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Metabolism of Carprofen, a Nonsteroidal Anti-Inflammatory Agent, in Rats, Dogs, and Humans

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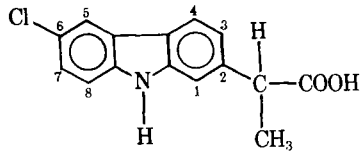
Abstract □ The metabolic disposition of ^{14}C-labeled carprofen [(±)-6-chloro-α-methylcarbazole-2-acetic acid] was investigated in rats, dogs, and humans. Carprofen is eliminated predominantly by biotransformation in these three species. In dogs and rats, the direct conjugation of carprofen to form an ester glucuronide and oxidation to the C-7 and the C-8 phenols followed by their conjugation represent the major metabolic pathways. Small amounts of the α-hydroxy derivative also are formed by these species and are excreted free in the urine. In dogs, biliary secretion predominates, and 70% of an intravenous dose of carprofen is excreted in the feces while 8–15% of the dose is excreted in the urine. In rats, fecal excretion due to biliary secretion varies from 60 to 75%, and urinary excretion accounts for 20–30% of an intravenous dose. In humans, direct conjugation of carprofen represents the only significant pathway of metabolism. Between 65 and 70% of the orally administered carprofen was found to be excreted as the ester glucuronide in the urine, and most of the remaining dose was estimated to be secreted as this metabolite in the bile. Due to enterohepatic circulation, only a fraction of the biliary metabolite was recovered in the feces in humans. Less than 5% of the dose was excreted in human urine as free, intact carprofen. In dogs and humans, plasma levels of carprofen and of total radioactivity exhibit a multiphasic decline. In the three human subjects studied, the terminal component declined with a 13–26-hr half-life; the terminal half-life was ~40 hr in dogs.

Keyphrases □ Carprofen—metabolism in rats, dogs, and humans □ Metabolism—carprofen in rats, dogs, and humans □ Anti-inflammatory agents—carprofen, metabolism in rats, dogs, and humans

Carprofen [(±)-6-chloro-α-methylcarbazole-2-acetic acid], a nonsteroidal drug, exhibits anti-inflammatory activity in several screening tests in rats (1, 2).

Fluorometric (3, 4) measurements of blood carprofen levels in human volunteers (5) and in dogs[1] receiving single doses of the drug showed a biexponential decline composed of a fast initial component and a very slow terminal component. Comparison of blood levels measured after single and multiple doses indicated predictable drug accumulation (6). As in dogs and rats, a small volume of distribution was consistent with the high drug concentration in the central plasma compartment that was observed in these subjects.



carprofen

The objectives of the present study were to establish and to compare the biotransformation of carprofen in dogs, rats, and human volunteers using ^{14}C-labeled drug and to determine further the disposition of the drug in these three species. A preliminary report on these investigations was published previously (7).

EXPERIMENTAL

Dose—[^{14}C]Carprofen, labeled in the α-position ($C_{15}H_{12}ClNO_2$, mol. wt. 273.2), was synthesized[2] with specific activities of 14.2 and 51.98 mCi/mmole. The radiopurity was at least 95%, and it was checked periodically by TLC. To minimize radiodecomposition during storage, the compound was dissolved in 10% ethanol in benzene. For the metabolic experiments, the specific activity was reduced to $1.0–6.0 \times 10^6$ dpm/mg for the dog and rat studies and to 0.5×10^6 dpm/mg for the human studies.

For the oral and intravenous dosing of dogs and rats, solutions were prepared containing 2.5 mg of [^{14}C]carprofen (0.0092 mEq)/ml in a mixture of five parts of propylene glycol, one part of 0.1 *N* NaOH (0.1 mEq), and four parts of distilled water.

[1] D. Maynard and S. A. Kaplan, Hoffmann-La Roche, Nutley, NJ 07110, reports on file.

[2] Syntheses were performed by Dr. Liebman and Dr. Muccino of the Roche Radiosynthesis Laboratory.

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Table I—TLC of Carprofen and Metabolites (R_f Values [a])

Solvent System	Carprofen	Carprofen Ester	I	I Ester	III	III Ester	IV	IV Ester
1 Chloroform–ethanol–formic acid (90:10:5)	0.48	0.69	0.27	0.55	0.36	0.53	0.36	0.51
2 Chloroform–ethanol–acetic acid (90:10:1)	0.38		0.0		0.28	0.53	0.22	
3 Chloroform–methanol–acetic acid (70:30:5)	0.79				0.76		0.76	
4 Chloroform–methanol–concentrated ammonia (70:30:1)	0.13		0.09 (trail)		0.13		0.13	
5 Chloroform–ethanol–formic acid (85:15:10)	0.65		0.44					
6 Chloroform–methanol–acetic acid (85:15:5)	0.60				0.51		0.51	

[a] These R_f values represent averages of several TLC runs and are reproducible within 10%. The metabolite esters all are methyl esters.

For oral administration to human subjects, radioactive carprofen was incorporated into tablets that were identical with the formulation used clinically. Each tablet contained 50 mg of [^{14}C]carprofen with a specific activity of 0.5 μCi/mg.

Animal Experiments—Two male beagle dogs, 12.8 and 10.1 kg, received a single intravenous dose of 1 mg of [^{14}C]carprofen/kg (Experiments D-I and D-V). Subsequently, the same dogs received a 1-mg/kg oral dose of this drug (D-II and D-VI). Two other dogs (D-III and D-XI) received single intravenous 10-mg/kg doses as a solution of 25 mg (0.092 mEq)/ml.

Blood specimens (5–10 ml, heparinized) were drawn by heart puncture or from the jugular vein at the indicated intervals. Aliquots of whole blood were taken for determination of the total carbon 14 content and for the hematocrit. The remaining blood was centrifuged. The radioactivity in the plasma was quantitated and fractionated, and the carbon 14 levels in the erythrocytes were calculated.

The dogs were kept in metabolism cages; urine (under toluene) and feces were collected for 96 hr at 24-hr intervals. Feces were homogenized in five volumes of 70% aqueous ethanol, and the radioactivity of these homogenates was determined using 10-fold dilutions.

In two additional male dogs weighing 10.5 (Experiment D-IV) and 7.6 (Experiment D-X) kg, the bile ducts were cannulated. The dogs were anesthetized by intravenous injection of 25–30 mg of pentobarbital sodium/kg. Anesthesia was maintained by additional 2.5-mg/kg pentobarbital sodium injections given about every 2 hr after the initial administration, as required. After intravenous injection of single 1-mg/kg doses of [^{14}C]carprofen, bile was collected for 5 hr at 0.5-hr intervals. Blood specimens were collected periodically from the cannulated femoral vein during the experiment.

After 5 hr, the dogs were sacrificed. The major tissues were excised, rinsed with ice-cold saline, and frozen. Aliquots (2–5 g) were homogenized with 10–20 volumes of ice-cold, aqueous, 0.2 *M* acetate buffer (pH 5) in a high-speed homogenizer.

Male 180–200-g Sprague–Dawley rats[3] were injected intravenously (tail vein) with 5 mg of [^{14}C]carprofen/kg in the described solution. Groups of three rats were sacrificed by decapitation after 10 min, 1 hr, and 4 hr, and plasma and the major tissues were collected. The tissues were homogenized as described, individually or as pools.

The bile ducts in two rats (320 g) were cannulated under light ether anesthesia, 5-mg/kg doses of [^{14}C]carprofen were administered intravenously, and bile was collected at 2-hr intervals for 6–7 hr, with the rats being in a conscious state and confined in restraining cages. Other animals were kept anesthetized with pentobarbital sodium (40 mg/kg), and their temperature was maintained at 37° during bile cannulation. In some experiments, Charles River CD rats of different ages and weights were used.

Urinary and fecal excretion (0–96 hr) of a 5-mg/kg iv dose of [^{14}C]-carprofen was determined in another group of three male rats.

Clinical Study[4]—Three male volunteers, 59–87 kg and 43–58 years old, were administered single 50-mg tablets of [^{14}C]carprofen orally with 240 ml of water. They fasted from 8 hr before until 4 hr after drug ingestion.

Blood specimens (10–15 ml, oxalated) were drawn before dosing and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hr and daily at 24-hr intervals after dosing for a minimum of 8 days or until no more radioactivity was detectable.

Urine was collected for intervals of 0–2, 2–4, 4–6, 6–9, 9–12, and 12–24 hr and as 24-hr pooled daily specimens thereafter until no more radioactivity was detected (for ~8–10 days). The creatinine content of each specimen was measured to provide an indication of completeness of urine collection. Feces were collected as 24-hr portions.

Quantification of Radioactivity—The isotope disintegration rates of all specimens were determined by liquid scintillation spectrometry[5] using the external standard channels ratio technique. Counting mixtures were prepared as follows. One milliliter of an aqueous tissue homogenate was mixed with 1.5 ml of distilled water and 10 ml of scintillation fluid[6] to produce a stable gelatinous suspension. Silica gel zones scraped from thin-layer chromatograms were suspended similarly in a mixture of 2.5 ml of water and 10 ml of scintillation fluid. Plasma aliquots of 0.1–1 ml were diluted to 2.5 ml with water and mixed with 10 ml of scintillation fluid. Aqueous solutions, diluted urine not exceeding 2 ml, and solvent extracts were mixed directly with the scintillation fluid.

Isotope disintegration rates in whole blood aliquots of 0.1–0.5 ml were determined following combustion[7].

Extraction and Fractionation of Drug Components from Urine, Bile, and Plasma—Intact carprofen and its unconjugated metabolites were extracted quantitatively (>95%) with 10 ml of ether from 0.5–1.0 ml of plasma diluted to 2 ml with 1.0 *M* acetate buffer (pH 5). The drug was extracted with two equal volumes of ether from urine titrated to pH 5. The extracted aqueous phase was titrated to pH 1 and was reextracted with ethyl acetate or with butanol at pH 5, using two extractions with equal solvent volumes. Diluted bile fractions, combined with 1 ml of 1.0 *M* acetate buffer (pH 5), were extracted twice with two volumes of ether to remove the free drug. The conjugates remaining in the aqueous phase were fractionated by cleaving first the ester glucuronide of carprofen at pH 11 (titration with 6 *N* NaOH) for 2 hr at room temperature and then the ethereal glucuronides by readjusting the pH to 5, adding 0.1 ml of glusulase enzyme[8], and incubating for 2 hr at 37°. Each treatment was followed by ether extraction of the freed metabolites at pH 5. The extracts were evaporated under nitrogen at 40–50°. The residues were taken up in 250 μl of ethanol immediately before aliquots were taken for measurement of the isotope disintegration rate and for TLC.

Suitable aliquots of these concentrated extracts were subjected to TLC on precoated silica gel 60 F254 plates[9] using the solvent systems listed in Table I. For preparative isolation (quantities up to 1 mg), each extract concentrate was streaked as a band across the plate; after development, the silica gel bands corresponding to the purified components were scraped off, suspended in a buffer, and extracted with a solvent.

The presence of intact [^{14}C]carprofen and other radioactive metabolites in plasma or tissue homogenates was determined by one-dimensional TLC of pH 5 ether extracts with System 1 and subsequent scanning[10]. These components were quantitated by two-dimensional TLC with System 2 and then System 4, scraping, and measurement of the isotope disintegration rates of the silica gel areas bearing the detected components.

Carprofen β-glucuronide ester in human plasma and urine was extracted (following removal of the free drug by ether extraction at pH 5) with ethyl acetate at pH 1. After cleavage of this ester at alkaline pH as

[3] Carworth Farm, N.Y.

[4] Special Treatment Unit, Newark Beth Israel Medical Center, Newark, N.J. (Dr. Robert Pocelinko, Scientific Director). The protocol of this study and the informed consent procedure were approved by the Institutional Review Committee, Newark Beth Israel Medical Center, Newark, N.J., on June 12, 1975. Use of radioactively labeled material in this human isotope study was approved as Amendment 14 of the Medical By-Product Material License 29-11847, assigned to Dr. Edward D. Fram, Newark Beth Israel Medical Center, by the U.S. Nuclear Regulatory Commission on March 13, 1975. It was approved by the Division of Oncology and Radiopharmaceutical Products of the Food and Drug Administration on February 5, 1976, as an addendum to IND 10,049 (carprofen) of Hoffmann-La Roche.

[5] Model 3380, Packard Instruments, Downers Grove, Ill.

[6] Aquasol, New England Nuclear Corp., Boston, Mass.

[7] Oxymat, Intertechnique Corp.

[8] Endo Laboratories, Garden City, N.Y.

[9] Brinkmann Instruments, Westbury, N.Y.

[10] Model 7201 scanner with a model 835 recording rate meter, Packard Instruments.

Table II—Excretion of Total Radioactivity in Urine and Feces of [^{14}C]Carprofen-Treated Dogs (Expressed as Cumulative Percent of Dose)

	Experiment					
	D-I	D-V	D-XI	D-III	D-II	D-VI
Dose, mg/kg	1.0	1.0	10.0	10.0	1.0	1.0
Route	Intravenous	Intravenous	Intravenous	Intravenous	Oral	Oral
Urinary excretion (at end of period)						
24 hr	10.29	8.02	5.52	9.41	17.34	8.07
48 hr	14.99	9.32	6.77	10.63	18.92	8.60
72 hr	15.20	9.50	—[a]	—[a]	19.07	8.72
96 hr	15.28	9.61	—[a]	—[a]	—[a]	—[a]
120 hr	15.31	—[a]	—[a]	—[a]	—[a]	—[a]
Fecal excretion (at end of period)						
24 hr	2.54	0.0	59.71	—[a]	32.0	45.01
48 hr	61.37	61.84	70.6	—[a]	66.7	65.71
72 hr	67.41	67.34	—[a]	—[a]	67.68	67.25
96 hr	67.97	68.26	—[a]	—[a]	—[a]	—[a]
Total excretion	83.28	77.87	77.37	—	86.75	75.97

[a] These specimens were not collected and analyzed.

Table III—Tissue Distribution of Total Radioactivity 5 hr after an Intravenous Dose of 1 mg of [^{14}C]Carprofen/kg in Two Bile Duct-Cannulated Dogs (D-IV and D-X)

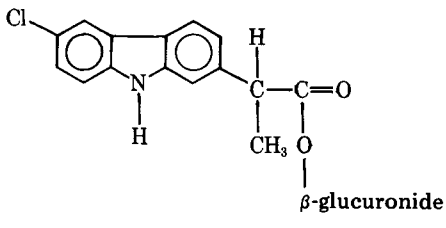
Sample	Carbon 14 Found[a]		Percent of Dose		Tissue Concentration/ Plasma Concentration	
	D-IV	D-X	D-IV	D-X	D-IV	D-X
Plasma[b]	5.85	5.45	29.3[b]	27.1[b]	1.00	1.00
Liver	2.24	1.95	6.1	6.3	0.38	0.36
Kidneys	1.24	1.16	0.79	0.94	0.21	0.21
Brain	0.09	0.08	0.07	0.06	0.016	0.015
Lungs	1.40	1.09	1.23	0.81	0.24	0.20
Spleen	0.25	n.m.[c]	0.15	n.m.	0.04	n.m.
Heart	1.24	0.7	0.79	0.51	0.21	0.13
Muscle[d]	0.22	0.25	8.7[d]	9.9[d]	0.037	0.045
Testes	0.33	0.12	0.05	0.02	0.056	0.021
Fat[e]	0.50	0.46	2.5[e]	2.3[e]	0.093	0.085
Small intestine tissue	0.96	0.82	1.19	2.47	0.16	0.15
Large intestine tissue	0.61	0.51	0.65	0.34	0.10	0.094
Stomach and contents	0.46	0.49	0.43	0.40	0.08	0.09
Small intestine contents	—	—	0.54	0.69	—	—
Large intestine contents	—	—	0.06	0.08	—	—
Bile	—	—	23.5	26.9	—	—
Total	—	—	76.6	78.8	—	—

[a] In microgram equivalents per gram. [b] Assuming that plasma is 5% of the total body weight. [c] n.m. = nonmeasurable. [d] Assuming that muscle tissue is 40% of the total body weight. [e] Assuming that fat tissue is 5% of the total body weight.

described, glucuronic acid was identified by TLC on cellulose-precoated plates with the solvent system *n*-butanol–acetic acid–water (2:1:1) followed by naphthoresorcinol spray. Glucuronic acid was quantitated by the naphthoresorcinol assay (8, 9) following cleavage of the conjugate by mammalian liver β-glucuronidase[11].

Representative portions of all human urine specimens collected during the first 24 hr from each subject were pooled. One 2-ml aliquot of these pools and of each subsequent 24-hr urine portion (up to 120 hr) were extracted directly with ether at pH 5. A second aliquot was cleaved with sodium hydroxide as described, and the deconjugated carprofen was extracted with ether at pH 5. Both extracts were subjected to one- and two-dimensional TLC, and carprofen was quantitated as described.

To check specifically for the presence of Metabolites I and III in human urine, 10-ml portions of 0–72-hr urine pools (1% of each fraction) were extracted after both alkaline and glusulase cleavage, and the two extracts were subjected to one- and two-dimensional TLC in the presence of



carprofen ester glucuronide

[11] Calbiochem, La Jolla, Calif.

nonlabeled standards of these metabolites. Since the recoveries of urinary carprofen varied from 85 to 95%, the values reported are uncorrected.

Analysis of Feces—Ten milliliters of a fecal homogenate in 70% ethanol was diluted with 10 ml of 1 *M* phosphate buffer (pH 7), and the dilution (35% ethanol) was extracted twice with 20 ml of ether followed by a backwash with 10 ml of water. The residue of the combined ether extracts was redissolved in 5 ml of ethanol–aqueous 0.1 *N* HCl (4:1). This solution was equilibrated twice with 10 ml of hexane. The hexane extracts were washed consecutively with a second 5-ml portion of the same ethanol–hydrochloric acid mixture and then were discarded. Both ethanol–hydrochloric acid solutions were diluted with 5 ml of 0.1 *N* HCl and extracted twice with 10 ml of a 3:2 mixture of ether and hexane. These extracts, containing 60–70% of the original radioactivity of a typical fecal homogenate, were combined, concentrated, and analyzed by one- and two-dimensional TLC essentially as described for the solvent extracts of urines.

Metabolite Characterization—UV spectra of solutions of ~5 μg/ml in 0.1 *N* HCl and in 0.1 *N* NaOH in 20% aqueous ethanol were measured using a microcell (0.5 ml) assembly in a spectrophotometer[12].

The fluorescence spectra of the individual metabolites of carprofen, dissolved in ethanol–1% acetic acid, were determined in a spectrofluorometer[13].

High-resolution and low-resolution mass spectra[14] were obtained. The ionizing energy was 70 ev, and the interface temperature was 270°. The

[12] DU, Beckman Instruments.

[13] Farrand.

[14] High-resolution mass spectra were obtained with a CEC 21-110 and low-resolution mass spectra were obtained with a Hitachi RMU-6L coupled to a Perkin-Elmer 990 gas chromatograph.

Table IV—UV and Fluorescent Spectra of Carprofen and Its Metabolites

Compound	Fluorescence (1% Acetic Acid in Ethanol)		UV Absorption			
			0.1 *N* HCl–20% Ethanol		0.1 *N* NaOH–20% Ethanol	
	$\lambda_{act}/\lambda_{emit}$, nm	TM[a]/μg/ml	A_{max}[b], nm	E[b], × 10^4	A_{max}[b], nm	E[b], × 10^4
I	295/375	123	240	5.10	240	5.21
			300	2.10	300	2.13
III	340/395	52 → 180[c]	245	5.35	255	5.02
			295	1.47	300	1.1
IV	310/360	422	240	5.05	245	4.37
			310	1.66	340	1.75
Carprofen	295/375	102	240	4.26	240	4.37
			300	1.67	300	1.75

[a] TM = fluorescence intensity reading. [b] E = molar absorptivity; A_{max} = wavelength of absorbance peak. [c] The first TM value represents the reading taken immediately after placing the sample into the light path of the fluorometer; the second TM value represents the final constant reading taken after leaving the sample in the light path for 5–10 min.

metabolite structures given in the text are based on integer masses combined with other information. GLC was performed on 3% OV-17 on 80–100-mesh Gas Chrom Q (183-cm × 4-mm i.d. column) at 250° or with temperature programming (4°/min to 300°) using a flame-ionization detector. The carrier gas was helium at 30 ml/min.

[^{14}C]Carprofen and its metabolites were esterified with diazomethane freshly prepared from *N,N*-nitrosomethylurea in ether in the presence of 40% KOH and distilled directly into the methanolic drug solution. Trimethylsilyl derivatives were prepared by reacting the compound under strictly anhydrous conditions with *N,O*-bis(trimethylsilyl)acetamide in silylation grade pyridine[15] for 30 min at room temperature. Methyl esters and trimethylsilyl methyl esters of carprofen and of some of its metabolites were separated and characterized by GLC[16] on a 122-cm column of 3% OV-17 on Gas Chrom Q (60–80 mesh) at a column temperature of 250° and a port temperature of 285°. The flame-ionization detector was operated at 300° at a hydrogen flow rate of 2.5 ml/min. The nitrogen carrier gas flow rate was 3.0 ml/min.

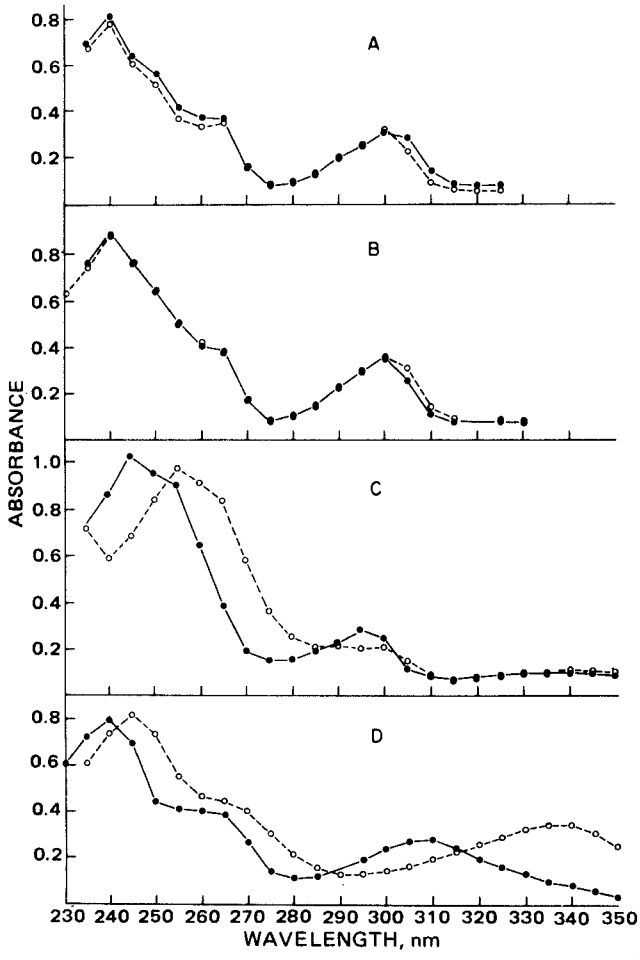


Figure 1—*Relative UV absorption spectra of carprofen (A), I (B), III (C), and IV (D) in acidic (●) and basic (○) media.*

[15] SIL BSA reagent, Pierce Chemical Co., Rockford, Ill.

RESULTS

Dog Experiments—*Excretion*—The cumulative excretion of total radioactivity by the dogs after intravenous and oral administration of 1 or 10 mg of labeled carprofen/kg accounted for 67–71% of the dose in the feces and for 7–19% in the urine (Table II). Twenty-four and 27% of the dose were excreted in the bile of two anesthetized bile duct-cannulated dogs within the first 5 hr after the intravenous administration of 1 mg of labeled carprofen/kg (Table III). Most of the radioactive fraction excreted in the feces by the intact dog after the intravenous dose (Table II) represented bile-secreted material since <1% of the dose was present in the intestinal contents in the bile duct-cannulated dogs within 5 hr after the dose of carprofen (Table III).

Identification of Urinary Metabolites of [^{14}C]*Carprofen*—The urinary metabolite patterns after a 1-mg/kg dose and after a 10-mg/kg dose were very similar. Dog urine excreted the 1st day after a 10-mg/kg iv dose of [^{14}C]carprofen (containing 9.41% of the dose) was fractionated. Direct extraction of the urine at pH 5 with ether removed 51% of the total urinary radioactivity. Subsequent extraction with butanol at pH 5 or with ethyl acetate at pH 1–2 removed an additional 28%. TLC of the ether extract in System 1 separated two quantitatively equal components of R_f 0.29 and 0.18, respectively. Intact carprofen (R_f 0.48) was absent.

UV-absorbing impurities were separated by preparative TLC with System 2, whereby the radioactive components remained at the origin. They were extracted together from the plate and separated from one another and from a remaining UV impurity by preparative TLC with System 5. Final purification, adequate for physical–chemical characterization, was achieved by extraction of each component from ether into 0.2 *N* NaOH, acidification of the alkaline extract, and reextraction into ether. Of these two radioactive excretion products, only the R_f 0.29 product represented a genuine metabolite of carprofen (Metabolite I); the R_f 0.18 component (Compound II) was an artifact[17].

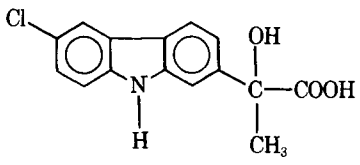
Metabolite I—The UV spectrum of Metabolite I was independent of pH, and the maxima and respective molar absorptivities were similar to those of intact carprofen (Table IV and Fig. 1A). The two fluorescence spectra also were indistinguishable. These characteristics pointed to an intact, unsubstituted carbazole moiety.

The methyl ester of Metabolite I differed by TLC (Table I) from the free acid; on GLC, it appeared as a homogeneous peak with an R_t value of 8.2 min, distinct from that of carprofen methyl ester (R_t 5.5 min).

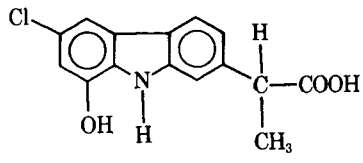
The mass spectrum of Metabolite I (the free acid) indicated a molecular ion at *m/e* 289 ($C_{15}H_{12}ClNO_3$), representing addition of one oxygen to carprofen, and a fragment at *m/e* 271 ($C_{15}H_{10}ClNO_2$), arising from the

[16] Model 402, Hewlett-Packard.

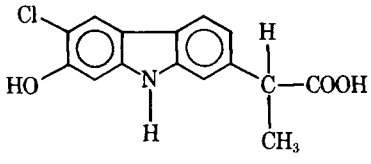
[17] Compound II proved to be derived from a labeled contaminant of the [^{14}C]-carprofen dose, which was not detected on TLC. Since the addition of carrier carprofen to prepare the doses did not reduce the specific activity of this contaminant to that of [^{14}C]carprofen, the specific activity of isolated Compound II, identified as α-hydroxy-α-desmethylcarprofen (by NMR, GLC–mass spectrometry, and synthesis), was several times that of the administered [^{14}C]carprofen. The fact that the same contaminant also is present, although to a lesser extent (0.5%), in unlabeled carprofen preparations is evidenced by the excretion of the same α-hydroxy-α-desmethylcarprofen derivative by dogs dosed with unlabeled drug.



Metabolite I



Metabolite III



Metabolite IV

loss of water. GLC–mass spectrometry of the methyl ester revealed a molecular ion of *m/e* 303 ($C_{16}H_{14}ClNO_3$), indicative of the addition of one oxygen atom to the carprofen ester ($C_{16}H_{14}ClNO_2$). The fragmentation pattern suggested an unsubstituted carbazole ring structure, consistent with the UV spectral evidence.

The GLC–mass spectral pattern, TLC properties, and NMR spectra of Metabolite I and the authentic α-hydroxy derivative of carprofen, whose structure had been unambiguously established as that of a tertiary α-hydroxy compound by its synthesis from the α-keto acid by the Grignard reaction, were identical.

Metabolites III and IV—The polar urinary radioactivity extracted from dog urine with *n*-butanol at pH 5 did not migrate on TLC with System 1. However, it was converted to a compound with a higher R_f value when exposed to either alkaline or acidic conditions and, partly, after incubation with glusulase at pH 5. The converted (high R_f) fraction was extractable with ether at pH 1 and 5. It was prepared for further characterization (from dog urine preextracted with ether at pH 5 to remove Metabolite I and Compound II) in 83% yield by slow, repeated equilibration of the acidified urine (0.4 *N* HCl) with ether.

UV-absorbing contaminants were removed by equilibrating the ether solution with 0.2 *M* phosphate buffer (pH 7) and by extracting the metabolites into 0.2 *N* NaOH and returning them to ether at pH 5 with a recovery of 85%. Preparative TLC with System 2 achieved complete resolution of Metabolites III (R_f 0.27–0.32) and IV (R_f 0.21–0.25), representing 16 and 2.4% of the urinary radioactivity, respectively. Metabolite III discolored when it was left on a TLC plate and was unstable in alkaline solution. With adequate precautions, preparative TLC produced both metabolites in sufficiently pure form for physical–chemical identification.

Metabolite III exhibited a major absorption maximum in acidic solution at 245 nm which was shifted to 255 nm at alkaline pH (Table IV and Fig. 1C). This bathochromic shift indicated a phenolic structure. Spectrofluorometric analysis revealed maxima at 290 and 340 nm, both with emission maxima at 395 nm. The fluorescence intensity increased during the exposure of the solution to the 290-nm light source to a value that remained stable.

Methylation resulted in a major reaction product, which was purified by preparative TLC (R_f 0.56 in System 1). Its high-resolution mass spectrum revealed a molecular ion of *m/e* 303.0688 ($C_{16}H_{14}ClNO_3$), compatible with a methyl ester of a monohydroxy derivative of carprofen. Substitution in the carbazole ring was supported by the fragment of *m/e* 217.0308 ($C_{12}H_8ClNO$), which retains the added oxygen and arises from loss of the $C_4H_6O_2$ side chain, consistent with the phenolic structure evidenced by the pH-dependent UV spectrum of this metabolite.

To determine the position of this phenolic function, Metabolite III methyl ester was dissolved in 0.35 ml of deuterochloroform, and its PMR spectrum was determined. A single scan revealed a methyl ester band at δ 3.68 and a CH_3CH doublet (J = 6.5 Hz) at 1.59, indicating that the side chain was intact. A time-averaged spectrum of 229 scans of the δ 5–10 region revealed the following parameters: δ 6.81 (d, 1H, J *meta* = 2 Hz,

Table V—Excretion of Carprofen and Metabolites by Humans, Dogs, and Rats

Species	Number of Animals or Subjects	Dose, mg/kg	Administration Route	Excretion Route (Collection Period)	Percent of Dose[a]								Total Percent of Dose per Fraction
					Carprofen		Free I	III		IV	Polar Component	Total Recovered	
					Free	β-Glucuronide		Free	β-Glucuronide	β-Glucuronide			
Human, male	3	0.74 ± 0.15	Oral	Urine (0–120 hr)	2.84 ± 0.47	63.1 ± 10.8	0.3	—[b]	0.4	—	—	66.6	67.7
				Feces[c] (0–120 hr)	7.3	—	0.95	0.7	—	—	8.0	17.0	23.7
Dog, male	2	1.0	Intravenous	Urine (0–24 hr)	—	—	2.28 ± 0.3	—	1.30 ± 0.35	0.20 ± 0.05	2.40 ± 0.7	6.2	10.3
	1	1.0	Intravenous	Feces (24–48 hr)	7.8	—	—	7.2[d]	—	—	33.0	48.0	59.0
	2	1.0	Intravenous	Bile (0–5 hr)	2.25 ± 0.52	12.6 ± 2.2	0.73 ± 0.04	0.25[d] ± 0.05	2.58 ± 0.17	0.34 ± 0.04	2.88 ± 0.18	21.6	24.0 ± 1.1
	1	1.0	Oral	Urine (0–24 hr)	—	—	3.67	—	2.70	0.39	4.35	11.1	17.3
Rat, male	3 pooled	5.0	Intravenous	Urine (0–72 hr)	0.79	—	1.55	5.23[d]	—	—	7.46	15.0	19.2
				Feces (0–40 hr)	7.19	—	—	22.9[d]	—	—	15.9	46.0	57.2
	1	5.0	Intravenous	Bile (0–7 hr)	—	34.1	—	—	3.1	5.0	3.5	45.7	53.7

[a] The mean ± range is given for two values, and the mean ± *SD* is given for three values. [b] The dash indicates that the amounts found were <0.1% of the dose. [c] Quantitative distribution of carbon 14 component in human feces represents an estimate in one subject. [d] In these specimens, the TLC system did not differentiate between free III and free IV.

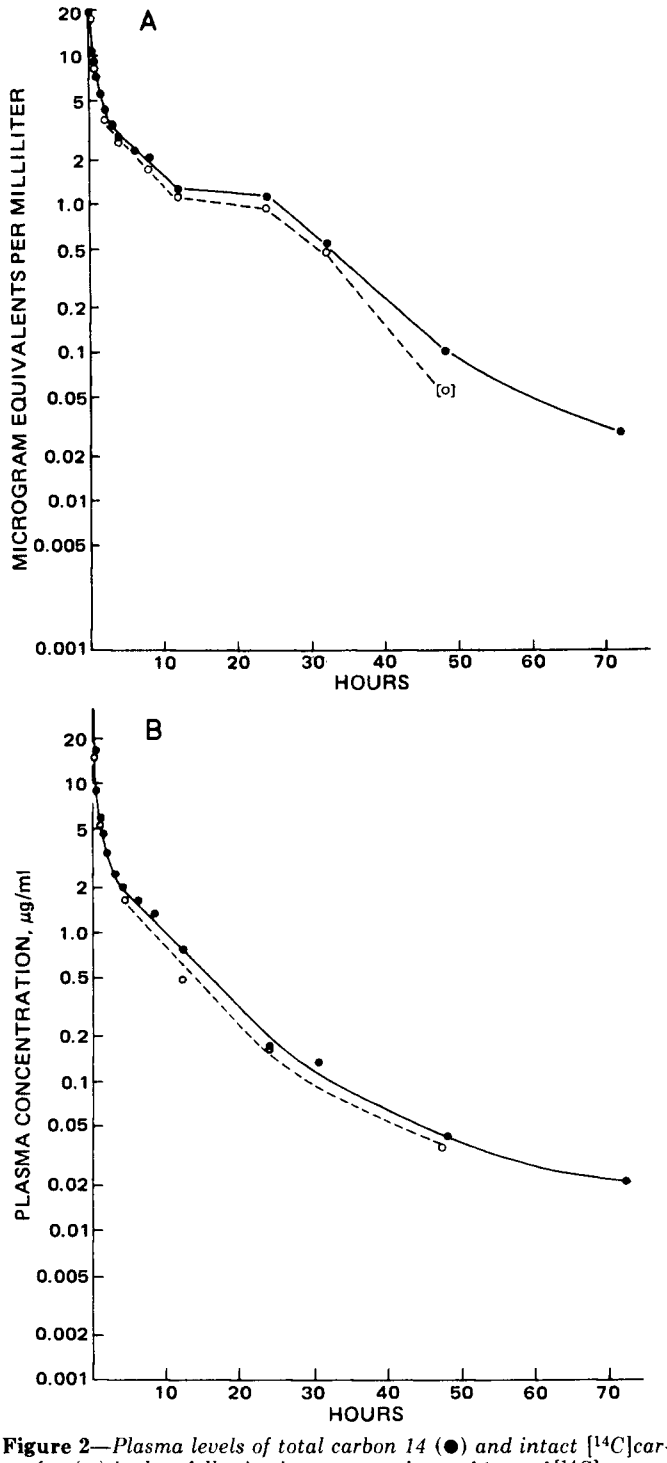


Figure 2—*Plasma levels of total carbon 14 (●) and intact [^{14}C]carprofen (○) in dogs following intravenous doses of 1 mg of [^{14}C]carprofen/kg. Key: A, Experiment D-I; and B, Experiment D-V.*

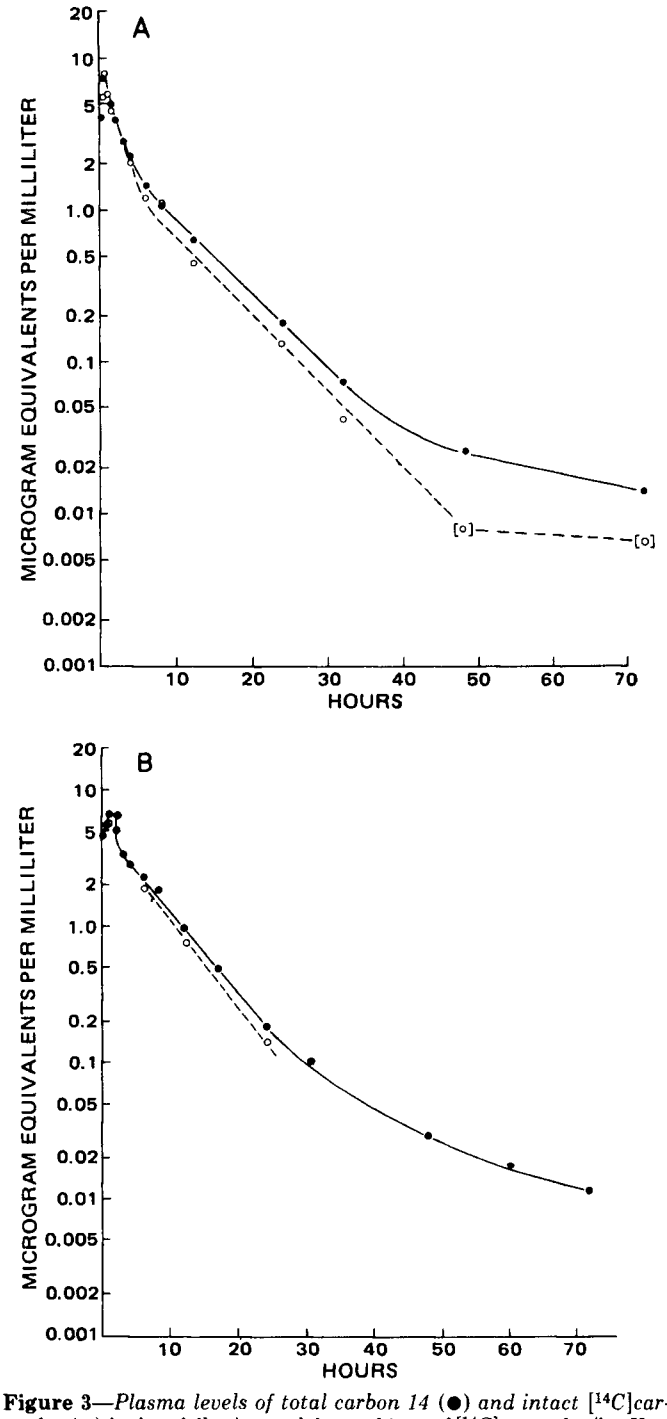


Figure 3—*Plasma levels of total carbon 14 (●) and intact [^{14}C]carprofen (○) in dogs following oral doses of 1 mg of [^{14}C]carprofen/kg. Key: A, Experiment D-II; and B, Experiment D-VI. Brackets indicate an estimated value.*

H-7), 7.17 (broad d, 1H, *J ortho* = 8 Hz, H-3), 7.38 (broad s, 1H, H-1), 7.59 (broad s, 1H, H-5), 7.90 (d, *J ortho* = 8 Hz, H-4), and 8.17 (broad, NH). The chemical shifts of the aromatic protons (H-1, H-3, and H-4) correspond (within ±0.02 ppm) with those of the parent ester and clearly showed that the alkyl-substituted ring was not hydroxylated. However, the bands at δ 6.81 and 7.59 were at δ 7.33 and 7.99 in the parent and indicated large significant upfield shifts of 0.52 (*ortho*) and 0.40 (*para*) ppm, respectively, which were attributed to the new hydroxyl group at C-8. This conclusion was confirmed by a resolved *meta*-splitting ($J_{5,7}$ = 2 Hz) observed in the H-7 signal. Thus, hydroxylation occurred definitely at C-8, and Metabolite III was characterized conclusively as 6-chloro-8-hydroxy-α-methylcarbazole-2-acetic acid.

The UV and fluorescence spectra of Metabolite IV differed from those of Metabolite III (Table IV and Fig. 1D). The UV spectrum of Metabolite IV exhibited a bathochromic shift from maxima at 240 and 310 nm to maxima at 245 and 340 nm. The fluorescence spectrum showed activation/emission maxima at 310/360 nm with a high intensity that was stable when exposed to light.

The high-resolution mass spectrum of the purified methyl ester of Metabolite IV indicated a molecular ion of *m/e* 303.0681 ($C_{16}H_{14}ClNO_3$), isomeric with that of Metabolite III methyl ester. The fragment of *m/e* 217.0206 ($C_{12}H_8ClNO$), arising from loss of the side chain ($C_4H_6O_2$), demonstrated oxygen addition in the carbazole moiety. These data support a structure for Metabolite IV of a phenolic monohydroxy derivative distinct from Metabolite III.

The position of the phenolic hydroxy function of Metabolite IV was elucidated by the NMR spectrum of its dimethyl derivative. Thus, the

Table VI—Excretion Patterns of [^{14}C]Carprofen and of Metabolites in Rats

Number of Rats	Strain (Sex)	Age, weeks	Weight, g	Dose mg/kg	Dose mg/Rat	Percent of Dose Excreted: Urine (0–72 hr)	Feces (0–72 hr)	Bile ^{14}C (0–4 hr)	Biliary Metabolites: Carprofen Glucuronide	III	IV	Polar Carbon 14
2	Carworth[a] Farm (M)	8	326	5.0	1.63	19.2	57.2	38.2[b]	24.3 ± 0.4	2.22 ± 0.38	3.55 ± 0.11	2.52 ± 0.11
3	Charles River (M)	10	410 ± 10	5.0	2.05 ± 0.05	—	—	34.8 ± 1.1	13.9 ± 0.44	11.0 ± 1.8	3.58 ± 0.21	2.96 ± 0.66
6	Charles River (F)	8–10	265 ± 14	5.0	1.33 ± 0.07	—	—	38.6 ± 6.7	12.6 ± 1.7	12.31 ± 3.1	4.75 ± 0.73	5.60 ± 2.1
3	Charles[c] River (M)	10	337 ± 32	3.0	1.04 ± 0.10	20.4 ± 2.0	74.6 ± 2.5	44.7 ± 6.3	15.4 ± 1.4	14.8 ± 3.8	5.9 ± 0.97	5.0 ± 2.0
3	Charles[c] River (M)	88	728 ± 90	3.0	2.27 ± 0.23	30.8 ± 6.7	58.3 ± 7.3	22.9 ± 3.8	11.0 ± 1.2	5.08 ± 1.8	3.23 ± 1.7	1.93 ± 0.74

[a] In these two experiments, rats used to measure excretion in urine and feces were different from those used to collect bile following bile duct cannulation. [b] Incomplete collection. [c] In these experiments, the same rats were used, first for the urinary and fecal excretion experiment and then, after 1 week, for the biliary secretion experiments.

Table VII—Tissue Distribution of Total Carbon 14 and [^{14}C]Carprofen in Rats at Different Times following Intravenous Administration of 5 mg of [^{14}C]Carprofen/kg

Tissue	10 Min: Carbon 14 Found[a]	Carbon 14 Ratio[b]	% of Carbon 14 Dose	μg of Carprofen/g	1 Hr: Carbon 14 Found[a]	Carbon 14 Ratio[b]	% of Carbon 14 Dose	μg of Carprofen/g	4 Hr: Carbon 14 Found[a]	Carbon 14 Ratio[b]	% of Carbon 14 Dose	μg of Carprofen/g
Plasma[c]	34.0	1.0	32.9[d]	29.6	20.2	1.00	19.5[d]	16.1	8.62	1.00	8.32[d]	5.9
Liver[c]	15.5	0.46	11.9	14.6	10.4	0.57	7.63	7.39	5.73	0.66	4.73	2.43
Kidneys[c]	21.7	0.64	3.3	20.5	16.3	0.81	2.45	13.5	7.44	0.86	1.09	5.19
Muscle[c]	1.9	0.056	—	1.81	1.65	0.082	—	1.33	0.64	0.07	—	0.45
Carcass[e]	2.36	0.069	32.9	2.18	2.28	0.110	32.4	1.64	0.87	0.10	12.3	0.55
Heart[f]	3.37	0.10	0.18	—	3.34	0.167	0.18	—	1.50	0.17	0.81	—
Brain[f]	0.66	0.019	0.11	—	0.44	0.022	0.07	—	0.19	0.02	0.03	—
Spleen[f]	2.18	0.063	0.09	—	1.74	0.086	0.08	—	1.04	0.04	0.04	—
Testes[f]	0.61	0.018	0.14	—	1.61	0.080	0.36	—	1.06	0.26	0.26	—
Fat[f]	0.77	0.022	—	—	1.31	0.065	—	—	0.82	0.14	—	—
Lungs[f]	3.57	0.11	0.63	—	9.25	0.46	0.64	—	2.86	0.33	0.23	—
GI tract[g] tissues	—	—	1.47	—	—	—	4.34	—	—	—	2.9	—
Small intestine[h]	—	—	3.26	—	—	—	20.5	—	—	—	19.0	—
Large intestine[h]	—	—	n.m.[i]	—	—	—	1.3	—	—	—	33.7	—
Total	—	—	86.88	—	—	—	89.45	—	—	—	83.41	—

[a] In microgram equivalents per gram. [b] Ratio of concentration in tissue to concentration in plasma. [c] Average of three individual values. [d] Total plasma carbon 14 estimated assuming plasma volume = 5% of body weight. [e] Value from one carcass at each time. [f] Value from three pooled tissues. [g] Stomach and intestinal tissue free of contents. [h] Value from three pooled contents. [i] n.m. = nonmeasurable.

methoxy methyl ester of Metabolite IV dissolved in dimethyl sulfoxide-d_6 provided a methyl ester band at δ 3.59 and a CH_3CH doublet (J = 7 Hz) at 1.45, indicating that the side chain was intact. The rest of the spectrum was assigned as follows: δ 3.91 (q, 1H, J = 7 Hz, CH_3CH), 3.93 (s, 3H, CH_3O ether), 7.04 (dd, 1H, J *meta* = 1.5 and J *ortho* = 8 Hz, H-3), 7.13 (s, 1H, H-8), 7.34 (d, 1H, J *meta* = 1.5 Hz, H-1), 7.96 (d, 1H, J *ortho* = 8 Hz, H-4), 8.13 (s, 1H, H-5), and 11.19 (s, 1H, NH). The chemical shifts and multiplicities of the bands assigned to H-1, H-3, and H-4 indicated definitely that the alkyl-substituted ring was not hydroxylated. The singlet character of the bands at δ 7.13 and 8.13 showed that oxygenation had occurred at C-7. Therefore, Metabolite IV is the 7-hydroxy derivative of carprofen.

The amounts of Metabolites I, III, and IV excreted in the 0–24-hr dog urines are included in Table V.

Metabolites in Bile and Feces of Dogs—The distribution of the biliary metabolites in two dogs (D-IV and D-X) is shown in Table V. A small portion of the biliary carbon 14 was present as pH 5 ether-extractable metabolites, predominantly as intact [^{14}C]carprofen. The other components appeared to be identical to the metabolites seen in urine according to TLC evidence (Systems 1 and 2). About 50% of the biliary carbon 14 consisted of the ester glucuronide of [^{14}C]carprofen, while 12% represented conjugates of Metabolites III and IV (in a ratio of 8:1) that were cleaved by glusulase.

About half of the fecal radioactivity was ether extractable at pH 5. This extract contained similar parts of carprofen and the phenolic fraction (mixture of Metabolites III and IV).

Plasma Levels and Tissue Distribution—The levels of total radioactivity and of intact [^{14}C]carprofen measured in the plasma of two dogs (D-I and D-V) following 1-mg/kg iv doses of [^{14}C]carprofen are presented in Fig. 2. In the first 48 hr, most of the radioactivity in the plasma represented intact drug. A minor ether-extractable metabolite with an R_f value (0.27–0.29) in System 1 corresponding to that of Metabolite I was detected in the 3–12-hr plasma samples of these intravenously dosed dogs. No radioactivity was found in the erythrocytes.

The plasma levels of carprofen showed a triexponential decline that was most distinct in Experiment D-V (Fig. 2B). In this study, the half-lives of the three phases were 0.5, 6, and 40 hr. In Experiment D-I (Fig. 2A), the plasma levels, after an initial decline, plateaued between 12 and 24 hr and then declined again.

Following oral administration of the same dose as a solution to the same two dogs (D-II and D-VI), plasma levels peaked at 0.5–1 hr, indicating that absorption of the drug was rapid (Fig. 3). Absorption, as estimated from comparison of the intravenous and oral areas under the total plasma carbon 14 level curves, was 106% in D-VI. In D-II, the lower value of 50% appeared to be related to a secondary drug uptake, reflected in the plateau of the intravenous curve.

Evaluation of the plasma levels obtained after a 10-times higher intravenous dose of 10 mg/kg (D-III and D-XI) indicated that in the 1–10-mg/kg dose range, the plasma levels were proportional to dose whereas the disposition rates were independent of the dose.

The distribution of total carbon 14 into the major tissues of two bile duct-cannulated dogs (D-IV and D-X) 5 hr after intravenous doses of 1 mg of [^{14}C]carprofen/kg is shown in Table III. Very low concentrations of carbon 14 in all of these tissues relative to plasma were evident. Of the radioactivity in the liver and in the kidneys, close to 70% represented intact carprofen. Both tissues contained trace levels of Metabolite I.

Rat Experiments—*Excretion*—After intravenous administration of [^{14}C]carprofen, the radioactivity was excreted predominantly in the

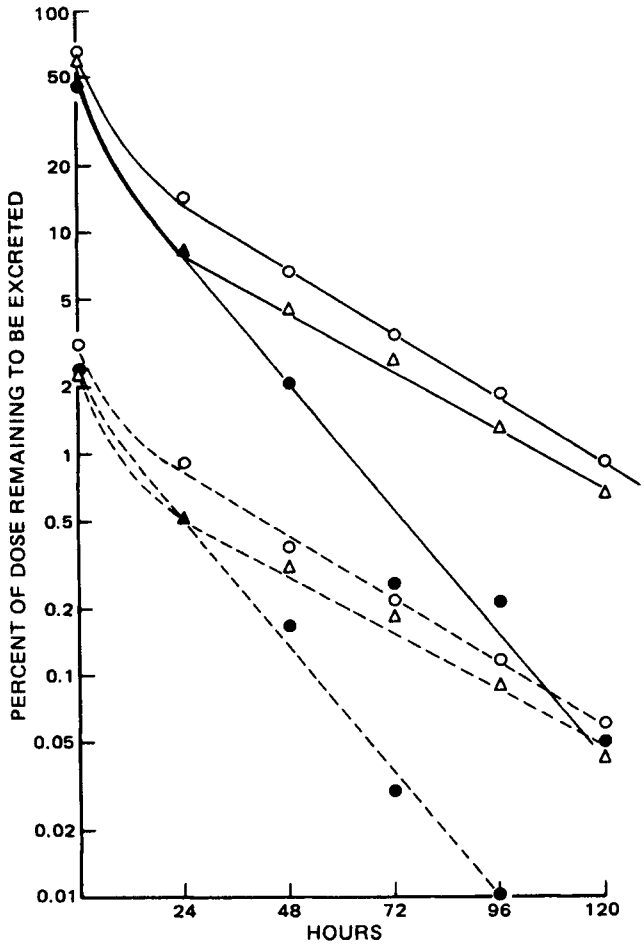


Figure 4—*Urinary excretion in three human subjects, following oral doses of 50 mg of* [^{14}C]*carprofen, of free intact carprofen* (- - -) *and of carprofen ester glucuronide* (—) *by Subject 1* (○), *Subject 2* (△), *and Subject 3* (●).

feces. Experiments in bile duct-cannulated animals showed this finding to result almost exclusively from biliary secretion of labeled carprofen metabolites.

The distribution of the radioactivity between feces (bile) and urine varied depending on the age and size of the rats (Table VI). In 337-g Charles River rats, 75% of a 3-mg/kg dose was excreted in the feces within 72 hr (45% in the bile in 4 hr) and 20% was excreted in the urine, with both forms of excretion being rapid and almost complete within 24 hr. In the larger and older Charles River rats (>700 g), only 58% of the same dose was excreted in the feces within 72 hr (23% in the bile in 4 hr) and 31%

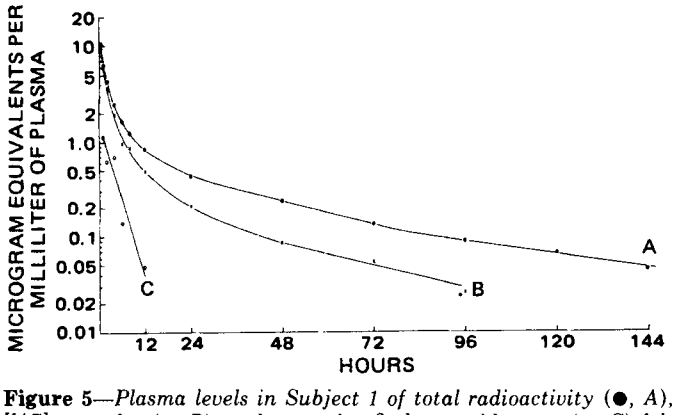


Figure 5—*Plasma levels in Subject 1 of total radioactivity* (●, *A*), [^{14}C]*carprofen* (×, *B*), *and carprofen β-glucuronide ester* (○, *C*) *following a 50-mg oral dose of* [^{14}C]*carprofen. Data expressed as log of microgram equivalents per milliliter of plasma are plotted* versus *time in hours.*

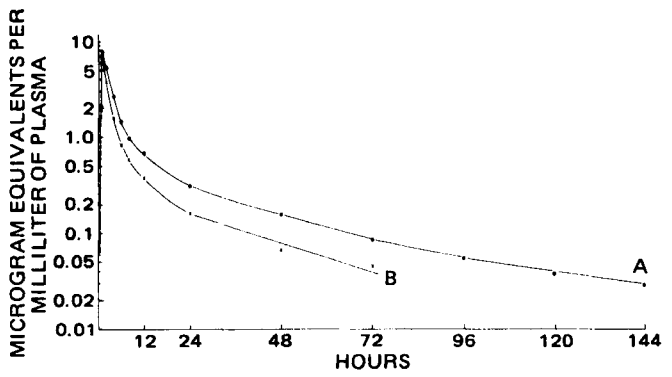


Figure 6—*Plasma levels in Subject 2 of total radioactivity* (●, *A*) *and of* [^{14}C]*carprofen* (×, *B*) *following a 50-mg oral dose of* [^{14}C]*carprofen. Data expressed as log of microgram equivalents per milliliter of plasma are plotted* versus *time in hours.*

was excreted in the urine, both at a slower rate of excretion. The biliary metabolite fraction contained exclusively conjugates, including the carprofen ester glucuronide and the ethereal glucuronides of the phenolic Metabolites III and IV, identified by cochromatography (in three systems) with the metabolites isolated from dog bile and urine. The quantitative distribution of the secreted metabolites appeared to vary depending on the strain and age (size) of the rats. Relatively less hydroxylated metabolites were secreted in the bile of the old (large) rats than in the bile of the young (small) rats. A somewhat larger fraction was secreted as the carprofen ester glucuronide by male rats of the Carworth Farm Sprague–Dawley (CF-D) strain than by the Charles River CD strain. No sex differences were evident.

Analysis of the intestinal and fecal carbon 14 fractions of the rats indicated a progressive deconjugation of the biliary metabolites and a decrease of the ratio of carprofen to the phenolic metabolites (III and IV) as the radioactivity moved from bile (ratio of 4.2) to the large intestine (ratio of 0.8) and feces (ratio of 0.31). This finding suggests a selective reabsorption of enterically deconjugated carprofen.

TLC of an extract obtained with ethyl acetate at pH 1 from a 0–24-hr rat urine specimen (containg 16.3% of a 5-mg/kg [^{14}C]carprofen dose) distinguished labeled components, which, from their R_f values in System 1, appeared to include intact carprofen, Metabolites III and/or IV, Metabolite I, and a polar component that remained at the origin (Table V).

Tissue Distribution—The tissue distribution of total carbon 14 and of [^{14}C]carprofen and the tissue to plasma carbon 14 ratios in rats at 10 min and 1 and 4 hr after intravenous administration of 5 mg of [^{14}C]-carprofen/kg are presented in Table VII. At each time, the carbon 14 levels were highest in the plasma. Intact carprofen prevailed in the plasma and tissues at all times. The residual radioactivity consisted mostly of ether-unextractable polar material.

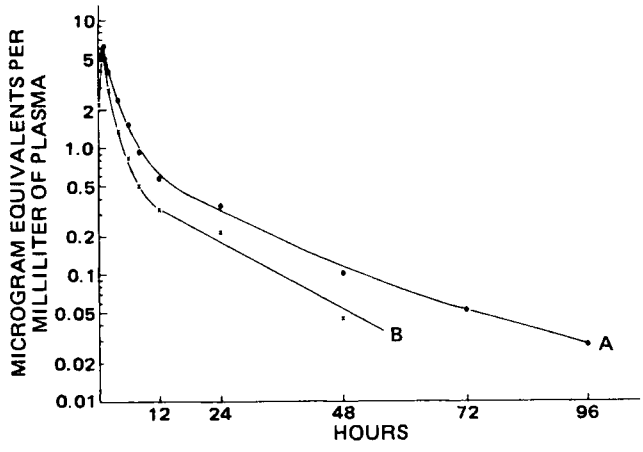


Figure 7—*Plasma levels in Subject 3 of total radioactivity* (●, *A*) *and of* [^{14}C]*carprofen* (×, *B*) *following a 50-mg oral dose of* [^{14}C]*carprofen. Data expressed as log of microgram equivalents per milliliter of plasma are plotted* versus *time in hours.*

Human Studies—*Excretion*—After oral administration of 50 mg of [^{14}C]carprofen, the main portion of the dose was excreted in the urine (75% of the dose in Subjects 1 and 2 and 55% in Subject 3). The ester glucuronide of carprofen represented >90% of the urinary carbon 14, and intact carprofen represented 3–5%. Only trace amounts of free Metabolite I and of conjugated Metabolite III could be detected (Table V). A semilog plot of intact carprofen and of its ester glucuronide, expressed as the percentages remaining to be excreted, *versus* time (Fig. 4) produced parallel curves for each component, reflecting biexponential excretion. The fast excretion rate was characterized by half-lives of 2.4–3.6 hr, whereas the terminal phase exhibited apparent half-lives of 24–25 hr in Subjects 1 and 2 and of 13 hr in Subject 3. These half-lives are in close agreement with the plasma half-lives for carprofen.

The fecal excretion of radioactivity represented 17–20% of the dose and extended from Day 2 to Day 10 in Subjects 1 and 2. In Subject 3, 33% of the radioactivity was excreted in the feces at a considerably faster rate than in the other two subjects.

The fraction extracted from the ethanolic homogenates of the human feces by the described procedures contained 60–70% of the fecal radioactivity. Of this fraction, 25–30% (~7% of the administered dose) was separated and identified as intact carprofen by TLC and by high-resolution mass spectrometry. The TLC pattern of the fecal extracts suggested the presence of small quantities of Metabolite I and two additional components not detected in dogs and rats. UV and mass spectral analysis of the two additional components yielded spectra similar to those of carprofen, suggesting that these metabolites may represent lipid-soluble addition products rather than true metabolites of carprofen.

Plasma Levels—Intact drug was the dominant radioactive component in human plasma during the first 12 hr. A polar component increased with time to more than half of the total radioactivity in plasma. One of these polar metabolites, identified in Subject 1 as the β-glucuronide ester of carprofen, declined rapidly with a half-life close to 2 hr.

The elimination rate in plasma of both carprofen and total radioactivity decreased with time (Figs. 5–7). A biphasic fall off of the plasma levels of carprofen occurred in all three subjects. The initial plasma carprofen levels declined with a half-life of 1.8–2.2 hr to reach values corresponding to ~5% of the peak levels within 12 hr. During the terminal phase, the levels declined with half-lives of 25–27 hr in Subjects 1 and 2 and ~13 hr in Subject 3. No drug-related radioactivity was detectable in the red cells throughout the experiment[18].

DISCUSSION

The disposition of [^{14}C]carprofen is similar in rats, dogs, and humans. It has many characteristics in common with the disposition of other acidic nonsteroidal anti-inflammatory compounds (10–16). Like most members of this class of drugs, carprofen is strongly bound to plasma proteins (17), and this binding is associated with a high ratio of the concentration of carprofen in plasma relative to its concentration in tissues. Intact carprofen accounts for most of the radioactivity in plasma and in the major tissues of rats and dogs. Drug elimination from plasma is characterized in dogs and humans by a bi- or multiphasic decline.

The main metabolites, primarily conjugates, do not accumulate and are rapidly eliminated, except for a small polar metabolite fraction that persists in human plasma.

Carprofen is eliminated from rats, dogs, and humans primarily by biotransformation. Probably <5% of the dose is cleared directly as the free, intact drug. The carprofen found in the feces probably arose from deconjugation of the ester glucuronide, which predominated in the bile (Table V).

The ester glucuronide of the intact drug represents the major metabolite in rats, dogs, and humans. The rapid formation and the characteristic rate and mode of elimination of this metabolite determine the pharmacokinetics of the drug. In rats and dogs but to a very minor extent in humans, oxidation in positions 7 and 8 of the carbazole moiety to phenolic derivatives (probably *via* a common arene oxide precursor) and their conjugation to form ethereal glucuronides represent alternate pathways. A small percentage of the dose also is metabolized by side chain oxidation to the α-hydroxy derivative, which is excreted free in the urine by dogs and rats. In rats and dogs, the ester glucuronide of carprofen and the ethereal glucuronides of the two phenolic metabolites are eliminated predominantly by biliary secretion.

Human subjects, in contrast to rats and dogs, excrete most of an oral carprofen dose as urinary ester glucuronide. However, the relatively small fecal ^{14}C-labeled component apparently represents only a fraction of a substantially larger biliary component because of enterohepatic circulation. A comparison of the initial 90% decline of the drug in plasma with the 50–60% of the dose excreted in the urine during the same 12-hr period suggests that the remaining 30–40% of the dose had been eliminated with the bile. This estimate of extensive biliary excretion of metabolites in humans was supported by analysis of biliary aspirates from an intubated human volunteer[19].

Extensive enterohepatic circulation in humans was suggested by the protracted fecal excretion of radioactivity in two of the three subjects discussed in the present report. Furthermore, the slow terminal decline of plasma levels and of the urinary excretion rates in these two subjects may be related to a continuous reabsorption of the biliary fraction. The extent of this reabsorption determines the fraction of the primary biliary portion that is excreted in the feces and the fraction that ultimately is excreted in the urine. In the third subject, the enterohepatic process may be concluded to have been less pronounced, resulting in a higher and more rapid fecal excretion and in a shorter apparent half-life of the radioactivity in plasma.

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[18] Because of the nonuptake of the drug by the red cells, plasma levels would be higher by a factor of $(1 - \text{hematocrit})^{-1}$ than the blood levels determined for the same specimen.

[19] Bile samples from collaborative study of Dr. C. Dujovne, Kansas City Medical Center, and Dr. J. Gustafson, Roche Department of Pharmacokinetics and Biopharmaceutics.