average of the results is not significantly different from 100%, so the method does not appear to have any bias.

Ophthalmic products that contain phenylmercuric nitrate have other substances in the solution such as the active drug, reducing agents, buffer systems, and surfactants. To assess the effectiveness of the assay for phenylmercuric nitrate in ophthalmic products, the effect of these other substances on the assay was determined. Table II shows the approximate concentration of the excipient used to determine its effect on the assay. Concentrations of buffer pairs were such that the solutions were isotonic and at pH 7.

Table II shows four excipients that interfered completely with the phenylmercuric nitrate assay; *i.e.*, no end-point could be obtained. Although they are used in commercial products (10) with phenylmercuric nitrate, the drugs naphazoline hydrochloride and phenylephrine hydrochloride probably cause precipitation of insoluble phenylmercuric chloride even before the titrating begins, as shown in Scheme III.

$$C_6H_5—Hg^+ + Cl^- \rightleftharpoons C_6H_5HgCl$$

Scheme III

The K_{sp} for this reaction is 5.0×10^{-10} (8); therefore, the solubility limit of phenylmercuric chloride is exceeded in the phenylmercuric nitrate solutions containing naphazoline hydrochloride and phenylephrine hydrochloride. This would no doubt also happen in solutions of other halide salts. It was reported that antipyrine complexes with mercury compounds (11). If this complexation occurs with phenylmercuric nitrate, it might prevent the formation of the insoluble phenylmercuric salt, which is crucial to the titration. In contrast to the other systems, the effect of fluorescein sodium on the assay and the significant decrease of phenylmercuric nitrate in the presence of chloramphenicol remain unexplained.

In summary, this method of analysis for phenylmercuric salts should prove useful in applications requiring phenylmercuric nitrate determination in dilute solution.

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Stereospecific Assay and Stereospecific Disposition of Racemic Carprofen in Rats

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Abstract □ A procedure was developed for the separation and selective quantitative determination of the (*S*)(+)- and (*R*)(−)-enantiomers of the racemic anti-inflammatory drug carprofen as their diastereomeric *l*-(−)-α-methylbenzylamides. These derivatives are obtained in equivalent yields by reacting purified ^{14}C-carprofen from biological specimens with *l*-(−)-α-methylbenzylamine *via* the 1,1′-carbonyldiimidazole intermediate, followed by extraction and differential radiometric quantitation of the TLC-separated diastereomers. In the rat, the (*R*)(−)-carprofen enantiomer was eliminated from blood and secreted in the bile as the ester glucuronide at a rate approximately twice that of the (*S*)-(+)-enantiomer, resulting in the accumulation of the pharmacologically more active (*S*)(+)-enantiomer in the rat blood. Evidence for an additional process favoring the elimination of the (*R*)(−)-enantiomer in the rat was derived from pharmacokinetic data evaluation.

Keyphrases □ Carprofen—racemic mixtures, stereospecific assay, stereospecific metabolism, rats □ Enantiomers—carprofen, stereospecific assay, stereospecific metabolism, rats □ Anti-inflammatory agents—carprofen, racemic mixtures, stereospecific assay, stereospecific metabolism, rats

The anti-inflammatory agent carprofen (1), (D,L)-6-chloro-α-methylcarbazole-2-acetic acid (I), is a racemic compound with a chiral center at the α-carbon. The optically active carprofen enantiomers have been resolved, and their confirmations have been identified by X-ray analysis[1].

The properties of the dextrorotatory (*S*)(+)-enantiomer (II) and the levorotatory (*R*)(−)-enantiomer (III) have been compared in biological *in vivo* and *in vitro* tests (1). In the acute adjuvant arthritis test in the rat, the (*R*)-(−)-enantiomer was less than one-tenth as active (ID_{30}) as the (*S*)(+)-enantiomer. In contrast to the latter, the former was virtually inactive as an inhibitor of platelet aggregation, prostaglandin synthetase, and arachidonic acid-induced diarrhea; it produced no ulcerogenesis nor any other *in vivo* manifestation of toxicity in the rat.

Therefore, a stereoselective disposition of the two carprofen enantiomers could result in a different pharmacokinetic profile of the biologically active enantiomer from that previously determined by nonstereospecific procedures following administration of the racemate (2, 3). Possible stereoselective drug disposition processes include selective biotransformation reactions, tissue uptake,

[1] The identification by X-ray crystallography of the chiral configuration of the carprofen enantiomers was performed by Dr. J. F. Blout of the Roche Department of Physical Chemistry.

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Table I—Optical Enantiomer Ratio of ^{14}C-Carprofen

Amount of ^{14}C-D,L-Carprofen Reacted, μg	Reacted ^{14}C Extracted, %	Microgram Equivalents per TLC[a]	(S)(+), %	(R)(−), %	(S)(+)/(R)(−) Ratio	(S)(+)/(R)(−) Average
2.43	89.2	0.542	32.4	32.6	0.994	1.022
	90.2	0.548	37.7	35.8	1.050	
4.86	89.8	1.091	35.0	34.0	1.029	1.015
	90.0	1.093	39.5	39.5	1.000	
9.73	98.8	2.40	35.0	36.1	0.970	0.981
	96.3	2.34	34.7	34.4	1.009	
19.46	92.6	4.50	34.8	35.5	0.980	0.986
	95.2	4.63	33.6	33.9	0.991	

[a] Chromatographing one-fourth of the total reaction product (a larger aliquot may be chromatographed).

Table II—^{14}C-Carprofen Enantiomer Ratio in Rat Bile

Concentration of ^{14}C-D,L-Carprofen, μg/ml	Bile Volume, ml	Carprofen Recovered from Bile, %	Microgram Equivalents ^{14}C per TLC[a]	(S)(+), %	(R)(−), %	(S)(+)/(R)(−) Ratio	(S)(+)/(R)(−) Average
22.6	0.5	87.1	1.44	43.2	43.1	1.002	1.014
22.6	0.5	90.8	1.62	39.6	38.6	1.026	
45.2	0.5	92.3	3.13	32.5	32.9	0.988	1.034
45.2	0.5	94.8	3.45	38.2	35.4	1.079	
14.1	—	—	3.08	32.8	32.7	1.000	1.026
14.1	—	—	3.25	34.9	34.2	1.052	

[a] Indicates the total microgram equivalents of the diastereoisomers applied to the TLC plate in each experiment using a 50–200-μl aliquot. One microgram equivalent represents 1086 dpm and is detectable on the plate.

protein binding, and elimination of the two enantiomeric forms (4).

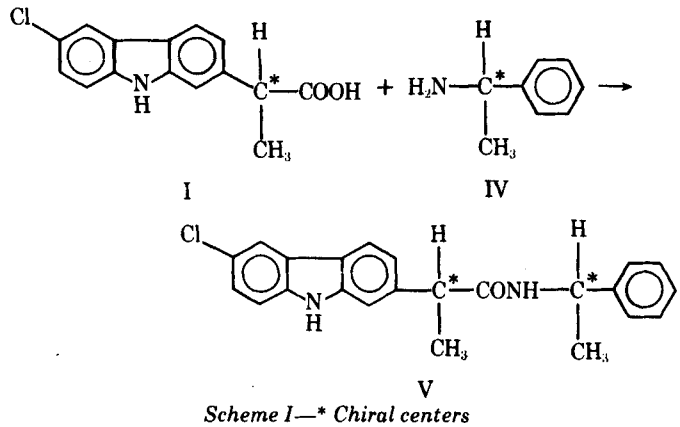
Closely related carprofen analogs, having in common an asymmetric α-phenyl propionic acid grouping, have been reported to undergo an enzymatic inversion at the chiral center at the α-carbon whereby the (R)(−)-enantiomer is converted to the (S)(+)-enantiomer. Lan *et al.* (5) demonstrated, in the rat and in the monkey, a conversion of the (R)(−)-form of cicloprofen (α-methylfluorene-2-acetic acid), to the (S)(+)-form accompanied by a stereoselective elimination process. Furthermore, the (R)(−)-enantiomer of the anti-inflammatory agent ibuprofen [2-(4-isopropylphenyl)propionic acid] underwent inversion to the (S)(+)-isomer in humans and, at the same time, was subjected to stereospecific oxidations at the isopropyl side chain leading to the formation of a second chiral center (6–9).

A procedure has been reported for the separate quantitation by GLC (7, 10) of the diastereomeric amides of ibuprofen obtained by reaction with *l*-(−)-α-methylbenzylamine. A similar, highly sensitive, selective procedure (4) involves the TLC separation and radiometric quantitation of the diastereomeric amides (peptides) obtained when reacting *d,l*-cicloprofen with ^{14}C-*l*-leucine.

This report describes a microprocedure that permits the selective determination of ^{14}C-carprofen diastereomers in biological fluids (11). Experiments applying this procedure to stereospecific pathways of carprofen metabolism in the rat (2) are described. In these studies, the chiral composition changes of free carprofen, the main drug component in the blood, and of carprofen ester glucuronide, a main biliary secretion product, were measured in carprofen-treated rats.

EXPERIMENTAL

Selective Determination of (S)(+)- and (R)(−)-Carprofen Enantiomers—The procedure relies on the: (*a*) reaction of racemic ^{14}C-carprofen (I) with *l*-(−)-α-methylbenzylamine (IV) to form the two diastereomeric *l*-methylbenzylamides (V) *via* the 1,1′-carbonyldiimidazole intermediate (7, 8) (Scheme I), (*b*) separation of the diastereomers by TLC, and (*c*) quantitation of radioactivity in the isolated TLC bands. From biological materials, I was extracted directly or after cleavage of its ester glucuronide and was purified by TLC prior to derivatization, as described previously (2).



Scheme I— Chiral centers*

Materials—*^{14}C-Carprofen (I)*—$C_{15}H_{12}ClNO_2$, mol. wt. 273.72. A stock solution of 1 mg/ml of ethanol was prepared. The original specific activity of the ^{14}C-labeled compound[2] of 9.91 μCi/mg was diluted in solution with appropriate amounts of unlabeled compound.

Table III—^{14}C-Carprofen Enantiomer Ratio in Pooled Rat Bile Specimen

^{14}C-Carprofen Specimen	^{14}C-Carprofen Reacted, μg	(S)(+), μg	(R)(−), μg	S(+)/R(−) Ratio
Standard added to rat bile	23.2	4.52	4.55	0.99
		6.04	5.72	1.06
Rat bile extract (see text)	14.52	2.26	4.48	0.510
		2.43	4.70	0.517
	29.04	4.84	8.89	0.540
		4.65	8.30	0.560
Mean ± *SD*				0.532 ± 0.023

[2] The labeled carprofen was synthesized by Dr. A. Liebman and Dr. R. Muccino of the Roche Department of Chemical Research.

Table IV—Secretion in the Bile of Total ^{14}C-Carprofen Ester Glucuronide and of Its (*S*)(+)- and (*R*)(−)-Enantiomers in Four Male Rats (Pooled Hourly Bile Fractions)

Biliary Fraction	0–1 hr		1–2 hr		2–3 hr		3–4 hr	
	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]
Total ^{14}C	14.40	14.40	12.30	26.7	9.45	36.15	7.55	43.70
Total ^{14}C-carprofen glucuronide	5.76	5.76	4.37	10.13	3.07	13.2	2.34	15.54
Hydroxy metabolites, conjugates plus polar components[c]	8.64	8.64	7.93	16.57	6.38	22.95	5.21	28.16
Ratio of carprofen (*S*)(+)/(*R*)(−)	0.46	—	0.56	—	0.69	—	0.81	0.58[d]
(*R*)(−)-Enantiomer	3.94	3.94	2.80	6.74	1.82	8.56	1.29	9.84
(*S*)(+)-Enantiomer	1.81	1.81	1.57	3.37	1.25	4.62	1.05	5.70
Excess (*R*)(−) [(*R*)(−) minus (*S*)(+)]	2.13	2.13	1.23	3.37	0.57	3.92	0.24	4.14

[a] Percent of dose per hour. [b] Cumulative percent of dose. [c] Unidentified polar component (after conjugate cleavage) represents ~20% of this fraction. [d] Calculated (*S*)(+)/(*R*)(−) ratio for 0–4-hr bile pool.

(S)(+)-*Enantiomer*—$C_{15}H_{12}ClNO_2$, mol. wt. 273.72, specific rotation in methanol $[\alpha]_D^{23}$ +53.7°.

(R)(−)-*Enantiomer*—$C_{15}H_{12}ClNO_2$, mol. wt. 273.72, specific rotation in methanol $[\alpha]_D^{23}$ −54.2°.

l-(−)-α-*Methylbenzylamine* (*IV*)—$C_8H_{11}N$, mol. wt. 121.18, bp 187°.

1,1′-Carbonyldiimidazole—$C_7H_6N_4O$, mol. wt. 162.15, mp 118–120°.

Solvent—A solution of 65 mg/ml of chloroform (stabilized with 0.75% ethanol) was prepared daily and was stable for 24–48 hr.

Reagents—The following reagent grade compounds were used: 6 *N* NaOH; 4 *N* HCl; pH 5 acetate buffer, 1 and 0.2 *M*; acetic acid; formic acid; methanol; ethanol (anhydrous); chloroform (stabilized); benzene; and ether (anhydrous).

TLC—TLC plates were precoated with silica gel 60 F-254[3]. Filter paper[4] was used for lining the TLC tanks. A shortwave UV lamp was used for visualization of spots on the TLC plates.

Derivatization—^{14}C-Carprofen standards (specific activity 0.5 μCi/mg) were processed with each series of unknowns in duplicate at 5 and 10 μg. The reaction was performed in 15-ml conical glass-stoppered centrifuge tubes. To the dried residues of the standards and the unknowns, 0.1 ml of the 1,1′-carbonyldiimidazole solution was added. Each tube was rotated to permit the reactant to contact the residue completely. The mixture was allowed to react at room temperature for 5 min; then 10 μl of acetic acid was added, and the solution was mixed. *l*-(−)-α-Methylbenzylamine, 50 μl, was added; then the solution was mixed well in the supermixer, centrifuged briefly to concentrate it at the tip of the tube, and allowed to react at room temperature for 20 min.

After completion of the reaction, 5 ml of ether and 3 ml of 0.2 *M* acetate buffer (pH 5) were added. The tubes were stoppered tightly, shaken for 10 min in a reciprocating shaker, and centrifuged for 5 min. As much ether as possible was transferred to a 15-ml conical centrifuge tube. The extraction was repeated with 5 ml of ether, which was combined with the previous extract and evaporated to dryness under nitrogen in a 30–40° water bath. The walls were rinsed, and the derivative was brought to the tip of the tube with 200 μl of ethanol. The solvent was evaporated, and the residue was dried thoroughly for 5 min in a desiccator under vacuum. The residue was redissolved in exactly 200 μl of ethanol, and duplicate 10-μl aliquots were counted.

Diastereomeric Derivative Quantitation—An aliquot of 50 or 100 μl was applied as a streak, 2–2.5 cm wide, on a precoated silica gel 60 F-254 plate (10 × 20 cm; two samples per plate). The solvent system was benzene–ether–methanol (60:35:5). The TLC tank was lined with solvent-soaked filter paper to achieve solvent saturation of the atmosphere. The solvent was allowed to ascend a distance of 14 cm from the origin to the front. The plate was examined under a shortwave UV lamp, and the absorbing bands corresponding to the diastereomeric methylbenzylamide derivatives (III) of the dextrorotatory (*S*)(+)-enantiomer at $R_f \simeq 0.36$ and the levorotatory (*R*)(−)-enantiomer at $R_f \simeq 0.41$ (carprofen R_f is

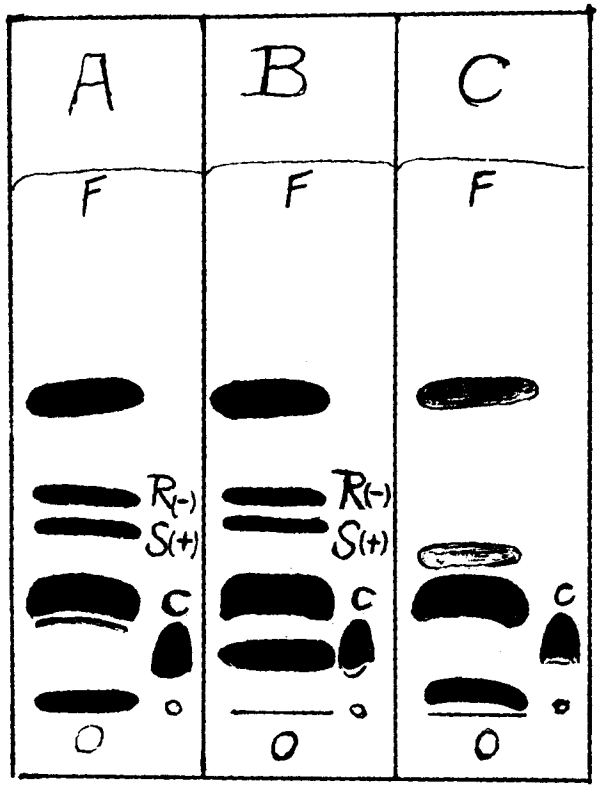


Figure 1—*TLC separation of the* (S)(+)- (R_f *0.47*) *and* (R)(−)- (R_f *0.55*) *diastereomeric* l-(−)-α-*methylbenzylamides of carprofen excreted in human urine. Key: A and B, duplicate aliquot of reaction mixture extract; C, control product; F, solvent front; O, origin; and c, carprofen standard.*

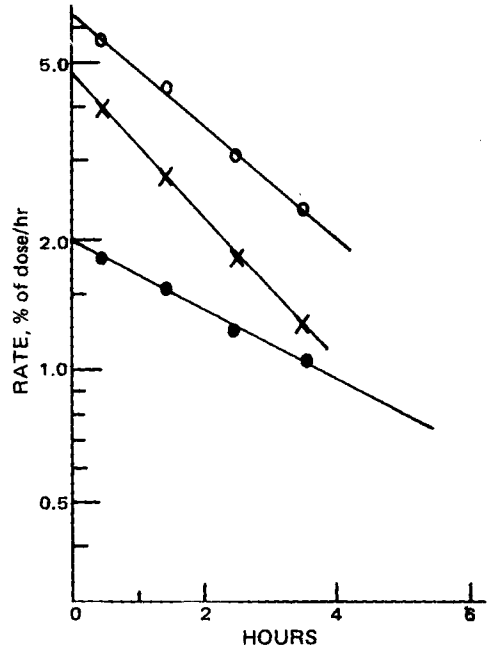


Figure 2—*Secretion of* ^{14}C-*DL-carprofen ester glucuronide* (O) *and of its individual* (S)(+)- (●) *and* (R)(−)- (×) *enantiomers. The dose was 3 mg/kg iv, measured in pooled bile from four male rats.*

[3] Brinkmann Instruments.
[4] Whatman No. 1.

Table V—Secretion in the Bile of Total ^{14}C, of ^{14}C-Carprofen Ester Glucuronide, and of Its (*S*)(+)- and (*R*)(−)-Enantiomers in Rat 1

Biliary Fraction	0–1 hr		1–2 hr		2–3 hr		3–4 hr		4–5 hr		5–6 hr	
	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]
Total ^{14}C	11.3	11.3	12.1	23.4	8.6	32.0	7.6	39.6	6.1	45.7	4.80	50.5
Total ^{14}C-carprofen glucuronide	4.56	4.56	3.98	8.54	2.68	11.22	1.95	13.17	1.39	14.56	1.09	15.65
Hydroxy metabolites, conjugates plus polar component[c]	6.74	6.74	8.12	14.86	5.92	20.78	5.65	26.43	4.71	31.14	3.71	34.85
Ratio of carprofen (*S*)(+)/(*R*)(−)	0.430		0.517		0.618		0.723		0.842		0.854	0.575[d]
(*R*)(−)-Enantiomer	3.19	3.19	2.62	5.81	1.66	7.47	1.13	8.60	0.755	9.35	0.588	9.93
(*S*)(+)-Enantiomer	1.37	1.37	1.36	2.73	1.02	3.75	0.82	4.57	0.635	5.21	0.502	5.71
Excess (*R*)(−) [(*R*)(−) minus (*S*)(+)]		1.79		3.06		3.69		4.02		4.14		4.22

[a] Percent of dose per hour. [b] Cumulative percent of dose. [c] Unidentified polar component (after conjugate cleavage) represents ~20% of this fraction. [d] Calculated (*S*)(+)/(*R*)(−) ratio for 0–6-hr bile pool.

0.18) were traced. Each band was scraped, and the scrapings were transferred to a counting vial; 2 ml of water plus 10 ml of counting cocktail solution[5] was added. Quantitation was by scintillation counting.

RESULTS AND DISCUSSION

Stereospecific Carprofen Assay—The linearity of the reaction and the relative reactivity of the two enantiomers were determined by directly reacting standard amounts of ^{14}C-carprofen, ranging from 2.43 to 19.46 μg of a specific activity of 1000 dpm/μg. The data presented in Table I indicate that the combined yield of the two disastereomeric derivatives was reproducible and ranged from 64 to 80%. When these standards of racemic ^{14}C-carprofen were reacted, the (*S*)(+)/(*R*)(−) ratio had a mean value of 1.009 (± 0.05 *SD*) for $n = 50$. For a 95% confidence level, the confidence limits for the (*S*)(+)/(*R*)(−) value were 0.91 and 1.11. This finding suggests equal reactivity of the two enantiomers. Doubling the *l*-(−)-α-methylbenzylamine concentration and the reaction time did not significantly increase the yield, indicating that the low yields were probably due to side-product formation rather than to incompleteness of the reaction.

Identical results were obtained with internal ^{14}C-carprofen standards added to control bile in the range 22–45 μg/0.5 ml and subjected to the alkaline condition for conjugate cleavage prior to extraction (Table II).

The separated diastereomers on the chromatogram were identified by separately derivatizing the unlabeled (*S*)(+)-enantiomer and the (*R*)(−)-enantiomer, along with the labeled racemate, and by cochromatographing the three products. The (*S*)(+)-derivative moved with the R_f 0.35 component, and the (*R*)(−)-derivative moved with the R_f 0.41 component.

Of a number of TLC solvent systems, benzene–ether–methanol (60:35:5), producing sharp and minimally trailing bands, appeared to give the best separation and the enantiomer ratios closest to unity (Fig. 1).

By extraction from the reaction mixtures with ether at pH 5, the methylbenzylamide derivatives (III) were quantitatively separated in sufficient purity for good TLC separation. Coextracted labeled side products did not interfere because of their much lower R_f value in this system. Attempts to remove putative remaining acidic material by an alkaline backwash of the ether extract proved not to be advantageous.

Good reaction yields and products could be achieved directly with carprofen extracted from bile after alkaline ester cleavage without further TLC purification. In the present study, however, all carprofen samples obtained from biological materials were purified routinely by preparative TLC prior to reacting to avoid unpredictable interference by coextracted material with the reaction and with the TLC diastereomer separation. Contamination by moisture and buffer salts accidentally transferred during the carprofen extraction into the ether interfered with the chemical reaction and led to the formation of polar reaction products.

In the described TLC assay, the sensitivity can be improved by increasing the specific activity of the labeled carprofen. By adding and coprocessing 10 μg of unlabeled carprofen as a carrier, the diastereomer detection on the TLC plate was facilitated without affecting the original enantiomer ratio that was measured radiometrically. For a specific activity of 1000 dpm/μg (0.5 μCi/mg) of ^{14}C-carprofen, the minimum amount of the compound that could be analyzed with accuracy was 0.6–1.0 μg, depending on the prevailing enantiomer ratio. To see clearly the diastereomer bands on the plate, at least 1.5 μg of total carprofen had to be reacted.

[5] Aquasol counting cocktail, New England Nuclear Corp., Boston, Mass.

Stereoselective Disposition of ^{14}C-D,L-Carprofen in Rats—In the rat, 80% of an intravenous carprofen dose (2) was eliminated by biliary secretion; the ester glucuronide of carprofen and the ethereal glucuronides of two phenolic metabolites represented the main biliary secretion products.

In an exploratory experiment, a specimen of ^{14}C-carprofen obtained by alkaline cleavage of the ester glucuronide and extraction from pooled 0–4-hr rat bile was analyzed. This bile originated from an experiment in which 5 mg of radioactive compound/kg (specific activity of 0.5 μCi/mg) had been injected into a group of male rats. The (*S*)(+)/(*R*)(−) ratio of this pooled bile specimen was 0.53 [35% (*S*)(+) and 65% (*R*)(−)], indicating a strong stereoselective effect (Table III).

To study this relative increase of the (*R*)(−)-enantiomer in bile as a function of time, consecutive hourly bile specimens that had been collected for 4 hr after dosing and pooled from four rats were examined. These young male rats (Charles River, 300–320 g) had received 3 mg of ^{14}C-carprofen/kg iv (specific activity of 0.5 μCi/mg). The relative amount of carprofen ester glucuronide secreted and the distribution of its enantiomers in the consecutive biliary fractions are listed in Table IV.

Calculating from these data the percentages of each enantiomer se-

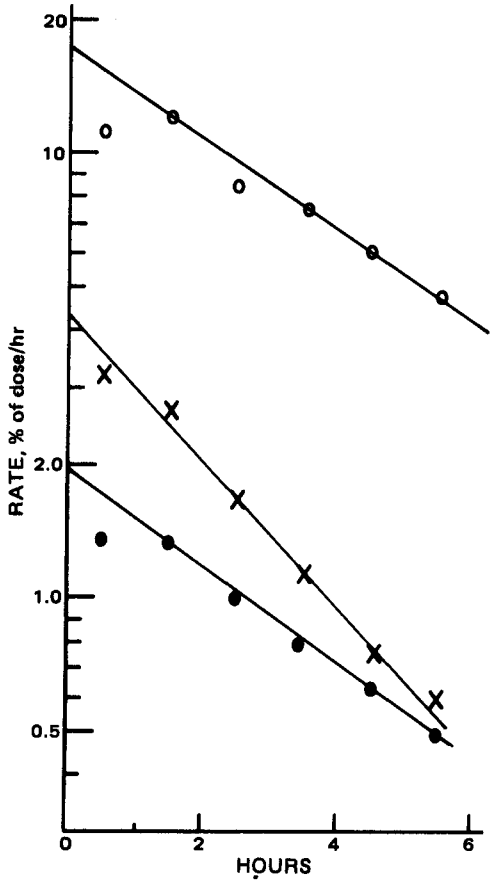


Figure 3—*Biliary secretion in Rat 1 of ^{14}C-DL-carprofen ester glucuronide* (O) *and of its individual* (S)(+)- (●) *and* (R)(−)- (×) *enantiomers. The dose was 3 mg/kg iv.*

Table VI—Secretion in the Bile of Total ^{14}C, of ^{14}C-Carprofen Ester Glucuronide, and of Its (*S*)(+)- and (*R*)(−)-Enantiomers in Rat 2

Biliary Fraction	0–1 hr		1–2 hr		2–3 hr		3–4 hr		4–5 hr		5–6 hr	
	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]	Per Hour[a]	Cumulative[b]
Total ^{14}C	12.8	12.8	12.7	25.5	9.4	34.9	7.3	42.2	5.7	47.9	4.4	52.3
Total ^{14}C-carprofen glucuronide	8.46	8.46	7.53	15.99	4.88	20.87	3.3	24.20	2.24	26.44	1.55	27.99
Hydroxy metabolites, conjugates plus polar component[c]	4.34	4.34	5.17	9.51	4.52	14.03	3.97	18.0	3.46	21.46	2.85	24.31
Ratio of carprofen (*S*)(+)/(*R*)(−)	0.494		0.612		0.718		0.858		0.994		1.083	0.66[d]
(*R*)(−)-Enantiomer	5.67	5.67	4.67	10.34	2.84	13.18	1.80	14.98	1.11	16.09	0.75	15.84
(*S*)(+)-Enantiomer	2.79	2.79	2.86	5.65	2.04	7.69	1.56	9.25	1.10	10.35	0.80	11.15
Excess (*R*)(−) [(*R*)(−) minus (*S*)(+)]		2.88		4.69		5.49		5.75		5.74		5.69

[a] Percent of dose per hour. [b] Cumulative percent of dose. [c] Unidentified polar component (after conjugate cleavage) represents ~20% of this fraction. [d] Calculated (*S*)(+)/(*R*)(−) ratio for 0–6-hr bile pool.

Table VII—Blood Levels and Elimination Rates of ^{14}C-D,L-Carprofen and Its Enantiomers in Rats

Rat	Hours	D,L-, μg/ml	Calc.[a]	(*S*)(+)/(*R*)(−) Ratio	(*S*)(+) Found, μg/ml	Calc.[a]	Percent Dose[b] Eliminated per Hour	(*R*)(−) Found, μg/ml	Calc.[a]	Percent Dose[b] Eliminated per Hour
1	0	—	11.40			5.83			5.74	
	1	9.81	9.53	1.143	5.23	5.11	6.17	4.58	4.45	11.24
	2	7.48	7.97	1.29	4.21	4.48	5.42	3.27	3.46	8.62
	3		6.69			3.94	4.62		2.721	6.45
	4	5.88	5.57	1.70	3.70	3.45	4.21	2.18	2.10	5.48
	5		4.72			3.04	3.52		1.643	3.92
	6	3.82	3.90	2.09	2.58	2.66	3.26	1.24	1.26	3.31
	k		0.179			0.131	0.131		0.253	0.247
	r^{2} [c]		0.992			0.980	0.996		0.997	
2	0		11.18			5.98			5.36	
	1	8.66	8.70	1.21	4.74	4.88	9.20	3.92	3.84	14.18
	2	6.67	6.67	1.52	4.02	3.99	7.44	2.65	2.75	10.17
	3		5.31			3.27	6.02		2.00	6.99
	4	4.27	4.10	1.95	2.82	2.66	5.10	1.45	1.41	5.51
	5		3.22			2.18	4.01		1.03	3.54
	6	2.42	2.48	2.39	1.71	1.77	3.43	0.72	0.73	2.84
	k		0.251	0.248		0.200	0.199		0.330	0.327
	r^{2} [c]		0.999			0.985	0.999		0.999	0.997

[a] $c = c_0e^{-kt}$, where c = blood level at time t, and c_0 = blood level at zero time. [b] Rate (percent dose per hour) = $(c_{n-1} - c_n)/c_0 \times 50$, where n = 1, 2, 3, 4, 5, and 6 hr, and c_n = blood level (micrograms per milliliter) at time n. [c] r^2 = multiple correlation coefficient.

Table VIII—Pharmacokinetic Parameters of Optically Selective Elimination of ^{14}C-D,L-Carprofen in Rats

Parameter	Rat 1				Rat 2			
	Total Carprofen	(*S*)(+)	(*R*)(−)	(*R*)(−)/(*S*)(+) Ratio	Total Carprofen	(*S*)(+)	(*R*)(−)	(*R*)(−)/(*S*)(+) Ratio
Biliary secretion								
k[a]	0.304	0.246	0.376	1.53	0.394	0.314	0.460	1.46
$t_{1/2}$	2.28	2.81	1.84	0.655	1.76	2.21	1.51	0.683
c_0	6.63	1.92	4.34	2.26	16.19	4.75	9.14	1.92
r^{2} [b]	0.995	0.994	0.994		0.996	0.999	0.999	
Blood elimination								
k[c]	0.174	0.131	0.248	1.89	0.247	0.199	0.327	1.64
$t_{1/2}$	3.98	5.29	2.81	0.53	2.78	3.48	2.12	0.61
c_0	17.67	6.55	12.50	1.91	24.8	10.1	17.0	1.68
r^2	0.991	0.996	0.997		0.992	0.999	0.997	
k bile/k blood elimination	1.75	1.88	1.52		1.60	1.58	1.41	

[a] Expressed as percent per hour. [b] Multiple correlation coefficient. [c] Blood level fall-off or elimination rate (percent per hour).

creted per hour and plotting the logarithms of these rates against time produced a linear fall-off curve (Fig. 2). The initially higher secretion rate of (*R*)(−)-ester glucuronide declined faster and approached the initially lower but more slowly declining secretion rate of the (*S*)(+)-enantiomer by the end of 4 hr. Furthermore, the data (Table IV) indicate that a 4.12% excess of the (*R*)(−)-enantiomer over the (*S*)(+)-enantiomer had been excreted during the 4-hr period.

This finding suggested the accumulation (retention) of a complementary amount of the (*S*)(+)-enantiomer during the same period and the development of an (*S*)(+)/(*R*)(−) ratio greater than unity in some other compartments, possibly in the plasma. Analysis of the carprofen fraction of a 4-hr plasma pool from the same four rats verified this conclusion, showing an (*S*)(+)/(*R*)(−)-enantiomer ratio of 2.24.

This change of the blood level ratio of the two carprofen enantiomers with time, together with the concurrent secretion rate of the enantiomeric ester glucuronides in the bile, was studied in two individual rats. Following bile duct cannulation, two male rats (200–300 g) were injected with 3 mg of D,L-^{14}C-carprofen/kg iv (specific activity of 2.5 μCi/mg) and were kept under continuous anesthesia (40 mg of pentobarbital sodium/kg) at controlled body temperature for 6 hr. Blood specimens of 0.4–0.6 ml were obtained at 1, 2, and 4 hr by tail clipping, in addition to a large terminal 6-hr specimen; bile was collected in six hourly fractions.

The biliary secretion data from both rats (Tables V and VI) and the semilogarithmic plots of the biliary secretion rates *versus* time (Figs. 3 and 5) were consistent with the results from the pooled rat specimens (Table IV and Fig. 2). In both rats, practically the same percentages of the dose were recovered as total biliary carbon 14. The fractions secreted as ^{14}C-carprofen glucuronide differed, being much higher in Rat 2 than in Rat 1. With it, a larger excess of (*R*)(−)-enantiomer was secreted by Rat 2.

The blood levels of (*S*)(+)- and (*R*)(−)-enantiomers (Table VII) of intact carprofen, when plotted semilogarithmically *versus* time (Figs.

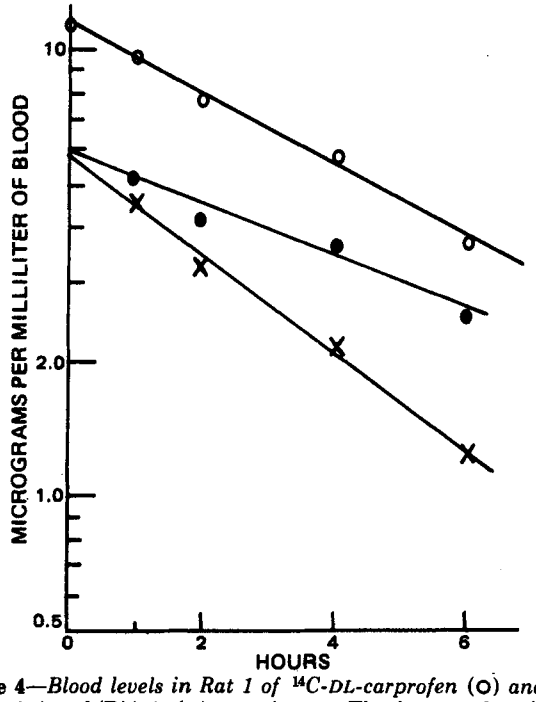


Figure 4—*Blood levels in Rat 1 of ^{14}C-DL-carprofen* (○) *and of its* (S)(+)- (●) *and* (R)(−)- (×) *enantiomers. The dose was 3 mg/kg iv.*

4 and 6), declined linearly. The elimination rate constants estimated for Rats 1 and 2 were higher for the (*R*)(−)-enantiomer than for the (*S*)-(+)-enantiomer by factors of 1.93 and 1.64, respectively.

If it is assumed that the distribution volume of intact free carprofen enantiomers remained constant over the experimental period, the blood level data can be characterized as elimination (disappearance) rates for each enantiomer, expressed as percent dose per hour (Table VII).

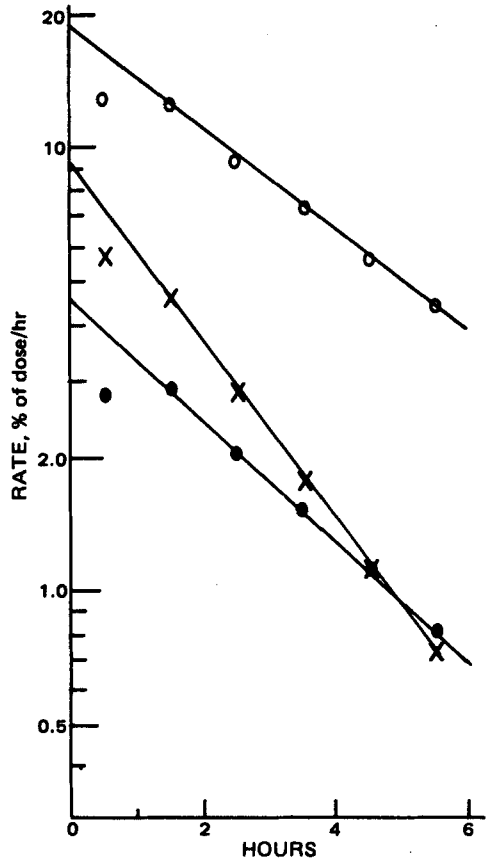


Figure 5—*Biliary secretion in Rat 2 of ^{14}C-carprofen ester glucuronide* (○) *and of its individual* (S)(+)- (●) *and* (R)(−)- (×) *enantiomers. The dose was 3 mg/kg iv.*

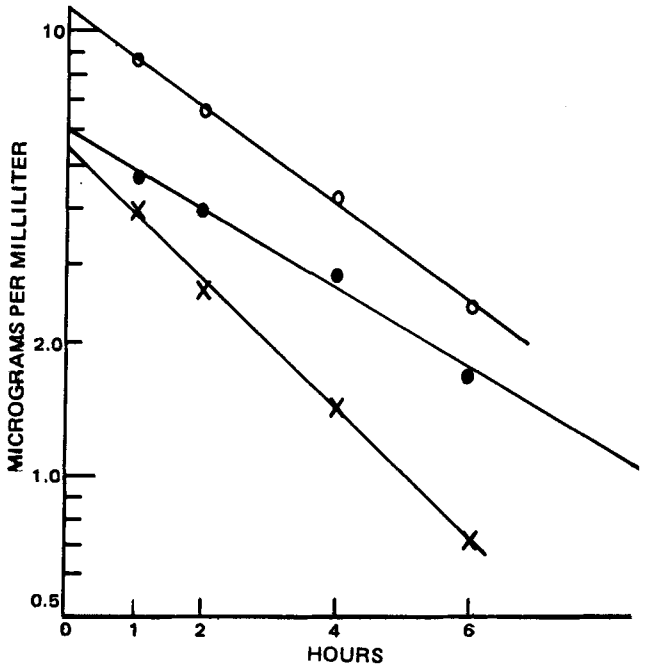


Figure 6—*Blood levels in Rat 2 of ^{14}C-DL-carprofen* (○) *and of its* (S)(+)- (●) *and* (R)(−)- (×) *enantiomers. The dose was 3 mg/kg iv.*

These elimination rate values for the (*R*)(−)- and (*S*)(+)-enantiomers in blood, when compared with the corresponding rates of biliary secretion of each enantiomer as the ester glucuronides (Tables V and VI), indicate that the excess (*R*)(−)-enantiomer secreted in the bile can account for only a fraction of the (*R*)(−)-excess eliminated from the blood during the same period. Based on an overall balance of both enantiomers, it can be estimated that ~64% of the (*R*)(−)-excess eliminated from the blood in Rat 1 was removed by an additional stereospecific process compared to ~37% in Rat 2, a difference that is related to the lower percentage secreted as the ester glucuronide of carprofen by Rat 1 as compared to Rat 2 (Tables V and VI). Evidence for an additional selective process is of interest in view of the reports of a metabolic chiral inversion of the (*R*)(−)- to the (*S*)(+)-enantiomer occurring in certain analogous compounds (3–6). While the data leave several possibilities open for such an additional process, including selective biotransformation, tissue uptake, and excretion, a chiral inversion process in which the (*R*)(−) is transformed to (*S*)(+)-enantiomer remains as a possibility equally compatible with the present data.

The pharmacokinetic constants derived from the data of the two rat experiments are presented in Table VIII. In both rats, the ratios of the (*R*)(−) to the (*S*)(+) rate constants for the elimination from blood and for the secretion into the bile are similar, ranging from 1.5 to 1.9. In both rats, however, the blood elimination constants for both enantiomers are significantly and consistently lower than the corresponding constants for biliary ester glucuronide secretion. This finding suggests that carprofen elimination from the blood into the bile as the ester glucuronide is a complex nonlinear process. The data at hand, therefore, do not permit a quantitative analysis of the elimination kinetics, nor do they allow quantitative predictions concerning other processes not directly measured but postulated from the data.

The main pharmacological conclusion from these stereoselective metabolic studies in the rat is that the biologically active (*S*)(+)-enantiomer is eliminated more slowly than the inactive (*R*)(−)-enantiomer and that, therefore, the half-life of the entity that counts pharmacologically is longer than that obtained when measuring total carprofen by a nonselective standard assay procedure.

The validity of this observation in respect to the disposition of this drug in humans and the possible effect of other stereoselective pathways on overall carprofen disposition in the rat and other species will be studied.

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ADS-12: An Automated Programmable 12-Tablet Dissolution Testing System

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Abstract □ A novel computerized automated system is described which conforms to the basic requirements set forth in the compendia for testing the dissolution characteristics of solid dosage forms. This modular system can test 12 tablets simultaneously. It can maintain sink conditions at all times and can handle any chemistry amenable to an automatic analyzer. Dissolution, sampling, chemistry, and readout are all accomplished simultaneously, reducing the complete test time for 12 tablets to the dissolution time itself.

Keyphrases □ Dissolution testing systems—automated programmable 12-tablet system □ Automated testing—dissolution testing system, automated, programmable, 12 tablet

In recent years, investigation of dissolution rates of solid dosage forms has become of prime importance to the pharmaceutical industry, due in part to the commitment by the Food and Drug Administration (FDA) to assure the bioavailability of active drugs from their various dosage forms. In a few cases, the FDA has already shown a correlation between *in vitro* dissolution results and *in vivo* bioavailability.

Dissolution testing can serve also as an important criterion of the effects of different constituents in drug formulations. In manufacturing, it can be useful for determining uniformity within and between different production batches. The USP considers this latter use to be so important that its 1980 revision will require a dissolution test for all tablet and capsule dosage forms. Guidelines for establishing dissolution requirements for oral solid dosage forms have been established already and are available from the USP.

BACKGROUND

Due to the increased testing workload that will result from this new USP requirement, many pharmaceutical companies have begun automating dissolution testing. Multiple automated dissolution testing apparatus have also been developed (1–3) and were reviewed critically in a previous paper (4), which pointed out that none of them met the requirements of a truly versatile, completely automated multiple dissolution testing system. The ADS-6 (4) (computerized six-tablet automatic dissolution testing system), which was developed in this laboratory in 1972, incorporates the USP-recommended dissolution apparatus (either the basket or paddle) and can test six tablets simultaneously. Unlike other systems, it is completely automated from tablet introduction to final readout of the raw data on punched paper tape. In addition, this system maintains sink conditions[1] at all times and performs any type of chemistry amenable to the automated analyzer. The raw data are computer analyzed in minutes, and final results are presented as a complete tabular report, a graphical dissolution profile, or both.

The ADS-12 has all of the features of the ADS-6, but it can test 12 tablets from one lot simultaneously, which is important in view of the latest USP requirement, or two different lots of six tablets each.

EXPERIMENTAL

Description of ADS-12—The ADS-12 is modular with seven functional components (Fig. 1).

Dual Six-Spindle Dissolution–Water Bath Assemblies[2] (*a*)—These assemblies contain the sample tablets in wire mesh baskets immersed in the dissolution flasks (USP Method I) or directly in the dissolution flasks (USP Method II). Basket rotation speeds are variable from 25 to 250 rpm. The bath temperature is maintained at 37°.

12-Channel Peristaltic Pump[3] (*b*)—The peristaltic pump has a pumping range of 0–50 ml/min. Each channel services a single dissolution kettle in the bath assembly. Silicone tubing is used to circulate liquid from the kettle continuously past the solenoid sampling valve assembly and to return it to the original kettle. In effect, a continuous sampling loop is formed.

If "induced" sink conditions are necessary due to very low solubility of the drug in the solvent used, then a second 12-channel pump is incorporated into the system. In this case, one pump continuously removes solution from the dissolution kettles at the proper rate while the second pump pumps fresh solvent back into the dissolution kettles at the same rate, thereby maintaining constant volume and sink conditions in the kettles.

Solenoid Sampling Valve Assembly[4] (*c*)—The valve assembly consists of 16 solenoid valves arranged in four blocks of four valves each. Three of the four valves per valve block are used in sampling the liquid from the dissolution kettles; the fourth valve is used for inserting an air segment between each liquid sample. The solenoid valves are of a single-channel design whereby the liquid sample passes from the sample loop *via* the T-connections, flows through the valve block into a five-pronged cross fitting, and finally flows to the sample pump tube of the automated chemistry system. The solenoid valves are operated automatically by an electrical signal put out by the system controller.

Figure 2 is a schematic view of two of the four solenoid valve blocks, showing the T-connections and the electrical hookup of the solenoid valves to the terminal bar. The solenoid valves are connected *via* this terminal bar to a circuit-indicating light box, which presents a visual

[1] Defined as never allowing the drug concentration in the dissolution flask to exceed 10–20% of saturation.
[2] Model QC-72R, Hanson Research Corp., Northridge, Calif.
[3] Model 7555-20 standard Ultramasterflex drive system, Cole-Parmer Co., Chicago, Ill.
[4] Catalog No. 18-4, General Valve Corp., East Hanover, N.J.

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