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Absorption, Excretion, Distribution and Metabolism of Perisoxal in Rats

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The drug was well absorbed from the intestinal tract, and the bulk of it was excreted in the urine and feces. Biliary excretion was significant, and the existence of enterohepatic circulation was considered likely. After intravenous injection, elimination of radioactivity from the blood and various tissues, except for fat, was rapid for 2 hr, then became slower. Elimination of unchanged drug from the blood was very fast.

Repeated oral doses did not change the excretion and distribution features as compared to a single oral dose. Significant accumulation of radioactivity was not caused by repeated doses of ^{14}C-labeled perisoxal.

Three oxidized metabolites, *p*-hydroxyperisoxal, *m*-hydroxyperisoxal and 4-hydroxyperisoxal, were identified. Excretion of hydroxyperisoxals in the urine (free and conjugates) was greater than that of perisoxal itself.

Keywords——3-(1-hydroxy-2-piperidinoethyl)-5-phenylisoxazole; perisoxal; basic drug; rat; absorption; excretion; distribution; metabolism

Perisoxal [3-(1-hydroxy-2-piperidinoethyl)-5-phenylisoxazole), an isoxazole analog,[2] possesses analgesic, anti-inflammatory and antitussive activities.[2a] Detailed pharmacology revealed that the analgesic and anti-inflammatory properties of perisoxal were more powerful than those of benzydamine or mefenamic acid but almost the same as those of mepirizole.[3] The antipyretic activity of perisoxal is a little weaker than that of benzydamine or aminopyrine. Very marked antitussive activity of perisoxal, almost comparable to that of codeine or dextromethorphane, was also observed.

The present paper deals with the absorption, excretion, distribution and metabolism of perisoxal in rats.

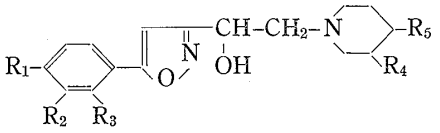
Experimental

Materials——Although perisoxal was administered as its citrate, all specific activities and doses of the drug are expressed in terms of the free base.

Three isotope-labeled perisoxal preparations were used for reasons of availability. Generally ^{3}H-labeled perisoxal ([^{3}H]perisoxal, 27.1 μCi/mg) and specifically ^{3}H-labeled perisoxal, substituted at 1-position of the ethyl chain moiety ([1-^{3}H]perisoxal, 31.1 μCi/mg), were purchased from Sinloihi Co., Ltd. (Kanagawa). The radiochemical purity of [^{3}H]perisoxal was more than 95% as determined by the reverse isotope dilution method. That of [1-^{3}H]perisoxal was determined by TLC, and no radioactive peak other than that corresponding to perisoxal was detected. ^{14}C-Labeled perisoxal, labeled at the 2-position of ethyl chain moiety ([2-^{14}C]perisoxal, 171.1 μCi/mg), was synthesized by Minato *et al.*[4] The radiochemical purity of [2-^{14}C]-perisoxal was more than 98% as determined by the reverse isotope dilution method.

1) Location: *a) 3-1 Tanabe-dori, Mizuho-ku, Nagoya; b) 5-12-4 Sagisu, Fukushima-ku, Osaka.*
2) *a)* H. Kano, I. Adachi, R. Kido, and K. Hirose, *J. Med. Chem.*, **10**, 411 (1967); *b)* H. Kano and I. Adachi, *Shionogi Kenkyusho Nempo*, **18**, 56 (1968).
3) K. Hirose, Y. Kojima, M. Eigyo, and H. Joyama, *Yakuri to Chiryo*, **2**, 1028 (1974); K. Hirose, Y. Kojima, M. Eigyo, and H. Sato, *Oyo Yakuri*, **6**, 1285 (1972).
4) H. Minato, T. Nagasaki, T. Yokoshima, K. Suga, and M. Yamaguchi, *J. Labelled Compounds*, **10**, 645 (1974).

Authentic samples of perisoxal metabolites, oxidized at the *p*-, *m*- and *o*-positions of the phenyl ring and 3- and 4-positions of the piperidinyl ring (*p*-, *m*-, *o*-, 3- and 4-hydroxyperisoxal), were synthesized by Hashimoto *et al.*[5]



	R_1	R_2	R_3	R_4	R_5
perisoxal	H	H	H	H	H
p-hydroxyperisoxal	OH	H	H	H	H
m-hydroxyperisoxal	H	OH	H	H	H
o-hydroxyperisoxal	H	H	OH	H	H
3-hydroxyperisoxal	H	H	H	OH	H
4-hydroxyperisoxal	H	H	H	H	OH

Chart 1

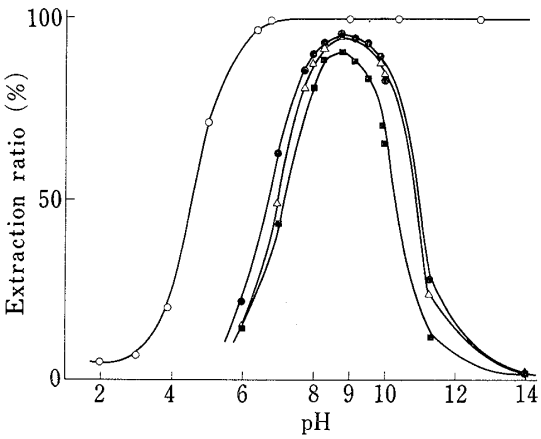


Fig. 1. Observed Extraction Ratios of Perisoxal and Hydroxyperisoxals by Ethylene Dichloride (EDC)

The μg of each compound was dissolved in 10 ml of EDC and shaken with the same volume of Britton–Robinson buffer solution (1/25 M H_3PO_4, 1/25 M CH_3COOH, 1/25 M H_3BO_3 and 1/5 N NaOH) or 1/10 N NaOH. The amounts of compounds in EDC were determined by measuring the absorbances at 262 nm (perisoxal), 281 nm (*p*-hydroxyperisoxal), 265 nm (*m*-hydroxyperisoxal) and 261 nm (*o*-hydroxyperisoxal). ○; Perisoxal, ●; *o*-hydroxyperisoxal, △; *m*-hydroxyperisoxal, ■; *p*-hydroxyperisoxal.

β-Glucuronidase (helix pomatia, Boehringer),[6] arylsulfatase (helix pomatia, Boehringer),[7] and other chemicals and solvents were of reagent grade.

Animals——Male Wistar rats weighing 230—270 g were used.

Excretion Study——[2-^{14}C]Perisoxal was administered to rats intravenously and orally at doses of 15.52 mg/kg in 0.9% NaCl solution and 73.93 mg/kg in aqueous solution, respectively. Intravenously dosed rats were held in a restraining cage and placed in a glass vessel attached to an air pump and CO_2-trap. Orally dosed rats were housed in a metabolic cage. Separate collections of urine, feces and/or CO_2 were made periodically for up to 24 hr after administration. Polyethylene catheters (Intramedic Polyethylene Tubing PE-50, Clay Adams) were introduced into the bile ducts of other rats and the same doses of [1-^{3}H]perisoxal were administered intravenously and orally. Bile and urine, collected periodically for up to 48 hr after administration, were diluted with H_2O and portions were used to count the radioactivity directly by liquid scintillation spectrometry using toluene-based scintillation cocktail (A), which contained 15% BBS-3 (Beckman), 0.6% 2,5-diphenyloxazole (PPO) and 0.01% 1,4-bis(5-phenyloxazole-2-yl)benzene (POPOP). Ethanolamine, which was used to absorb the exhaled $^{14}CO_2$, was mixed with methanol and toluene-based scintillation cocktail (B), containing 1.5% PPO and 1% bis-MSB (Packard) in a ratio of 3: 9: 7 (v/v), respectively, then the radioactivity was counted. Feces were mixed with H_2O and assayed as follows; about 100 mg of sample was combusted in a Packard 305 sample oxidizer. The $^{14}CO_2$ evolved was absorbed in ethanolamine and followed by the method used for the counting sample described above. The carcass was solubilized in 250 ml of 6 N HCl by heating at 106° according to Kozatani *et al.*[8] The solubilized carcass was made up to 600 ml with H_2O. A portion of this solution (200 μl) was treated with 200 μl of $HClO_4$ and 400 μl of 30% H_2O_2 according to Mahin *et al.*,[9] then analyzed for radioactivity by adding 6 ml of 2-ethoxyethanol and 10 ml of toluene cocktail (0.6% PPO).

Distribution Study——Rats were sacrificed by decapitation at 0.5, 1, 2 and 6 hr after an intravenous dose of [^{3}H]perisoxal in 0.9% NaCl solution, and the liver, kidney, lung, heart, spleen, fat (perirenal fat pads), muscle (gastrocnemius), testis and brain were isolated. Blood or tissues were taken into counting vials and assayed as described for the solubilized carcass.

In order to determine the blood level of unchanged perisoxal, 0.2 ml of blood was taken from the orbital vessel at 5, 10, 30 min and 1, 2, 4, 6 and 8 hr after administration. Blood samples were hemolyzed with 0.1 M NaOH and extracted with cyclohexane. The extract was evaporated to dryness under a stream of N_2 and the residue was dissolved in 150 μl of ethanol. This solution (100 μl) was chromatographed on a pre-

5) S. Hashimoto, M. Shizu, and S. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1757 (1976).
6) Arylsulfatase activity was less than 2%.
7) β-Glucuronidase activity was less than 2%.
8) J. Kozatani, M. Okui, K. Noda, T. Ogino, and H. Noguchi, *Chem. Pharm. Bull.* (Tokyo), **20**, 1105 (1972).
9) D.T. Mahin and R.T. Lofberg, *Anal. Biochem.*, **16**, 500 (1966).

coated thin-layer chromatography (TLC) plate (Silica gel F_{254}; 0.25 mm, Merck) in acetone–benzene–diethylamine (15: 15: 1, v/v) [solvent system (A)]. The area of perisoxal was removed from the plate, and extracted with ethanol. The ethanol extract was placed in a counting vial and evaporated to dryness. The residue was dissolved in a toluene-based scintillation cocktail (A).

Repeated Administration Study——[2-^{14}C]Perisoxal was administered orally to rats once a day for 10 days at a daily dose of 73.93 mg/kg in aqueous solution. Each rat was housed in a metabolic cage and urine and feces were separately collected every 24 hr after administration. Rats were sacrificed at 0, 1, 2, 4, 6, 8 and 24 hr after the last dose by exsanguination at the femoral artery and vein, and organs and tissues were isolated. Radioactivity in blood, tissue and feces was assayed as described above. Unlabeled perisoxal was administered orally to another three rats once a day for 10 days at a daily dose of 73.93 mg/kg in 0.9% NaCl solution, then 24 hr after the 10th dose [2-^{14}C]perisoxal was administered intravenously at a dose of 15.52 mg/kg in 0.9% NaCl solution. Five, 10, 30 min and 1, 2 and 4 hr after the injection 0.2 ml of blood was taken from the orbital vessel and the unchanged drug level was determined essentially as described above.

Metabolic Study——Rats were given 136.2 mg/kg of unlabeled perisoxal in aqueous solution subcutaneously. The urine excreted in 24 hr was combined and extracted twice with ethylene dichloride (EDC) after being adjusted to pH 10. The extract was evaporated to dryness *in vacuo* at 40°. The residue was dissolved in a small amount of methanol and chromatographed on a preparative TLC plate (Silica gel F_{254}; 0.75 mm or 0.5 mm) in solvent system (A). Metabolites separated by TLC were eluted with methanol. This procedure was repeated until each of the metabolites on the TLC plates gave one spot. These final eluates were subjected to various analyses for identification.

Determination of Urinary Metabolites——Dosing and Urine Collection: [2-^{14}C]Perisoxal was intravenously injected into rats at a dose of 15.52 mg/kg in 0.9% NaCl solution. Urine was collected during 0—8 and 8—24 hr after administration and diluted to 25 ml with H_2O.

Extraction of Free Base: The pH profiles of the extraction ratios of perisoxal and *p*-, *m*- and *o*-hydroxyperisoxal by EDC are shown in Fig. 1. Perisoxal was extracted by EDC almost completely above pH 6.8. On the other hand, hydroxyperisoxals were about 90—95% extracted at pH 8.9, but only 2—3% extracted at pH 14. Utilizing these properties, perisoxal and hydroxyperisoxals were extracted and separated. Fifteen ml of diluted urine was added to 5 ml of ammonium buffer (0.2 M, pH 8.9), then shaken with 15 ml of EDC three times. The EDC extracts were combined and made up to 50 ml. The aqueous layer was neutralized with HCl followed by acid or enzyme hydrolysis for the measurement of conjugates. Half of the extract was subjected to TLC separation for the measurement of perisoxal. The rest of the extract was shaken with 0.1 N NaOH to remove perisoxal followed by TLC separation for measurement of hydroxyperisoxals.

TLC Separation and Radioactive Counting: Twenty ml aliquots of extracts (both intact and treated with 0.1 N NaOH) were evaporated to dryness *in vacuo* at 40°. The residue was dissolved in a small amount of ethanol and subjected to TLC on a precoated Silica gel F_{254} plate using solvent system (A). Spots of perisoxal and hydroxyperisoxals were scraped off the plate and radioactivity was counted by the procedure described above.

Acid Hydrolysis: Three ml of aqueous layer was added to 2 ml of 4 N HCl and heated in a water bath for 1 hr. After neutralization with NaOH, it was extracted with EDC by the procedure described above.

Enzyme Hydrolysis: Three ml of aqueous layer was added to 2 ml of acetate buffer (0.2 M, pH 4.5) containing 1750 units of β-glucuronidase, then the mixture was incubated at 37° for 24 hr. A further 1750 units of enzyme was added to the mixture 4 hr after the beginning of incubation. Sulfatase hydrolysis was carried out following the above procedure with another acetate buffer (0.2 M, pH 6.2) and 1400 units of arylsulfatase. Hydrolyzed mixtures were subjected to EDC extraction.

Instruments——Radioactivity was measured with a Beckman DPM-100 or Nuclear Chicago system 720 liquid scintillation counter. The external standardization method was used to correct for quenching. Ultraviolet (UV) absorption spectra were recorded with a Hitachi EPS-3T spectrophotometer. Gas chromatography (GC) was carried out with a Shimadzu 4APTF gas chromatograph fitted with a flame-ionization detector, utilizing a 1.5 m × 0.4 cm i.d. glass column with 1% SE-30 on 100 mesh Gas chrom Q. The temperatures of the column, the injection port, and the detector were 200°, 240° and 250°, respectively. The carrier gas (N_2) flow rate was 50 ml/min. The samples were injected after trimethylsilylation with N,O-bis(trimethylsilyl)acetamide (Applied Science). Combined gas chromatography–mass spectrometry (GC-MS) was carried out with a NEVA 1740 gas chromatograph and a Hitachi RMU-6 mass spectrometer.

Results and Discussion

Excretion

Excretion and recovery of radioactive materials from rats after intravenous and oral administration of [2-^{14}C]perisoxal are shown in Table I.

Urinary excretion of oral doses was lower up to 8 hr, but became almost equal to that of intravenous doses at 24 hr. Approximately 50% of the radioactivity was excreted in feces

Table I. Excretion and Recovery of Radioactivity from Rats after an Intravenous or Oral Dose of [2-^{14}C]Perisoxal

Time (hr)	Excretion and recovery (% of dose)					
	Intravenous dose			Oral dose		
	Urine	Feces	Expired	Urine	Feces	Carcass
0— 4	20.40±1.23	n.d.	n.d.	11.11±0.92	n.d.	n.d.
0— 8	31.33±3.56	n.d.	n.d.	19.51±1.90	n.d.	n.d.
0—24	40.00±3.71	50.66±2.43	0.38±0.09	43.51±2.00	49.23±5.07	4.23±4.25
Total		91.04±6.04			96.97±3.34	

Values are means ±S.D. for 2 or 3 rats.
The dose was 15.52 mg/kg (intravenous) or 73.93 mg/kg (oral). n.d., not determined.

within 24 hr in either case. The excretion of $^{14}CO_2$ in the breath after an intravenous dose was less than 0.5% and the recovery from the carcass after oral dose in 24 hr was 4.23%. These findings indicate that the drug was well absorbed and the radioactivity excreted in the feces was not due to unabsorbed drug.

Table II shows that significant radioactivity was excreted in the bile, and the sum of biliary and urinary excretion in 48 hr was not significantly different between oral and intravenous doses. The good absorbability of perisoxal was also supported by this result.

Table II. Cumulative Excretion of Radioactivity in the Bile and Urine after an Intravenous or Oral Dose of [1-^{3}H]Perisoxal to Rats

Time (hr)	Excretion (% of dose)			
	Intravenous dose		Oral dose	
	Bile	Urine	Bile	Urine
0— 4	65.6±3.4	13.9±2.8	8.4	1.9
0— 8	71.0±2.2	16.5±2.5	18.6	4.0
0—24	73.5±1.7	18.0±2.1	62.5	13.2
0—48	74.0±1.7	18.5±2.0	80.7	18.2
Total	92.5±1.3		98.9	

Values are means ±S.D. for 5 rats (intravenous) or those obtained from 1 rat (oral).
The dose was 15.52 mg/kg.

Table III. Concentration of Radioactivity in Rat Tissues or Organs after an Intravenous Dose of [^{3}H]Perisoxal

Tissues or organs	μg equivalent of perisoxal/g wet tissue			
	0.5 (hr)	1 (hr)	2 (hr)	6 (hr)
Brain	17.9	7.1	3.0	1.6
Fat	13.1	6.0	3.7	0.5
Heart	7.8	4.7	2.6	1.6
Kidney	32.0	17.2	14.8	7.6
Liver	39.1	22.4	15.5	10.3
Lung	40.5	38.2	7.7	3.2
Muscle	5.6	3.1	1.9	1.7
Spleen	18.8	10.4	4.4	2.3
Testis	12.7	7.3	3.4	2.2
Whole blood	4.5	3.1	2.6	2.6
Blood plasma	6.3	3.7	3.2	3.3

Values are means for 2 rats.
The dose was 15.52 mg/kg.

The marked difference in excretion patterns between intact and bile-cannulated rats, as shown in Table I and II, suggests the existence of enterohepatic circulation of radioactive materials.

Distribution after Intravenous Administration

Table III shows the blood and tissue levels of radioactivities after intravenous administration of [^{3}H]perisoxal.

Radioactivity was rapidly taken up into all the tissues examined, and the decreasing order of concentrations at 30 min was: lung, liver, kidney, spleen, brain, fat, testis, heart, blood plasma, muscle and whole blood. The time courses of decreasing radioactivity in these tissues, except for fat, whole blood and blood plasma, were similar; for the first 2 hr there was a very rapid decrease, followed by a slower decrease. Radioactivity in fat decreased rapidly until 6 hr after the injection. On the other hand, the radioactivity in whole blood and blood plasma decreased relatively slowly, especially after 2 hr.

In another experiment, the radioactivity of unchanged perisoxal in whole blood was measured.

Figure 2 shows that the radioactivity of unchanged perisoxal in whole blood disappeared very quickly and that most of the perisoxal was transformed into several metabolites which were eliminated more slowly than unchanged perisoxal.

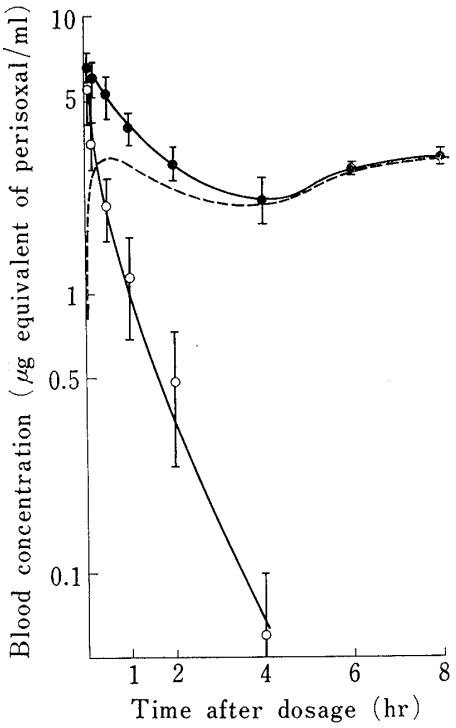


Fig. 2. Blood Concentration of Perisoxal in Rats after an Intravenous Administration of [^{3}H]Perisoxal

The dose was 15.52 mg/kg. Values are means ±S.D. for 3 rats.
—○—: perisoxal (P).
—●—: total radioactivity (T)
————: metabolites (M) (calculated using M=T−P).

Pepeated Administration

The urinary and fecal excretions of radioactivity during repeated daily oral doses of [2-^{14}C]perisoxal are shown in Table IV.

Table IV. Cumulative Excretion of Radioactivity during and after Repeated Oral Doses of [2-^{14}C]Perisoxal to Rats

Day	Excretion (% of total dose)	
	Urine	Feces
1	38.8±2.9	44.2± 7.1
2	40.0±3.1	44.8±16.0
3	39.9±4.8	49.8±12.4
4	40.2±6.1	45.2± 6.9
5	41.3±4.9	44.8± 6.1
6	42.2±4.3	46.8± 7.2
7	40.8±4.4	46.4± 4.5
8	39.1±5.8	45.8± 5.4
9	38.9±6.1	49.4± 8.1
10	41.0±4.0	43.6± 6.3

Values are means ±S.D. for 29 rats or 11 rats (10 th day).
The dose was 73.93 mg/kg once a day.

Daily excretions of radioactive materials in urine and feces were 38.8—42.2% and 43.6—49.8%, respectively, and no change due to repeated doses was observed.

The tissue distribution of radioactivity after repeated oral doses is listed in Table V compared with that after a single oral dose.

Tissue concentrations following repeated administration were higher than those after a single dose. However, except for the pancreas and spleen, these values fell essentially to those seen for a single dose, if the 0 hr values (the values 24 hr after the 9th dose) were

Table V. Concentration of Radioactivity in Rat Tissues or Organs after Repeated and Single Oral Doses of [2-^{14}C]Perisoxal

Tissue or organ		μg equivalent of perisoxal/g wet tissue						
		0 (hr)	1 (hr)	2 (hr)	4 (hr)	6 (hr)	8 (hr)	24 (hr)
Brain	a)	3.3	9.2	6.6	5.6	7.5	7.0	3.4
	b)		4.7	3.8	3.1	2.3	3.0	0.8
Spleen	a)	14.4	36.8	45.8	34.8	36.2	32.2	17.7
	b)		9.8	9.1	16.6	16.2	12.4	3.5
Heart	a)	6.8	21.9	13.7	9.6	13.6	11.7	7.0
	b)		7.8	5.9	4.4	4.6	6.4	1.4
Lung	a)	10.4	41.8	21.9	18.1	27.5	22.6	11.3
	b)		12.4	11.6	8.3	8.7	11.1	2.4
Kidney	a)	22.9	75.9	62.6	61.1	83.0	74.8	30.7
	b)		38.0	38.8	35.3	40.9	58.0	7.9
Liver	a)	39.3	112.1	88.1	94.7	118.6	98.6	49.9
	b)		70.5	65.3	46.6	58.9	58.4	14.4
Thymus	a)	6.0	14.3	10.1	8.3	14.2	10.8	5.6
	b)		4.6	4.5	3.6	6.4	4.8	1.4
Adrenal	a)	12.0	38.4	25.9	22.0	39.0	28.0	13.7
	b)		14.8	14.1	10.7	11.8	15.0	3.8
Testis	a)	3.3	9.2	6.7	6.8	8.2	7.5	3.4
	b)		3.7	3.6	3.3	2.7	3.8	0.9
Stomach	a)	12.2	409.9	681.0	685.9	490.2	153.9	16.3
	b)		513.4	473.8	297.2	216.6	156.7	3.7
Intestine	a)	14.5	244.1	283.9	295.4	287.0	228.1	24.9
	b)		252.7	383.2	427.3	300.7	287.7	9.3
Pancreas	a)	11.0	129.8	124.9	75.5	82.1	65.5	22.0
	b)		38.7	31.1	37.6	55.2	51.5	8.3
Hypophysis	a)	10.0	17.9	19.7	14.9	41.6	17.1	10.9
	b)		6.8	12.4	7.4	7.0	10.6	3.5
Submaxillary g.	a)	7.5	31.0	22.9	24.0	32.7	26.5	10.0
	b)		13.8	17.0	16.8	12.4	15.0	3.6
Thyroid	a)	18.3	19.8	29.0	24.8	35.9	35.3	19.2
	b)		3.4	7.5	4.8	7.5	7.5	3.9
Seminal vesicle	a)	14.4	31.4	80.1	80.0	211.9	89.4	20.0
	b)		22.8	94.5	127.9	59.1	172.7	3.5
Prostate	a)	6.9	16.6	28.5	40.5	39.2	30.6	7.3
	b)		17.0	34.2	58.9	13.0	82.6	2.5
Muscle	a)	3.2	6.9	7.1	6.0	7.8	6.6	3.0
	b)		3.0	3.2	3.1	2.8	3.4	0.7
Fat	a)	6.4	30.8	17.2	16.4	18.3	24.7	5.8
	b)		11.1	6.7	10.9	7.5	9.8	1.2
Whole blood	a)	9.1	13.2	12.2	11.8	14.4	13.8	9.6
	b)		4.9	6.5	4.3	5.0	6.8	1.9
Blood plasma	a)	4.8	10.0	8.0	8.2	10.8	10.5	5.0
	b)		5.8	7.7	4.6	6.3	7.8	1.6

Values are means for 3 rats.
a) Repeated oral administration of [2-^{14}C]perisoxal at a daily dose of 73.93 mg/kg.
b) Single oral administration of [2-^{14}C]perisoxal at a dose of 73.93 mg/kg.

subtracted from the actual values. These results suggest that the elimination of radioactivity from tissues is rather slow, but this did not cause significant accumulation of radioactivity.

The time courses of tissue levels, after both repeated and single doses, showed two peaks at 1 or 2 hr and 6 or 8 hr after administration in all tissues and organs. A similar tendency was also observed in intravenous doses as a slow decline of radioactivity from the tissues after 2 hr. The appearance of secondary peaks could be attributed to the enterohepatic circulation of metabolites and slow elimination of some kinds of metabolites.

Though the stomach and intestine levels were relatively low, the liver, kidney and pancreas showed very high levels. Comparable levels were seen in the heart, lung, adrenal, hypophysis, submaxillary gland, seminal vesicle and prostate. The elimination of radioactivity from the thyroid and spleen was very slow. Low concentrations were found in the brain, testis and muscle.

The highest concentration was found in the content of the alimentary tract. As shown in Fig. 3, the radioactivity in the stomach contents was the same as that of the unchanged drug but that in the small intestine was higher. Therefore it is suggested that the radioactivity in the stomach was due to unabsorbed drug whereas that in the small intestine originated from metabolites which were excreted *via* the bile.

The effects of repeated administrations of perisoxal on the elimination of unchanged drug were examined. Rats were dosed with unlabeled perisoxal orally once a day for 10 days and finally with [2-^{14}C]perisoxal intravenously. The blood level and pharmacokinetic parameters, *i.e.*, first-order elimination rate constant (k_{el}), biological half-life ($t_{1/2}$) and distribution volume (V_d), of the unchanged drug after the final dose are shown in Table VI.

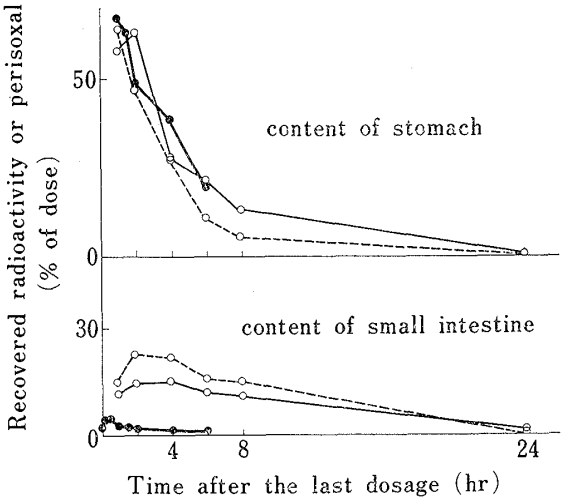


Fig. 3. Radioactivity Recovered in the Contents of the Stomach and Small Intestine after Repeated or Single Oral Administration of [2-^{14}C]Perisoxal in Rats

—○—: recovered radioactivity after repeated oral administration of [2-^{14}C]perisoxal for 10 days at a daily dose of 73.93 mg/kg.
—○—: recovered radioactivity after a single oral administration of [2-^{14}C]perisoxal at a dose of 73.93 mg/kg.
—●—: recovered unchanged drug after a single oral administration of unlabeled perisoxal at a dose of 73.93 mg/kg.

Table VI. Effects of Repeated Doses on the Blood Level of Unchanged Perisoxal after an Intravenous Injection of [2-^{14}C]Perisoxal into Rats at a Dose of 15.52 mg/kg

Rat		Blood concentration of perisoxal (μg/ml)						Pharmacokinetic parameters[a]		
		5 (min)	10 (min)	30 (min)	60 (min)	120 (min)	240 (min)	$k_{el}\pm$S.E.[b] (hr^{-1})	$t_{1/2}$ (min)	$V_d\pm$S.E.[b] (ml/kg)
Pretreated[c] with unlabeled perisoxal	1	3.33	3.26	2.22	0.91	0.32	0.14	1.23±0.12	33.8	5451±215
	2	4.12	3.65	1.88	0.77	0.22	0.05	1.84±0.08	22.6	4327±809
	3	3.90	3.50	2.22	1.27	0.26	0.03	1.29±0.04	32.2	4844± 64
	Mean	3.78	3.47	2.10	1.00	0.27	0.07	1.43±0.10	29.1	4860±140
Control[d]	4	3.47	3.18	1.63	0.69	0.22	0.05	1.76±0.11	23.6	5115±141
	5	3.57	2.92	1.63	0.70	0.23	0.05	1.61±0.10	25.8	5272±149
	6	4.19	2.83	1.87	0.76	0.23	0.05	1.85±0.33	22.5	4679±375
	Mean	3.74	2.98	1.71	0.72	0.23	0.05	1.74±0.12	23.9	5017±150

a) First-order elimination rate constant (k_{el}), biological half-life ($t_{1/2}$), and distribution volume (V_d) were calculated by fitting the blood concentrations to the following equations; $C=D/V_d\exp(-k_{el}t)$, $t_{1/2}=0.693/k_{el}$, where C is the blood concentration, D is the dose and t is time.
b) Standard error.
c) Pretreated by oral administration of unlabeled perisoxal for 10 days at a daily dose of 73.93 mg/kg in 0.9% NaCl solution.
d) Pretreated by oral administration of the vehicle (0.9% NaCl) for 10 days.

The parameters of both pretreated and control rats were similar. A slight difference of k_{el} between pretreated and control rats was not considered to be significant, since if the induction of a metabolizing enzyme was caused by repeated administration, k_{el} should be larger than in control rats.

Metabolism

In the preliminary study it was noted that about 40% of the radioactivity could be extracted by EDC at pH 10 from rat urine collected after a subcutaneous dose of [^{3}H]perisoxal, but only 3% could be extracted at pH 3. TLC of the extract showed the presence of unchanged drug and metabolic products. Thus, separation and identification of the basic metabolite was carried out using rat urine collected after a subcutaneous dose of unlabeled perisoxal.

After extraction by EDC, metabolites were purified as far as possible by successive TLC separations. Then, they were subjected to TLC analysis, color reaction analysis for phenolic OH, determination of UV absorption spectra and gas chromatography with authentic reference standards.

Table VII. Identification of Rat Urinary Metabolites of Perisoxal

Compound	*Rf*[a]				Color[b] reaction	λ_{max} in EtOH	Relative[c] retention time on GLC
	A	B	C	D			
Metabolite 1	0.94	0.77	0.68	0.72	−	262	0.40
Metabolite 2	0.49	0.37	0.34	0.27	+	265.5, 302	1.00
Metabolite 3	0.43	0.34	0.36	0.36	+	282	1.37
Metabolite 4	0.15	0.39	0.30	0.20	+	265, 303	
Metabolite 5	0.83	0.45	0.16	0.24	−	263	
Metabolite 6	0.52	0.27	0.16	0.20	±	255, 355	
Metabolite 7	0.56	0.32	0.17	0.18	−	262	1.00
Metabolite 8	0.90	0.56	0.38	0.48	−	269	
Metabolite 9	0.56	0.39	0.43	0.33	−	268	
Perisoxal	0.94	0.77	0.69	0.72	−	262	0.40
p-Hydroxyperisoxal	0.43	0.34	0.36	0.36	+	281	1.37
m-Hydroxyperisoxal	0.49	0.37	0.34	0.27	+	265, 303	1.00
o-Hydroxyperisoxal	0.51	0.36	0.30	0.30	+	261, 271.5, 307	0.80
3-Hydroxyperisoxal	0.61	0.47	0.37	0.30	−	268	0.77
4-Hydroxyperisoxal	0·56	0.32	0.17	0.18	−	261, 345	1.00

a) Plate; silica gel precoated sheet (Eastman chromagram sheet 6061), solvent; A, acetone–benzene–diethylamine (15: 15: 1); B, chloroform–benzene–methanol (5: 5: 1), C, benzene–methanol–ethyl acetate (6: 0.5: 3.5); D, chloroform–acetone (3: 2).
b) Diazobenzenesulfonic acid.
c) 1% SE-30, 1.5 m×0.4 cm i.d. column, 200°.

From the data listed in Table VII, metabolite 1, metabolite 2 and metabolite 3 were identified as unchanged drug, *m*-hydroxyperisoxal and *p*-hydroxyperisoxal, respectively. The data for metabolite 7 matched those for 4-hydroxyperisoxal, except for the UV absorption spectra. It is possible that the slight difference in UV absorption spectra between metabolite 7 and the reference 4-hydroxyperisoxal is due to the presence of some impurity in metabolite 7, and most of metabolite 7 might be 4-hydroxyperisoxal. To confirm this, metabolite 7 was subjected to GC–MS analysis. As shown in Fig. 4, the mass spectrum of the main peak of derivatized metabolite 7 was in accordance with that of 4-hydroxyperisoxal. Furthermore, Fig. 4 shows that metabolite 2, which had the same retention time on GC as metabolite 7, had the same mass spectrum as *m*-hydroxyperisoxal.

Based on the UV absorption spectrum having peaks at 265 nm and 303 nm, and its positive color reaction, it was considered that metabolite 4 might be a compound closely related to *m*-hydroxyperisoxal. For example, dihydroxyperisoxal, a hydroxylated product of *m*-hydroxyperisoxal, was considered as a possible structure, but a detailed study was unsuccessful.

The structures of metabolites 5, 6 and 8 were not elucidated, but these appear to be extremely minor metabolites on the basis of visual evaluation of the spots on TLC.

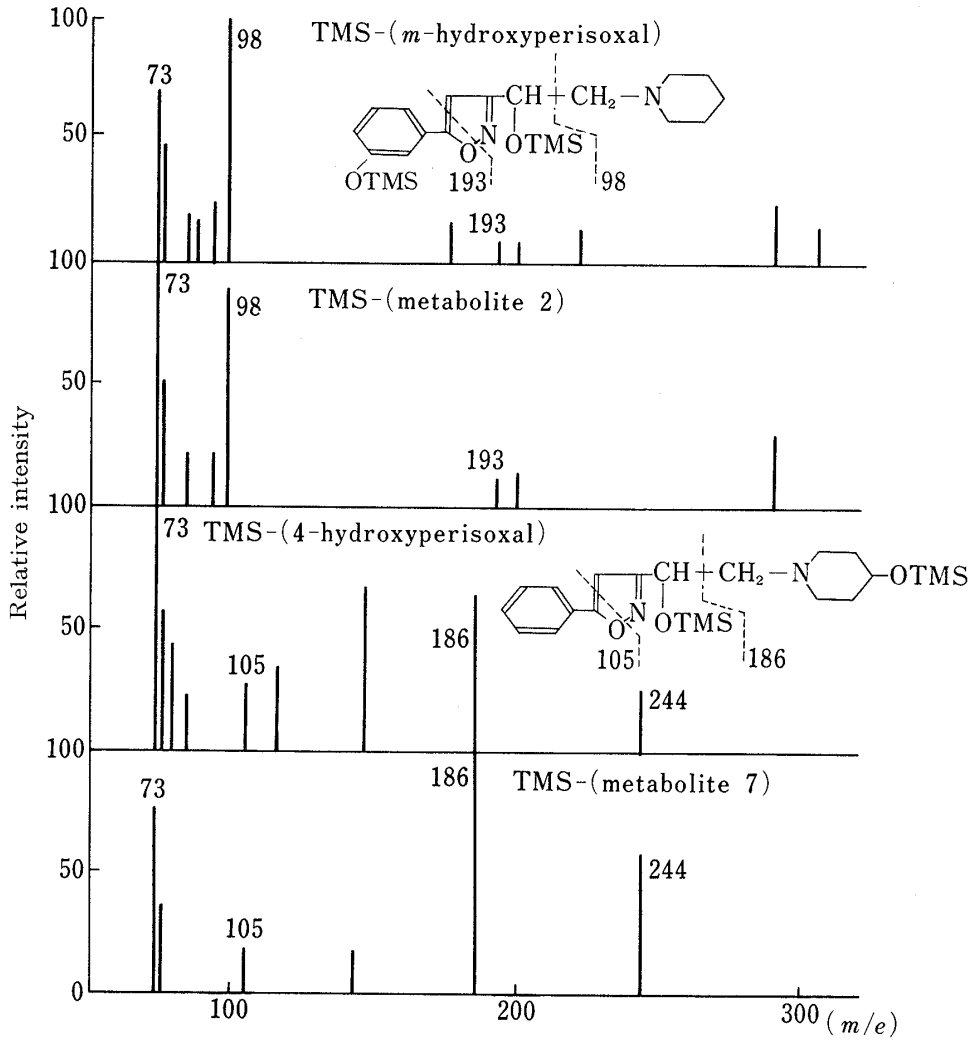


Fig. 4. Mass Spectra of Metabolite 2, Metabolite 7 and Reference Compounds

Each metabolite and reference compound was derivatized with N,O-bis-(trimethylsilyl)acetamide and subjected to GC–MS analysis.

TABLE VIII. Excretion of Perisoxal and Its Metabolites in Rat Urine[a] after an Intravenous Dose of [2-^{14}C]Perisoxal

Metabolite	Percent of urinary excretion
Perisoxal	
Free base	3.1
Glucuronide	2.2
Sulfate	0.9
Unknown conjugate (MX)	5.3
Hydroxyperisoxal (*p*, *m* or *o*-)	
Free base	2.2
Glucuronide	16.6
Sulfate	9.1
Others	
Free base	4.0
Glucuronide	7.1
Sulfate	4.9

Dose was 15.52 mg/kg.
a) 0—24 hr urine.

In this study, all the identified and possible metabolites appeared to be oxidized compounds. Hashimoto *et al.*[5] have reported on the *in vitro* rabbit metabolism, giving 3-[1-hydroxy-2-(N-oxidopiperidino)ethyl]-5-phenylisoxazole (perisoxal-N-oxide) and *p*-hydroxyperisoxal as major metabolic products and *m*-, 3- and 4-hydroxyperisoxal as minor products. Although the formation of perisoxal-N-oxide was not detected in this study, it was considered that oxidative reaction was the main metabolic route in the rat, because *m*-, *p*- and 4-hydroxyperisoxal were detected in rat urine, as in rabbit urine.

Urinary excretions of the unchanged drug, hydroxyperisoxals and their conjugates following intravenous injection of [2-^{14}C]perisoxal were measured. MX is an unknown conjugate of perisoxal and is readily hydrolyzed by acid. On the other hand, the glucuronide of perisoxal is hardly hydrolyzed by acid. Hydroxyperisoxals (*p*-, *m*- or *o*-) were measured as the total values because TLC separation of each isomer was very difficult.

As shown in Table VIII, approximately 50% of the radioactive material excreted in urine was identified as perisoxal, hydroxyperisoxal(s) and their conjugates. Excretion of total perisoxal was lower than that of hydroxyperisoxal. Excretion of hydroxyperisoxal glucuronide was predominant among identified materials. These results indicate that perisoxal is extensively metabolized, and that oxidation is one of the main metabolic routes of perisoxal.