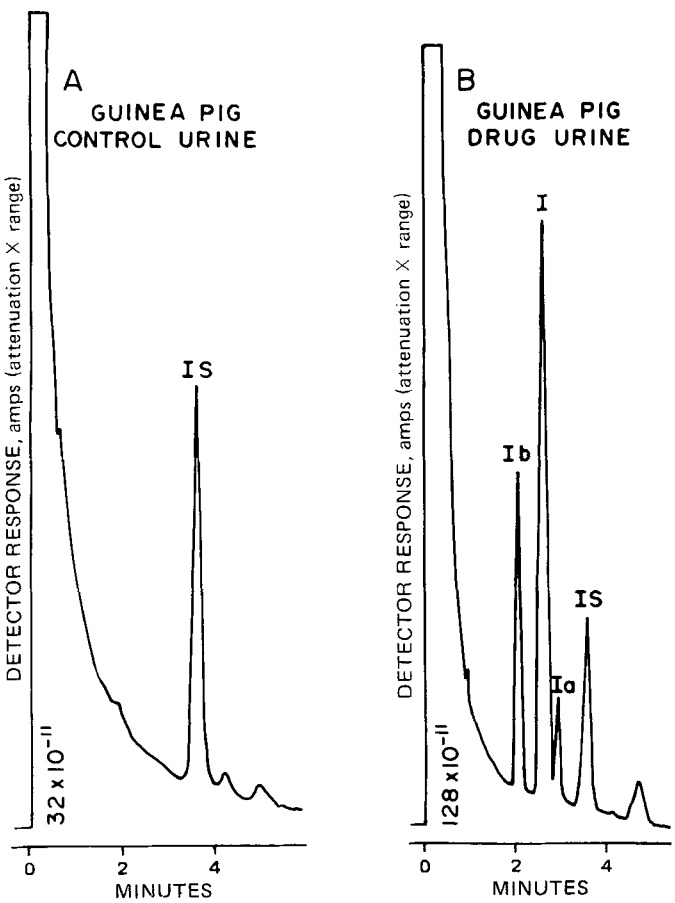


Figure 3—*Gas chromatograms of unhydrolyzed guinea pig urinary extracts derivatized with trifluoroacetic anhydride (IS is the internal standard morphine.)*

Table IV—Timed Urinary Excretion of Free and Conjugated Hydromorphone in Five Human Subjects[a]

	Subject J		Subject W		Subject I		Subject M		Subject D	
Hours	Free	Conjugated	Free	Conjugated	Free	Conjugated	Free	Conjugated	Free	Conjugated
2	1.7	6.7	—[b]	—	—	—	0.5	3.2	4.6	4.5
4	—	—	2.2	17.7	6.3	11.9	0.8	4.6	5.7	4.8
8	2.1	16.8	2.1	6.3	1.0	4.4	0	8.1	0	10.9
12	0	3.3	0	4.2	—	—	0	1.8	0.7	0.8
24	0	6.0	0	3.5	0	19.7	0	7.4	2.2	1.0
48	0	8.4	0	11.0	0	15.1	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0
Total	3.8	41.2	4.3	42.7	7.3	51.1	1.3	25.1	13.2	22.0

[a] Values represent the means of triplicate determinations and are expressed as the percent of the administered dose. Levels of conjugated drug and metabolite were determined by subtraction of free from total concentrations after acid hydrolysis. [b] Sample was not produced.

aqueous ammonia. The zone containing I*b* (R_f 0–0.1) was removed and extracted with methanol. Evaporation followed by derivatization and injection of the extract produced a chromatogram of I*b* free of interferences. No interferences were encountered in the determination of I and I*a*.

Solvent and pH effects on the extractability of the drug and metabolites were determined prior to the metabolic study. Optimal extraction efficiency was found for a chloroform–2-propanol mixture (9:1) where about 50 and 65% recoveries were observed for I and the 6-hydroxy metabolites, respectively. The optimal pH for extraction was 9.5–10.5.

The results of the urinary excretion study of single doses of I in animals and humans are shown in Table II. Both free and conjugated I and the 6-hydroxy metabolites I*a* and I*b* were detected in the urine of all species. As shown in Fig. 4, I was excreted predominantly as an acid-hydrolyzable conjugate (presumably glucuronide); in the guinea pig, however, free I (34.0%) was highest in amount followed by free I*b* (19.1%) and conjugated I (11.3%). The order of excretion of drug and metabolites is illustrated in Table III.

Levels of free or conjugated I*b* were generally higher than or equal to those of the epimeric I*a* for all species. This result was reversed only for the rat, where levels of free I*a* exceeded those of I*b* in the Day 1 and 2 samples (Table II). Apparently, the reduction pathway leading to formation of 6-hydroxy metabolites of hydromorphone is relatively minor for the rat, dog, and human. Greater amounts of free and conjugated I*b* were encountered for the rabbit (2.2 and 4.7%) and guinea pig (19.1 and 0.2%). Levels of I*a* were also highest for the guinea pig (8.3%). Overall total recoveries of drug and metabolite from urine ranged from lows of 28.6 and 31.9% for the rabbit and rat to 65.4 and 72.9% for the dog and guinea pig. Total recovery of drug from humans was intermediate at 43.5%.

The time course of urinary excretion of drug and metabolite was similar for all species, with the major portion being excreted in the first 24 hr. Levels of drug and metabolite in the Day 2 samples were generally lower, ranging from 10 to 50% of the levels in Day 1 samples. The rat was an exception; levels of conjugated I*a*, unlike I*b*, increased in the Day 2 sample. The apparent longer half-life of I*a* *versus* I*b* in the rat might be a result of enterohepatic recirculation of I*a*.

Excretion of free and conjugated I in human subjects was examined at timed intervals during Day 1 through Day 5. Table IV contains the excretion data for free and conjugated I for five subjects. Considerable intersubject variability existed. The initial urine sample of all subjects contained the highest drug concentration. Levels dropped rapidly, and I was undetectable in the free form after 8 hr for four of five subjects. The remaining subject had free I through 24 hr. Conjugated I was detectable in Day 2 samples for three of five subjects.

With TLC for the elimination of the interfering substance, I*b* was quantitatively determined for one subject. Only 1.0% of the administered dose was found. GLC–mass spectral screening of the human samples indicated that both I*a* and I*b* were present in all Day 1 pooled urine samples. The levels of I*a* were consistently below the lower limits of sensitivity of the GLC method (Fig. 1) and were estimated to represent approximately 0.1% of the administered dose.

The relatively small amounts of I*a* and I*b* (Fig. 4) found in rat, dog, rabbit, and human urine suggest that the contribution to pharmacological activity from these active metabolites would be minimal (I*a* and I*b* are approximately two-thirds and one-fifth as potent as I in producing analgesia) (6). However, the amounts of free I*b* and I*a* in the guinea pig were considerably higher, and some pharmacological contribution by these metabolites is likely. This study demonstrated that the species studied

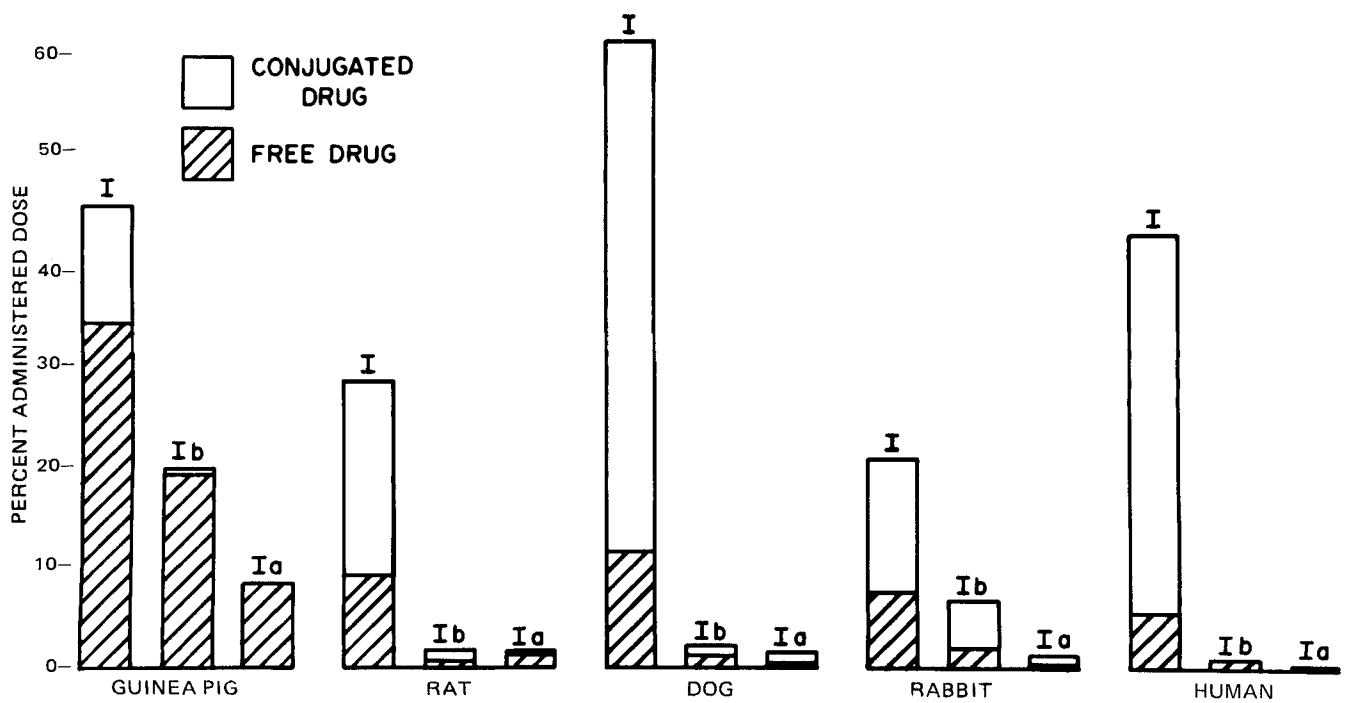


Figure 4—*Overall mean urinary excretion levels of free and conjugated hydromorphone and 6-hydroxy metabolites in humans and animals.*

have the capability of metabolic reduction of I to both epimeric 6-hydroxy metabolites. However, considerable stereospecificity by the drug-metabolizing enzyme(s) favoring formation of the 6β-hydroxy metabolite was observed for all species. This result is consistent with metabolic data reported for other 6-oxo compounds containing the hydromorphone structure (2, 4).

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[x] To whom inquiries should be directed.

Generic Propoxyphene: Need for Clinical Bioavailability Evaluation

K. A. DeSANTE [x], R. G. STOLL [*], D. G. KAISER, and A. R. DiSANTO

Abstract □ Plasma level data on two investigational capsule formulations of propoxyphene with similar physicochemical parameters demonstrate that the formulations have different *in vivo* bioavailabilities. The potential for bioavailability problems with water-soluble drugs and the lack of correlation of *in vitro* and *in vivo* parameters for equivalent drug formulations are discussed.

Keyphrases □ Propoxyphene hydrochloride—different formulations, bioavailability compared □ Bioavailability—propoxyphene hydrochloride, different formulations compared □ Analgesics—propoxyphene hydrochloride, different formulations, bioavailability compared

With the recent expiration of the propoxyphene hydrochloride patent, several pharmaceutical manufacturers have marketed a generic formulation of this drug. These manufacturers were required to assess *in vitro* parameters such as drug content and dissolution rate to assure equivalence to the innovator's product. To date, no *in vitro* dissolution rate standard that has been shown to correlate with *in vivo* bioavailability has been developed.

However, with digoxin, for which *in vitro* standards were used to assure product equivalence (through batch certification), the Food and Drug Administration (FDA) clearly recognized that *in vitro* testing alone often does not ensure bioequivalence (1). This knowledge recently resulted in written bioavailability requirements for marketed digoxin products by the FDA (2).

Propoxyphene hydrochloride is a readily soluble drug. A simple capsule formulation of this drug would generally be considered as having a very low potential for bioavailability problems. Usually, only drugs with low water solubility are considered potential problems in formulating a readily available dosage form (3–5). The converse of this hypothesis (*i.e.*, soluble drugs have low potential for bioavailability problems) has not been investigated adequately. To ascertain possible bioavailability problems of drugs with high risk potential, the low risk potential drugs are rarely studied (6).

Although propoxyphene hydrochloride is a very soluble drug, comparative bioavailability data should be obtained prior to marketing any new formulation. Even the finding of equivalent *in vitro* parameters should not be sufficient justification for assuming bioequivalence of two different formulations of the same parent drug without *in vivo* data.

This paper reports the results of two bioequivalence studies in which two proposed marketed formulations with nearly identical *in vitro* parameters of potency and dissolution rate were compared to the recognized standard propoxyphene product.

EXPERIMENTAL

Bioavailability Studies—The design for both studies was identical. In Study 1, 21 healty adult volunteers[1] (14 males and seven females), averaging 23 years of age (range of 22–33 years) and weighing 68.6 kg (range of 52.2–86.4 kg), were selected. In Study 2, 24 healthy adult male volunteers, averaging 24.5 years (range of 19–51 years) and weighing 77.5 kg (range of 61.4–89.1 kg), were selected.

All subjects had normal screening vital signs (blood pressure, pulse, respiration, and temperature) and laboratory parameters (complete blood count, urinalysis, blood urea nitrogen, serum alkaline phosphatase, serum glutamic–oxaloacetic transaminase, serum bilirubin, and glucose). No subject received any barbiturates or other enzyme-inducing drugs for 30 days, or any other medication for 7 days, preceding the start of the study. All subjects received only the medication prescribed in the protocol for the duration of the study.

At zero time of each treatment period, each subject received single oral doses of medication[2] as follows: Treatment A, one 65-mg hard-filled capsule of propoxyphene hydrochloride (test formulation); or Treatment

[1] Study was initiated with 22 subjects, but one subject was dropped before completion of the clinical phase.

[2] Identity of products is available from authors upon request.