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Absorption, Distribution, and Metabolic Fate of 7-Chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one in Rats, Dogs, and Humans

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Abstract □ The absorption and metabolic fate of 7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one (I) was studied in rats, dogs, and humans. Orally administered I was readily absorbed by all species. In the rat, orally administered I was converted to its metabolite, 5-chlorosalicylic acid, by the intestinal wall. The half-lives of blood radioactivity, after the oral administration of I-9-^{14}C, were about 18 and 12 hr in the rat and beagle hound, respectively. In human subjects, no intact I was detected in the bloodstream; however, the clearance of the metabolite, 5-chlorosalicylic acid, had a half-life of about 33 hr. Cleavage of the oxazine ring of I generated 5-chlorosalicylic acid, which was excreted both in the free form and conjugated with glycine and glucuronic acid. The isoxazole moiety was converted to β-hydroxybutyric acid and its metabolites carbon dioxide and fumaric, citric, α-ketoglutaric, succinic, and malic acids. Binding of I to plasma proteins was extensive but was less than that of 5-chlorosalicylic acid.

Keyphrases □ 7-Chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one—absorption, distribution, and metabolic fate, rats, dogs, and humans □ 5-Chlorosalicylic acid—identified as major metabolite of 7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one in rats, dogs, and humans □ GLC—analysis, 7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one in plasma

7-Chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one (I) is a new orally active anti-inflammatory agent, which is chemically dissimilar to the steroids. Unlike most other clinically effective nonsteroidal anti-inflammatory agents, I is not ulcerogenic in the rat and does not induce GI blood loss as measured with ^{51}Cr-labeled rat blood cells (1). Studies on the absorption, distribution, metabolism, and excretion of I in rats, dogs, and human subjects are presented in this report.

EXPERIMENTAL

Materials—I-9-^{14}C and I-3-^{14}C were synthesized using a recently patented procedure (2). Since I has asymmetric carbon atoms at positions 2 and 3a and is obtained as a mixture of diastereoisomers, the chromatographic systems employed (Table I) were chosen carefully in order not to resolve the diastereoisomeric pairs.

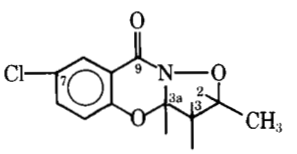
Absorption in Rats—The small intestine of male Sprague–Dawley rats, 230–250 g, was rapidly filled with a solution of 90 μg/ml of I-9-^{14}C (2.13 μCi/ml), using a previously described technique (3). Constant blood volume was maintained by transfusion with blood from a donor animal. At appropriate intervals, samples were taken of hepatic portal and systemic blood, and portions of the contents of the intestinal lumen were removed. These materials were assayed for radioactivity and subjected to TLC. The developed plates were scanned for radioactivity, and the area under the trace was determined by integration to measure the ^{14}C in each zone.

Rat Intestine Preparation—Mature male Sprague–Dawley rats were exsanguinated, and the entire small intestine was excised. A fine mince was prepared by cutting the tissue with scissors and scalpels, and the preparation was centrifuged at 48,000×*g* at 4°. Three milliliters of the supernatant fluid was incubated at 37° with 2.0 ml of I-9-^{14}C (57 μg/ml, 1.35 μCi/ml) for 1.5 hr with shaking. Then an equal volume of acetone was added, and the mixture was again centrifuged. The supernatant fluid, containing over 95% of the radioactivity, was spotted on thin-layer plates and treated as already described.

Concentration in Circulating Blood—A solution containing 5 mg of I-9-^{14}C (129 μCi) dissolved in 0.5 ml of polyethylene glycol 400 was administered by stomach tube to each of six male Sprague–Dawley rats, weighing an average of 293 g. Six other rats received 0.5 mg of I-3-^{14}C (7.43 μCi) dissolved in 0.5 ml of the same vehicle. Blood samples were taken from the tail vein at appropriate intervals, and radioactivity was determined by liquid scintillation counting (4).

Beagle hounds, 7.6–10.2 kg, received single oral doses of I-9-^{14}C of 30.8 mg/kg (three animals, 42.9 μCi each), 66 mg/kg (one animal, 495 μCi), and 490 mg/kg (one animal, 1.98 μCi). At suitable intervals, blood was taken from the jugular vein and the plasma radioactivity of each sample was determined.

Human Studies—Six human volunteers each received 1500 mg of nonradioactive I. Heparinized blood samples were taken at various times postadministration and immediately centrifuged. The plasma was frozen until assayed by GLC.



I

Table I—TLC of Urinary End-Products of I

Compound	R_f[a] Solvent System A[b]	Solvent System B[c]
I	0.58	0.63
5-Chlorosalicylic acid	0.47	0.47
5-Chlorosalicyluric acid	0.35	0.16
5-Chlorosalicylic acid glucuronide	—	0.06

[a] Silica gel (Merck). [b] Benzene–methanol–acetic acid (180:32:16). [c] *n*-Butanol saturated with 2 *N* ammonia.

Urine was collected from each subject for 48 hr. A urine aliquot was adjusted to pH 6.4 with hydrochloric acid; then four volumes of 0.07 *M* pH 6.4 phosphate buffer, containing 100 units of β-glucuronidase[1], was added. The mixture was incubated for 18 hr at 37° and then assayed for 5-chlorosalicylic acid.

GLC Assays—The amount of I present in plasma was measured in concentrations of 1–10 μg/ml. Chloroform, 0.2 ml, containing 1.2 μg of dibutyl phthalate as the internal standard, was added to a 1-ml aliquot of plasma. The mixture was agitated on a vortex mixer for 30 sec and centrifuged for 10 min. The aqueous phase was discarded, and the remaining emulsion was disrupted by freezing in dry ice–acetone. The liquid phases were separated by centrifugation, and 1.4 μl of the chloroform extract was injected into a gas chromatograph[2] equipped with a flame-ionization detector.

The GLC conditions were: column, 1.2-m (4-ft) glass, 3-mm i.d. packed with 3.8% W-98 on Diatoport S, 80–100 mesh; column temperature, 198°; flash heater temperature, 250°; detector temperature, 252°; helium flow rate, 86 ml/min; hydrogen flow rate, 34 ml/min; oxygen flow rate, 600 ml/min; and range, 10 with an attenuation of 16×.

Freshly prepared samples of plasma containing known amounts of I were used as standards. The 5-chlorosalicylic acid content of plasma and urine samples was determined by GLC. The conditions for the extraction and assay were reported separately (5).

Stability in Blood and Plasma—Heparinized rat blood was obtained by cardiac puncture. A solution of 60 μg of I-9-^{14}C (136 μCi), dissolved in 1.0 ml of 0.07 *M* pH 7.4 phosphate buffer, was added to 4.0 ml of whole rat blood; the resulting mixture was incubated at 37° with stirring. Aliquots, 1.0 ml, were taken at the start and after 4 hr. The cells were removed by centrifugation, washed, and assayed for radioactivity. An equal volume of acetone was added to the plasma, and the mixture was again centrifuged. The clear supernatant fluid, containing essentially all radioactivity, was examined by TLC. Only one radioactive zone, corresponding to I, was detected.

One-milliliter aliquots of a solution of I in normal dog plasma, 10 μg/ml, were placed in stoppered tubes and stored at 4° or frozen and stored at −18°. At intervals, samples were removed and assayed for I by the described GLC procedure.

Tissue Distribution in Rats—A male Sprague–Dawley rat, 215 g, received 4.0 mg of I-9-^{14}C (95.8 μCi), dissolved in 1.0 ml of polyethylene glycol 400, by stomach tube. A second rat, 198 g, received a solution containing 2.9 mg of I-3-^{14}C (39.8 μCi) orally. Each animal was placed in a metabolism chamber for 24 hr and then sacrificed. The excreta, soft tissue organs, and remainder of the carcass were processed and analyzed for radioactivity as previously reported (4). Respiratory carbon dioxide was collected in 2.5 *N* sodium hydroxide, and the ^{14}C content of an aliquot of this solution was determined.

Isolation of 5-Chlorosalicylic Acid—Nonradioactive I was administered, 50 mg/kg ip, to two male beagle hounds. Pooled 24-hr urine was continuously extracted with carbon tetrachloride for 18 hr. The organic phase was separated, washed with water, and concentrated, and the residue was examined for unchanged I by TLC; no free drug was detected.

The urine remaining from the initial extraction was acidified with concentrated hydrochloric acid to pH 1 and continuously extracted for an additional 24 hr with carbon tetrachloride. The organic phase was separated, washed with acidified water, and evaporated to dryness under vacuum. The residue, which was recrystallized from methanol–water and then from water, was identified as 5-chlorosalicylic acid by melting point (171–172°), TLC characteristics, and IR spectrum, which were identical to those of authentic 5-chlorosalicylic acid.

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Hewlett-Packard model 402.

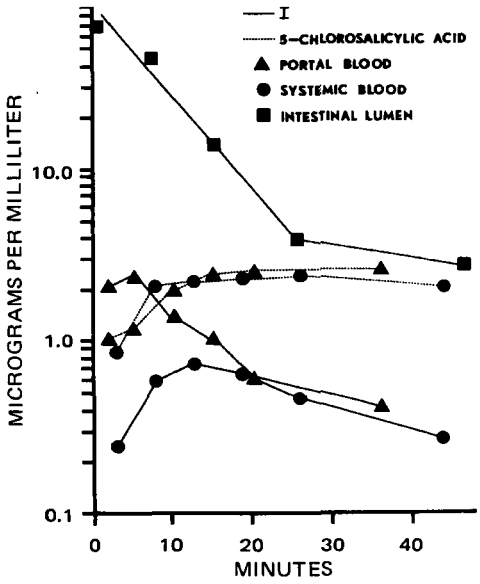


Figure 1—*Disappearance of I-9-^{14}C from rat intestine and the concentration of I and 5-chlorosalicylic acid in systemic and hepatic portal plasma, as determined by the area under a radiochromatogram trace.*

Inverse Isotope Dilutions—Inverse isotope dilution studies were performed using the urine of animals that had received labeled I. Typical procedures are described here.

I-9-^{14}C—A male Sprague–Dawley rat, about 250 g, received 2.5 mg of I-9-^{14}C (63.1 μCi), dissolved in 0.5 ml of polyethylene glycol 400, by stomach tube. Urine was collected for 24 hr and its radioactivity was measured. Inverse isotope dilutions were carried out on separate aliquots of urine.

1. Nonradioactive I, 1000 mg, was added to a 1.0-ml aliquot. Sodium carbonate solution, 5%, was added to adjust the pH to 9–10 and to prevent the extraction of 5-chlorosalicylic acid; the mixture was extracted three times with six volumes of chloroform. The chloroform phases were combined and evaporated to dryness. The

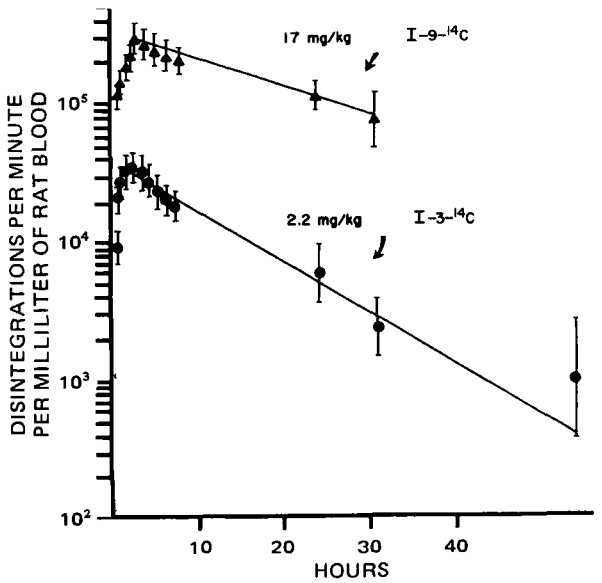


Figure 2—*Blood radioactivity after oral administration of I-^{14}C to rats.*

Table II—Comparison of Theoretical and Actual Concentrations of I in Dog Plasma

Hours	I, μg/ml[a]	
	Observed[b]	Calculated[c]
0	0	0
0.5	0.6	216
1	2.2	761
2	2.6	886
4	4.8	1034
7	8.0	830
24	0.6	341
48	0	88
72	0	3

[a] Dog received a capsule containing I-9-^{14}C, 490 mg/kg, 1.98 μCi. [b] By a GLC procedure. [c] From the amount of radioactivity in the plasma.

residue, consisting of crude I, was repeatedly crystallized from ethyl acetate to constant specific activity, mp 150–151°.

2. Details of the procedures for quantitating 5-chlorosalicylic acid and 5-chlorosalicyluric acid by the isotope dilution technique were reported previously (6).

3. The metabolites in the urine present in a bound form were converted to the free form by hydrolysis. An aliquot was treated with an equal volume of concentrated hydrochloric acid, and the mixture was heated on a steam bath for 10 min. Nonradioactive carrier was then added, and the inverse isotope dilutions were carried out as with the free metabolites. Conjugated urinary end-products were calculated by difference.

4. Similar studies were performed on the urine of a dog that had received 502.2 mg of I-9-^{14}C orally.

I-3-^{14}C—A male Sprague–Dawley rat, 251 g, received 4.6 mg of I-3-^{14}C (62.6 μCi), dissolved in 1.0 ml of polyethylene glycol 400, by stomach tube. Urine was collected for 24 hr and assayed for radioactivity.

1. Nonradioactive sodium β-hydroxybutyrate, 1000 mg, was added to a 0.5-ml aliquot. A homogeneous solution resulted from the addition of 5 ml of water, 4 drops of 1 *N* hydrochloric acid, and 10 ml of ethanol. 2,4′-Dibromoacetophenone, 1.0 g, was added, and the mixture was heated at reflux for 2 hr. The resulting *p*-bromophenacyl ester was precipitated by the addition of water, washed with water, dried, and recrystallized from ethyl acetate–heptane to constant specific activity, mp 82–83°. The specific activity was corrected for the difference in the formula weight between the crystalline derivative and the sodium β-hydroxybutyrate added. This ester, *p*-bromophenacyl β-hydroxybutyrate, has not previously been reported.

Anal.—Calc. for $C_{12}H_{13}BrO_4$: C, 47.86; H, 4.35. Found: C, 48.11; H, 4.29.

2. Nonradioactive succinic acid, 1000 mg/kg, was added to another 0.5-ml aliquot of urine, and 10 ml of ethanol was added to give a homogeneous solution. This solution was evaporated to dryness under vacuum, the residue was triturated with hot ethyl acetate, and the insoluble material was discarded. The addition of heptane to the ethyl acetate solution separated crystals of succinic acid. These crystals were removed by filtration and recrystallized from ethyl acetate–heptane to constant specific activity, mp 185–186°.

3. The techniques for the isolation of α-ketoglutaric, fumaric, malic, and citric acids were similar to those for succinic acid.

Plasma Binding—The extent of binding was determined using a previously described procedure (3), except that the dialysis bags were placed in a solution of either 0.4 mg of I-9-^{14}C or 0.5 mg of 5-chlorosalicylic-7-^{14}C acid dissolved in 5 ml of buffer. The flasks were shaken at 20 ± 2° for 18 hr to ensure equilibration. TLC confirmed the stability of both I and 5-chlorosalicylic acid under the experimental conditions.

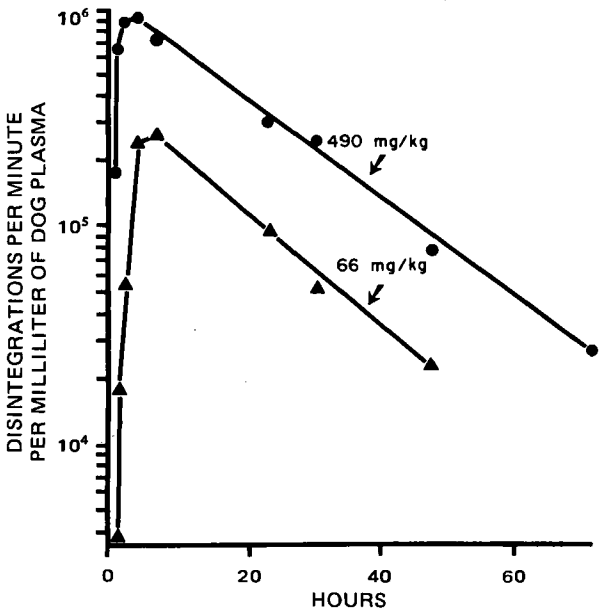


Figure 3—*Blood radioactivity after oral administration of I-9-^{14}C to dogs.*

Table III—Comparison of Theoretical and Actual Concentrations of 5-Chlorosalicylic Acid in Dog Plasma

Hours	5-Chlorosalicylic Acid, μg/ml					
	Dog 695[a]		Dog 795[a]		Dog 727[a]	
	Observed[b]	Calculated[c]	Observed[b]	Calculated[c]	Observed[b]	Calculated[c]
0	0	0	0	0	0	0
0.25	0	0	0	0	2	3
0.5	0	0	8	9	9	10
1	7	8	19	19	16	16
2	14	16	28	31	14	16
4	18	18	35	36	14	17
6	14	16	31	34	15	17
24	10	13	7	9	10	13

[a] Dogs received capsules containing I-9-^{14}C, 30.8 mg/kg, 42.9 μCi each. [b] By a GLC procedure. [c] From the amount of radioactivity in the plasma.

RESULTS

Absorption in Rats—The disappearance of radioactivity from the lumen of the intestine of an anesthetized rat, which had been rapidly infused with a solution of I-9-^{14}C, followed apparent first-order kinetics with a half-life of about 6 min. Results with a typical rat are shown in Fig. 1. After 25 min, more than 95% of the radioactivity of the administered dose had been absorbed and the clearance of ^{14}C from the gut lumen declined thereafter. The intestinal lumen contained intact drug for at least 20 min. After 46 min, in a single rat, less than 13% of the I remaining in the intestine had been hydrolyzed.

When the contents of the hepatic portal vein were investigated,

Table IV—Effect of β-Glucuronidase Treatment on Human Urine

Subject	Urine Sample Time Period, hr	5-Chlorosalicylic Acid, mg		
		Initial	After Treatment	Difference
1	0—24	38.3	57.5	19.2
	24—48	21.5	33.3	11.8
2	0—24	48.0	67.2	19.2
	24—48	38.8	46.7	7.9
3	0—24	15.5	62.1	46.6
	24—48	10.7	26.7	16.0
4	0—24	45.9	72.1	26.2
	24—48	9.6	10.6	1.0
Average	0—24	36.9	64.7	27.8
	24—48	20.2	29.4	9.2

Table V—Tissue Distribution of Radioactivity in Rat after Oral Administration of I-^{14}C[a]

Specimen	Radioactivity Recovered			
	I-9-^{14}C		I-3-^{14}C	
	dpm/g	% of Dose[b]	dpm/g	% of Dose[c]
Urine	13,470,000[d]	61.1	3,190,000[d]	62.0
Carcass	324,000	26.8	76,000	13.5
GI tract (including contents)	610,000	5.55	308,000	6.21
Liver	828,000	4.28	321,000	2.51
Kidneys	870,000	1.03	302,000	0.61
Lungs	793,000	0.56	143,000	0.18
Heart	500,000	0.21	131,000	0.13
Spleen	276,000	0.13	146,000	0.13
Respired Carbon Dioxide	—	0.08	—	16.1
Feces	189,000	0.003	377,000	0.29
Recovery, %	99.7		101.6	

[a]Animals sacrificed 24 hr after drug administration. [b]Animal received 4.0 mg of I-9-^{14}C, 95.8 μCi. [c]Animal received 2.9 mg of I-3-^{14}C, 39.8 μCi. [d]Disintegrations per minute per milliliter.

however, substantial quantities of I had been converted into 5-chlorosalicylic acid. About 8 min after the drug solution filled the intestine, the portal plasma contained equal concentrations of the metabolite and I. Thereafter, more 5-chlorosalicylic acid than intact I was present. Examination of the blood in the systemic circulation showed that the concentration of intact I was less in systemic blood than in portal blood during the initial 20 min. However, after absorption from the intestine had declined, the concentrations of I in the systemic circulation and in the hepatic portal vein were the same (Fig. 1). These results suggest that the I was metabolized during its passage through the intestinal wall. The ability of a preparation of minced intestine to convert 41.5% of the I present into 5-chlorosalicylic acid during a 90-min incubation period confirmed the metabolic activity of intestinal tissue.

Concentration in Circulation—Peak levels of blood radioactivity were found about 2 hr after the oral administration of I-9-^{14}C to the rat. The clearance of ^{14}C from the bloodstream followed apparent first-order kinetics, with a half-life of about 18 hr (Fig. 2). Maximal blood radioactivity values were attained within 90 min of the oral administration of I-3-^{14}C to rats, and the first-order half-life was about 8 hr (Fig. 2).

The absorption and elimination of the radioactivity of 9-labeled drug in two beagle hounds are shown in Fig. 3. Apparent first-order kinetics were followed for the decline in blood radioactivity. After a 0.5-g dose, 66 mg/kg, the half-life was about 11.5 hr; the feces contained only about 5% of the ^{14}C of the dose after 48 hr. After a 5.0-g dose, 490 mg/kg, the half-life was about 13.2 hr; only 27% of the radioactivity of the dose was found in the feces after 48 hr.

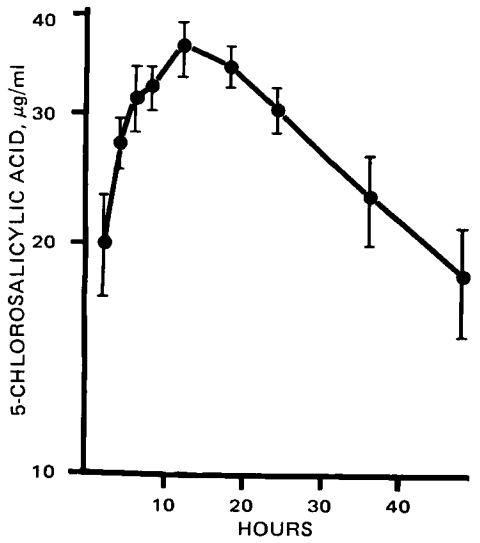


Figure 4—*Concentration of 5-chlorosalicylic acid in human plasma after oral administration of I (average of six subjects).*

Plasma samples from the dog that had received the larger dose were found by the GLC procedure to contain small amounts of I (Table II). Three other beagle hounds received 30.8 mg/kg of I-9-^{14}C; five of the samples, chosen at random, contained less than 1 μg/ml of intact I, as determined by GLC. The radioactivity of the samples was used to calculate the amount of 5-chlorosalicylic acid that would be present theoretically if all of the radioactivity were in that chemical form. The actual concentration of 5-chlorosalicylic acid was determined by GLC; the agreement between the theoretical and actual values is shown in Table III, which vividly demonstrates the extent of degradation of I to 5-chlorosalicylic acid.

Human Studies—Nonradioactive I was readily absorbed after oral administration to humans. No intact I could be detected in the plasma from any of the six subjects at either 2 or 4 hr after drug administration. However, the clearance of 5-chlorosalicylic acid, the metabolite of I, followed apparent first-order kinetics, with an estimated half-life of 33 hr (Fig. 4).

Incubation of the urine from four men who had ingested I with β-glucuronidase resulted in substantially higher levels of 5-chlorosalicylic acid, as determined by GLC (Table IV). Incubation of urine with buffer, in the absence of enzyme, had no effect. The nature of the bond between glucuronic acid and 5-chlorosalicylic acid was not determined.

GLC Assays—The relationship between peak height and the concentration of I was linear in the 1–10-μg/ml range. The repro-

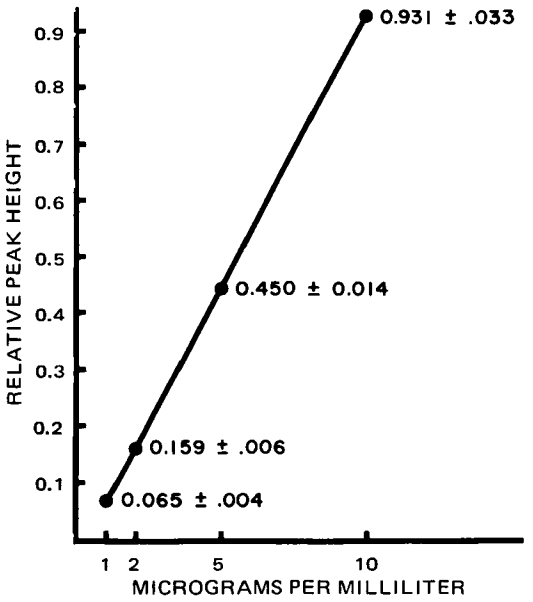
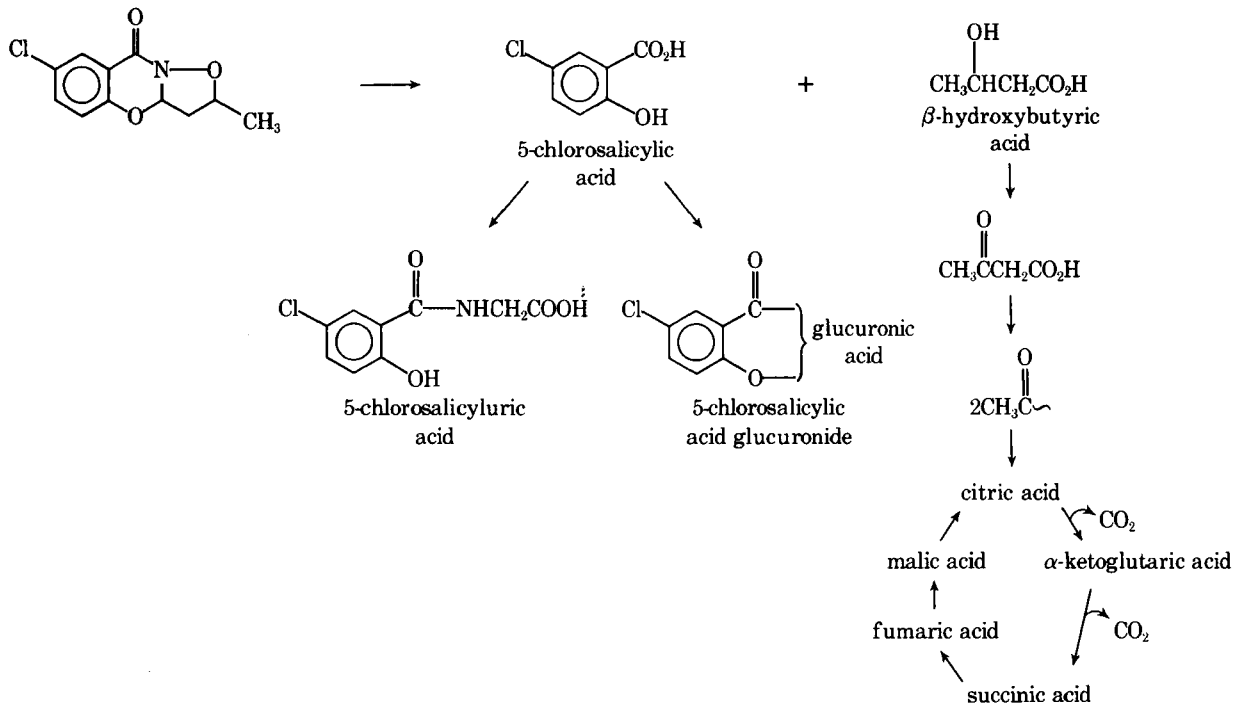


Figure 5—*Relationship between relative peak height and plasma concentration of I. Results are the means of quadruplicate determinations and are shown with their standard errors.*



Scheme I

ducibility of the technique was shown by the standard errors of quadruplicate analyses (Fig. 5).

Stability in Blood and Plasma—Compound I was not degraded by incubation with whole rat blood for 4 hr. Furthermore, the compound was stable in dog plasma for at least 51 days when stored in the freezer and for at least a week when refrigerated.

Tissue Distribution in Rats—A study of the distribution of radioactivity after the oral administration of I-9-^{14}C showed that the kidneys were the major route of elimination of ^{14}C within the 24-hr experimental period. Significant amounts of radioactivity were also found in the carcass, GI tract, and liver at that time (Table V).

A similar study with the 3-labeled drug showed that the radioactivity of the isoxazole moiety is also eliminated in rat urine. About one-sixth of the ^{14}C was exhaled as respired carbon dioxide. The location of the remainder of radioactivity is shown in Table V.

Identification and Quantitation of Urinary End-Products—Four radioactive substances were detected by TLC in the urine of dogs that had received I-9-^{14}C (Table I). Assignment of the glucuronide structure to the 5-chlorosalicylic acid conjugate was based upon the liberation of additional compound upon treatment of the urine with β-glucuronidase.

The identities and amounts of the urinary metabolites of I-9-^{14}C in the rat and dog are shown in Table VI. The major end-product was 5-chlorosalicylic acid. In the dog, this metabolite occurred in an unbound form and accounted for three-quarters of the urinary radioactivity. In the rat, there were about equal amounts of free 5-chlorosalicylic acid and its glucuronic acid conjugate. In both species, lesser amounts of the glycine derivative of 5-chlorosalicylic acid, 5-chlorosalicyluric acid, were found. Small quantities of unchanged drug were excreted in the urine of both species.

Isotope dilution experiments on the urine of a rat that had received I-3-^{14}C showed the presence of β-hydroxybutyric acid and other acids of the tricarboxylic acid cycle (Table VII). The presence of these compounds suggests that the labeled carbon dioxide found in respired air (Table V) came from the decarboxylation of the oxidation products of β-hydroxybutyric acid by Krebs' cycle enzymes.

Plasma Binding—Compound I was bound appreciably to the plasma proteins of all species studied (Table VIII), but it was bound less extensively than 5-chlorosalicylic acid.

DISCUSSION

Part of orally administered I is hydrolyzed upon passage through the intestinal wall during the absorption of the drug. The ability of the gut to hydrolyze I was verified by *in vitro* studies. No intact drug could be detected in the systemic circulation, unless the administered dose of I was very large; at a dose of 490 mg/kg, the maximum plasma concentration attained was only 8 μg/ml.

Since I was stable in blood and plasma, the unaltered drug that was absorbed into the circulation and subsequently converted into 5-chlorosalicylic acid was not hydrolyzed by plasma enzymes but was probably degraded by liver enzymes. In any event, labeled 5-chlorosalicylic acid was the metabolite of I-9-^{14}C that was present in the bloodstream, and this metabolite accounted for virtually all of the radioactivity of the administered drug.

Table VI—Quantitation of I-9-^{14}C Urinary End-Products in Rat and Dog

Urinary End-Product	Urinary Radioactivity, % Rat[a]	Dog[b]
5-Chlorosalicylic acid		
Free	46.1	73.6
Conjugated	47.7	7.3
5-Chlorosalicyluric acid		
Free	1.3	4.2
Conjugated	n.d.[c]	5.4
I	0.3	6.2
Total radioactivity recovered	95.4	96.7

[a] This rat received 2.5 mg of drug orally. [b] The dog received 502.2 mg of I-9-^{14}C orally. [c] None detected.

Table VII—Quantitation of I-3-^{14}C Urinary End-Products in Rat

Urinary Metabolite	Urinary Radioactivity[a], %
β-Hydroxybutyric acid	19.1
Citric acid	10.9
α-Ketoglutaric acid	24.9
Succinic acid	15.8
Fumaric acid	18.4
Malic acid	12.9
Total radioactivity recovered	102.0

[a] This rat received 4.6 mg of I-3-^{14}C orally.

Table VIII—Binding of I and 5-Chlorosalicylic Acid to Plasma

Species	Percent Bound[a]	
	I[b]	5-Chlorosalicylic Acid[c]
Human	80.5	99.4
Sprague—Dawley rat	54.6	71.1
Rhesus monkey	83.2	97.2
Beagle dog	78.9	97.5
Control, buffer only	−1.7	0.3

[a] Average of duplicates. [b] Initially there was 0.4 mg of I to be partitioned between 5 ml of plasma and 5 ml of buffer. [c] Initially there was 0.5 mg of 5-chlorosalicylic acid to be distributed between 5 ml of plasma and 5 ml of buffer.

In all three species, rat, dog, and human, cleavage of the oxazine ring of I yielded 5-chlorosalicylic acid, which was excreted into the urine in the free form and as conjugates with glucuronic acid and glycine.

The radioactivity from 3-labeled I appeared as respiratory carbon dioxide in exhaled breath and as β-hydroxybutyric acid and its metabolites fumaric, citric, α-ketoglutaric, succinic, and malic acids in the urine.

The metabolic fate of I is summarized in Scheme I.

CONCLUSIONS

The present study showed that I was converted to 5-chlorosalicylic acid by rats, beagle hounds, and humans and that the chemical entity present in the systemic circulation after the oral administration of I was not the intact drug but was the metabolite, 5-chlorosalicylic acid. The latter compound was excreted through the kidneys either free or conjugated with glucuronic acid and glycine. Elements of the isoxazole ring were converted to β-hydroxybutyric acid, which was metabolized by enzymes of the citric acid pathway.

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Comparative Anti-Inflammatory, Analgesic, and Antipyretic Activities of 7-Chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one and 5-Chlorosalicylic Acid in Rats

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Abstract □ Evidence is presented which indicates that 7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one (I) and 5-chlorosalicylic acid, its major metabolic end-product, are equally effective as anti-inflammatory and antipyretic agents, while the former is a somewhat more effective analgesic than its metabolite in the rat. However, at the equimolar doses used in this study, I is not ulcerogenic, while 5-chlorosalicylic acid does possess this untoward effect in the fasted rat. Moreover, the LD_{50} for 5-chlorosalicylic acid (261.0 mg/kg) is approximately 6.5 times less than that of I (1710.0 mg/kg) in the nonfasted rat. These results support the postulation that 5-chlorosalicylic acid is most likely responsible for the pharmacological activity displayed by I; *i.e.*, the latter acts as a carrier or delivery system, allowing attenuation of the toxic properties of its active metabolite.

Keyphrases □ 7-Chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one—anti-inflammatory, analgesic, and antipyretic activities compared to 5-chlorosalicylic acid, rats □ 5-Chlorosalicylic acid—anti-inflammatory, analgesic, and antipyretic activities compared to 7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one, rats □ Anti-inflammatory activity—7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)-(1,3)-benzoxazin-9-one and 5-chlorosalicylic acid compared in rats □ Analgesic activity—7-chloro-3,3a-dihydro-2-methyl-2*H*,-9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one and 5-chlorosalicylic acid compared in rats □ Antipyretic activity—7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3) - benzoxazin-9-one and 5-chlorosalicylic acid compared in rats

7-Chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)-benzoxazin-9-one[1] (I) has been reported to be an orally effective nonsteroidal anti-inflammatory, analgesic, and antipyretic agent in rats (1). Unlike most other clinically useful nonsteroidal anti-inflammatory drugs, I is not ulcerogenic at markedly effective doses nor does it promote GI blood loss. In addition, I has a mild diuretic action, which does not contribute to its anti-inflammatory action.

The preceding study (2) showed that, in both the

[1] Wallace Laboratories W-2395.