[the phenylmethyl derivative, Ip, 7.1–7.8 ppm (10H)], and the vinyl proton (1H) appeared as a broad singlet at 6.0–6.3 ppm. The 6-phenyl group of II compounds was in the same range downfield, and the 2-methylene group ($OCH_2N$) appeared as a doublet of doublet, 4.41 and 4.58 ppm and 4.80 and 4.95 ppm, probably corresponding to the axial and equatorial protons, respectively[4].

**1-Methyl-1-octyl-4-phenyl-1,2,3,6-tetrahydropyridinium Bromide (Ic)**—A solution of 3.5 g (0.02 mole) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (1) and 10 g (0.05 mole) of 1-bromooctane in 50 ml of anhydrous acetone was heated on a steam bath for 12 hr. The mixture obtained upon final cooling in an ice bath was filtered. Then the crystalline solid was washed with cold acetone and diethyl ether and purified by recrystallization, 1 g/50 ml of acetone, to provide colorless needles.

**1-Decyl-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium *o*-Sulfobenzimide (Ih)**—One gram (0.0025 mole) of 1-decyl-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium bromide (Ie) was suspended in 100 ml of warm (35°) deionized water with stirring, and a solution of 3.0 g (0.012 mole) of sodium *o*-sulfobenzimide dihydrate in 10 ml of deionized water was dripped in (10 min). The product separated initially as a colorless oil and solidified upon mixing an additional 2 hr, followed by cooling in an ice bath. The water-washed and air-dried colorless solid was recrystallized by solution of 1 g in 2 ml of acetonitrile and addition of approximately 18 ml of ether until incipient turbidity. The colorless plates obtained were washed with cold acetonitrile–ether (1:9 v/v).

## REFERENCES


(1) C. J. Schmidle and R. C. Mansfield, *J. Am. Chem. Soc.*, **78**, 425, 1702 (1956). *Ibid.*, **77**, 5698 (1955). C. J. Schmidle and R. C. Mansfield, U.S. pats. 2,847,414 (1958) and 2,750,385 (1956).

(2) M. Rajšner, E. Adlerová, and M. Protiva, *Coll. Czech. Chem. Commun.*, **28**, 1031 (1963).

(3) T. Urbański, D. Guene, S. Šlopek, H. Modarska, and M. Modarska, *Nature*, **187**, 426 (1960).

(4) A. N. Petrocci, in "Disinfection, Sterilization, and Preservation," 2nd ed., S. S. Block, Ed., Lea & Febiger, Philadelphia, Pa., 1977, p. 325.

(5) J. DeMare, H. W. Rossmore, and T. H. F. Smith, *Dev. Ind. Microbiol.*, **13**, 341 (1972).

(6) N. Grier, U.S. pat. 3,920,642 (Nov. 18, 1975).

(7) N. Grier and R. Strelitz, *J. Pharm. Sci.*, **65**, 616 (1976).

(8) J. M. Tanzer, A. M. Slee, and B. A. Kamay, *Antimicrob. Agents Chemother.*, **12**, 721 (1977).

(9) P. L. Block, C. L. Dooley, and E. E. Howe, *J. Periodont.*, **40**, 41 (1969).

(10) R. J. Fitzgerald, *Antimicrob. Agents Chemother.*, **1**, 296 (1972).

(11) H. B. Woodruff, K. H. Nollstadt, R. B. Walton, and T. H. Stoudt, *Dev. Ind. Microbiol.*, **17**, 405 (1976).

(12) Y. E. Kazantsev, I. P. Boiko, Y. F. Malina, O. I. Zhuk, Y. Y. Samitov, and B. V. Unkovskii, *Zh. Org. Khim.*, **9**, 2616 (1972) (in English).

(13) H. Booth and R. U. Lemieux, *Can. J. Chem.*, **49**, 777 (1971).


## ACKNOWLEDGMENTS


The author thanks Microanalytical Services for the elemental analyses, the Basic Microbiology Department for the *in vitro* antimicrobial assays, Dr. R. B. Walton for the *in vitro* periodontal disease screen, and Dr. E. E. Howe and associates for the *in vivo* dental study.


[4] Detailed NMR studies of the tetrahydro-1,3-2*H*-oxazine ring system were reported (12, 13).

# Plasma Naltrexone Kinetics after Intravenous Bolus Administration in Dogs and Monkeys


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Received April 14, 1978, from the **College of Pharmacy, Ohio State University, Columbus, OH 43210,* and the ‡*Pharmaceutical Research Division, Warner-Lambert/Parke-Davis, Ann Arbor, Mich.* Accepted for publication September 6, 1978.


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**Abstract** □ This investigation generated data characterizing a specific electron-capture GLC assay reported previously for naltrexone and applied the method to a determination of naltrexone pharmacokinetics. Extraction efficiencies are reported for the assay, and mass spectral evidence indicates that naltrexone forms a triester when derivatized for electron-capture GLC with pentafluoropropionic anhydride and a base catalyst. Plasma level–time data for intravenous naltrexone at two dose levels in monkeys yielded no evidence of dose-dependent kinetics. A two-compartment open pharmacokinetic model was fitted to plasma level–time data for naltrexone in two dogs and yielded a total body clearance of 51–55 ml/min/kg. Urine collected for 0–24 hr contained 36% of the dose as naltrexone conjugates with less than 1% as unchanged naltrexone. Plasma level–time data for intravenous naltrexone in six monkeys yielded an average terminal half-life of 7.8 hr and a total body clearance of 64 ml/min/kg. The total body clearance for naltrexone was greater than the hepatic plasma or blood flow in both dogs and monkeys. This finding, together with the extremely low renal excretion of naltrexone, suggests the existence of elimination mechanisms besides liver metabolism and renal excretion.



**Keyphrases** □ Naltrexone—plasma kinetics after intravenous bolus administration, dogs and monkeys, electron-capture GLC assay □ Pharmacokinetics—naltrexone after intravenous bolus administration, dogs and monkeys, electron-capture GLC assay □ GLC, electron capture—specific assay for naltrexone □ Narcotic antagonists—naltrexone, pharmacokinetics after intravenous bolus administration, dogs and monkeys, electron-capture GLC assay


Naltrexone, a narcotic antagonist, has been investigated for use in the treatment of opioid dependence (1). In humans, most of the typical responses to a heroin challenge (25 mg iv) are blocked by naltrexone for 48–72 hr after a 100-mg oral dose (2). Because the duration of action of naltrexone is short in relation to that considered optimal


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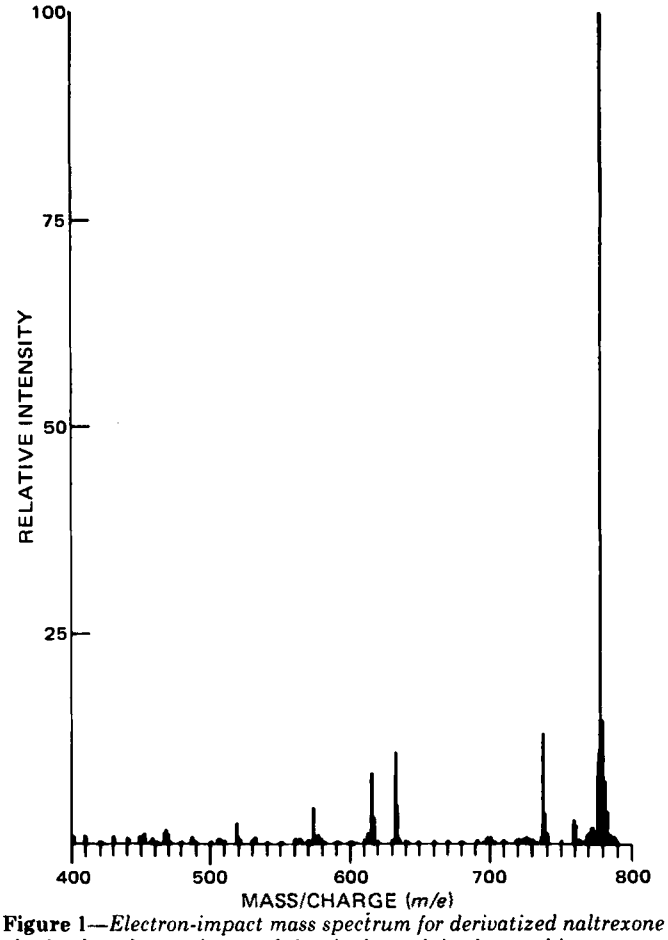



**Figure 1**—*Electron-impact mass spectrum for derivatized naltrexone obtained at the maximum of the single peak in the total ion current* versus *scan number plot.*

from a treatment standpoint, an effort to develop and evaluate sustained-release delivery systems for naltrexone was initiated (3). One prerequisite to a pharmacokinetic evaluation of *in vivo* release rates of naltrexone from such delivery systems is a knowledge of its distribution and elimination kinetics in the species of interest.

Cone *et al.* (4) were the first to report kinetic data on naltrexone in humans. Their data on urinary naltrexone excretion after a single 50-mg po dose indicated a urinary excretion half-life of 1.1 hr (mean of six subjects). However, since the urinary excretion data were obtained after oral administration, it is not possible to ascertain whether the half-life observed is representative of absorption or elimination (5). In addition, the half-life estimate appears to be based on only two time points (4).

Another study (2) reported the plasma level and urinary excretion kinetics of naltrexone in four postaddict human volunteers. The plasma naltrexone data indicated a half-life of 10 hr for the time period up to 24 hr and a half-life of 96 hr from 24 to 72 hr after an oral naltrexone dose. In the absence of intravenous data, however, it is difficult to ascertain the meaning of these half-life values.

The values reported by Verebey *et al.* (2) were much longer than those of Cone *et al.* (4). It is possible that kinetic parameters measured over the first 24 hr are influenced by continuing naltrexone absorption over part or all of this time period. The subsequent 96-hr half-life at very low drug levels probably represents either slow

**Table I—Efficiency of Extraction of Naltrexone from Plasma** [a]

| Naltrexone Added, ng | Blank Plasma, ml | Percent Extracted with Benzene[b] Dog Plasma[c] | Percent Extracted with Benzene[b] Monkey Plasma[c] | Percent Extracted with Ethyl Acetate[b] Dog Plasma[d] | Percent Extracted with Ethyl Acetate[b] Monkey Plasma[c] |
|---|---|---|---|---|---|
| 5[e] | 0.5 | 70 | 82 | — | — |
| 10[e] | 0.5 | — | — | 107 | 78 |
| 50[e] | 0.5 | 68 | 76 | 96 | 90 |
| 100[e] | 0.5 | 72 | 81 | 95 | 92 |
| 2[f] | 2.0 | 64 | 64 | — | — |
| 10[f] | 2.0 | 71 | 76 | — | — |
| 20[f] | 2.0 | 77 | 67 | — | — |
| Mean (*SD*) | — | 70 (4) | 74 (7) | 99 (7) | 87 (8) |

[a] Blank plasma samples containing the added naltrexone were basified and supplemented with sodium chloride, extracted with either benzene or ethyl acetate, back-extracted into 0.1 *N* $H_2SO_4$, basified and supplemented with sodium chloride, and then reextracted into the same organic solvent. For the controls, naltrexone was added after the complete extraction procedure. [b] Results are for the entire extraction procedure and are corrected for volume losses. [c] Single determinations. [d] Mean of duplicate determinations. [e] Internal standard, naloxone (60 ng), added after extraction. [f] Internal standard, naloxone (12 ng), added before extraction because inconsistent results were obtained when it was added after extraction.

mobilization of a small amount of naltrexone from a tissue depot or assay interference at very low naltrexone concentrations. Thus, past studies have provided important data and raised pertinent questions regarding naltrexone kinetics in humans. No studies have been published on naltrexone pharmacokinetics in animals.

To understand naltrexone pharmacokinetics, it is necessary to measure its pharmacokinetic parameters after intravenous administration as well as to perform quantitative studies of naltrexone absorption, distribution, excretion, and metabolism. This paper reports the results of the initial stage of a comprehensive investigation of naltrexone pharmacokinetics in animals and humans. The plasma level kinetics determined after intravenous administration in monkeys and dogs were obtained using an assay previously developed in these laboratories[1] (6).

## EXPERIMENTAL

**Reagents**—Naltrexone hydrochloride[2], naltrexone reference standard[2], naloxone reference standard[2], GLC–spectrophotometric quality methanol[3], and benzene[4] were used without further treatment. Pentafluoropropionic anhydride[5] was distilled prior to use, and 4-dimethylaminopyridine[6] was recrystallized. All other chemicals were reagent grade.

**Extraction**—An appropriate amount of a solution of naloxone (internal standard) in methanol was placed in each siliconized, screw-capped test tube. Samples of plasma or urine, 0.05–2.0 ml, were added and then extracted according to a reported procedure (7) with either benzene (dog study and initial study in four monkeys) or ethyl acetate (final study in six monkeys) as the organic solvent.

The efficiency of extraction of naltrexone from plasma was determined by assaying samples in which known amounts of naltrexone were added before and after extraction. Expression of the assayed amount in samples in which naltrexone was added prior to extraction as a percentage of the assayed amount in samples in which an equal amount of naltrexone was added after extraction yielded the extraction efficiency.

**Derivatization and GLC**—The residue obtained after extraction was dried by storage for at least 16 hr in a vacuum desiccator. Derivatization with pentafluoropropionic anhydride and subsequent electron-capture GLC were carried out according to the procedure described by Sams and Malspeis (6) with several modifications. The derivatization was done in

[1] Hopefully, similar studies in humans will be carried out when naltrexone is approved for intravenous use.
[2] Endo Laboratories, Garden City, N.Y.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Burdick & Jackson, Muskegon, Mich.
[5] Pierce Chemical Co., Rockford, Ill.
[6] Aldrich Chemical Co., Milwaukee, Wis.

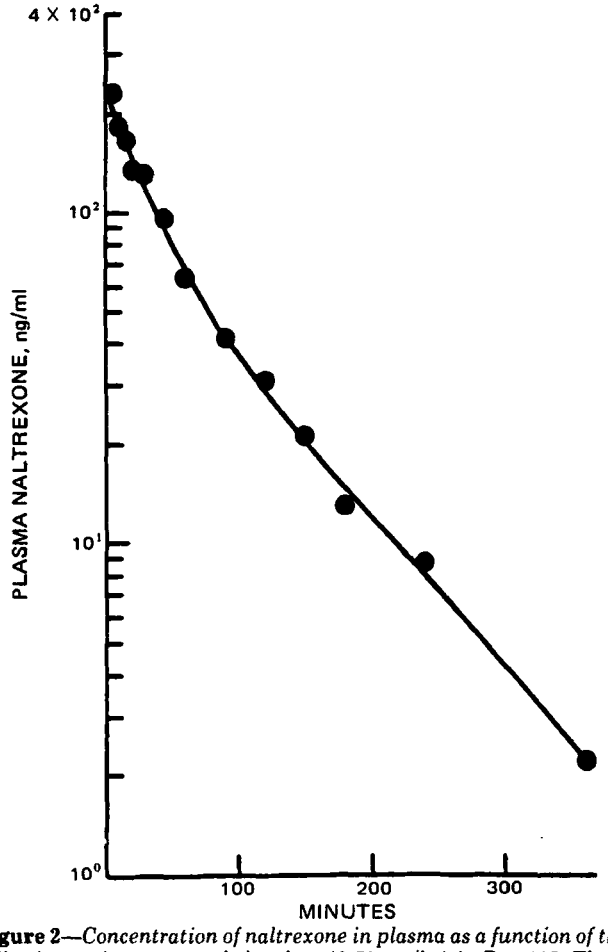



**Figure 2**—*Concentration of naltrexone in plasma as a function of time following an intravenous bolus dose (0.72 mg/kg) in Dog 105. The regression line was obtained by a nonlinear least-squares method using all data points. The fitting procedure and equations were described previously (12).*

benzene at 68–70° for 1 hr with 1% 4-dimethylaminopyridine as the catalyst (8).

The derivatives were chromatographed on a gas chromatograph equipped with a $^{63}$Ni-electron-capture detector[7]. The coiled glass column was 2 mm i.d., either 1.8 m (6 ft) or 2.4 m (8 ft) in length, and was packed with 3% OV-17 on 100–120-mesh support[8]. The oven temperatures were 205° for the 1.8-m column (dog study and initial study in four monkeys) and 203° for the 2.4-m column (final study in six monkeys). The argon–methane (95:5) carrier gas flow rates were 40 ml/min for the 1.8-m column and 24–30 ml/min for the 2.4-m column.

**Mass Spectrometry**—The derivatization of naltrexone was done on a much larger scale than that described for assay purposes. Naltrexone base (2.0 mg) was placed in a siliconized culture tube with a polytef-lined cap and derivatized with 100 μl of pentafluoropropionic anhydride, using 200 μl of 1% dimethylaminopyridine in benzene as a catalyst. The mixture was heated at 68° for 1 hr and then cooled in an ice bath. The excess pentafluoropropionic anhydride was removed by washing the mixture with 10 ml of aqueous saturated sodium tetraborate.

A sample of the organic phase was transferred to another siliconized tube, and 2 μl of the organic phase was injected into a gas chromatograph[9] that had been interfaced through a glass jet separator to a mass spectrometer[10]. The gas chromatograph contained a 1.8-m coiled glass column, 2 mm i.d., packed with 3% OV-17 on 100–120-mesh support[8]. The carrier gas was helium. The spectrometer was operated in the electron-impact mode at 70 ev. A plot of total ion current *versus* scan number revealed only one peak, and mass spectra were recorded at the maximum of this peak.

[7] Model 5700A, Hewlett-Packard, Palo Alto, Calif.
[8] Gas Chrom Q, Applied Science Laboratories, State College, Pa.
[9] Model 2700, Varian Instrument Division, Palo Alto, Calif.
[10] Model 21-491, Dupont Instruments, Wilmington, Del.

**Pharmacokinetic Studies in Animals**—Two male foxhound dogs, 20 kg, were fasted for 24 hr prior to drug administration. Naltrexone hydrochloride was dissolved in normal saline and administered *via* the cephalic vein at 0.72 mg of naltrexone/kg over 30 sec. Blood was obtained at frequent intervals by venipuncture using a heparinized syringe. Total 0–24-hr urine was collected either by catheterization of the dog or by use of a metabolism cage.

Monkeys were supplied with a polyethylene venous catheter, which extended into the inferior vena cava *via* the saphenous vein. Naltrexone, 1.0 or 10.0 mg/kg, was administered through the catheter as a bolus dose followed by a saline rinse. At predetermined times after dosing, blood samples were drawn from the catheter into heparinized syringes.

Plasma was separated from dog and monkey blood samples by centrifugation, and all plasma and urine samples were stored frozen prior to analysis. Total body clearance was calculated by dividing the dose by the area under the plasma naltrexone level–time curve from time zero to infinity. The area was estimated from the macroconstants of a biexponential equation (9).

**Enzymatic Hydrolysis and Dilution of Urine**—After thawing and mixing the urine samples, a 0.5-ml aliquot of each sample was mixed with 1 ml of a 1:10 dilution of a commercial enzymatic mixture[11] of β-glucuronidase (~177,000 units/ml) and sulfatase (~35,000 units/ml) in 0.2 *M* acetate buffer, pH 5.0. Each sample was incubated in a water bath at 37° for 24 hr. These conditions result in a maximal degree of enzymatic hydrolysis of naltrexone conjugates (10). Hydrolyzed and unhydrolyzed urine samples were diluted 10–1000-fold with 0.9 *M* NaCl prior to extraction.

## RESULTS

**Analytical Aspects**—Derivatization and chromatography of naltrexone, either at the nanogram level for assay purposes or at the milli-

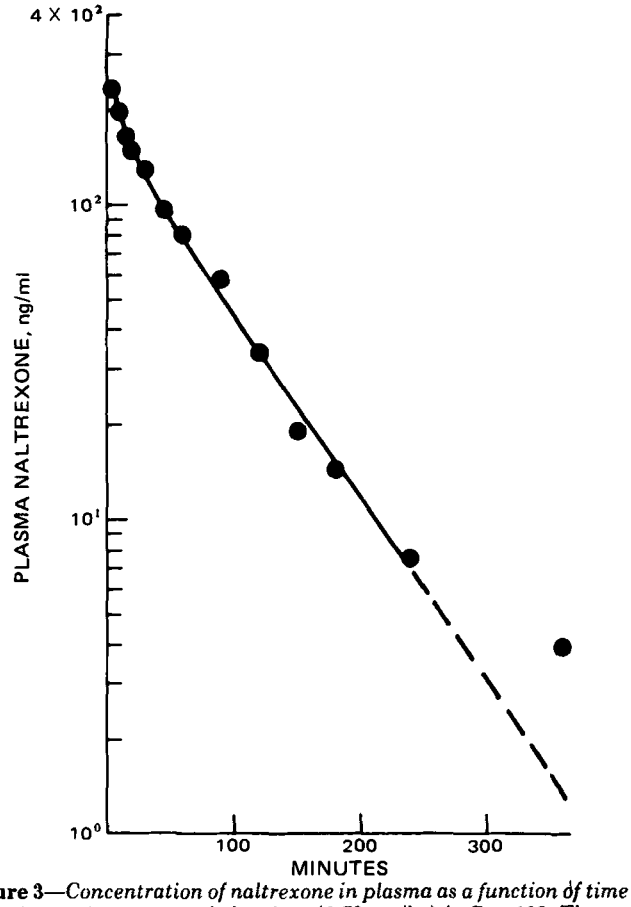
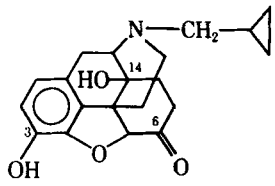



**Figure 3**—*Concentration of naltrexone in plasma as a function of time following an intravenous bolus dose (0.72 mg/kg) in Dog 103. The regression line was obtained by a nonlinear least-squares method using the data from 5 to 240 min.*

[11] Glusulase, Endo Laboratories, Garden City, N.Y.



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gram level for mass spectrometry studies, yielded a single peak with the same retention time in each case. Thus, the GLC results indicate that the same derivative was formed at either level. The mass spectrum, obtained at the maximum of the single peak in the plot of total ion current *versus* scan number, is illustrated in Fig. 1. The base peak at *m/e* 779 corresponds to the molecular ion of the pentafluoropropionyl triester derivative of naltrexone. This finding is consistent with formation of a completely derivatized naltrexone molecule esterified at the 3-, 6-, and 14-positions (I). The only significant fragment ions observed were at *m/e* 738 (12.9% of base peak), corresponding to a loss of a $C_3H_5$ moiety from the *N*-cyclopropylmethyl group, *m/e* 632 (10.8% of base peak), corresponding to a loss of a $CF_3CF_2CO$ moiety, and *m/e* 616 (8.3% of base peak), corresponding to a loss of a $CF_3CF_2COO$ moiety from the molecular ion.

The efficiency of benzene extraction of naltrexone from plasma averaged 70% in the dog (range of 64–77%) and 74% in the monkey (range of 64–82%) (Table I). There was no discernible concentration dependence of the extraction efficiency over the concentration ranges encountered in routine assays of plasma samples. These extraction efficiencies compare favorably to the 72.5% reported previously for extraction of naltrexone from dog urine with this procedure (7). When ethyl acetate was substituted for benzene, these efficiencies increased to an average of 99% for dog plasma and 87% for monkey plasma (Table I).

Standard curves for the assay were obtained by: (*a*) adding varying known amounts of naltrexone and a constant amount of naloxone (internal standard) to a series of blank plasma samples, (*b*) measuring the chromatographic peak heights after completion of the extraction, derivatization, and GLC assay, and (*c*) plotting the peak height ratio, naltrexone to naloxone, as a function of the amount of added naltrexone. Linear standard curves were obtained and a new standard curve covering the range of expected concentrations was prepared on each day of analysis.

**Pharmacokinetic Studies**—The plasma naltrexone concentration–time curves obtained after administration of an intravenous bolus dose to each of two foxhound dogs are illustrated in Figs. 2 and 3. Because the plasma naltrexone levels declined with time in a biexponential manner, the classical two-compartment open model (Scheme I) was chosen for initial attempts at fitting a model to the data (5). The results of the nonlinear least-squares fit (11, 12) are indicated in Figs. 2 and 3. The terminal half-life values of the fitted curves averaged 1.0 hr (range of 52–67 min), and the total body clearance was in the range of 51–55 ml/min/kg.

Assay of both enzymatically hydrolyzed and unhydrolyzed 0–24-hr urine collections provided an estimate of the urinary excretion of naltrexone and its conjugates. An average of 37% of the dose was excreted as total (unconjugated plus conjugated) naltrexone during the first 24 hr after the intravenous dose with less than 1% excreted as unconjugated naltrexone (Table II). No attempt was made to analyze for the small amounts of other metabolites that may have been present (7).

The initial experiment on naltrexone pharmacokinetics in the monkey was designed to determine the concentration ranges in plasma encountered at various intravenous dosage levels and to detect any pronounced dose dependence of naltrexone pharmacokinetics. The limited data obtained (Table III) indicate that, at each point in time, a 10-fold increase in dosage yielded about a 10-fold increase in the average plasma level.

**Table II—Naltrexone and Conjugates in Dog Urine Collected 0–24 hr following an Intravenous Bolus of Naltrexone**

| | Percent of Dose | | |
|---|---|---|---|
| Dog | Naltrexone[a] | Naltrexone Conjugates[b] | Total[c] |
| 103 | 0.90 | 35.7 | 36.6 |
| 105 | 0.73 | 37.2 | 37.9 |

[a] Determined by assay of unhydrolyzed urine samples. [b] The difference between total and unchanged naltrexone. [c] Determined by assay of urine samples subsequent to enzymatic hydrolysis of naltrexone conjugates.

**Table III—Initial Study of Naltrexone Levels in Monkey Plasma as a Function of Time after an Intravenous Bolus Dose**

| | Plasma Level, ng/ml | | | |
|---|---|---|---|---|
| | 10 mg/kg | | 1 mg/kg | |
| Minutes | 6715[a] | 6700[b] | 6705[c] | 6719[d] |
| 10 | 2275[e] | 3440 | 244 | 252 |
| 20 | 1880 | 2075 | 166 | 167 |
| 40 | 1280 | 1447 | 150 | 120 |
| 80 | 666 | 810 | 91.5 | 46.7 |
| 160 | 212 | 288 | 44.8 | 11.8 |
| 320 | 31.2 | 52.8 | 11.8 | — |
| *AUC*[f] | 171 | 224 | 23.0 | 13.8 |

[a] Monkey 6715 was a 3.8-kg female. [b] Monkey 6700 was a 6.4-kg male. [c] Monkey 6705 was a 4.7-kg female. [d] Monkey 6719 was a 3.6-kg male. [e] Each value is the mean of duplicate assays. [f] Area under the plasma naltrexone level–time curve from zero time to the last data point (μg min/ml) determined by the trapezoidal method.

When plotted as a function of time, the plasma levels declined in a biexponential manner. The average area under the plasma level–time curve for the 10-mg dose was 10.7 times that for the 1-mg dose.

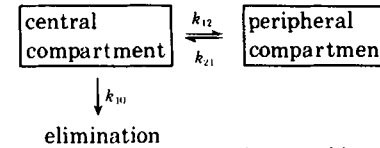
Following completion of this initial experiment, another investigation was carried out in six monkeys at the 10-mg/kg dosage. Blood samples were obtained at more frequent intervals over 48 hr. The results of the nonlinear least-squares fit (11, 12) of the two-compartment open model (Scheme I) to the plasma level–time data are shown in Figs. 4 and 5 for two monkeys. The close proximity of the data points to the regression curve indicate that an excellent fit was obtained whether measurable levels were found over 24 hr (Fig. 4, representative of four monkeys) or over 48 hr (Fig. 5, representative of two monkeys). The pharmacokinetic parameters obtained from the nonlinear least-squares fit of the data for all six monekys are summarized in Table IV. The terminal half-life of the plasma naltrexone level–time curve averaged 7.8 hr, and the total body clearance averaged 64 ml/min/kg.

## DISCUSSION

Mass spectral evidence that the base peak of the derivative formed during the assay corresponds to the molecular weight of the pentafluoropropionyl triester of naltrexone indicates that the completely derivatized naltrexone moiety is the compound being chromatographed. The fragmentation pattern is also consistent with this conclusion. Sams and Malspeis (6) presented mass and IR spectral evidence that naltrexone reacted with heptafluorobutyric anhydride in the presence of a base catalyst forms the analogous heptafluorobutyryl triester. Although derivatization of an aliphatic enol such as that at the 6-position of naltrexone is unusual (13), Cone *et al.* (14) presented evidence consistent with the formation of a similar acyl derivative of hydromorphone. This complete degree of derivatization in the presence of a base catalyst in the present assay permits maximal sensitivity and avoids any variability due to an inconsistent degree of partial derivatization.

With benzene as the organic solvent for extraction, possible interference from the blank and from unknown polar naltrexone metabolites is minimized. However, when ethyl acetate is used, the extraction efficiency of naltrexone is close to 100% and the extraction and chromatographic assay of certain polar metabolites may also be possible. It was previously shown that this procedure is specific for naltrexone (7) and that the known metabolites, *i.e.*, α-naltrexol and β-naltrexol, as well as the potential metabolite noroxymorphone do not interfere. Thus, the evidence on specificity indicates that the analytical method is suitable for pharmacokinetic studies.

The fit of the two-compartment open model to the plasma naltrexone level–time data was excellent for both dogs (Figs. 2 and 3), with the exception of the last sample in one dog (Fig. 3). The deviation of this last point from the extrapolation of the curve fitted to the other data points could be due to experimental error or may indicate a very slow terminal phase for a small fraction of the dose. Because of the limitation of assay sensitivity, it was not possible in this experiment to investigate further




*Scheme I—Two-compartment open pharmacokinetic model. The* k's *are rate constants for the transfer processes indicated.*

**Table IV—Pharmacokinetic Parameters [a] for Intravenous Naltrexone in the Monkey**

| Monkey[b] | $k_{12}$, min$^{-1}$ | $k_{21}$, min$^{-1}$ | $k_{10}$, min$^{-1}$ | $t_{1/2}$, min | $V_{d_\beta}$, liters/kg | Total Body Clearance, ml/min/kg |
|---|---|---|---|---|---|---|
| 6726 (4.36 kg) | 0.00109 (0.00011)[c] | 0.00095 (0.00018) | 0.01278 (0.00043) | 797 | 63 | 55 |
| 6695 (5.26 kg) | 0.00102 (0.00025) | 0.00184 (0.00040) | 0.01104 (0.00070) | 418 | 61 | 101 |
| 6763 (5.76 kg) | 0.00111 (0.00025) | 0.00085 (0.00026) | 0.00984 (0.00078) | 910 | 94 | 71 |
| 428 (5.55 kg) | 0.00060 (0.00009) | 0.00211 (0.00022) | 0.01089 (0.00029) | 351 | 26 | 50 |
| 6706 (5.08 kg) | 0.00117 (0.00020) | 0.00110 (0.00018) | 0.01302 (0.00079) | 691 | 68 | 69 |
| 6761 (4.90 kg) | 0.00111 (0.00011) | 0.00308 (0.00014) | 0.00886 (0.00016) | 265 | 15 | 41 |
| Mean | 0.00102 | 0.00166 | 0.01107 | 468[d] | 54 (29) | 64 (21) |

[a] The parameters $k_{12}$, $k_{21}$, and $k_{10}$ are the rate constants defined in Scheme I and were obtained by nonlinear least-squares regression (9, 10). The $t_{1/2}$ is the half-life of the terminal exponential phase of the plasma naltrexone level–time curve; $V_{d_\beta}$, the apparent volume of distribution, was calculated by an accepted method. [b] Monkeys were all female. [c] Standard deviation in parentheses. [d] Harmonic mean. The coefficient of variation of the mean of the reciprocal $t_{1/2}$ values was 50%.

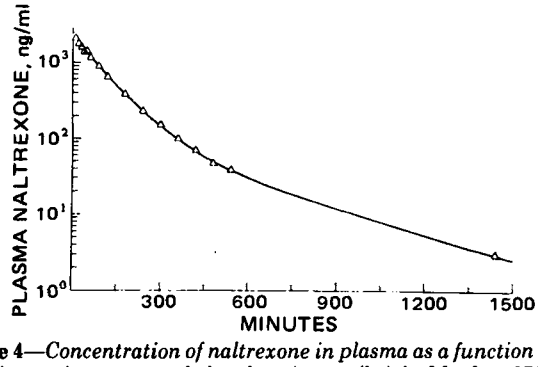



**Figure 4**—*Concentration of naltrexone in plasma as a function of time following an intravenous bolus dose (10 mg/kg) in Monkey 6761. The regression line was obtained by a nonlinear least-squares method.*

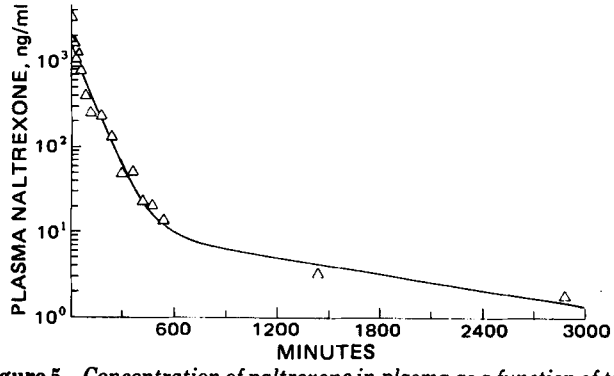



**Figure 5**—*Concentration of naltrexone in plasma as a function of time following an intravenous bolus dose (10 mg/kg) in Monkey 6763. The regression line was obtained by a nonlinear least-squares method.*

the possible existence of a terminal phase of the plasma level–time curve having a much longer half-life. This possibility will be examined in the dog using alternative experimental designs.

The recovery of 37% of the naltrexone dose from 0 to 24 hr in dog urine, mostly as conjugated naltrexone, is consistent with literature data. Malspeis *et al.* (7) found only small amounts of β-naltrexol in enzyme[11]-hydrolyzed dog urine after intravenous administration and could not detect any α-naltrexol. Only 38% of an oral dose was accounted for in humans as unchanged drug and metabolites in 0–24-hr urine (2), and 53% was accounted for in 0–6-day urine (4). Thus, there is incomplete recovery of naltrexone in the urine in both humans and dogs. Possible additional modes of elimination include fecal excretion of naltrexone and/or its metabolites, formation of unknown metabolites, and urinary excretion of conjugates not hydrolyzed by the enzyme preparation[11]. The relative importance of these potential elimination pathways is currently under investigation.

The initial experiment on plasma naltrexone level kinetics at two different dose levels in the monkey did not yield any evidence of nonlinear pharmacokinetics since plasma naltrexone levels and areas under the curve were approximately proportional to dose (Table III) even though the experiment was not done in crossover fashion. However, this evidence is not conclusive and will be investigated in a more definitive manner.

The experiment designed to determine the pharmacokinetic parameters of naltrexone in the monkey from plasma level–time data yielded evidence of a very long terminal half-life in comparison to that found in the dog. However, when the terminal data point for Dog 103 was included in the data analysis, a half-life of 356 min for the terminal phase was obtained; this value is within the range of the terminal half-life values observed in the six monkeys (Table IV). Thus, these findings suggest that the terminal half-life for naltrexone is at least several hours in duration in both the dog and monkey. This result agrees with the results of Verebey *et al.* (2) obtained after oral administration of naltrexone in humans. A more accurate quantitation of this long half-life will require more plasma samples at various times in the terminal phase and perhaps either a more sensitive assay for plasma naltrexone or the inclusion of urinary excretion data in the pharmacokinetic analysis. This terminal phase probably represents a rather small, slowly equilibrating compartment for naltrexone.

The total body clearance of naltrexone was in the range of 51–55 ml/min/kg in the dog and 40–100 ml/min/kg in the monkey. The calculation of total body clearance was influenced mainly by the contribution of the initial (alpha) phase of the plasma level–time curve (Figs. 2–5) to the area under the curve and thus was not appreciably affected by the uncertainty of the estimation of the terminal phase half-life in the dog. Each of these values is higher than the respective liver plasma flow of 15–25 ml/min/kg in the dog and 11–17 ml/min/kg in the monkey as well as the liver blood flow of 29–45 ml/min/kg in the dog and about 25 ml/min/kg in the monkey (15).

The fact that the total body clearance of naltrexone is greater than the liver plasma flow or blood flow in both species, coupled with the extremely low renal excretion of unchanged drug in monkeys (16) and dogs (Table II), suggests the existence of elimination pathways besides liver metabolism and renal excretion. If such alternative eliminating organs are quantitatively important in humans, the calculations by Kogan *et al.* (17) of total body clearance and extent of first-pass metabolism of naltrexone (based on plasma level data after oral administration of naltrexone in humans) are erroneous since the calculation method is applicable only to drugs eliminated exclusively by renal excretion and hepatic metabolism (18).

## REFERENCES

(1) W. R. Martin, D. R. Jasinski, and P. A. Mansky, *Arch. Gen. Psychiatry*, **28**, 784 (1973).

(2) K. Verebey, J. Volavka, S. J. Mule, and R. B. Resnick, *Clin. Pharmacol. Ther.*, **20**, 315 (1976).

(3) "Narcotic Antagonists: The Search for Long-Acting Preparations," R. Willette, Ed., Monograph Series 4, National Institute on Drug Abuse, Rockville, Md., 1975.

(4) E. J. Cone, C. W. Gorodetzky, and S. Y. Yeh, *Drug Metab. Dispos.*, **2**, 506 (1974).

(5) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, pp. 41, 42, 48–55.

(6) R. A. Sams and L. Malspeis, *J. Chromatogr.*, **125**, 409 (1976).

(7) L. Malspeis, M. S. Bathala, T. M. Ludden, H. B. Bhat, S. G. Frank, T. D. Sokoloski, B. E. Morrison, and R. H. Reuning, *Res. Commun. Chem. Pathol. Pharmacol.*, **12**, 43 (1975).
(8) K. A. Connors and K. S. Albert, *J. Pharm. Sci.*, **62**, 845 (1973).
(9) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1975, p. 83.
(10) T. M. Ludden, M. S. Bathala, L. Malspeis, M. A. Drum, and R. H. Reuning, *Drug Metab. Dispos.*, **6**, 321 (1978).
(11) C. M. Metzler, NONLIN, Technical Report 7292/68/7292/005, The Upjohn Co., Kalamazoo, Mich., 1969.
(12) W. G. Kramer, R. P. Lewis, T. C. Cobb, W. F. Forester, Jr., J. A. Visconti, L. A. Wanke, H. G. Boxenbaum, and R. H. Reuning, *J. Pharmacokinet. Biopharm.*, **2**, 299 (1974).
(13) C. F. Poole, in "Handbook of Derivatives for Chromatography," K. Blau and G. S. King, Eds., Heyden, London, England, 1977, pp. 160–163.
(14) E. J. Cone, B. A. Phelps, and C. W. Gorodetzky, *J. Pharm. Sci.*, **66**, 1709 (1977).
(15) "Biology Data Book," vol. III, 2nd ed., P. L. Altman and D. S. Dittmer, Eds., Federation of American Societies for Experimental Biology, Bethesda, Md., 1974, pp. 1702–1705.
(16) H. E. Dayton and C. E. Inturrisi, *Drug Metab. Dispos.*, **4**, 474 (1976).
(17) M. J. Kogan, K. Verebey, and S. J. Mulé, *Res. Commun. Chem. Pathol. Pharmacol.*, **18**, 29 (1977).
(18) D. P. Vaughan, *J. Pharm. Pharmacol.*, **27**, 458 (1975).

### ACKNOWLEDGMENTS

Supported by Contract HSM 42-73-182 (Ohio State), Grant DA-00473 (Ohio State), and Contract HSM-72-72-167 (Warner-Lambert/Parke-Davis) from the National Institute on Drug Abuse, U.S. Department of Health, Education, and Welfare, Rockville, Md.

The authors thank Dr. E. H. Fairchild for the mass spectrum.

# Pharmacokinetic Description of Drug Interactions by Enzyme Induction: Carbamazepine–Clonazepam in Monkeys

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**Abstract** □ The applicability of a pharmacokinetic model for drug interactions by enzyme induction was tested in rhesus monkeys using a design in which both the inducer (carbamazepine) and the induced agent (clonazepam) were infused chronically. Two types of studies were conducted. Studies I and II examined the kinetic behavior of plasma clonazepam levels during induction and postinduction, respectively. The addition of carbamazepine (Study I) caused the preinduction clonazepam steady-state level to decrease exponentially to a lower (induced) steady state after lag times of 14.0–60.5 hr, and the removal of carbamazepine (Study II) caused induced clonazepam steady-state levels to climb exponentially to a higher steady state after lag times of 34.0–81.0 hr. The extent of induction ranged between 23 and 54%. The time course of clonazepam levels was described in terms of a one-compartment induction model with zero-order input and a metabolic clearance that increased (Study I) or decreased (Study II) exponentially with time. In both studies, induced clonazepam half-lives (3.7–7.7 hr) were significantly shorter ($p < 0.0005$) than control values (5.2–12.2 hr). Apparent enzyme turnover half-lives were shorter in Study II (2.7–19.3 hr) than in Study I (6.9–66.4 hr). A two- to threefold increase in urinary excretion of D-glucaric acid during carbamazepine administration provided additional evidence that the present interaction was due to enzyme induction.

**Keyphrases** □ Interactions—carbamazepine and clonazepam in monkeys by enzyme induction, pharmacokinetic description □ Carbamazepine—interaction with clonazepam by enzyme induction, pharmacokinetic description, monkeys □ Clonazepam—interaction with carbamazepine by enzyme induction, pharmacokinetic description, monkeys □ Enzyme induction—pharmacokinetic description of carbamazepine–clonazepam interaction, monkeys □ Pharmacokinetics—description of carbamazepine–clonazepam interaction by enzyme induction, monkeys □ Models—drug interactions by enzyme induction, carbamazepine–clonazepam, monkeys

Many drugs possess enzyme-inducing properties and thereby stimulate their own metabolism (autoinduction) and/or that of other drugs (heteroinduction). In humans and animals, experimental evidence for enzyme induction is often associated with decreases in plasma concentrations of the induced agent. Recently, a pharmacokinetic theory was proposed to describe the kinetics of plasma drug levels during auto- and heteroinduction (1). This theory proposes that the metabolic clearance of the induced species increases exponentially during induction. The time course of this increase in clearance is governed by the apparent turnover half-life of the induced metabolic enzyme(s).

The simplest and most critical experimental validation of this theory entails administration of both an inducer and an induced agent by constant rate intravenous infusion. Preliminary studies with this design were performed in monkeys using carbamazepine as the inducer and valproic acid and ethosuximide as the induced agents (2, 3). Following addition of carbamazepine, plasma levels of these two drugs decreased in an exponential fashion to a lower steady state, as expected.

The present study, in addition to investigating shifts of steady-state levels during induction, focused on determining control and induced half-lives of clonazepam. Furthermore, the hypothesis that removal of an inducer causes a return of metabolic clearance from an induced state to the basal state was tested by following increases in plasma clonazepam levels upon removal of carbamazepine.

## EXPERIMENTAL

**Animal Preparation**—Five healthy male rhesus (*Macaca mulatta*) monkeys, 4.0–5.6 kg, were chair adapted for 1 month prior to surgical implantation of catheters at two different sites. The jugular and femoral veins were catheterized for drug infusion and blood sampling, respectively. Each monkey was maintained in a three-level restraining chair during individual studies and was given cage rest at appropriate intervals. Patency of catheters was assured by a slow, continuous saline infusion (1 ml/hr). The daily diet consisted of fresh fruit (bananas, oranges, and apples) and monkey chow.



0022-3549/79/0400-0416$01.00/0