of a perturbation. Despite this limitation, the competitive indicator method has one notable feature that will often make it a valuable resource, namely, it allows observations to be made of a substrate whose own properties do not provide quantitative measures of complex formation; the indicator spectral change is a surrogate property.

REFERENCES

(1) W. Saenger, *Angew. Chem. Intern. Ed.*, **19**, 344 (1980).

(2) J. Szejtli, Ed., "Proceedings of the First International Symposium on Cyclodextrins," Akadémiai Kiadó, Budapest, 1982 (D. Reidel Publishing Co., Dordrecht, Holland).

(3) J. Szejtli, "Cyclodextrins and their Inclusion Complexes," Akadémiai Kiadó, Budapest, 1982.

(4) V. Lautsch, W. Bandel, and W. Broser, *Z. Naturforsch.*, **11B**, 282 (1956).

(5) B. Casu and L. Ravà, *Ric. Sci.*, **36**, 733 (1966).

(6) S.-F. Lin and K. A. Connors, *J. Pharm. Sci.*, **72**, 1333 (1983).

(7) F. J. C. Rossotti and H. S. Rossotti, *Acta Chem. Scand.*, **9**, 1166 (1956).

(8) F. J. C. Rossotti and H. S. Rossotti, "The Determination of Stability Constants," McGraw-Hill, New York, N.Y., 1961, p. 108.

(9) D. D. Pendergast, Ph.D. thesis, University of Wisconsin-Madison, 1983.

(10) T. W. Rosanske and K. A. Connors, *J. Pharm. Sci.*, **69**, 564 (1980).

(11) K. A. Connors, S.-F. Lin, and A. B. Wong, *J. Pharm. Sci.*, **71**, 217 (1982).

(12) J. S. Coleman, L. P. Varga, and S. H. Mastin, *Inorg. Chem.*, **9**, 1015 (1970).

(13) F. R. Hartley, C. Burgess, and R. M. Alcock, "Solution Equilibria," Ellis Horwood Ltd., Chichester, 1980, chap. 2.

(14) T. Higuchi and K. A. Connors, *Adv. Anal. Chem. Instrum.*, **4**, 117 (1965).

(15) K. Iga, A. Hussain, and T. Kashihara, *J. Pharm. Sci.*, **70**, 108 (1981).

(16) R. Gelb, L. Schwartz, B. Cardelino, H. Fuhrman, R. Johnson, and D. Laufer, *J. Am. Chem. Soc.*, **103**, 1750 (1981).

ACKNOWLEDGMENTS

Some of the potentiometric data were contributed by Stuart Sonnedecker. This work was supported by a grant from The Upjohn Company.

Metabolism of Meperidine in Several Animal Species

S. Y. YEH

Received June 4, 1983, from the *National Institute on Drug Abuse, Addiction Research Center, Lexington, KY 40583.* Accepted for publication February 8, 1984. Present address: NIDA, P.O. Box 5180, Baltimore, MD 21224.

Abstract □ Four new meperidine metabolites were identified by GC–MS in the urine of rats, guinea pigs, rabbits, cats, and dogs. In addition to known meperidine metabolites, 4-ethoxycarbonyl-4-phenyl-1,2,3,4-tetrapyridine (dehydronormeperidine; IV, the *N*-hydroxydehydro derivative of normeperidine (X), the dihydroxy derivative of meperidine (XII), and the dihydroxy derivative of normeperidine (XIII) were identified. The possible role of the *N*-hydroxy derivative of normeperidine (IX) in the pharmacological interaction of meperidine (I) with MAO inhibitors, seen selectively in the rabbit (and humans), is discussed. Following the administration of the *p*-hydroxy derivative of meperidine (VII), the major metabolite was conjugated VII. Trace amounts of the *p*-hydroxy derivative of normeperidine (VIII), the methoxy hydroxy derivative of meperidine (XI), XII, and XIII also were detected as metabolites of VII. The degree of *N*-demethylation of VII, both *in vitro* and *in vivo*, was small.

Keyphrases □ Meperidine—TLC, GC–MS, identification of metabolites, interaction with monoamine oxidase inhibitors □ Monoamine oxidase inhibitors—metabolites of meperidine, TLC, GC–MS

Meperidine is extensively metabolized by humans and animals. The major metabolites of meperidine identified in the urine of rats, guinea pigs, dogs, and humans are normeperidine and (1-methyl-4-phenyl-4-piperidine carboxylic) and (4-phenyl-4-piperidine carboxylic) acids (1–12), while the minor metabolites are the *p*-hydroxy derivative of meperidine (5), the *N*-oxide of meperidine (5, 6), the methoxy hydroxy derivative of meperidine (7), and the *N*-hydroxy derivative of normeperidine (7). In the urinary excretion studies of meperidine and its metabolites [normeperidine, and (1-methyl-4-phenyl-4-piperidine carboxylic) and (4-phenyl-4-piperidine carboxylic) acid], the amount of each excreted by the rabbit was found to be low (16–53% of the administered dose) when compared with other species (13). Of this total, meperidine and normeperidine accounted for only ~0.4 and 4–8.5%, respectively, of the administered dose. The remainder of the dose excreted represented unidentified metabolites. Severe interaction between monoamine oxidase inhibitors (MAOI) and meperidine have been observed in rabbits and humans, but seldom observed in other species (14–16). The purpose of this study was to identify new metabolites in rabbits and other species which might contribute to the severe interaction of meperidine and MAOI observed in the rabbit. The metabolism of the *p*-hydroxy derivative of meperidine (VII) was also investigated in a preliminary study in the rat in an attempt to determine if VII is metabolized *in vivo* to VIII.

EXPERIMENTAL SECTION

Drugs—Meperidine hydrochloride[1], normeperidine hydrochloride[1], and the *p*-hydroxy derivative of meperidine[2] (5) were obtained commercially. The *N*-oxide of meperidine was synthesized as follows. Meperidine hydrochloride (200 mg) was dissolved in 2 mL of water, basified with ammonium hydroxide, and extracted with benzene. After removal of the solvent under reduced pressure, the residue was dissolved in 1 mL of absolute ethanol, and 1 mL of 30% H_2O_2 was added. The solution was heated in a water bath at 70°C for 2 h. At that time, TLC showed no starting material. The solution was transferred to a dish and evaporated to near dryness on a steam bath. The residue was dissolved in isopropyl alcohol and transferred to a 15-mL centrifuge tube. The solvent was removed under a stream of nitrogen, to give a white liquid. Ether was added to the residue to give a white precipitate. The mixture was refrigerated and centrifuged, and the liquid phase was transferred to another centrifuge tube. By repeating this treatment, two products were obtained: ether-soluble material and a white precipitate. The solid material was recrystallized from absolute ethanol to yield 18 mg of white crystals, mp 173–175°C. The solid material showed a single spot on TLC (R_f 0.37 and

[1] Sterling-Winthrop Co., Rensselaer, N.Y.

[2] Professor C. Lindberg, Biomedical Center, University of Uppsala, Uppsala, Sweden.

0022-3549/84/1200-1783$01.00/0

0.40) after development with the respective solvent systems as stated below. Elemental analyses of the crystalline product showed: C, 58.18; H, 7.12; N, 4.90. The respective theoretical values for the derived *N*-oxide VI were: C, 68.44; H, 7.98; N, 5.32.

The solvent was removed under a stream of nitrogen to give 95 mg of a white oil. TLC of the oil on instant thin-layer chromatography (ITLC)[3] showed a single spot [R_f 0.70, ethyl acetate–ammonium hydroxide (17:1) or R_f 0.54, benzene–methanol–diethylamine (48:1:1)] and visualized by spraying with iodoplatinate reagent. Treatment with 10% sodium bisulfite solution of both the liquid and the crystalline materials gave meperidine as evidenced by TLC R_f values.

GC–MS identification of VI was performed by solid probe or by injection on a 0.9-m column packed with 3% OV-17 coated on 60/80-mesh Gas Chrom Q after treatment with or without trimethylsilylimidazole in pyridine. The MS of the aforementioned oil (solid probe) showed a molecular ion at *m/z* 264 (M + H), and ions at *m/z* 276 (M + CH_3), *m/z* 292 (M + C_2H_5), *m/z* 248 (M − O_2), *m/z* 246 (M − H_2O), and *m/z* 234, indicative of the *N*-oxide of meperidine. The MS of the aforementioned solid material, mp 173–175°C (solid probe) showed a molecular ion at *m/z* 246 (M + H), and ions at *m/z* 262 (M + CH_3), *m/z* 274 (M + C_2H_5), and *m/z* 232, indicative of 4-ethoxycarbonyl-1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine, a thermal-dehydrated product of the *N*-oxide of meperidine. Landberg and Bogentoft (17) have synthesized 4-ethoxycarbonyl-1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine, and compared its MS with that of VI. In addition, the MS of meperidine, *m/z* 248 (M + H), *m/z* 264 (M + CH_3), *m/z* 276 (M + C_2H_5), and *m/z* 234, 218, and 149, were also observed. This appears to indicate that VI underwent thermal decomposition and loss of an oxygen atom. Chromatograms of both the aforementioned oily and solid materials, treated with or without trimethylsilylimidazole and injected on the column, showed two peaks. The retention time and the MS of the major peak corresponded to meperidine. The minor peak showed the MS of 4-ethoxycarbonyl-1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine. These observations confirmed the reports in the literature (6, 7, 17). The IR[4] of the oily VI showed strong bonds at 2970, 1710 (ester C=O), 1430, 1213 [N—O stretching as shown in 4-methylpyridine *N*-oxide (18)], 1182, and 1130 cm^{-1}. ^{1}H-NMR ($CDCl_3$)[4] of the oily VI showed peaks at δ 2.6, 4.6, 6.4, and 6.7 ppm. Mitchar *et al.* (6) reported that the NMR spectrum of VI showed a characteristic downfield shift of the CH_3 group from δ 7.7 ppm of meperidine base to δ 6.4 ppm due to the inductive influence of the oxygen atom. Due to the limited amount of the material, a boiling point was not measured. Phillipson *et al.* (19) have investigated *N*-oxide of morphine, codeine, and thebaine and observed that each alkaloid forms two *N*-oxides, which have been separated and characterized by NMR and MS and reduced to the parent alkaloid.

The *N*-hydroxy derivative of normeperidine (IX) was synthesized by oxidation of normeperidine with *m*-chloroperbenzoic acid (7, 20). TLC of the product [benzene–methanol–diethylamine (48:1:1), visualized by spraying with iodoplatinate or ammoniacal silver nitrate reagent] showed two spots, one with an R_f value (equal to 0.24) corresponding to that of authentic normeperidine. The other spot (R_f 0.44), a black spot after spraying with ammoniacal silver nitrate, was due to the *N*-hydroxy derivative of normeperidine. Chromatography of the product, treated with trimethylsilylimidazole and injected on a 0.9-m column packed with 3% OV-17 coated on 60/80-mesh Gas Chrom Q and column temperature programmed from 160°C to 250°C at 10°C min, showed two major peaks with several minor peaks. One of the major peaks had a retention time and MS corresponding to that of authentic normeperidine. The other major peak (R_f 4.5) had a molecular ion at *m/z* 322 (M + H), with ions at *m/z* 350 (M + C_2H_5), *m/z* 306 (M − CH_3), *m/z* 232 [M − $(CH_3)_3SiOH$], and *m/z* 214, indicative of IX (7). Attempts to purify the IX failed. The crude product was used qualitatively to identify IX in the biological fluids.

Animal Experiments—Collection of urine samples from rats, guinea pigs, rabbits, dogs, and cats administered meperidine (20–35 mg/kg) has been described (13). The *p*-hydroxy derivative of meperidine (40 mg/kg sc) was administered to one rat (250 g), and urine was collected from a stainless steel metabolism cage for 24 h. All urine was frozen until drug analysis.

Extraction of Meperidine, the *p*-Hydroxy Derivative of Meperidine, and Their Metabolites—Meperidine, VII, and their metabolites were extracted from urine according to described procedures (20). On evaporation of the extract to dryness, the residues were used for TLC and GC–MS studies. For GC–MS studies, the residue was derivatized with trimethylsilylimidazole in pyridine[5] according to the procedure described previously (21).

Hydrolysis of Conjugated Meperidine Metabolites—Aliquots (0.5–2 mL) of urine samples were adjusted to pH 6 with acetic acid, buffered with 0.5 mL of 0.5 M sodium acetate buffer at pH 4.8, and incubated with 50 μL a solution of 9725 U of β-glucuronidase–4210 U of sulfatase[6] at 37°C for 18–20 h. After incubation, the solution was adjusted to pH 9–10 with sodium hydroxide solution, buffered with 0.1 mL of concentrated ammonium hydroxide, and extracted with ether as described previously (20).

Thin-Layer Chromatography—TLC identification of meperidine and its metabolites was by silica gel pre-coated TLC plates[7]. The chromatograms were developed with benzene–methanol–diethylamine (48:1:1) and visualized by spraying with ammoniacal silver nitrate solution for detection of IX or developed with ethyl acetate–methanol–ammonium hydroxide (17:2:1) and sprayed with iodoplatinate reagent for detection of I, II, VII, and VI (20).

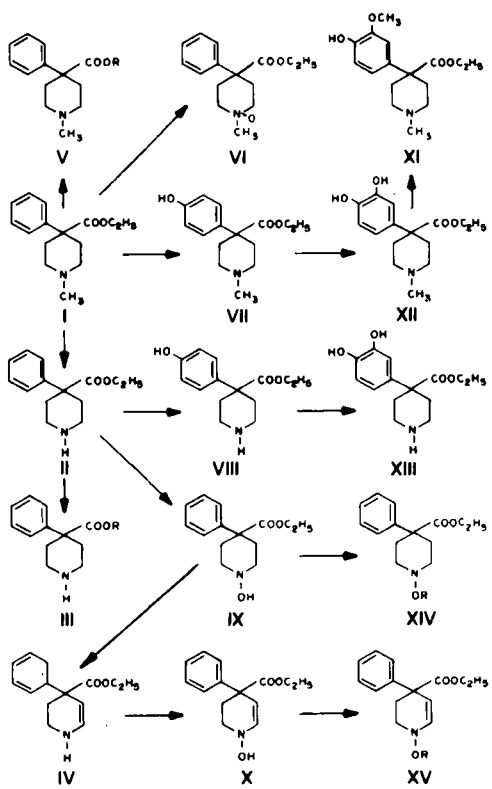
Gas Chromatography–Mass Spectrometry—Chemical-ionization MS data were obtained from a gas chromatograph–mass spectrometer[8] equipped with an interactive data system[9]. The GC column consisted of a 91 cm × 2 mm glass column packed with 3% OV-17 coated on 80/100-mesh Gas Chrom Q. The temperature of the injector and ion source were 160°C and 100°C, respectively. Two temperature programs were employed: hold at 150°C for 3.33 min, followed by programming to 250°C at 6°C/min (referred to as column A) and program from 160°C to 250°C at 10°C/min (referred to as column B). Methane, with a flow rate giving an ion chamber pressure of ~1000 μm, was used as the carrier gas as well as the chemical-ionization reagent gas in the ion source. Other specifications of the MS were described elsewhere (21).

Drug and metabolites were identified by comparison of spectra with authentic standards. Other metabolites (Scheme I) were tentatively assigned structures based on MS.

RESULTS AND DISCUSSION

Thin-Layer Chromatographic Identification of Meperidine Metabolites—The limit of detection of I, II, VI, VII, and IX by TLC was ~10–25 μg/sample. In general, meperidine was found in the urinary extracts of all species studied, except the rabbit. Normeperidine was found in the urine of the rat and guinea pig, but not in the urine of the rabbit, cat, and dog. Metabolites VI and VII were not observed by TLC analysis.

TLC of extracts of rabbit urine visualized with ammoniacal silver nitrate solution showed two black spots with R_f values of 0.50 and 0.44, whereas that of a dog showed one black spot, R_f 0.50. The spot at R_f 0.50 corresponds to



Scheme I—Metabolic pathways of meperidine.

[3] Gelmen Instrument Co., Ann Arbor, Mich.

[4] The author thanks Mr. William F. Rencher, Jr., College of Pharmacy, University of Kentucky, for the excellent IR and NMR spectra.

[5] Pierce Chemical Co., Rockford, Ill.

[6] Glusalase; Endo Laboratories, Garden City, N.Y.

[7] Quantum Industries, Fairfield, N.J.

[8] Model 3300; Finnegan, Sunnyvale, Calif.

[9] Model 6000; Finnegan.

Table I—TLC Identification of Meperidine Metabolites in the Urine of Several Species [a]

Compounds	R_f
I	0.78
II	0.33
VI	0.03
VII	0.17
IX	0.50
Extracts from urine	
Rat	0.78, 0.33
Guinea pig	0.78, 0.33
Rabbit	0.50, 0.44
Cat	0.78
Dog	0.78, 0.50

[a] Quantum Industries, Fairfield, N.J. Chromatogram was developed with benzene–methanol–diethylamine (48:1:1), or ethyl acetate–methanol–ammonium hydroxide (17:2:1).

that of authentic IX. The other spot was possibly due to X (detailed in the GC–MS identification). Metabolite IX was not seen in the TLC analysis of the urine of the rat, guinea pig, and cat (Table I).

Gas Chromatographic–Mass Spectrometric Identification of Meperidine Metabolites—Chemical-ionization GC–MS characteristics of peaks observed in the urinary extracts are compared with known standards, and previously unidentified peaks were assigned to new metabolites (Table II). Meperidine metabolites, the number of times the metabolite was identified, and the number of urine samples examined are listed in Table III.

Identification of Metabolites of Meperidine—Meperidine (I) and metabolites [*i.e.*, II, VI (detected as its thermal decomposition product, 4-ethoxycarbonyl-1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine), IX, and VII (as the trimethylsilyl derivatives)] were identified in urine samples by comparison of retention times and spectra with authentic materials. The *N*-hydroxy metabolite of normeperidine was detected in urine samples only after enzymatic hydrolysis, suggesting that this material is excreted only in the conjugated form. In addition to the known metabolites, some new metabolites of meperidine were tentatively identified.

4-Ethoxycarbonyl-4-phenyl-1,2,3,4-tetrahydropyridine (Dehydronormeperidine) (IV)—This metabolite appeared at 3.75 min in the chromatogram of the extract of rabbit urine. The MS of the metabolite contains a pseudomolecular ion at *m/z* 232 (M + H) and ions at *m/z* 260 (M + C_2H_5), *m/z* 186 (232 − C_2H_5OH), and *m/z* 158 (232 − C_2H_5OH − CO). Also, the retention time of this metabolite bears the same relationship to normeperidine as does dehydromeperidine to meperidine. This metabolite was detected in all urine samples except those of the female rat, cat, and guinea pig (Table III). Based on the structure of the *N*-hydroxy derivative of normeperidine, the olefinic bond of dehydronormeperidine was arbitrarily assigned to positions 2 and 3.

Formation of dehydronormeperidine *in vivo* could occur either through the dehydration of the *N*-hydroxy derivative of normeperidine, observed to be a major metabolite in the urine of the rabbit, or by dehydration of the 2-hydroxy or 3-hydroxy derivatives of normeperidine. *In vivo* formation of the latter metabolites is plausible since analogues of these metabolites [the 2-hydroxy-*N*-nitro derivative of piperidine, a metabolite of the *N*-nitro derivative of piperidine (22) and the 3-hydroxy and 4-hydroxy derivatives of piperidine, major metabolites of piperidine in the rat (23)] have been reported. Formation of dehydronormeperidine by dehydration of the *N*-hydroxy derivative of normeperidine during GC–MS was considered possible; however, this was ruled out since the *N*-hydroxy derivative of dehydronormeperidine also has been observed in the urine (see below). If dehydronormeperidine is formed by dehydration of the *N*-hydroxy derivative of normeperidine, then the *N*-hydroxy derivative of dehydronormeperidine should not be detected.

N-Hydroxy Derivative of Dehydronormeperidine (X)—In the GC–MS analysis of rabbit urine, this metabolite (as the trimethylsilyl derivative) appeared at 5.2 min (Fig. 1). The MS of the metabolite shows a molecular ion at *m/z* 320 with abundant ions at *m/z* 348 (M + C_2H_5), *m/z* 304 (M − CH_3), *m/z* 246 (320 − C_2H_5OH − CO), *m/z* 230 [320 − $(CH_3)_3SiOH$], *m/z* 202 (230 − C_2H_4), and *m/z* 156 [246 − $(CH_3)_3SiOH$]. This metabolite was not detected in the urine without enzymatic hydrolysis, suggesting that the metabolite exists only in the conjugated form. Formation of the *N*-hydroxy de-

Table II—Chemical Ionization GC–MS Characteristics of Peaks Observed in the Urinary Extracts

Compound	Retention Time (R_t), min[a] Column A	Column B	Ions, *m/z*[b] Molecular $(m + H)^+$	Fragment $(m + C_2H_5)^+$	Fragment $(m - CH_3)^+$	Others
I	2.0	1.1	248(100)	276(15)		174(10)
VI[c]	2.8	1.6	246(100)	274(15)		172(80)
II	3.2	1.9	234(100)	262(15)	218(30)	160(10)
IV	3.8	2.1	232(100)	260(18)		158(45)
IX	4.8	2.5	322(80)	350(15)	306(70)	232(100), 158(25)
X	5.2	3.1	320(100)	348(10)	304(20)	246(35), 202(30), 156(15)
VII	7.7	3.8	336(100)	364(18)	304(25)	262(5)
VIII	8.5	4.5	322(100)	350(18)	306(20)	248(4), 212(15)
XII		4.8	424(70)	452(3)	408(60)	
XIII		4.9	410(85)	438(15)	394(100)[d]	320(80)
XI		4.9	366(95)	394(100)[d]	350(40)	

[a] Column A temperature was held at 150°C for 3.33 min and then raised to 250°C at 6°C/min. Column B was programmed from 160°C to 250°C at 10°C/min. [b] Relative intensity is in parentheses. [c] This compound was thermally decomposed through GC–MS to form 4-ethoxycarbonyl-1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (17). [d] The relative intensity appeared to be the sum of two metabolites.

Table III—Meperidine Metabolites Identified in the Urine of Several Species by GC–MS

Species	Metabolites II	IV	VI	IX	X	VII	VIII	XI	XII	XIII
Rat										
Male	5/5[a]	2/5	4/5	5/5	2/5	5/5	5/5	2/3	2/3	0/3
Female	5/5	0/5	2/5	5/5	0/5	5/5	5/5	3/5	1/5	0/5
Guinea pig										
Male	5/5	2/3	5/5	5/5	3/5	2/3	2/3	0/3	0/3	0/3
Female	4/4	0/4	3/4	3/4	2/4	1/4	—[b]	—	—	—
Rabbit										
Male	2/2	2/2	0/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Female	2/2	2/2	0/2	2/2	2/2	2/2	2/2	—	—	—
Cat										
Male	2/2	2/2	2/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2
Female	2/2	0/2	2/2	0/2	0/2	1/2	1/2	0/2	0/2	0/2
Dog										
Male	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	0/1
Female	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	0/1

[a] Number of times the metabolite was detected/number of urine samples examined. [b] Not examined.

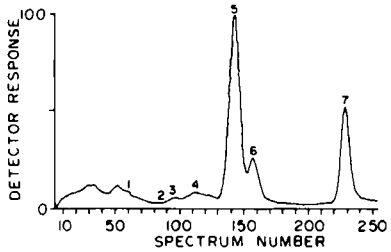


Figure 1—*GC-MS analysis of the extract of enzymatic hydrolyzed urine of a rabbit administered meperidine. The chromatogram was obtained using column A (see Table II). Key: (1) I; (2) VI; (3) II; (4) IV; (5) IX; (6) XV; (7) VII.*

rivative of dehydronormeperidine may be through *N*-hydroxylation of dehydronormeperidine.

p-*Hydroxy Derivative of Normeperidine* (*VIII*)—This metabolite (as the trimethylsilyl derivative) has the same molecular ion as the trimethylsilyl derivative of the *N*-hydroxy derivative of normeperidine, but the mass spectral characteristics and retention time (R_t = 4.5 min) are different. The MS of this peak showed a molecular ion at *m/z* 322 (M + H) and ions at *m/z* 350 (M + C_2H_5), *m/z* 306 (M − CH_3), *m/z* 248 (322 − C_2H_5OH − CO), and *m/z* 212. Since the retention time of this material is longer than that of the *p*-hydroxy derivative of meperidine, and *p*-hydroxylation of aromatic rings is a predominant metabolic pathway for this type of compound, it is reasonable to assume that this material is VIII. It is reasonable to assume that the hydroxyl group is in the *para* position based on the isolation of the *p*-hydroxy derivative of meperidine from urine (5).

The *in vitro* synthesis of VIII for use as a standard was attempted by incubating VII with rat liver homogenate and the NADPH-generating system used for *N*-demethylation of meperidine (24). After incubation, the medium was pooled, adjusted to pH 10 with sodium hydroxide solution, buffered with 1 mL of 40% K_2HPO_4 solution, and extracted. The extracted residue was dissolved in methanol and chromatographed on TLC. No spots other than those of VII and NADP were observed.

Biosynthesis of VIII has also been attempted without success by administration of VII to a rat. An aliquot of the urine of rat administered VII was hydrolyzed with β-glucuronidase-sulfatase[6] and extracted. The residue was derivatized with trimethylsilylimidazole and analyzed by GC-MS. No peak corresponding to VIII was observed.

Dihydroxy Derivative of Meperidine (*XII*)—The integrated total-ion-current chromatogram obtained by column B was scanned for *m/z* 424 the molecular ion of XII (as the trimethylsilyl derivative). A peak was localized at retention time 4.8 min (Fig. 2). The MS of this peak represents a mixture of at least two, probably three compounds, including XII and VIII both as the trimethylsilyl derivatives. The MS showed a molecular ion at *m/z* 424 (M + H) and ions at *m/z* 452 (M + C_2H_5) and *m/z* 408 (M − CH_3), which were tentatively identified as XII as the trimethylsilyl derivative. The formation of XII could result from oxide formation and hydroxylation of both the aromatic and piperidyl rings of the meperidine molecule. Based on the MS, it is difficult to assign positions to the hydroxyl groups in XII; however, *in vitro* hydroxylation of other opiates and phenolic compounds leads to *meta-para* hydroxylation (25). The MS showed a molecular ion at *m/z* 322 (M + H) and ions at *m/z* 350 (M + C_2H_5) and *m/z* 306 (M − CH_3), identified as VIII as the trimethylsilyl derivative. The MS also showed a molecular ion at *m/z* 234 (M + H) and ions at *m/z* 262 (M + C_2H_5) and *m/z* 248 (M − CH_3), which appear to be from normeperidine.

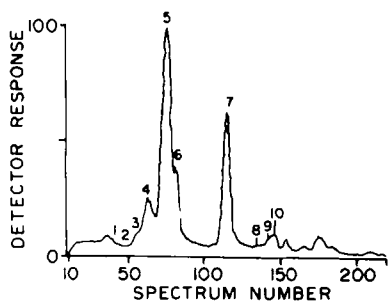


Figure 2—*GC-MS analysis of the extract of enzymatic hydrolyzed urine of a rabbit administered meperidine. The chromatogram was obtained using column B (see Table II). Key: (1–7) as in Fig. 1 legend; (8) VIII; (9) XII; (10) XI and XIII.*

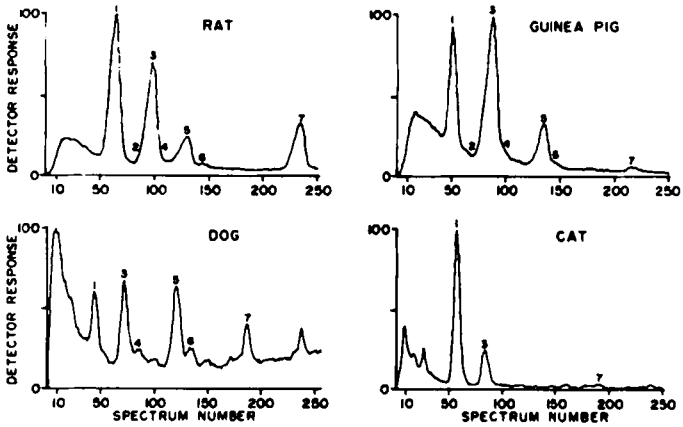


Figure 3—*GC-MS analysis of the extract of enzymatic hydrolyzed urine samples of animals administered meperidine, obtained using column A. The peaks are as listed in Fig. 1.*

Dihydroxy Derivative of Normeperidine (*XIII*) *and Methoxy Hydroxy Derivative of Meperidine* (*XI*)—The integrated total-ion-current chromatogram was scanned for *m/z* 410, indicative of XIII as the trimethylsilyl derivative. A peak with retention time at 5.0 min was located which overlapped another metabolite. One is due to XIII as the trimethylsilyl derivative, which has molecular ion at *m/z* 410 (M + H) and ions at *m/z* 438 (M + C_2H_5) and *m/z* 320 [410 − $(CH_3)_3SiOH$]. The other is due to XI as the trimethylsilyl derivative, which has molecular ion at *m/z* 366 (M + H) and ions at *m/z* 394 (M + C_2H_5) and *m/z* 350 (M − CH_3).

Formation of XI could result from hydroxylation to form a catechol, followed by methylation with catechol methyltransferase. Evidence for this metabolic pathway has been demonstrated *in vitro* for opiates and other phenolic compounds (25) and *in vivo* for naltrexone (26, 27).

Although the position of the hydroxyl and methoxy groups could not be assigned based on the MS, a reasonable assignment could be made based on the structure of the methoxy hydroxy derivative of naltrexone (isolated from urine), whose structure is secure (26). Therefore, it is reasonable to assign the structure for XI as illustrated in Scheme I.

Based on the ratio of meperidine to its metabolites observed in the total-ion-current chromatogram, it appears that IX was a major metabolite in the rabbit, while normeperidine was found in minor amounts (Fig. 1). On the other hand, normeperidine was found to be a major metabolite in the other species studied, while IX was minor (Fig. 3). As mentioned previously, severe interaction between MAOI and meperidine has been observed in humans and rabbits, but has seldom been observed in other species. The role of IX in the interaction between MAOI and meperidine observed in rabbits is not well understood.

Other major metabolites of meperidine, the corresponding free and conjugated carboxylic acids of V and III (4, 13), are not extracted by the assay used here.

Identification of Metabolites of the *p*-Hydroxy Derivative of Meperidine in the Urine of the Rat—The integrated total-ion-current chromatogram and MS of the extract (as the trimethylsilyl derivative) of an enzymatically hydrolyzed urine sample of a rat administered VII are shown in Fig. 4. The chromatogram showed a major peak and several small peaks. The retention time and MS of the major peak correspond to that of VII as the trimethylsilyl derivative. Minor metabolites of VII were also identified.

p-*Hydroxy and Dihydroxy Derivatives of Normeperidine*—The peak with retention time at 4.7 min was apparently due to a mixture of the *p*-hydroxy and dihydroxy derivatives of normeperidine, both as the trimethylsilyl derivatives (Fig. 4). The MS with a molecular ion at *m/z* 322 (M + H) and ions

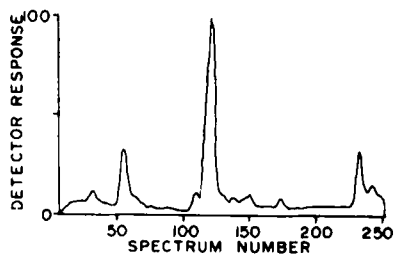


Figure 4—*GC-MS analysis of the extract of enzymatic hydrolyzed urine of a rat administered VII, obtained using column A. The peaks were identified as VII, VIII and XIII, and XI and XII.*

at m/z 350 (M + C_2H_5), m/z 306 (M − CH_3), m/z 276 (322 − C_2H_5OH), and m/z 248 (322 − C_2H_5OH − CO) was attributed to VIII as the trimethylsilyl derivative. The MS with a molecular ion at m/z 410 (M + H) and ions at m/z 438 (M + C_2H_5) and m/z 394 (M − CH_3) was attributed to XIII as the trimethylsilyl derivative. The concentration of XIII was low but detectable. Formation of VIII was apparently through *N*-demethylation of VII. Compound XIII appears to be formed *in vivo* by hydroxylation of VIII.

Methoxy Hydroxy and Dihydroxy Derivatives of Meperidine -The peak with retention time at 5.0 min was apparently due to a mixture of XI and XII, both as the trimethylsilyl derivatives (Fig. 4). The MS with a molecular ion at m/z 366 (M + H) and ions at m/z 394 (M + C_2H_5), m/z 350 (M − CH_3), and m/z 292 (366 − C_2H_5OH − CO) was attributed to XI as the trimethylsilyl derivative. The MS with a molecular ion at m/z 424 (M + H), and ions at m/z 452 (M + C_2H_5) and m/z 408 (M − CH_3) was attributed to XII as the trimethylsilyl derivative. Formation of XII and XI *in vivo* apparently occurs first by hydroxylation of VII and then by methylation of one of the hydroxyl groups. Evidence for this metabolic pathway has been mentioned above (26, 27).

Metabolites XII, VIII, XIII, and XI were not detected in the urine of rats administered either meperidine or VII without enzymatic hydrolysis, suggesting that these metabolites exist only in the conjugated form. The peak with retention time at 1.0 min has a MS with a molecular ion at m/z 306 (M + H) and ions at m/z 334 (M + C_2H_5) m/z 290 (M − CH_3), and m/z 232 (306 − C_2H_5OH − CO). The peak with retention time at 8 min showed a molecular ion at m/z 364 (M + H) and ions at m/z 392 (M + C_2H_5) and m/z 348 (M − CH_3). Peaks at 1.9, 3.6, 5.8, and 7.7 min were also observed in the extract of the control urine, indicating that these are due to natural urinary products and from the enzyme source.

N-Demethylation is a major metabolic pathway for biotransformation of meperidine, but plays only a minor role on the metabolism of VII. This is probably due to the fact that VII has a phenolic group available for conjugation. Conjugation has been shown to be a major metabolic pathway for compounds having both *N*-methyl tertiary amine and phenolic groups, such as morphine (28). In addition, *N*-demethylation of a compound depends on its lipid solubility, *N*-demethylation taking place to a greater degree with more lipid-soluble compounds (29). That the *p*-hydroxy derivative of meperidine (VII), a more polar compound than meperidine, would yield less of VIII than meperidine would yield normeperidine is understandable.

REFERENCES

(1) J. J. Burns, B. L. Berger, P. A. Lief, A. Wollack, E. M. Papper, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **114,** 289 (1955).

(2) N. P. Plotnikoff, E. L. Way, and H. W. Elliott, *J. Pharmacol. Exp. Ther.*, **117,** 414 (1956).

(3) A. M. Asatoor, D. R. London, M. D. Milne, and M. L. Simenhoff, *Br. J. Pharmacol.*, **20,** 285 (1963).

(4) I. W. Wainer and J. E. Stambaugh, *J. Pharm. Sci.*, **67,** 116 (1978).

(5) C. Lindberg, C. Bogentoft, U. Bondesson, and B. Danielsson, *J. Pharm. Pharmacol.*, **27,** 975 (1975).

(6) M. Mitchard, M. J. Kendall, and K. Chan, *J. Pharm. Pharmacol.*, **24,** 915 (1972).

(7) W. G. Stillwell, C. S. Myron, and J. T. Steward. *Res. Commun. Chem. Pathol. Pharmacol.*, **14,** 605 (1976).

(8) N. P. Plotnikoff, H. W. Elliott, and E. L. Way, *J. Pharmacol. Exp. Ther.*, **104,** 377 (1952).

(9) V. R. Jenkins II, W. M. Talbert, and P. V. Dilts, Jr., *Obs. Gyn.*, **39,** 254 (1972).

(10) T. J. Goehl and C. Davison, *J. Pharm. Sci.*, **62,** 907 (1973).

(11) L. E. Mather and G. T. Tucker, *J. Pharm. Sci.*, **63,** 306 (1974).

(12) U. Klotz, T. S. McHorse, G. R. Wilkinson, and S. Schender, *Clin. Pharmacol. Ther.*, **16,** 667 (1974).

(13) S. Y. Yeh, H. A. Krebs, and A. Changchit, *J. Pharm. Sci.*, **70,** 867 (1981).

(14) S. Y. Yeh and C. L. Mitchell, *J. Pharmacol. Exp. Ther.*, **179,** 642 (1971) and references cited therein.

(15) S. Y. Yeh and C. W. Gorodetzky, *Toxicol. Appl. Pharmacol.*, **24,** 387 (1973) and references cited therein.

(16) S. Y. Yeh, B.-L. Chang, H. A. Krebs, and C. W. Gorodetzky, *J. Pharmacol. Exp. Ther.*, **209,** 125 (1978) and references cited therein.

(17) C. Lindberg and C. Bogentoft, *Acta Pharm. Suec.*, **12,** 507 (1975).

(18) K. Nakanishi, "Infrared Absorption Spectroscopy-Practice," Holden-Day, San Francisco, Calif., 1962, p. 210.

(19) T. D. Phillipson, S. S. Handa, and S. W. El-Dabbas, *Phytochemistry*, **15,** 1297 (1976).

(20) S. Y. Yeh and H. A. Krebs, *J. Pharm. Sci.*, **70,** 482 (1981).

(21) S. Y. Yeh, H. A. Krebs, and C. W. Gorodetzky, *J. Pharm. Sci.*, **68,** 133 (1979).

(22) K.-H. Leung, K. K. Park, and M. C. Archer, *Res. Commun. Chem. Pathol. Pharmacol.*, **19,** 201 (1978).

(23) Y. Okano, T. Miyata, S. H. Hung, T. Motoya, M. Kataoka, K. Takahama, and Y. Kase, *Jpn. J. Pharmacol.*, **28,** 41 (1978).

(24) D. H. Clouet, *J. Pharmacol. Exp. Ther.*, **144,** 354 (1964).

(25) J. Daly, J. K. Inscoe, and J. Axelrod, *J. Med. Chem.*, **8,** 153 (1965).

(26) E. J. Cone, C. W. Gorodetzky, W. D. Darwin, F. I. Carroll, G. A. Brine, and C. D. Welch, *Res. Commun. Chem. Pathol. Pharmacol.*, **20,** 413 (1978).

(27) K. Verebely, M. A. Chedekel, S. J. Mule, and D. Rosenthal, *Res. Commun. Chem. Pathol. Pharmacol.*, **12,** 67 (1975).

(28) S. Y. Yeh, *J. Pharmacol. Exp. Ther.*, **192,** 201 (1975).

(29) R. E. McMahon, *J. Med. Pharm. Chem.*, **4,** 67 (1961).

ACKNOWLEDGMENTS

The author sincerely thanks Mr. H. A. Krebs for technical assistance and Ms. W. M. Roberts for her aid in the preparation of this manuscript.