Metabolism of [^{14}C]Verapamil

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The metabolism of [^{14}C]verapamil was studied in rats and dogs at dose levels similar to those employed during human therapy. Verapamil is well absorbed, rapidly metabolized, and eliminated largely in feces of both species. Little tissue accumulation occurs in rats. The 7 major drug-related substances in the urine and feces of dogs were isolated and identified by mass spectrometry as various O- and N-dealkaylated metabolites of drug. Major urinary and plasma metabolites represent N-dealkylated products of verapamil which retain the α-isopropylphenylacetonitrile portion of the molecule. A gas chromatographic assay was developed which measures 2 of these metabolites, 1-isopropyl-1-*N*-methylpropylamino-(3,4-dimethoxyphenyl)acetonitrile and 1-isopropyl-1-propylamino-(3,4-dimethoxyphenyl)acetonitrile, in plasma. Major fecal metabolites reflect the importance of hepatic O- and N-demethylation processes. It was concluded from comparative studies that pathways of metabolism of verapamil are similar in rats, although different rates of metabolism are indicated. The major metabolite in the blood stream of dogs was also found in the plasma of several angina patients receiving verapamil.

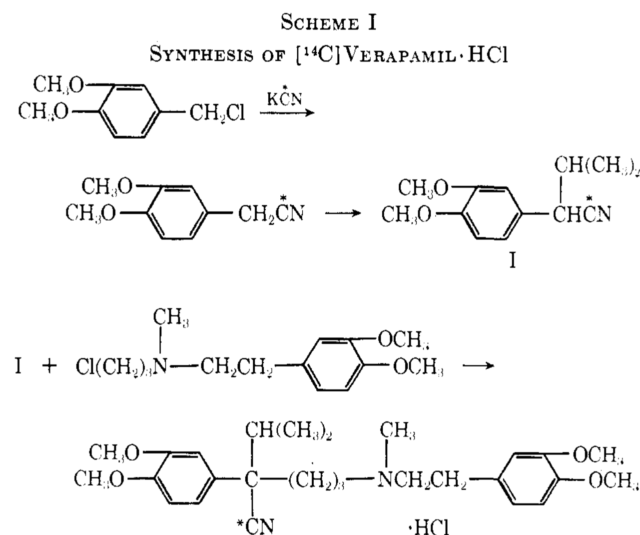
Verapamil (Isoptin, Cordilox, Knoll A.-G., Ludwigshafen, Germany), 5-[*N*-(3,4-dimethoxyphenethyl)-methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride, is a phenethylamine coronary vasodilator which has shown clinical usefulness in the management of angina pectoris.[1-5] This paper describes the absorption, biotransformation, distribution, and excretion of verapamil labeled with ^{14}C in the nitrile C upon administration to rats and dogs at dose levels similar (on a mg/kg basis) to those used in human therapy. Limited blood level data were also obtained from angina patients.

Experimental Section

Synthesis of ^{14}C-Labeled Verapamil.—^{14}C-labeled verapamil·HCl was synthesized from veratryl chloride by an adaptation of established methods[6] according to the sequence shown in Scheme I. Labeling was accomplished with [^{14}C]KCN during nitrile formation.

Scheme I

Synthesis of [^{14}C]Verapamil·HCl



(1) G. Knoch, M. Schlepper, and E. Witzleb, *Med. Klin. (Munich)*, **58**, 1485 (1963).

(2) J. H. Atterhog and G. Porje, *Sv. Laekartidn.*, **63**, 2071 (1966).

(3) M. Neumann and A. A. Luisada, *Amer. J. Med. Sci.*, **251**, 552 (1966).

(4) G. Sandler, G. A. Clayton, and S. G. Thornicroft, *Brit. Med. J.*, **3**, 224 (1968).

(5) *Arzneim.-Forsch.*, **20**, 1277 (1970).

(6) Knoll A.-G. Chemische Fabriken, U. S. Patent 2,695,319 (1954), *Chem. Abstr.*, **49**, 15963*a* (1955); Knoll Akt.-Ges. Chemische Fabriken, Belgian Patent 615,861 (1962), *Chem. Abstr.*, **59**, 13892*d* (1963).

The product and an authentic sample of drug were identical by comparison of mp, uv, ir, and chromatographic data. Radiochemical purity was estimated to be greater than 99% by tlc in 2 solvent systems. Drug metabolism studies were conducted in mature beagle dogs and male Charles River rats with a preparation of specific activity 3.98 μCi/mg of verapamil base. Dose levels and concentrations of drug are expressed in terms of verapamil base equivalent.

Radiochemical Analyses. Total radioactivity levels in plasma, tissues, and excreta of animals administered [^{14}C]verapamil were determined in duplicate by standard liquid scintillation spectrometric methods. Plasma and tissue samples required predigestion with strong base solubilizers (Hyamine or Soluene 100, Packard Instrument Co.) before assay. Fecal specimens and the carcass and GI tract of rats were homogenized with water, lyophilized, and combusted to ^{14}[C]CO_2 which was collected in ethanolamine–ethyl cellosolve (1:2) for assay. The expired air of a rat administered labeled drug and maintained in a sealed drug metabolism apparatus was monitored for [^{14}C]CO_2 content by collection in 10% NaOH traps.

Verapamil levels in dog plasma and urine were determined by reverse isotope dilution. Unlabeled drug was equilibrated with plasma or urine of medicated animals, extracted with EtOAc at alkaline pH, and recrystallized as the hydrochloride to constant specific activity.

The distribution of verapamil metabolites in the excreta of rats and dogs was estimated by radioassay following a tlc separation procedure. EtOAc extracts of urine and feces were applied to precoated silica gel glass plates (Brinkmann Instruments, Inc.) and developed in $CHCl_3$–MeOH–AcOH (90:5:5; system 1). The metabolite zones were visualized by autoradiography (Kodak Blue Brand medical X-ray film), individually scraped from the plate, and eluted from the gel in a capillary tube with 0.1 *N* HCl in 90% MeOH. It was necessary to repeat this procedure for the separation of metabolites D, E, F, and G, using the solvent system 5% Et_2NH in PhH (system 2). Final silica gel eluates were directly radioassayed; percentage of dose calculations assume 100% recovery from excreta.

Whole-body radioautographic investigations were conducted (Arthur D. Little, Inc., Cambridge, Mass.) in male and female albino Fischer rats after drug administration by stomach intubation of a saline soln (5.4 mg/kg, 6.7 μCi/mg). Animals were frozen in liq N_2 at intervals of 45 min, 2 hr, and 6 hr. Whole-body sections and X-ray film were apposed for development of radioactivity density, according to the method of Liss, *et al.*[7]

Isolation and Identification of Verapamil Metabolites.—For the isolation and identification of metabolites A, B, and C, day 1 urine from a dog which received a single oral dose of drug, 5 mg/kg, was rendered strongly alk with KOH and shaken with EtOAc. The extract contained largely metabolites A and B. The recovery of metabolite C required enzymatic hydrolysis with a mixture of β-glucuronidase and sulfatase (Glusulase, Endo Products, Inc.). Following the removal of ether-sol impurities the hydrolysate was made basic with KOH and extracted with EtOAc. The extract contained metabolite C.

(7) R. H. Liss, D. W. Yesair, G. P. Watts, F. A. Cotton, and C. J. Kensler, *Pharmacologist*, **10**, 154 (1968).

Metabolites D, E, F, and G were isolated from a lyophilized day 1 fecal sample from the same dog. A slurry was prepared in 1 *N* KOH and extracted with EtOAc. The extract was evaporated to dryness, reconstituted with 1 *N* HCl, and freed of ether-soluble impurities. The aq phase was made basic with KOH and extracted with EtOAc to yield a fraction which contained the 4 important fecal metabolites D, E, F, and G

TABLE I
TLC DISTRIBUTION OF THE MAJOR METABOLITES OF VERAPAMIL IN DOGS

Metabolite	$R_{verapamil}$[a] System 1	$R_{verapamil}$[a] System 2	Relative abundance by autoradiography, system 1: 0–24 hr urine, 1–7 hr plasma	Relative abundance by autoradiography, system 1: 0–24 hr feces, 0–24 hr bile
D, E	0.92	0.28, 0.17	+	++++
F, G	0.83	0.43, 0.28	+	+++++
A	0.63	0.37	+++++	+
B	0.47	0.42	++	+
C	0.30	0.09	++++	++

[a] As detd from precoated silica gel tlc plates using the solvent systems: $CHCl_3$-MeOH-AcOH (90:5:5; system 1, mean of 3 values) and 5% Et_2NH in PhH (system 2). Small amounts of verapamil were detectable in all samples (R_f 0.52, system 1).

ization potentials of 70 and 12–13.5 eV. Useful solid probe spectra at programmed inlet temps were obtained from verapamil and metabolites A and E (underivatized) as supportive data. Trimethylsilyl (TMS) ether derivatives of metabolites D–G were prepared using an on-column technique with the simultaneous injection of metabolite and bis(trimethylsilyl)acetamide (Pierce Chemical Co.) contained in a single syringe. The analysis of metabolite C was supplemented by the examination of bis(trimethylsilyl)acetamide-silylated and CH_2N_2-methylated (Me) derivatives.

Pathways of verapamil metabolism following oral administration were also characterized in 0- to 24-hr excreta of 3 rats administered 3 mg of verapamil/kg, and in 0- to 24-hr bile of a female beagle established with a biliary fistula and administered 5 mg of verapamil/kg, orally in soln. The standard procedures of EtOAc extraction at alkaline pH, before and after Glusulase hydrolysis, followed by tlc analysis were employed. A substantial portion of radioactivity in rat and day 2 dog feces could not be recovered in this manner. Tlc examination of these samples was therefore performed with exhaustive MeOH extracts (Soxhlet, 16 hr) of lyophilized specimens after removal of ether-sol impurities.

Mass spectral evidence (see Table II) for verapamil and its metabolites are given below: verapamil, *m/e* 454 (calcd M^+ 454), 303 [$-3,4-(CH_3O)_2C_6H_3CH_2$; ion a^+], 260 (ion a^+ $-CH_3N=$

TABLE II
PROPOSED STRUCTURES OF THE 7 MAJOR METABOLITES OF VERAPAMIL IN DOGS AND THEIR RELATIVE ABUNDANCE IN DAY 1 EXCRETA OF DOGS AND RATS

R^3—O, R^4—O–C_6H_3–C($CH(CH_3)_2$)(*CN)–CH_2–CH_2–CH_2–N(R^1)–R^2

Metabolite	R^1	R^2	R^3	R^4	Per cent of day 1 urinary radioactivity[a]: Dog 2[b]	Per cent of day 1 urinary radioactivity[a]: Rats[c]	Per cent of day 1 fecal radioactivity: Dog 2[b]	Per cent of day 1 fecal radioactivity: Rats[c]
A	CH_3	H	CH_3	CH_3	26	4	5	A–G, 15
B	H	H	CH_3	CH_3	7	3	2	
C	CH_3	H	CH_3	H	19	40	6	
D	CH_3	CH_2CH_2–C_6H_3(3-OCH_3)(4-OH)	CH_3	H	5 (D + E)	4 (D + E)	5	
E	H	CH_2CH_2–C_6H_3(3-OCH_3)(4-OCH_3)	CH_3	H			15	
F	CH_3	CH_2CH_2–C_6H_3(3-OCH_3)(4-OH)	CH_3	CH_3	4 (F + G)		11	
G	CH_3	CH_2CH_2–C_6H_3(3-OCH_3)(4-OCH_3)	CH_3	H			16	

[a] Metabolites C-G were present largely as Glusulase-sensitive conjugates in the urine of dogs and rats. [b] Dog 2 received 5 mg of verapamil/kg in aq soln. Day 1 urinary and fecal radioactivity represented 17% and 31% of the dose, respectively. Results are based on a single analysis and are presumed typical. [c] Percentages are derived from a single analysis of the collective excreta of 3 rats, each receiving verapamil orally in aq soln, 3 mg/kg. Day 1 urinary and fecal radioactivity represented 8.4% and 59.5% of the dose, respectively.

All urine and fecal metabolites were next subjected to tlc separation (see Table I) and purification by successive development in solvent systems 1, 2, and 1, in that order. Final silica gel eluates were evaporated under a stream of N_2 and reconstituted with dil aq KOH. The soln was extracted with EtOAc, reduced to dryness under N_2, and dissolved in acetone for analysis with a LKB 9000 combined gas chromatograph-mass spectrometer equipped with flame ionization and radioactivity detector systems as described by Hobbs.[8] A 1.5 mm id × 2 m glass column containing 1% OV-1 on Gas-Chrom Q with a He carrier gas flow of 40 ml/min was employed. The mass spectrometer was operated at ion-

CH_2), 165 [$3,4-(CH_3O)_2C_6H_3CH_2CH_2^+$; ion b^+], 151 [$3,4-(CH_3O)_2C_6H_3CH_2^+$; ion c^+], 58 [$(CH_3)_2\overset{+}{N}=CH_2$]; metabolite A,† *m/e* 290 (calcd M^+ 290), 275 ($-CH_3$), 262 ($-C_2H_2$),[9] 259 ($-NH_2$-, CH_3),[10] 247 ($-CH_3$, $-CO$); metabolite B,† *m/e* 276 (calcd M^+ 276) 261 ($-CH_3$), 259 ($-NH_3$), 233 ($-CH_3$, $-CO$) 216 (233-NH_3); metabolite C, *m/e* 276 (calcd M^+276), 261 ($-CH_3$), 248 ($-C_2H_2$),

(8) D. C. Hobbs, American Society for Mass Spectrometry, Annual Meeting, June 15–19, 1970, San Francisco, Calif.

(9) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, p 409.

(10) D. L. Smith and M. F. Grostic, *J. Med. Chem.*, **10**, 375 (1967).

† Spectra obtained from reference samples of proposed metabolites A and B (Knoll A.-G.) under the same conditions were identical with those of metabolites A and B, respectively.

245 ($-NH_2CH_3$), 233 ($-CH_3$, $-CO$); C-TMS, m/e 348 (calcd M^+ 348), 333 ($-CH_3$), 147 [$(CH_3)_2Si{=}\overset{+}{O}Si(CH_3)_3$], 57 ($CH_3NHCH{=}CH_2^+$), 43 ($CH_3N{=}CH_2^+$); C-Me, m/e 290 (calcd M^+ 290), same as metabolite A; metabolite D-$(TMS)_2$, m/e 570 (calcd M^+ 570), 555 ($-CH_3$), 361 [$-3,4-(CH_3O)(TMSO)C_6H_3CH_2$; TMSO ion a^+] 209 ($3,4-(CH_3O)(TMSO)C_6H_3CH_2^+$; TMSO ion c^+]; metabolite E, m/e 426 (calcd M^+ 426), 275 [$-3,4-(CH_3O)_2C_6H_3CH_2$; ion $a^+ - 2CH_3$], 165 (ion b^+), 151 (ion c^+); E-TMS, m/e 498 (calcd M^+ 498), 347 [$-3,4-(CH_3O)_2C_6H_3CH_2$; TMSO(NH) ion a^+], 165 (ion b^+), 151 (ion c^+); metabolite F-TMS, m/e 512 (calcd M^+ 512), 497 ($-CH_3$), 303 (ion a^+), 209 [$3,4-(CH_3O)(TMSO)C_6H_3-CH_2^+$; TMSO ion c^+], (512 + 510):303::1:12, 223:209:179::2:1:1.5; 3'-demethylverapamil (D628, Knoll A.-G.)-TMS, m/e 512 (calcd M^+ 512), (512 + 510):303::1:>200, 223:209:-179::1:2:3; metabolite G-TMS, m/e 512 (calcd M^+ 512), 497 ($-CH_3$), 361 [$-3,4-(CH_3O)_2C_6H_3CH_2$; TMSO ion a^+], 165 (ion b^+), 151 (ion c^+).

Gas Chromatographic Assay for Metabolites A and B in Plasma.—Five ml of plasma contg 500 mg of $NaHCO_3$ was extd in a 50-ml centrifuge tube with 20 ml of EtOAc‡ by mechanical shaking for 15 min. After centrifugation the EtOAc layer was withdrawn, dried (Na_2SO_4, 2.5 g), and evapd under N_2 to 1.0 ml. The concentrate was then filtered through a prewashed (EtOAc) column of silica gel (silica gel, 0.05–0.2 mm, 70–325 mesh ASTM, for column chromatography, E. Merck, Darmstadt), 0.5 g, contd in a 13.1-cm heavy-wall Pasteur capillary pipette. The filtrate including 3 ml of EtOAc wash soln was rejected. Metabolites A and B were eluted from the column with 5 ml of EtOAc–MeOH–*N*-methylpyrrolidine (88:10:2). The eluate was collected in a 15-ml centrifuge tube and evaporated to 0.1 ml. To the sample was added 0.2 ml of 0.1 *M* $Na_2B_4O_7$ and 0.5 ml of a a 1% soln of 4-fluoro-3-nitrobenzotrifluoride (Pierce Chemical Co.) in acetone. The tube contents were mixed and heated in a water bath (70–75°) for 30 min, then cooled, and dild to 1.0 ml with distd H_2O. The corresponding 2-nitro-4-trifluoromethylaniline derivatives[11] of metabolites A and B were extd from the reaction medium with three 1-ml portions of hexane. The combined hexane exts were then evapd to dryness under N_2 and reconstituted with 50 μl of EtOAc immediately prior to assay. Portions (1 μl) were injected into a 6 mm od × 2 m glass column maintained at 260° and containing a support of silanized Corning glass beads glc 110 (Chemical Research Services, Inc.), 60–80 mesh, coated with 0.05% OV-1. A Hewlett-Packard F & M Scientific Model 5750 gas chromatograph equipped with a flame ionization detector (280°) was employed. With a He carrier gas flow rate of 100 ml/min, typical retention times of chromatographic peaks corresponding to metabolites A and B were 3.8 min and 5.1 min, respectively. Untreated dog plasma from control animals in acute and chronic studies, and pooled normal human serum (NHS) were essentially free of gas chromatographic background interferences at these retention times under assay conditions.

The metabolite peak height was referred to a standard curve derived from NHS spiked with increasing concns of each metabolite (generally ranging from 0.1 to 1.0 μg/ml of plasma for therapeutic dose levels, and to 3 μg/ml for high multiple dose level studies in dogs) and carried through the assay procedure. Metabolites A and B could be measured with sensitivities of 5 and 10 ng, corresponding to 0.05 and 0.10 μg/ml of plasma (5-ml sample), respectively. With each compd, response was linear and suitably accurate up to 3.0 μg/ml of plasma; efficiencies of recovery averaged about 70%. Serum vols of less than 5 ml were dild to 5 ml with H_2O prior to assay, and corrected for by insertion of the corresponding diln factor in final calcns. The potential for increased assay sensitivity exists in principle with the use of an electron capture detector.

Verapamil in NHS at levels of 0.3 μg/ml or greater can be measured simultaneously in the same assay injection sample, with retention time of about 8.1 min in an area free of plasma constituents. Spiked NHS standards for the metabolite assays usually included verapamil for this reason. Detector response to verapamil was linear from 0.3 to 1.0 μg/ml of NHS, and drug recovery was in the order of 70%. Levels representing 0.1 to 0.3 μg of verapamil/ml could only be estimated due to a poorly defined peak response in this range.

Blood levels of metabolites A and B were measured in dog 1 from 25 min through 6 hr following the oral administration of a single dose of 10 mg (unlabeled) of encapsulated drug/kg, and in 16 beagles that had received a daily regimen of verapamil for 8 months (40, 62.5, 70, and 85 mg/kg per day, 2 animals/sex per dose level). Samples were taken from the chronically treated animals at 1, 2, and 4 hr after the first of 3 divided daily doses. Selected dog plasma samples during single dose ^{14}C studies were also assayed. Blood levels were detd in 6 patients with angina pectoris, generally 1–2 hr after the last dose, during daily therapy of 320 mg of verapamil administered in capsules in 4 divided doses.

‡ All organic solvents used in the assay were freshly distilled.

(11) D. G. Crosby and J. B. Bowers, *J. Agr. Food Chem.*, **16**, 839 (1968).

Results

Excretion of Metabolites in the Dog.—Three-day cumulative radioactivity in the urine of 3 dogs§ contained from 21 to 33% of the dose after oral as well as im administration of labeled drug. This radioactivity was composed essentially of metabolites, since negligible quantities of verapamil were found (less than 0.5% of dose). About 80% of total urinary label was excreted by 2 dogs within the first 24 hr after oral administration. Three-day cumulative fecal collections contained the balance of ingested radioactivity (58%, 65%), about one-half of which was excreted during the first day of treatment. High levels of label in feces were measured following im injection (40% in 3 days) to reveal the probable significance of biliary excretion.

EtOAc extraction of 24-hr dog (dog 2) urine at alk pH removed 63–69% of the radioactivity present. An additional 27% was extracted following hydrolysis with a β-glucuronidase–sulfatase mixture. At least 13 urinary metabolites were detected by radiochromatography. Three of these substances designated as metabolites A, B, and C were predominant; the most polar metabolite C largely represented the conjugated substances freed by enzyme treatment. The major metabolites in urine were also the major metabolites in plasma by comparative tlc radiography (Table I).

The extraction of radioactivity from an alk slurry of 24-hr dog (dog 2) feces was essentially quant. Only small quantities of label were excreted in conjugated form (7%) as determined by hydrolysis. Tlc analysis of fecal extract indicated less than 1% of dose (oral and im) as unchanged drug, and a mixture of metabolites similar to those in urine in number and mobility but markedly different in terms of relative proportion. The preponderant zones of radioactivity in feces comprised 4 metabolites labeled D, E, F, and G, each of which was more polar than verapamil (Table I). Relatively smaller amounts of radioactivity represented metabolites A, B, and C. Metabolites A–G comprised about 45% of radioactivity in second-day fecal collections; the remaining radioactivity was not extractable at alk pH with EtOAc. An additional 25–45% could be extracted following acidification of the raffinate; acid hydrolysis released little additional label. Tlc analysis of purified MeOH extracts of day 2 feces, freed of metabolites A–G, revealed substances of increased polarity ($R_{verapamil} < 0.3$, system 1).

A comparative tlc examination of metabolites in the excreta of a dog dosed im indicated that pathways of drug metabolism were the same by either the oral or parenteral route of administration. All metabolites recognized in dog feces were also found in similar rela-

§ Dog 1, female, 5 mg/kg, oral capsule; dog 2, male, 5 mg/kg, oral soln in H_2O; dog 3, male, 5 mg/kg, im injection.

tive abundances in extracts of Glusulase-treated fistula bile. This confirmed the operation of hepatic biotransformation and biliary elimination processes as an important source of fecal metabolites D–G, which are largely transported as conjugates and presumably hydrolyzed by enzymes in the gut[12] prior to excretion.

An isolation procedure which utilized the resolution and purification capabilities of 2 tlc systems was successful in providing enriched μg quantities of each of the 7 major verapamil metabolites (A–G) in dog 2. Identification was accomplished by combined gas chromatog–mass spectrometric analysis. Structural assignments are summarized in Table II. Included are the relative abundances of each metabolite following their isolation and radiochemical assay. The major urinary metabolites A, B, and C represent N-dealkylated products derived by loss of the 3,4-dimethoxyphenethyl moiety of verapamil. The principal metabolite A comprised at least 26% of 24-hr urinary radioactivity and was identified as the substituted *N*-Me secondary amine, 1-isopropyl-1-*N*-methylpropylamino-(3,4-dimethoxyphenyl)acetonitrile. Metabolites B and C represented the *N*-demethyl and *O*-monodemethyl analogs of A, respectively, consistent with the finding that only C was excreted in urine largely in conjugated form. The identification of metabolites A and B was confirmed when authentic reference samples were made available and shown to be indistinguishable by tlc, mass spectrometric, and gas chromatographic analysis.

Structures of the important fecal metabolites D, E, F, and G were deduced from mass spectra of trimethylsilylated derivatives as various O- and N-demethylated products of verapamil which otherwise maintained the structural integrity of drug: metabolites F and G represented O-monodemethylated substances; F was demethylated in the substituted phenethylamine ring, while G was metabolized in the dimethoxyphenylacetonitrile portion of the molecule. Metabolite D was O-monodemethylated in each ring system. Metabolite E underwent N-demethylation in addition to O-monodemethylation in the substituted phenylacetonitrile ring.

The position of aromatic ring O-demethylation in metabolites C–G was not firmly established due to the small quantities isolated and the absence of appropriate model compounds. It is presumed however, that at least in the phenethylamine ring O-demethylation occurred in the para position, based on analogy to the metabolism of 3,4-dimethoxyphenethylamine[13] and on mass spectral information derived from a reference sample of 3′-demethylverapamil, a positional isomer of the proposed structure for metabolite F. A careful comparison of major ion peak ratios revealed the 2 substances were not identical.

Plasma Levels of Drug and Metabolites in the Dog.—Plasma levels of radioactivity in dogs after oral and parenteral [14C]verapamil administration, 5 mg/kg, are presented in Figure 1. Max plasma levels of total drug-related substances were attainable 2 hr after dose by either an oral or im dose and ranged from 0.5 to 0.9 μg of verapamil equiv/ml. Reverse isotope dilution and tlc methods indicated that plasma radioactivity represented both unchanged drug and a metabolite mixture in proportions that were dependent upon sampling time and route of administration. Label was detected as early as 15 min in a dog after an im dose and essentially consisted of unchanged drug. Thereafter, the portion of total plasma radioactivity which corresponded to metabolite content increased and became predominant after 2 hr. Verapamil levels, after an initial peak at 45 min, appeared to diminish with fluctuation and prevented accurate half-life determinations (estimated apparent $t_{1/2}$ = 70 min). Residual plasma levels of metabolite radioactivity in the order of 0.1 μg of drug equiv/ml were maintained 24 hr after the dose and fell to about 40 ng/ml at the 48-hr mark. Oral drug administration to dog 2 afforded plasma levels of radioactivity which at no time interval contained more than 10–15% of unchanged drug, to reveal the efficient metabolism of verapamil following absorption.** The predominance of a metabolite mixture in early plasma (1–7 hr) was supported by tlc radiography of a composite extract. Verapamil metabolites accounted therefore for most of the radioactivity observed through 7 hr. Metabolite assays in 2 additional dogs dosed orally were consistent with these findings (Table III).

TABLE III

PLASMA LEVELS OF METABOLITES IN (A) DOGS AFTER VERAPAMIL ADMINISTRATION AND IN (B) PATIENTS WITH ANGINA PECTORIS RECEIVING VERAPAMIL THERAPY, 80 mg QID[a]

Study	Hr after dose	Metabolite A, μg/ml	Metabolite B, μg/ml
	A. Dogs		
Dog 1, Figure 1	1–5 composite	0.60	<0.1
Dog 1,	0	0	0
10 mg/kg, po	0.42[b]	0	0
(capsule)	0.66[b]	0.15	0
unlabeled drug	1[b]	0.24	<0.1
	2[b]	0.38	0.10
	4[b]	0.33	0.10
	6[b]	0.30	<0.1
Dog 3, Figure 1	7	0.30	0
	B. Angina Patients		
1	1[b]	0.40	<0.1
2	1[b]	0.60	<0.1
3	1.5[b]	0	0
4	2[b]	0	0
5	b	0.05	<0.1
6	b	0.37	0.15

[a] Levels were detd by gas chromatog assay. [b] Levels of verapamil estimated to be 0.1–0.2 μg/ml of plasma were present in these samples. Collection times from patients 5 and 6 are unknown.

Periodic radioassays of whole blood indicated there was no concn of drug-related substances in erythrocytes.

In view of the predominance of low levels of metabolites in dog plasma following oral administration of verapamil, a gas chromatographic assay was developed for the proposed major substances, metabolites A and B. The method was used to determine plasma levels in dog 1 after oral verapamil administration (Table III). The data obtained support previous tlc results that the secondary amine metabolite A is the major drug-related substance that persists in plasma after treatment.

(12) R. S. Scheline, *J. Pharm. Sci.*, **57**, 2021 (1968).

(13) K. D. Charalampous and L. W. Tansey, *J. Pharmacol. Exp. Ther.*, **155**, 318 (1967).

** Absorption of intact drug is presumed from the results of dissoln rate studies in gastric juice, and from indications of drug stability in dil acid and base.

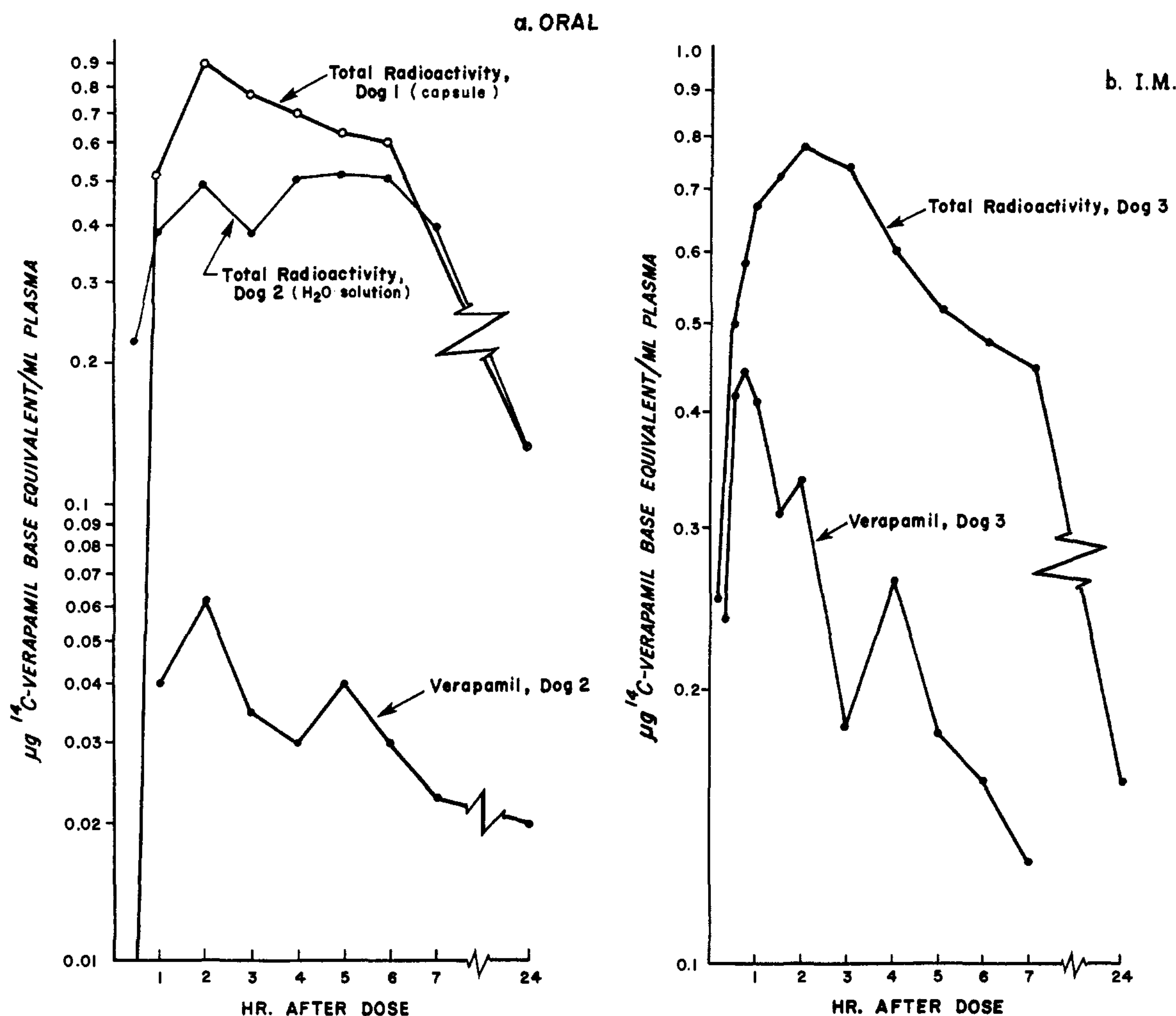


Figure 1.—Plasma levels of verapamil and total radioactivity after oral (plot a) and intramuscular (plot b) administration of [^{14}C]verapamil to female (dog 1) and male (dogs 2 and 3) beagles, 5 mg/kg.

Table IV

Plasma Levels of Drug and Metabolites A and B in Dogs after Repeated Daily Oral Administration of Verapamil for Eight Months[a]

Total daily dose level, mg/kg	Verapamil, μg/ml			Metabolite A, μg/ml			Metabolite B, μg/ml		
	1 hr	2 hr	4 hr	1 hr	2 hr	4 hr	1 hr	2 hr	4 hr
40	0.57 ± 0.23	0.47 ± 0.20	0.42 ± 0.25	1.9 ± 0.5	1.8 ± 0.5	1.7 ± 0.5	0.84 ± 0.29	0.77 ± 0.25	0.92 ± 0.28
62.5	1.3 ± 0.6	1.2 ± 0.3	0.77 ± 0.20	2.8 ± 0.4	2.7 ± 0.5	2.4 ± 0.1	0.78 ± 0.26	0.83 ± 0.26	0.87 ± 0.15
70	0.67 ± 0.21	0.57 ± 0.05	0.60 ± 0.30	2.8 ± 0.9	3.1 ± 0.5	2.9 ± 0.6	1.1 ± 0.4	1.2 ± 0.2	1.1 ± 0.30
85	0.83 ± 0.28	1.1 ± 0.2	0.79 ± 0.26	2.4 ± 0.4	2.6 ± 0.5	2.6 ± 0.5	0.78 ± 0.08	0.80 ± 0.16	0.77 ± 0.16

[a] Plasma was drawn at 1, 2, and 4 hr following the first of 3 divided daily doses. Levels are expressed as the mean ±S.D. from 4 animals (2/sex) at each dose level.

The metabolite appears early (within 1 hr) after dose, achieves maximum levels of about 0.4 μg/ml after 2 hr and persists through a 6-hr period with an apparent half-life of about 10 hr. The metabolite is predominant in 7-hr dog plasma (0.3 μg/ml, dog 3) after im administration. Levels of the primary amine metabolite B, thought to be the second most abundant metabolite in plasma, are significantly lower. The low levels of verapamil also found in the dog dosed orally minimized the usefulness of unchanged drug assays at dose levels comparable to those used in human therapy.

Plasma levels of both verapamil and metabolites A and B following the repeated daily administration of drug to dogs for 8 months were measurable and in proportions that maintained metabolite A as the predominant drug-related substance (1–4 μg/ml, Table IV).

Distribution and Excretion of Metabolites in the Rat.—The distribution of radioactivity was determined in the rat 1.5 hr, 24 hr, and 5 days after the oral administration of [^{14}C]verapamil, 3 mg/kg. Concns were low (<0.1 μg of verapamil equiv/g of tissue, mean of 3 rat detns) in the major tissues with the exception of kidney at 1.5 hr (0.16 μg/g), and liver at 1.5 hr (1.3 μg/g) and 24 hr (0.66 μg/g). Liver to plasma ratios of radioactivity were at least 20:1 at these time intervals. Efficient tissue clearance was indicated by total carcass radio-

assays which accounted for an average of 0.3% of an oral dose in 2 animals, 5 days after drug administration. In support of distribution studies, whole-body radioautography investigations revealed no unusual tissue localizations through a 6-hr period after oral drug administration. Radioactivity was most evident in the liver and intestinal tract, while only very low levels of generally distributed label were observed in 6-hr radioautographs.

Similar patterns of low urinary (15%) and high fecal radioactivity (68%) were observed in 2 orally dosed rats over a 5-day period. Excretion of label was largely completed during the first 24 hr (9.2% and 62.4% in urine and feces, respectively, mean values from 2 groups of 3 animals). The respiratory air of a rat given 3 mg of labeled drug/kg by oral intubation contained no $[^{14}C]CO_2$ through 21 hr after the dose, indicating *in vivo* stability of the CN group in verapamil.

In a comparative tlc study, the major urinary (A–C) and fecal (D–G) metabolites in dogs were also recognized in the combined excreta of 3 treated rats but in different relative proportions (Table II). Negligible quantities of unchanged drug were found. The predominant urinary metabolite in these rats was metabolite C which was largely excreted in conjugated form and accounted for 40% of 24-hr urinary radioactivity. Only very minor amounts of the remaining 6 metabolites were present while a significant portion of urinary label (30–50%) represented more polar unidentified substances. Metabolites A–G comprised about 15% of day 1 rat fecal radioactivity. The remaining drug-related substances in rat feces were not identified, however, their extraction properties and relative chromatographic distribution were very similar to polar radiolabeled components characterized in late (day 2) dog feces.

Plasma Levels of Drug and Metabolites in Man.—Serum samples from 6 angina patients receiving verapamil therapy were examined for drug and metabolite content. Three of the subjects exhibited metabolite A levels of 0.4–0.6 μg/ml while verapamil and metabolite B levels were 0.2 μg/ml or less in all patients (Table III).

Discussion

The rapid disappearance of verapamil-related substances, measured by spectrophotometry, from the blood stream of rats, dogs, and rabbits following parenteral administration was previously observed (E. Spector, communications to Knoll A.-G., 1967, and ref 14). Furthermore, verapamil exhibited significant binding to canine serum *in vitro* and *in vivo*. Drug was absent from the 24-hr urine of rats given drug iv, 10 mg/kg, but no metabolites were identified. The respiratory air of 4 rats that received $[^{14}C]NCH_3$-labeled verapamil ip (25 mg/kg) contained no more radioactivity than 1% of the dose through the first 24 hr after administration. Systemic levels of label during the first 3 hr were largely confined to liver, followed by cardiac tissue, and decreased sharply with time from both organs. Persistent levels of radioactivity measured in bile and kidneys suggested their role in the elimination of drug. Excretion of label in the urine and feces during the first 24 hr reportedly ranged from 18 to 26% and 1 to 4% of the dose, respectively, in 4 rats. It was concluded that N-demethylation was insignificant in the metabolism of verapamil although the majority of administered radioactivity was not accounted for.

The present investigation with [14C]CN-labeled verapamil has examined the fate of this drug in dogs and rats at dose levels comparable to those used in human therapy. Verapamil can be rapidly absorbed following oral administration to dogs, as judged by the early achievement of maximum plasma levels of drug-related substances. A comparison of plasma levels and excretion patterns of total radioactivity following both oral and parenteral drug administration also indicates that drug can be well absorbed from the gastrointestinal tract. In support of this finding, the feces of orally dosed animals contained little or no unchanged drug.

The urinary excretion by 3 dogs of drug-related substances did not exceed 35% of the dose regardless of the route of administration; the majority of excreted radioactivity was found in feces. Negligible quantities of unchanged drug were present in urine and feces. The secretion of drug-related substances into the intestinal tract was suggested when appreciable quantities of radioactivity were found in the feces of a dog dosed im (40% in 3 days), and confirmed with an examination of bile collected from a cannulated beagle which had received an oral solution of [14C]verapamil. All important drug-related substances normally found in feces were also recognized in bile, and in the presence of little unchanged drug.

The most important drug-related substances excreted by the kidney of a dog dosed orally are N-dealkylated metabolites which retain the α-isopropyl-(3,4-dimethoxyphenyl)acetonitrile portion of the molecule. Two of these substances are further O- or N-demethylated prior to excretion. Their elimination in bile and feces was of only minor significance. The 4 major metabolites in dog feces represent various N- and/or O-demethylated products of verapamil. These metabolites are also prominent in bile in bound form, but are excreted in only small quantities in urine. It would appear therefore that O-conjugated metabolites D–G are preferentially secreted into the gut probably by the biliary route, hydrolyzed by intestinal enzymes, poorly reabsorbed, and excreted with feces. Verapamil may be recirculated by enterohepatic cycling during its short-lived duration, however, as suggested from secondary peaking in blood level patterns[15] of 2 dogs. Verapamil levels in bile were not determined in this regard, although the presence of small amounts of drug in bile extracts was suggested chromatographically.

The significance of verapamil metabolism is clearly demonstrated from an analysis of blood levels of radioactivity after oral and im drug administration. Total drug-related substances in plasma from 1 to 7 hr after dose comprise little unchanged drug particularly after oral dosing. The higher proportion of plasma metabolites following oral administration of verapamil would be consistent with the fact that more drug traverses the liver after absorption by this route.

The rapid disappearance of verapamil from plasma following im injection was accompanied with increasing levels of metabolite which became predominant after

(14) W. Appel, *Arzneim.-Forsch.*, **12**, 562 (1962).

(15) J. G. Wagner, *Annu. Rev. Pharmacol.*, **8**, 67 (1968).

2 hr. Persistent plasma levels of radioactivity therefore are attributed to the rapid emergence and slow elimination of metabolites.

The metabolites present in plasma of a single dog dosed orally were found to be similar in distribution and relative abundance to metabolites identified in the urine, with metabolite A as the major radiolabeled component. This was consistent with an analysis of plasma from a second dog following oral drug administration (5, 10 mg/kg). Metabolite A rapidly appeared in the blood stream and persisted with a relatively long apparent half-life. Significantly smaller amounts of unchanged drug and metabolite B were estimated. Metabolite A was important in 7-hr plasma after an im drug injection. Plasma levels of verapamil and metabolites A and B were also assayed following chronic drug administration to dogs at high dose levels. After 8 months of repeated dosing, predominant levels of metabolite A were maintained in plasma, with no evidence to suggest drug accumulation or altered rates of metabolism.

[^{14}C]Verapamil studies in rats indicate that similar absorption and elimination kinetics are probably operative, as high fecal and low urinary levels of radioactivity were noted after drug administration. Tissue assays revealed that liver contains the highest levels of radioactivity in rats following an oral dose of drug, suggesting an important role for hepatic biotransformation and elimination processes in the metabolism of verapamil. Retention of label in the rat carcass is negligible after 5 days. A more comprehensive assessment of drug distribution in rats was provided by whole-body radioautography studies following ^{14}C-labeled verapamil administration. Tissue localization was largely confined to the liver and intestinal tract during a 6-hr postdose test period. Generally distributed radioactivity was discernible throughout the body of rats and progressively declined to a very low level by 6 hr.

It was shown by comparative chromatographic studies that verapamil is subjected to similar pathways of metabolism in rats and in dogs. Little unchanged drug was present in urine and feces. All metabolites identified in dogs were also recognized in rat excreta, however a sizable portion of additional unknown polar drug-related substances were present in rats which probably arise by further metabolism of known drug-related substances. Although their identities were not established, these unknown metabolites resembled radioactive substances characterized in dog feces collected after the first 24-hr period of the study. Drug biotransformations in rats therefore may be more advanced than in dogs over a given period of time, but there was no evidence for the operation of different pathways of metabolism.

The unknown group of polar metabolites of verapamil may represent corresponding carboxylic acids and their conjugates derived from well-established pathways of oxidative deamination of aryl-substituted aliphatic amines.[16,17] The most important secondary amine metabolite A may serve as an intermediate in the formation of these acids. Minor pathways of nitrile metabolism cannot be discounted with verapamil, although this group is sterically hindered and does not give rise to expired radioactivity in rats. If nitrile metabolism is operative to any extent it likely would involve slow hydrolysis to the corresponding amide or carboxylic acid; chemically stable nitriles of this nature (α-tertiary C) are not expected to give rise to HCN by metabolic processes.[18-20]

There were indications in the current study that similar pathways of metabolism are operative in man, as metabolite A levels exceeded metabolite B and unchanged drug levels in the blood stream of several angina patients receiving verapamil. N-Dealkylated metabolites A and B are recognized as pharmacologically active agents in dogs (H. Haas, Knoll A.-G., personal communication) but are less active than verapamil. In view of the fact that metabolite A can achieve rather persistent plasma levels in a several fold excess of verapamil levels, this substance may contribute significantly to the prolonged cardiovascular activity of the drug.

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