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Metabolism of Flurazepam, a Benzodiazepine, in Man and Dog

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Abstract □ The metabolism of ^{14}C-flurazepam hydrochloride, 7-chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one-5-^{14}C dihydrochloride, was studied in a dog administered oral and intravenous 2-mg./kg. doses and in two human subjects who each received a 28-mg. oral dose. In both species, evidence was obtained for rapid and essentially complete absorption followed by a rapid elimination of plasma flurazepam. Biotransformation of the drug was rapid and virtually complete in man and dog. Pathways of biotransformation were similar in both species. All the metabolites identified either showed some alteration in the N_1-diethylaminoethyl moiety or lacked the N_1-substituent altogether. The major metabolite in the dog was a carboxylic acid, the N_1-acetic acid analog of flurazepam. In man, the analogous alcohol (the N_1-ethanol analog) predominated.

Keyphrases □ ^{14}C-Flurazepam HCl metabolism—humans, dogs □ Metabolites, flurazepam HCl—isolation, identification □ Urinary, fecal excretion—^{14}C-flurazepam □ Plasma levels—^{14}C-flurazepam □ TLC—separation □ UV spectrophotometry—analysis □ Scintillometry—analysis

The synthesis (1) and the pharmacology (2) of flurazepam hydrochloride,[1] 7-chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one dihydrochloride, have been reported as well as the clinical use of this compound (also designated Ro 5-6901) as a hypnotic (3, 4).

In a previous report (5), the isolation by TLC and the characterization by high-resolution mass spectrometry of five urinary metabolites from the dog and one human urinary metabolite were described. The structures of these metabolites are shown in Table I, together with those of flurazepam, the 3-hydroxy derivative of flurazepam (F-3-OH), and Compound III. While neither of the latter two compounds had been detected as metabolites, F-3-OH was of interest as a reference compound to test for 3-hydroxylation of intact drug, and Compound III was a likely metabolic precursor of metabolites III-OH and IV. All the compounds of Table I, except the incompletely characterized III-OH, have been synthesized (6).

The synthesis of ^{14}C-labeled flurazepam hydrochloride allowed for a study of the disposition of this drug in terms of absorption, distribution, biotransformation, and excretion.

EXPERIMENTAL

Labeled Compound and Counting of ^{14}C—Flurazepam-5-^{14}C hydrochloride[2] was diluted with unlabeled compound to a specific activity of 3.12 μc./mg. flurazepam base for the dog studies and 2.10 μc./mg. base for the human studies. Before administration to the human subjects and the dog, the labeled drug was shown to be radiochemically pure by TLC with System A (described later).

All samples were counted in a Nuclear-Chicago Corp. Mark I liquid scintillation spectrometer equipped with a ^{133}Ba external standard; the external standard-channels ratio technique was used to determine counting efficiency. Aliquots of urine and extracts of both urine and plasma were counted in Phosphor I (7), while plasma (0.2–1.0 ml.) and silica gel segments from chromatoplates were counted as suspensions in Phosphor II (7) which contained a thixotropic gel. The ^{14}C in aliquots of 50% ethanol homogenates of feces was determined by the Schoniger combustion, carbon dioxide trapping, and counting procedures of Kelly *et al.* (8).

Dog Studies—A nonfasted 13-kg. male dog (beagle) was first given an oral dose of 2 mg./kg. of ^{14}C-flurazepam HCl in a gelatin capsule. Two months later, it was given the same dose as a solution in 2.6 ml. of physiological saline by rapid intravenous injection. Urine and feces were collected until the excretion of radioactivity reached the limits of detection. Blood (10 ml., heparinized) was drawn at 0, 1, 2, 3, 4, 7, 12, and 24 hr. after oral dosing and at 0, 5, 15, and 30 min., and 1, 2, 3, 4, 7, 11, and 24 hr. after intravenous dosing of the dog.

For fractionation of the plasma radioactivity, aliquots (0.2–0.5 ml.) of plasma were brought to 1 ml. with water, mixed with 1 ml. of 0.5 *M* borate buffer, pH 9.0, and extracted twice with 5 ml. of ether. This procedure quantitatively removed flurazepam (9). The aqueous phases were adjusted to pH 7.0 and were extracted twice with 5 ml. of ether and then twice with 5 ml. of ethyl acetate. Finally, the aqueous phases were brought to pH 3.0 and again extracted twice with 5 ml. of ethyl acetate. These extracts were concentrated and counted.

The urinary radioactivity was fractionated in a similar manner. Aliquots (10 ml.) of urine were extracted twice with equal volumes of ethyl acetate at various pH's before incubation at pH 5.5 and 37° for 3 hr. with a commercial preparation[3] containing β-glucuronidase

[1] Flurazepam hydrochloride is the active ingredient in the trademarked product Dalmane of Hoffmann-La Roche Inc., Nutley, N. J.

[2] The labeled flurazepam hydrochloride synthesized by H. H. Kaegi and G. Bader, Isotope Synthesis Laboratory, Hoffmann-La Roche Inc., was labeled with ^{14}C at the C-5 position. The synthesis has not been published but is available from these chemists.

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

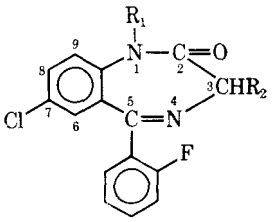


Table I—Flurazepam and Metabolites

Compound Designation	R_1	R_2	Metabolic Status from Previous Work (5)
Flurazepam	$-CH_2CH_2N(C_2H_5)_2$	H	Intact drug
F-3-OH	$-CH_2CH_2N(C_2H_5)_2$ H	OH	Not found in urine
I	$-CH_2CH_2NC_2H_5$	H	Urinary metabolite in dog
I-DE[a]	$-CH_2CH_2NH_2$	H	Urinary metabolite in dog
II	CH_2CH_2OH	H	Urinary metabolite in man and dog
III	H	H	Not found in urine
III-OH[b]	H	H	Urinary metabolite in dog
IV	H	OH	Urinary metabolite in dog

[a] DE signifies that this compound is the desethyl derivative of I. [b] Compound III-OH was not completely characterized; mass spectral analysis indicated the addition of a phenolic group to Compound III.

and sulfatase; they were reextracted at pH 7.0 after incubation. This incubation previously yielded maximum hydrolysis of benzodiazepine conjugates (7). Further details of these extractions are included with the results in Table II. Fecal homogenates in 50% ethanol were first evaporated to remove the ethanol before two extractions at pH 9.0 with an equal volume of ether.

Human Study—Two women, H-17 and H-18, were each given 28.0 mg. of labeled flurazepam hydrochloride (specific activity of 3.90×10^6 d.p.m./mg.) in a gelatin capsule. H-17, a 57-year-old hypertensive weighing 91 kg., was treated with 50 mg. of hydrochlorothiazide per day before and during the study. H-18, 61 years old and weighing 73 kg., suffered from labile hypertension but was normotensive during the study and required no therapy.

Both subjects were given a glass of water every 2 hr. during the 1st day to promote diuresis; intervals of urine collection were 0–2, 2–4, 4–6, 6–9, 9–12, and 12–24 hr. and daily thereafter until the end of the study. Feces were collected as daily pools. Oxalated blood (10 ml.) was drawn at 0, 1, 2, 4, 7, 12, and 24 hr. and at 24-hr. intervals thereafter. Aliquots of plasma were extracted at pH 9.0 with ether, as described for dog plasma, and the extracts were counted. Aliquots of urine were serially extracted at pH 9.0 with ether, at pH 7.0 with ethyl acetate before and after treatment with the β-glucuronidase–sulfatase preparation, and finally at pH 3.0 with ethyl acetate; equal volumes of solvent were used twice for each extraction. The extracts were evaporated to dryness, and the residues were dissolved in 5 ml. of ethanol; aliquots were counted and, if sufficient ^{14}C was present, were examined by TLC.

Separation and Estimation of Drug and Metabolites—The technique used previously (7) involves the determination of the amount of chromatographed radioactivity that migrates on two-dimensional TLC as an authentic reference compound. Internal reference compounds chromatographed with extracts of plasma, urine, and feces included flurazepam, F-3-OH HCl, I 2HCl, I-DE 2HCl, II HCl, III, and IV. Also used as a reference compound was the dehydrated derivative of I-DE which was shown (5) to be an artifact arising on TLC of I-DE; any ^{14}C separated as this compound was considered to have been present in the biological media as I-DE. The recovery of plasma ^{14}C-flurazepam on extraction and TLC was estimated at roughly 90%.

Silica gel containing a fluorescent indicator[4] was used for TLC, together with the following solvent systems: System A, ethyl acetate–ethanol–concentrated ammonia (95:5:0.5); System B, isopropanol–ethanol–concentrated ammonia (90:10:1); System C, benzene–ethyl acetate–ethanol–concentrated ammonia (80:20:10:0.2); System D, chloroform–acetone–concentrated ammonia (80:20:1); System D′, chloroform–acetone–concentrated ammonia (95:5:0.1); System E, benzene–*n*-butanol–methanol–water (1:1:2:1); System F, benzene–methanol–acetic acid (90:10:1); System G, heptane–chloroform–ethanol (10:10:2.5); System G′, heptane–chloroform–ethanol–concentrated ammonia (50:50:25:1); System H, benzene–*n*-butanol (90:10); System I, methylene chloride–*n*-butanol (90:10); System J, heptane–ethyl acetate–ethanol–concentrated ammonia (50:50:5:0.2); and System K, heptane–benzene–ethanol (5:5:2).

In many instances, these solvent systems were used as pairs for two-dimensional TLC. For example, System AC signifies that the chromatoplate was developed first in A and then, after being turned 90°, in C. Compounds were located on the plates under shortwave UV light.

Metabolites of II HCl in the Dog—The biotransformation in the dog of unlabeled metabolite II was studied to elucidate further the pathways of flurazepam metabolism. Three milliliters of plasma separated from blood drawn 1, 2, and 3 hr. following oral administration of 22 mg./kg. of II HCl in a gelatin capsule was combined and extracted at pH 9.0 with ethyl acetate. The extracted metabolites were separated by TLC. Urine excreted the day before and the day after the intravenous administration in propylene glycol–water (1:1) of 6 mg./kg. of II HCl was separately extracted by the Series 2 procedure of Table II. The extracts were dried over anhydrous sodium sulfate, concentrated, and examined by TLC. Identification was accomplished by the demonstration that the metabolite and an authentic reference compound migrated as a single compound.

RESULTS

Metabolism in the Dog—*Excretion of Labeled Drug and Metabolites*—The radioactivity excreted after the oral and intravenous administration of ^{14}C-flurazepam hydrochloride is shown in Table III. After each route of administration, roughly the same amount of ^{14}C was excreted in the feces as in the urine during the 1st day. Fecal excretion of ^{14}C was significant for the first 3 days, and the total amount excreted exceeded that found in the urine. These findings suggest that a considerable secretion of labeled material into the gastrointestinal tract occurred. The feces excreted in the first 2 days after intravenous drug administration were analyzed by ether extraction at pH 9.0 and TLC of the extracts with System DB. No flurazepam was detected in this extract. While I-DE and IV were found, the fecal excretion of each was less than 1% of the dose. The remaining fecal radioactivity remained unidentified.

The composition of the urinary radioactivity was first studied by solvent extraction. The results of three separate series of extractions of urine from the orally treated dog (Table II) demonstrated the following: (*a*) no more than 5% of the urinary ^{14}C, that extracted at pH 9.0, could be intact drug; (*b*) roughly 60% of the urinary metabolites was acidic (the Series 3 extraction ruled out the possibility that the ^{14}C removed at pH 1.0 and 3.0 in the first two extraction

[4] Mallinckrodt Silicar 7-GF-5.

Table II—Extractability of the Radioactivity Excreted by a Dog in the 0–36-hr. Urine after Oral and Intravenous Doses of ^{14}C-Flurazepam HCl[a]

Serial Extraction with Ethyl Acetate[b]	—Percent of Urinary ^{14}C Extracted— After Oral Dose— Series 1	Series 2	Series 3	After Intravenous Dose Series 2
pH 1.0	58.1	—[c]	—	—
pH 9.0	—	4.8	—	5.2
pH 7.0	3.5	2.6	6.1	2.0
pH 3.0	—	60.6	[d]	45.4
pH 7.0	—	0.3	4.4	0.4
After β-glucuronidase-sulfatase treatment				
pH 7.0	11.0	10.2	—	11.6
Remaining aqueous	25.7	20.0		30.2
	98.3	98.5		94.8

[a] After the oral dose, 33.0% of the ^{14}C was excreted in the urine in 36 hr.; after the intravenous dose, 24.3% was so excreted. [b] An aliquot (10 ml.) of the 0–36-hr. pooled urine was subjected to consecutive ethyl acetate extraction at the pH values shown. [c] A dash indicates that the urine was not adjusted to, nor extracted at, the designated pH. [d] The sample was adjusted to pH 3.0, allowed to stand for 15 min., and then adjusted to the next pH (pH 7.0) before being extracted.

series represented nonacidic artifacts formed on exposure to these acidic conditions); (*c*) only 10–11% of the metabolites were excreted as conjugates susceptible to hydrolysis by the β-glucuronidase–sulfatase preparation; and (*d*) 20–26% of the metabolites were of such a polar nature that they were not extractable with ethyl

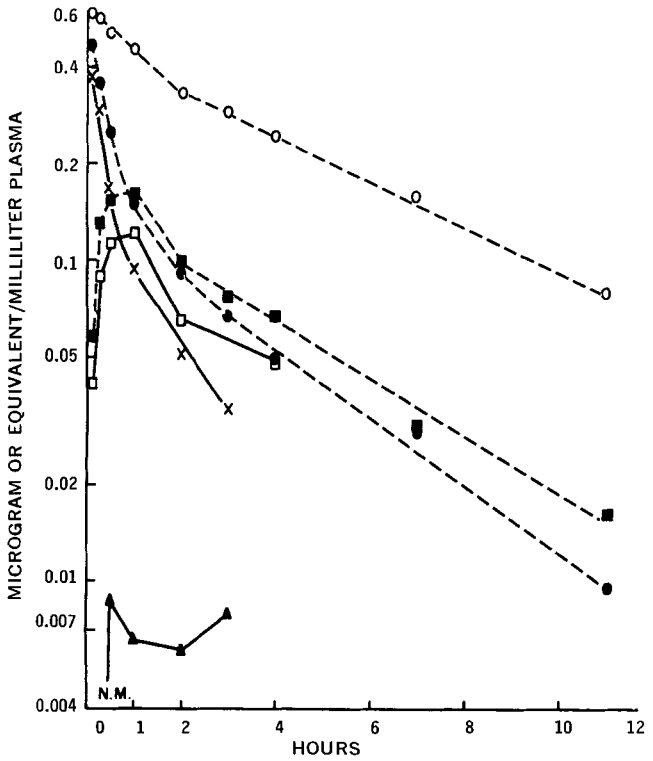


Figure 1—*Plasma levels of flurazepam-derived ^{14}C and labeled metabolites in a dog given an intravenous dose of 2 mg./kg. of ^{14}C-flurazepam hydrochloride. The concentrations of plasma total ^{14}C (○- - -○), the ^{14}C extracted at pH 9.0 into ether (●- - -●), and the ^{14}C extracted at pH 3.0 into ethyl acetate (■- -■) are expressed as microgram equivalents flurazepam per milliliter of plasma; the concentrations of flurazepam (× — ×), III (▲ — ▲), and V (□ — □) are expressed as micrograms per milliliter of plasma. The basic metabolites were quantitated by TLC of the pH 9.0 ether extract in System DB; the acidic metabolite V was quantitated by TLC of the pH 3.0 ethyl acetate extract in System EF. N.M. = not measurable.*

Table III—Excretion of Radioactivity by a Dog after Oral and Intravenous 2-mg./kg. Doses of ^{14}C-Flurazepam Hydrochloride

Time Interval after Dose, Days	—Excretion of ^{14}C, % of Dose— Urine	Feces	Total
	Oral 2-mg./kg. Dose		
1	30.4	29.4	59.8
2	4.1	14.1	18.2
3	1.1	4.0	5.1
4	0.3	0.6	0.9
5	0.2	0.2	0.4
6	0.1	0.2	0.3
7	0.04	—[a]	0.04
8	0.08	—	0.08
	36.3	48.5	84.8
	Intravenous 2-mg./kg. Dose		
1	22.8	19.9	42.7
2	2.3	28.4	30.7
3	1.1	3.9	5.0
4	0.5	0.8	1.3
5	0.3	0.4	0.7
6	0.2	0.3	0.5
7	0.07	0.2	0.27
8	0.1	—	0.1
9	0.05	—	0.05
	27.4	53.9	81.3

[a] The dash indicates that the feces were not collected.

acetate under any condition listed in Table II. It is also evident from Table II that the urine from the intravenously treated dog contained metabolites similar in extractability to those excreted after oral drug administration.

To identify the acidic metabolites, 180 ml. of the 0–36-hr. urine from the orally treated dog was extracted according to Series 2, Table II. At pH 3.0, 57.4% of the urinary ^{14}C was extracted. TLC of the concentrated extract with System H yielded a UV-absorbing band at R_f 0.3–0.4, which was associated with roughly 80% of the chromatographed radioactivity. This labeled component was eluted from the silica gel, and an aliquot of the concentrated ethanol eluate was rechromatographed with System I alongside authentic 2-[7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2-oxo-2*H*-1,4-benzodiazepin-1-yl]acetic acid (Compound V). Compound V was chosen as the reference compound because the analogous alcohol, II, was a known urinary metabolite of flurazepam hydrochloride in the dog (5). The labeled metabolite migrated as a single band (R_f 0.75), which contained 94% of the ^{14}C chromatographed with System I and did not separate from authentic V. Although TLC with System I of greater amounts of labeled material yielded a diffuse band, this diffuse component, after elution from the silica gel, and authentic V were shown by high-resolution mass spectral analysis[5] to be identical. It was estimated that roughly 45% of the urinary ^{14}C was present as V.

Although this new metabolite was quantitated successfully in plasma extracts by two-dimensional TLC, this technique could not be used with extracts of urine because of excessive tailing. It was, therefore, necessary to convert metabolite V into its methyl ester prior to TLC. Freshly prepared diazomethane (10) was added in excess to the urinary extract and, after standing 15 min. at room temperature, the solution was evaporated to dryness and the residue dissolved in ethanol. Authentic V was found to be completely esterified under these conditions.

The urinary excretion of drug and metabolites, quantitated by solvent extraction and TLC, is summarized in Table IV. After oral administration, no flurazepam was detected in the urine; the major metabolite was V, while lesser amounts of conjugated IV and free I–DE were excreted. The same three metabolites were excreted after intravenous drug administration in amounts similar to those found after oral dosing. In addition, the excretion of small amounts of intact drug (0.2% of the dose), Compound I, and conjugated II was

[5] Performed by F. M. Vane, Physical Chemistry Department, Hoffmann-La Roche Inc., Nutley, NJ 07110

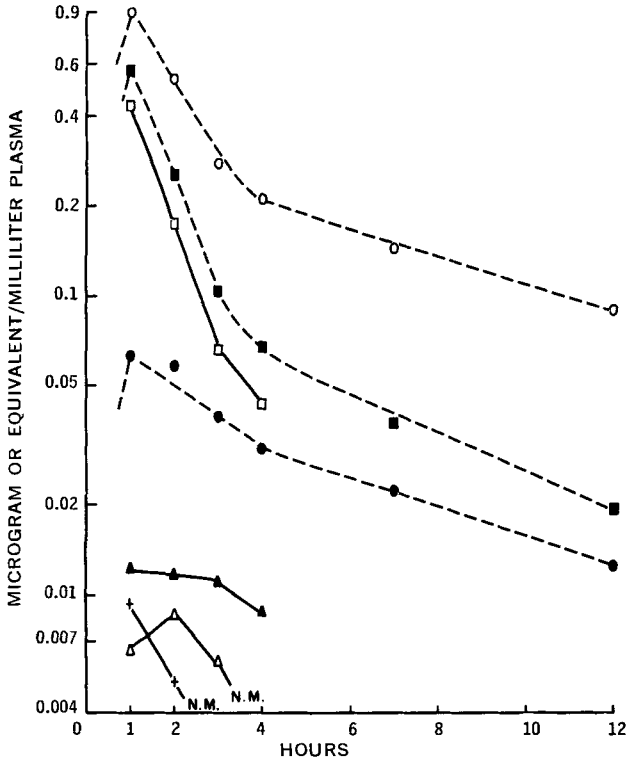


Figure 2—*Plasma levels of flurazepam-derived ^{14}C and labeled metabolites in a dog given an oral dose of 2 mg./kg. of ^{14}C-flurazepam hydrochloride. The concentrations of plasma total ^{14}C (O- - -O), the ^{14}C extracted at pH 9.0 into ether (●- - -●), and the ^{14}C extracted at pH 3.0 into ethyl acetate (■- -■) are expressed as microgram equivalents flurazepam per milliliter of plasma; the concentrations of III (▲ − ▲), I-DE (△ − △), IV (+ − +), and V (□ − □) are expressed as micrograms per milliliter of plasma. The metabolites were quantitated by TLC as described in the legend of Fig. 1. N.M. = not measurable.*

seen only after intravenous drug administration. Although not shown in this table, no excretion of F-3-OH, either as a free or conjugated metabolite, was found.

Plasma Levels of Drug and Metabolites—The plasma levels of undifferentiated ^{14}C and of specific labeled compounds following the intravenous administration of ^{14}C-flurazepam hydrochloride are shown in Fig. 1. Intact drug was the major labeled compound extracted at pH 9.0, and its rapid biexponential elimination was characterized by half-lives of 11 min. and 1.4 hr. In addition, its apparent volume of distribution in terms of the central compartment of a two-compartment open system was calculated (11) to be 44.2 l. or 340% of body weight, a value indicating extensive tissue uptake of drug. Compound III was found in the same extract as flurazepam, but its plasma levels remained below 0.01 mcg./ml. At 5 min., the

Table IV—Urinary Excretion of Drug and Metabolites by a Dog after Oral and Intravenous ^{14}C-Flurazepam Hydrochloride

Urinary Components	Amount Excreted: After Oral Drug[a] (% of Urinary ^{14}C)	After Oral Drug[a] (% of Dose)	After Intravenous Drug[b] (% of Urinary ^{14}C)	After Intravenous Drug[b] (% of Dose)
Basic				
Flurazepam	<0.17	Nil	0.57	0.2
I	<0.2	Nil	0.15	<0.1
I-DE	1.2	0.4	0.82	0.2
Unknown	3.6	1.2	2.68	0.7
Acidic				
V	45	15	41.5	11.4
Unknown	15	5.0	9.3	2.5
Conjugates				
IV	3.8	1.3	6.9	1.9
II	<0.3	Nil	0.32	0.1
Unknown	6.4	2.1	8.5	2.3
Polar nonconjugates				
Unknown	20	6.6	31.0	8.5

[a] The urinary excretion of metabolites in 36 hr. is shown; over 90% of the total urinary ^{14}C excreted was excreted in this time interval. The appropriate extracts of Series 2, Table II, were used for the quantitation of the basic and conjugated metabolites. Basic metabolites were separated by TLC with System AC, while both Systems AC and BD were used to separate the deconjugated metabolites. The amount of acidic metabolite was estimated during its isolation. The "polar nonconjugates" represent the nonextractable ^{14}C of Series 2, Table II. [b] The total urinary excretion of drug and metabolites is shown. Each urine collected during the first 3 days was analyzed by solvent extraction (Series 2, Table II) and TLC. Basic and deconjugated metabolites were separated by TLC with System DB, while the acidic metabolite was esterified with diazomethane and separated in two one-dimensional systems, G and D′.

acidic metabolite (V) was present at a plasma level of 0.041 mcg./ml.; at 1 hr., it reached a peak level of 0.12 mcg./ml. Since no appreciable amounts of intact drug were found in the urine and feces, it appears that the rapid elimination of plasma flurazepam resulted from tissue uptake and rapid biotransformation.

These two processes were also apparently responsible for the absence of detectable plasma levels of drug after oral administration. As shown in Fig. 2, the highest level of plasma ^{14}C was observed at 1 hr., suggesting that rapid absorption had occurred. But even at this early time the major portion of the plasma radioactivity resided in acidic metabolites. Metabolite V accounted for over 70% of the acidic metabolite fraction of 1, 2, 3, and 4-hr. plasma, and its levels fell rapidly from 0.43 mcg./ml. with a half-life of 0.76 hr. Also found in the plasma were relatively low concentrations of I-DE, III, and IV.

Metabolism of II HCl—Intact II and Compound III were both identified in the ethyl acetate extract of dog plasma by TLC with Systems D and J. In the urine, both free and conjugated II were found (System DB), while the presence of conjugated IV was demonstrated with Systems AD and G′B. The acidic Compound V was also shown to be a urinary metabolite by formation of its methyl ester and subsequent TLC with System GK.

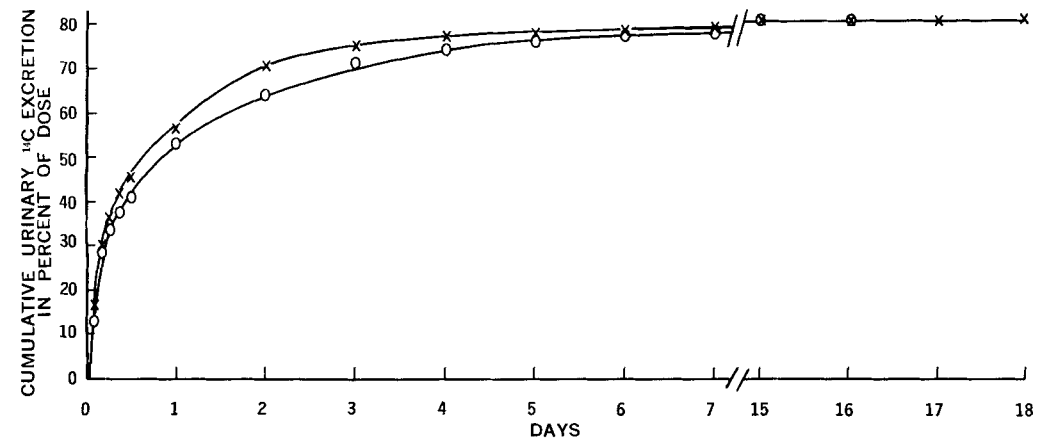


Figure 3—*Cumulative urinary excretion of radioactivity by Subject H-17 (O − O) and Subject H-18 (× − ×) following an oral dose of 28 mg. of ^{14}C-flurazepam hydrochloride.*

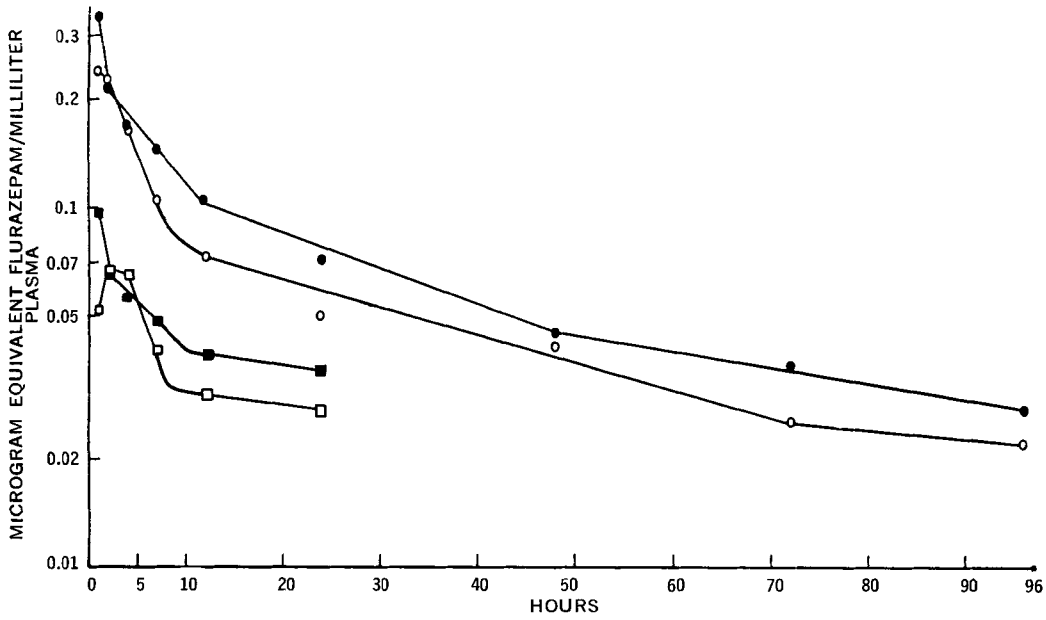


Figure 4—*Falloff of ^{14}C levels expressed as microgram equivalent flurazepam per milliliter in plasma (○) and ether extracts of plasma (□) of Subject H-17 and in plasma (●) and ether extracts of plasma (■) of Subject H-18.*

Administered II was apparently biotransformed *via* two pathways. One involved the removal of the *N*-ethanol moiety to give plasma III; this was subsequently hydroxylated at C-3 to form IV which, as a conjugate, was excreted in the urine. The second pathway involved oxidation of the alcohol moiety of II to form the carboxylic acid, V, which was found in the urine.

Metabolism in Man—The cumulative excretion of radioactivity in urine and feces was almost the same for both subjects. More than half of the administered ^{14}C was excreted by each subject in the urine during the 1st day; each excreted a total of 81% of the dose in the urine, with 9% of the dose excreted in the feces by H-17 and 8% of the dose so excreted by H-18. The similarity of the excretion of urinary radioactivity for both subjects is clear from Fig. 3, which shows the cumulative excretion of urinary ^{14}C as a function of time.

In the plasma, the highest levels of total radioactivity, 0.24 mcg. equivalents of flurazepam per ml. for H-17 and 0.34 mcg. equivalents/ml. for H-18, were seen at 1 hr. (Fig. 4). These early peak levels indicated that absorption of labeled drug had been rapid. While the levels of ether-extracted radioactivity were markedly lower than those of total ^{14}C, the falloff of this extracted radioactivity closely paralleled that of total plasma ^{14}C in each subject.

Only in the plasma samples containing the highest levels of ^{14}C was it feasible to search for drug and metabolites. As seen in Table V, intact flurazepam was detected only in the 1-hr. plasma of H-17. Furthermore, intact drug in these early samples accounted for roughly 1% or less of the total plasma ^{14}C shown in Fig. 4. In view of the evidence for rapid absorption of labeled drug, this finding indicates that the absorbed drug was rapidly biotransformed into metabolites. With respect to the plasma metabolites identified (Table V), II was the major metabolite in both subjects at 1 hr. while roughly equivalent levels of II, III, and I were found at 2 hr. In addition, trace levels of I-DE and IV were found in the plasma, while F-3-OH was not detected and 33–47% of the extracted ^{14}C was not identified.

The nature of the urinary radioactivity was investigated with pooled urine (0–4 days) representing over 90% of the total urinary ^{14}C eliminated by each subject. Only 15% (H-17) and 11% (H-18) of the urinary ^{14}C was extractable as intact drug and/or basic metabolites (Table VI). The major portion, 49% in each subject, was extracted at pH 7.0 after β-glucuronidase–sulfatase treatment; *i.e.*, it was apparently excreted as glucuronide and/or sulfate conjugates. Less than 7% of the excreted radioactivity represented acidic metabolites extractable at pH 3.0, and roughly 20% was comprised of polar nonextractable compounds.

The composition of the urinary radioactivity extractable directly at pH 9.0 and, following β-glucuronidase–sulfatase treatment, at pH 7.0 is shown in Table VII. While no intact drug was found, both subjects excreted I and I-DE as unconjugated metabolites. The major urinary metabolite was conjugated II, which accounted for 25% of the dose in H-17 and 22% of the dose in H-18. A small amount of conjugated IV was also excreted by both subjects.

The rate of excretion of metabolites was studied in H-18. The unconjugated and conjugated metabolites of each urine specimen collected during the first 4 days were extracted as described previously, and the metabolites of each extract were separated and quantitated by TLC with System DB. The total urinary excretion of each metabolite in milligrams was readily estimated (I, 0.21; I-DE, 0.76; II, 4.69; and IV, 0.30), and the amount of each compound remaining to be excreted was plotted as a logarithmic function of time (Fig. 5) to

Table V—Composition of the Plasma Radioactivity Extractable at pH 9.0 into Ether following Oral ^{14}C-Flurazepam Hydrochloride Administration to Two Subjects

Compound	Plasma Concentration, mcg./ml.[a] H-17 1 hr.	H-17 2 hr.	H-18 1 hr.	H-18 2 hr.
Flurazepam	0.004	N.S.	N.S.	N.S.
II	0.014	0.013	0.020	0.009
III	0.005	0.008	0.010	0.010
I	0.003	0.008	0.012	0.010
I-DE	N.S.	0.004	0.004	0.004
IV	0.003	N.S.	N.S.	0.004
F-3-OH	N.S.	N.S.	N.S.	N.S.
Unaccounted[b]	0.014	0.027	0.041	0.028

[a] The distribution of the ether-extracted ^{14}C was determined by two-dimensional TLC (System DB), using the compounds listed in the table as internal standards. Limit of sensitivity for each compound was roughly 0.003 mcg./ml. plasma; N.S. indicates that levels of ^{14}C were below 10 c.p.m. above background. [b] The ^{14}C unaccounted for is expressed as microgram equivalent flurazepam/milliliter.

Table VI—Extraction of the Radioactivity in 0–4-Day Pooled Urine[a] from Subjects H-17 and H-18

Serial Extraction	Radioactivity Extracted—Subject H-17 (% of Urinary ^{14}C)	Subject H-17 (% of Dose)	Subject H-18 (% of Urinary ^{14}C)	Subject H-18 (% of Dose)
pH 9.0, ether	15.0	11.1	10.9	8.4
pH 7.0, ethyl acetate	2.6	1.9	2.5	1.9
After β-glucuronidase–sulfatase treatment				
pH 7.0, ethyl acetate	49.0	36.3	49.2	37.9
pH 3.0, ethyl acetate	4.1	3.0	6.1	4.7
Remaining aqueous	21.1	15.6	17.7	13.6
Recovery	91.8		86.4	

[a] The 0–4-day urine pool represented the excretion of 74% of the administered ^{14}C dose by H-17 and 77% of the dose by H-18.

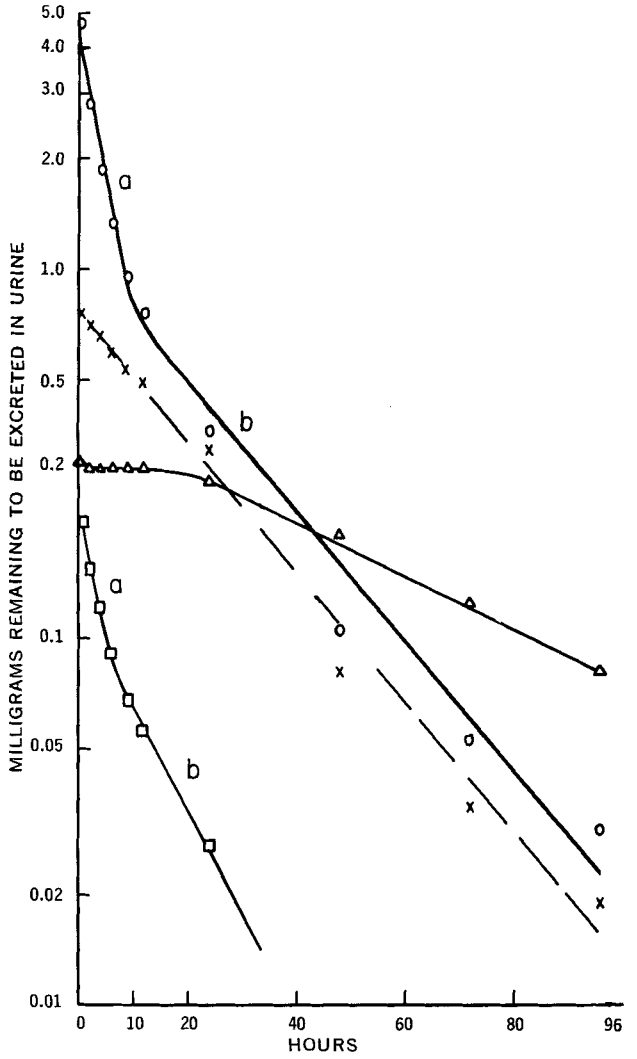


Figure 5—*Urinary excretion of metabolites by Subject H-18. The milligrams of I* (□ — □), *I-DE* (×- -×), *II* (○ — ○), *and IV* (△ — △) *remaining to be excreted are plotted logarithmically as a function of time. (Metabolites II and IV were excreted as conjugates.) The half-life in hours for each rate [with rate (a) obtained by the "residuals" method] was:*

I		*I-DE*	*II*		*IV*
(*a*)	(*b*)		(*a*)	(*b*)	
2.0	*11*	*17*	*2.5*	*17*	*42*

give the pseudo-first-order elimination rates expressed as half-lives in the legend. The major urinary metabolite, conjugated II, was eliminated biexponentially at rates characterized by half-lives of 2.5 and 17 hr. Metabolite I was also eliminated biexponentially at similar rates. Both I-DE and conjugated IV were eliminated monoexponentially; the latter metabolite exhibited a long lag period before appreciable excretion occurred and had the longest half-life.

DISCUSSION

The evidence presented indicated that in both man and dog the rate of labeled drug absorption was relatively fast. Furthermore, the dog excreted more of the labeled dose in the urine after oral ^{14}C-flurazepam administration than after intravenous administration, and the human subjects excreted over 80% of the oral dose in the urine. These findings suggest that absorption was fairly complete in both species.

Since orally administered flurazepam hydrochloride was not excreted intact by dog or man, it is clear that the drug was eliminated solely by biotransformation. Evidence for the elimination being a rapid process was provided by the fact that, in the intravenously injected dog, drug disappeared rapidly from the plasma exhibiting a biexponential decline with half-lives of 11 min. and 1.4 hr. Furthermore, in both species, metabolites predominated even in the earliest plasma samples. The rapid biotransformation, together with tissue uptake of intact drug, appeared to be responsible for keeping plasma flurazepam below measurable levels after oral administration to man or dog.

The postulated pathways of flurazepam biotransformation are shown in Scheme I. Flurazepam was metabolized by successive *N*-dealkylation to yield I and I-DE, both of which were excreted in the urine by man and dog. The dog produced V as a major metaboli e of plasma and urine and II as a minor urinary metabolite. It is likely that both arose from the same precursor, an aldehyde formed by oxidative deamination of I-DE. In the dog, oxidation of the aldehyde was preferred and V was the dominant metabolite; in man the reduction to II, which was excreted as the major urinary metabolite in the form of a conjugate, was the preferred pathway.

The study of the metabolism of II in the dog revealed that III was formed by removal of the N–CH_2CH_2OH group, a pathway shown in Scheme I. Since III was also a metabolite of flurazepam in man and dog and has not been shown to be formed exclusively *via* II as the intermediate, possible pathways (broken arrows) leading from flurazepam, I, and I-DE are also shown.

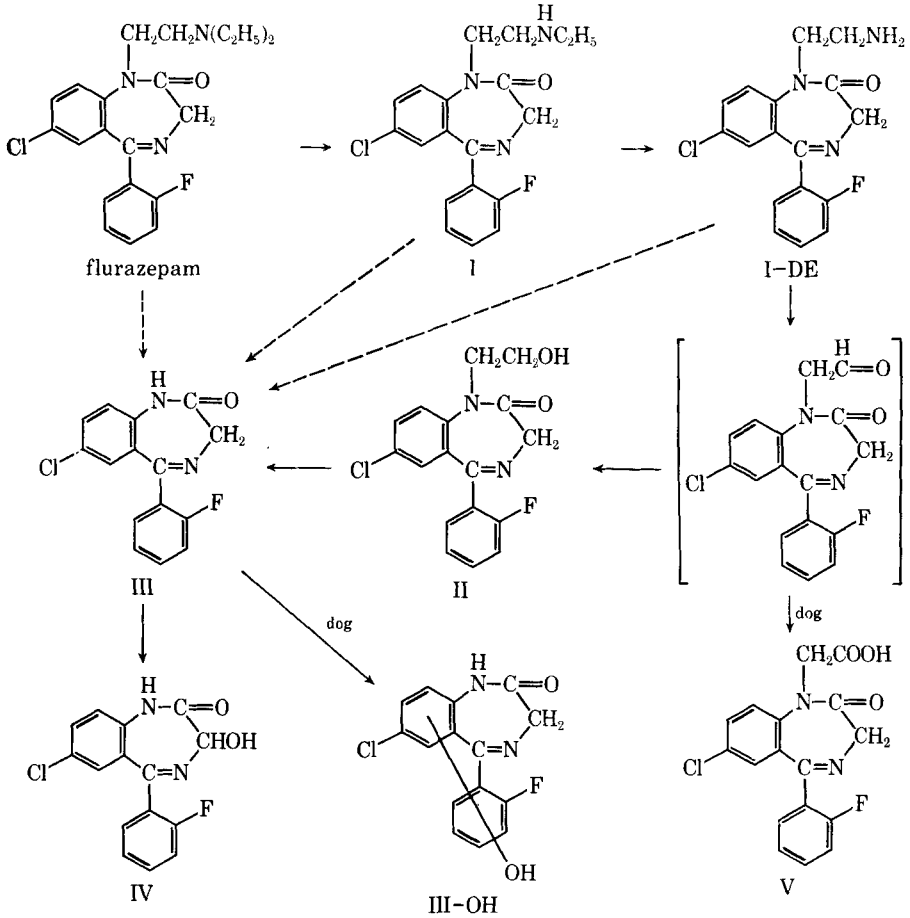
The 3-hydroxy derivative of flurazepam, F-3-OH, was not found as a metabolite in either man or dog, suggesting that metabolic attack at the N-1 side chain was preferred over 3-hydroxylation of intact drug. Therefore, the conjugated IV found in the urine of both species probably was formed *via* a pathway in which *N*-dealkylation preceded 3-hydroxylation, thus indicating that III was the immediate precursor of IV. Scheme I also shows III-OH, the incompletely characterized phenolic metabolite previously found (5) as a urinary conjugate in a dog chronically treated with flurazepam, arising from III. Although this metabolite may have been excreted in the present experiments in very small amounts, it definitely was not a significant metabolite in man or dog following single doses of flurazepam.

The three ether-extractable metabolites present in human plasma in greatest amounts were I, II, and III. I and conjugated II were excreted fairly rapidly in the urine, while III was not excreted *per se* but was biotransformed to IV. Conjugated IV did not appear in the urine in any appreciable amounts until the 2nd day, and it was excreted relatively slowly. This suggests that the conversion of III to IV may be slow and that III, on repeated administration of flurazepam, may tend

Table VII—Estimation by Two-Dimensional TLC of the Conjugated and Unconjugated Urinary Metabolites of Subjects H-17 and H-18

Source of 0–4-Day Urine	Metabolite Form	Urinary Excretion of Drug and Metabolites in Percent of Administered Dose[a]						Unaccounted for
		Flurazepam	I	I-DE	II	III	IV	
Subject H-17	Unconjugated	N.D.[b]	0.4	1.3	N.D.	0.5	N.D.	9.0
	Conjugated	N.D.	N.D.	0.8	25.0	N.D.	0.9	9.6
Subject H-18	Unconjugated	N.D.	1.2	4.1	N.D.	N.D.	N.D.	3.1
	Conjugated	N.D.	N.D.	N.D.	22.0	N.D.	1.5	14.4

[a] The values presented were based on the average distribution of ^{14}C on two-dimensional TLC in at least two systems. The "pH 9.0, ether" extract of H-18 was chromatographed in Systems DB and AD; the other extracts were each chromatographed in Systems DB, AD, and DC. [b] N.D. indicates that the compound was not detectable. The detection limits were 0.2% of the dose for unconjugated metabolites and 0.8% of the dose for conjugated metabolites. Compound F-3-OH was also used as an internal reference compound but was not detected as a metabolite in any of the extracts.



Scheme I—Postulated pathways of flurazepam metabolism in man and dog. The compound shown in parenthesis was not detected but is a presumed intermediate which would result from oxidative deamination of I-DE. The pathways labeled "dog" denote that the resulting metabolites were definitely established solely in the dog

toward higher plasma concentrations. Further studies are required to test this supposition.

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