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GAS—LIQUID CHROMATOGRAPHIC RESOLUTION AND ASSAY OF TOCAINIDE ENANTIOMERS USING A CHIRAL CAPILLARY COLUMN AND STUDY OF THEIR SELECTIVE DISPOSITION IN MAN

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SUMMARY

Tocainide is a new antiarrhythmic agent that is used clinically as the racemic mixture. In order to study the disposition of the individual enantiomers in man, a gas—liquid chromatographic assay was developed based on the resolution of the *R*-(—)- and *S*-(+)-enantiomers as their heptafluorobutyryl derivatives on a capillary column coated with a chiral stationary phase. Two healthy male volunteers ingested an oral solution dose of racemic tocainide hydrochloride at a dose of 3 mg/kg and plasma and urine were collected at intervals for up to 54 h. Analysis of the plasma samples revealed a stereoselective disappearance of the *R*-(—)-enantiomer, such that the apparent half-life for the *S*-(+)- and *R*-(—)-enantiomers were 25.6 and 20.5 h, respectively in one subject and 11.1 and 9.0 h in the second subject. Similar relationships were observed in urine, where the ratio of the *S*-(+)- to *R*-(—)-enantiomers varied from 0.98 in 1 h to 3.03 in the 54-h samples in one subject over the same time period.

INTRODUCTION

Tocainide, 2-amino-2′,6′-propionoxylidide (I), is a relatively new antiarrhythmic agent presently undergoing clinical trials in Canada and the United States. Tocainide is a structural analogue of lidocaine (II), but unlike the latter agent is effective by the oral route. Several studies [1—4] have reported the pharmacokinetic parameters of tocainide in humans and have clearly demonstrated its high bioavailability by the oral route (90—100%), its rapid peak plasma levels (90 min) and its comparatively long apparent elimination half-life ranging from 11.7 to 29.1 h among subjects. Tocainide has been reported [4, 5] to undergo a novel biotransformation to tocainide carbamoyl-O-β-D-glucuronide (III) and 2-hydroxy-2′,6′-propionoxylidide (IV). Urinary excretion of the unchanged drug varies between 28—55% after an oral dose [4].

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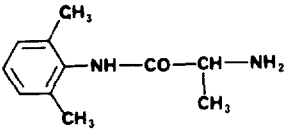

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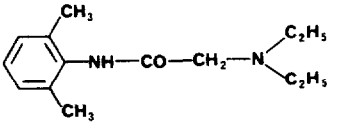

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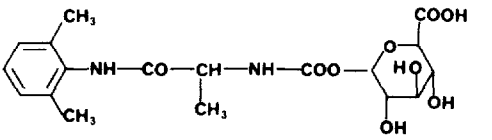

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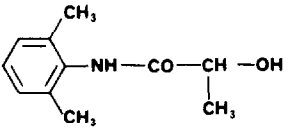

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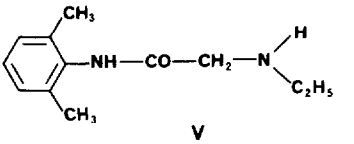

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Tocainide has a chiral centre and is used therapeutically as the racemic mixture. To date, the antiarrhythmic activity of the individual *R*-(—)- and *S*-(+)-enantiomers have only been tested in dogs and mice [6]. The results indicated that the *S*-(+)-isomer exhibits higher antiarrhythmic activity than its antipode. The possibility that the two enantiomers exhibit different pharmacokinetic parameters has been investigated in rats and mice [7] and the findings indicate that a preferential excretion of one isomer occurs, and that this is species dependent. In a preliminary study [8] conducted on two human subjects, plasma level ratios of *R*- and *S*-tocainide were found to differ in one of the volunteers. Unfortunately, the levels of the two enantiomers were only measured at 2 h and at 3 days following drug administration; hence firm conclusions could not be drawn on the stereoselective disposition of tocainide enantiomers in humans.

The present study was therefore undertaken to determine if sequential sampling of the absorption, distribution and elimination phases of tocainide enantiomers would reveal distinct differences in the ratios of these enantiomers in plasma and urine after oral administration of the racemic drug to human volunteers.

EXPERIMENTAL

Materials

R,S-Tocainide hydrochloride (Astra Pharmaceuticals, Mississauga, Canada), 2-ethylamino-2′,6′-acetoxylidide hydrochloride (Astra Pharmaceuticals Products, Worcester, MA, U.S.A.), carbobenzyloxy-D-alanine, N-*tert.*-butoxycarbonyl-L-alanine (Sigma, St. Louis, MO, U.S.A.), 2,6-dimethylaniline, N,N′-

dicyclohexylcarbodiimide, (+)-di-*p*-toluoyl-*d*-tartaric acid monohydrate, 30—32% hydrogen bromide in acetic acid (Aldrich, Milwaukee, WI, U.S.A.) and heptafluorobutyric anhydride (Pierce, Rockford, IL, U.S.A.) were used without further purification. Water and *n*-hexane were of HPLC grade (Fisher Scientific, Vancouver, Canada) and dichloromethane and benzene were distilled in glass quality (Caledon, Georgetown, Canada). All other chemicals and reagents were of analytical quality. Supplies required for blood collection were obtained from Becton-Dickinson and Co. (Mississauga, Canada).

Melting points were determined on a capillary tube melting point apparatus, Model 6406 (Thomas Hoover, Philadelphia, PA, U.S.A.) and are uncorrected. Optical rotation studies were conducted on a Perkin-Elmer Model 142 polarimeter (Perkin-Elmer, Norwalk, CT, U.S.A.) in a 10-cm tube at 25°C.

All analyses were carried out on a Model 5830A reporting gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with split/splitless injection modes and a ^{63}Ni electron-capture detector operated in the pulsed mode (150 μsec). Helium carrier gas flow-rate was maintained through the capillary columns at 1 ml/min with 25 ml/min passing through the split vent. Argon—methane (95:5) was used as the make-up gas at the detector at a flow-rate of 60 ml/min. The injection port and detector temperatures were 240°C and 350°C, respectively and the oven was operated isothermally at the conditions required for each assay procedure.

The 50 m × 0.2 mm fused silica capillary column coated with Carbowax 20M was prepared in the laboratory by a procedure reported earlier [9]. The oven temperature for this column was maintained at 180°C and samples were introduced in the split injection mode of the gas chromatograph.

The Chirasil-Val® 25 m × 0.25 mm borosilicate capillary column (Applied Science, State College, PA, U.S.A.) was operated at an oven temperature of 180°C for urine samples and 183°C for plasma samples. The latter temperature was found useful to facilitate the resolution of a small peak due to an endogenous substance in plasma from the two peaks for the enantiomers of tocainide.

Synthesis of R-(—)-tocainide hydrochloride

To a solution of 13.38 g (0.06 mole) of carbobenzyloxy-D-alanine and 7.3 g (0.06 mole) of 2,6-dimethylaniline in 150 ml of dichloromethane were added 13.6 g (0.066 mole) of N,N′-dicyclohexylcarbodiimide in 60 ml of dichloromethane. After the solution was left standing at room temperature for 1 h, the precipitated N,N′-dicyclohexylurea was filtered off and the solvent was evaporated from the filtrate under reduced pressure leaving 12.3 g of white solid, m.p. 167—168°C.

To remove the carbobenzyloxy-group, 70 ml of a solution of 30—32% hydrogen bromide in acetic acid were added to 12.3 g of the above reaction product, and the mixture was stirred until dissolved. To this solution, 200 ml of dry diethyl ether were added and the precipitated tocainide hydrobromide was filtered off and dried, yielding 8.2 g of white solid, m.p. 267°C. This material was converted to a hydrochloride salt and recrystallized from ethanol—diethyl ether to yield *R*-(—)-tocainide hydrochloride, m.p. 265—266°C, and $[\alpha]_D$ —42.16° (*c*, 2.63 in methanol). Literature [6] gave $[\alpha]_D$ —44.1° (*c*,

2.63 in methanol). A sample of this material was converted to its base and reacted with heptafluorobutyric anhydride as described below. Evaluation of the enantiomeric ratio using the Chirasil-Val column indicated that this material had a 95:5 ratio of the *R*:*S* enantiomers.

Synthesis of S-(+)-tocainide hydrochloride

To a solution of 18.9 g (0.1 mole) of N-*tert.*-butoxycarbonyl-L-alanine and 12.1 g (0.1 mole) of 2,6-dimethylaniline in 200 ml of dichloromethane were added 20.6 g (0.1 mole) of N,N′-dicyclohexylcarbodiimide. After the mixture was stirred at room temperature for 1 h, the precipitated N,N′-dicyclohexylurea was filtered off and the solvent was evaporated from the filtrate under reduced pressure, leaving 16.1 g of a creamy white solid, m.p. 131°C.

To remove the N-*tert.*-butoxycarbonyl group, 50 ml of 30—32% hydrogen bromide in acetic acid was added to 10 g of the above reaction product and the mixture was stirred until dissolved. To this solution 200 ml of dry diethyl ether were added and the precipitated tocainide hydrobromide was filtered off and dried, yielding 7.8 g of white solid, m.p. 274°C. This material was converted to a hydrochloride salt and recyrstallized from ethanol—diethyl ether to yield *S*-(+)-tocainide hydrochloride, m.p. 266°C. A sample of this material was evaluated as its heptafluorobutyryl derivative on the Chirasil-Val column and was found to consist of a 81:19 ratio of the *S*:*R* enantiomers.

To increase the optical purity of this product, the tocainide base obtained from 1 g of the above material was added to a warm solution of 1.4 g of di-*p*-toluoyl-*d*-tartaric acid in 10 ml of 95% ethanol. The diastereoisomeric salt was crystallized five times at room temperature to yield fine needles. This material was converted to its hydrochloride salt and recrystallized from ethanol—diethyl ether to yield *S*-(+)-tocainide hydrochloride, m.p. 266°C, $[\alpha]_D$ +42.35° (*c*. 2.64 in methanol). Literature [6] gave m.p. 264.5—265.5°C, $[\alpha]_D$ +41.7° (*c*. 2.64 in methanol). An evaluation of the enantiomer ratio of the heptafluorobutyryl derivative on the Chirasil-Val column indicated a ratio of 91:9 of the *S*:*R* enantiomers.

Stock solutions

Racemic tocainide hydrochloride and the internal standard, 2-ethylamino-2′,6′-acetoxylidide hydrochloride (V), were dissolved in HPLC grade water at concentrations of 1 μg/ml and 10 μg/ml, respectively.

In-vivo study

Two healthy male volunteers (38 years, 68 kg and 39 years, 98 kg) fasted overnight for 12 h and were administered 100 ml of an oral solution of racemic tocainide hydrochloride at a dose of 3 mg/kg body weight under the supervision of a physician. Food was allowed after 3 h and water was allowed ad libitum. An indwelling butterfly cannula was inserted in the cubital vein of the arm by a medical laboratory technologist and was used to collect blood samples for the first 7 h. Subsequent blood samples were collected in 10-ml heparinized Vacutainers®. Blood samples (approximately 8 ml) were collected at 15-min intervals for the first 2 h and at 3, 5, 7, 24, 48 and 72 h thereafter. The cannula was flushed with sterile isotonic heparin solution (50 units in 1 ml) after each

collection). Blood samples were centrifuged at 2300 *g* and the plasma was separated and stored at −20°C until required for assay. Urine samples were collected in polyethylene bags at 0, 1, 2, 3, 5 and 7 h and as convenient for the subject thereafter up to 54 h. Urine samples were stored at −20°C without treatment until required for assay. Samples of plasma and urine were obtained before drug ingestion to serve as blanks and for determination of calibration curves.

Gas chromatographic assay

To 0.5 ml of plasma or 0.1 ml of urine were added, 0.1 ml of the internal standard solution, 0.2 ml of 0.1 *N* sodium hydroxide solution and 5 ml of dichloromethane. The tubes were tumbled for 15 min on a Model 343 Roto-Rak tube tumbler (Fisher Scientific). After centrifugation at 1600 *g* for 10 min, 4 ml of the organic phase were transferred to a 10-ml PTFE-lined screw-capped culture tube and the contents were evaporated under a gentle stream of clean dry nitrogen which had been passed through a gas purifier, Model 451 cartridge (Matheson, Edmonton, Canada). The residue was dissolved in 100 μl of *n*-hexane, 20 μl of heptafluorobutyric anhydride were added and the tightly capped tubes were heated at 55°C for 30 min in an aluminum block (Thermolyne Dri-Bath, Fisher Scientific). The excess reagent and solvent were removed under a stream of clean dry nitrogen and the residue was reconstituted in 200 μl of *n*-hexane. A 1—2 μl aliquot was used for chromatographic assay on the Chirasil-Val column. The procedure was repeated for assay on the Carbowax column. The stability of the derivatives stored in hexane at 4°C were tested over four weeks and evidenced no detectable change in peak area ratios between enantiomers and their relationship to the internal standard.

Determination of calibration curve and detector linearity

To 0.5 ml of blank plasma were added 100, 250, 500, 750, 1000, 1500 ng of racemic tocainide hydrochloride contained in the stock solution and 0.1 ml of (1000 ng) the internal standard solution. In a similar fashion, 100, 200, 400, 1000, 2000 ng of the racemic tocainide hydrochloride stock solution were added to 0.1 ml of urine along with 0.1 ml of the internal standard solution. Triplicate samples of plasma and urine thus prepared, were extracted and derivatized as described above.

RESULTS AND DISCUSSION

Direct resolution of enantiomers of chiral amino compounds on optically active stationary phases offers several advantages compared to other methods which require the preparation of diastereoisomers by reaction with asymmetric reagents such as N-trifluoroacetyl-(*S*)-prolyl chloride [9,10] or (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride [7, 8]. In the latter methods, however, the optical purity of the resolving agent, its stability during synthesis, storage or derivatization, and volatility of the diastereoisomers thus formed, are frequent problems in the routine and accurate assay of enantiomeric amines by the use of such reagents. The readily prepared and stable monoheptafluorobutyryl derivative of tocainide enantiomers has excellent chromatographic

properties and the resulting isomer derivatives are well resolved on a chiral column coated with N-*tert.*-butyl-L-valine-*tert.*-butylamide. This chiral phase, also known as Chirasil-Val, has been reported to resolve *R,S*-mixtures of amino acids [11]. The mechanism of resolution of such optical isomers on this phase appears to be due to the transient formation of diastereoisomers in the column by hydrogen bonding [12].

In the resolution of the optical isomers of tocainide using the Chirasil-Val phase, the identities of the enantiomers were established by comparison with retention times of these enantiomers prepared by a stereoselective synthesis starting from *S*-(+)- or *R*-(—)-alanine based on the method originally described by Byrnes et al. [6]. The synthetic approach is similar to that used in peptide synthesis, and from examination of the resulting products by their optical rotation, and comparison with literature values [6], it was observed that the optical purity was retained to a large extent. Accordingly, the first peak to elute from the Chirasil-Val column was designated the *S*-(+)-enantiomer.

For purposes of analysis of human plasma and urine samples, detector linearity and assay precision were determined from calibration curves constructed from blank plasma and urine samples obtained from the two volunteers prior to ingestion of the drug. Accordingly, linearity was established over the range of 0.2 to 3 μg/ml of *R,S*-tocainide hydrochloride in plasma and from 1.0 to 20 μg/ml in urine. Each calibration curve was constructed from triplicate samples of each of five concentrations of *R,S*-tocainide hydrochloride. The data given in Table I show the mean slopes and correlation coefficients. The mean correlation coefficients for plasma and urine were 0.9998 and 0.9989, respectively.

TABLE I

CALIBRATION CURVE DATA FOR PLASMA AND URINE

Quantity of each tocainide enantiomer (ng)*	Plasma area ratios (± S.D.)**		Urine area ratios (± S.D.)**	
	S-(+)	*R*-(—)	*S*-(+)	*R*-(—)
50	0.0449 ± 0.0026	0.0383 ± 0.0075	0.0719 ± 0.0100	0.0487 ± 0.0116
100	—	—	0.0965 ± 0.0141	0.0965 ± 0.0141
125	0.1129 ± 0.0136	0.1048 ± 0.0150	—	—
200	—	—	0.2339 ± 0.0509	0.2092 ± 0.0573
250	0.2181 ± 0.0284	0.2088 ± 0.0273	—	—
375	0.3404 ± 0.0030	0.3115 ± 0.0074	—	—
500	0.4515 ± 0.0136	0.4110 ± 0.0165	0.6823 ± 0.0811	0.6318 ± 0.0789
750	0.6748 ± 0.0090	0.6121 ± 0.0080	—	—
1000	—	—	1.2987 ± 0.1444	1.2833 ± 0.1746
Correlation coefficient	0.9998	0.9998	0.9989	0.9996
Mean slope	0.9024	0.8171	1.3210	1.3161
Y-Intercept	—0.0012	0.0018	—0.0108	—0.0330

*Tocainide hydrochloride used as the racemate mixture.

**Area ratio determined for drug/internal standard. Standard deviations calculated for three aliquots for each weight of *R,S*-tocainide hydrochloride.

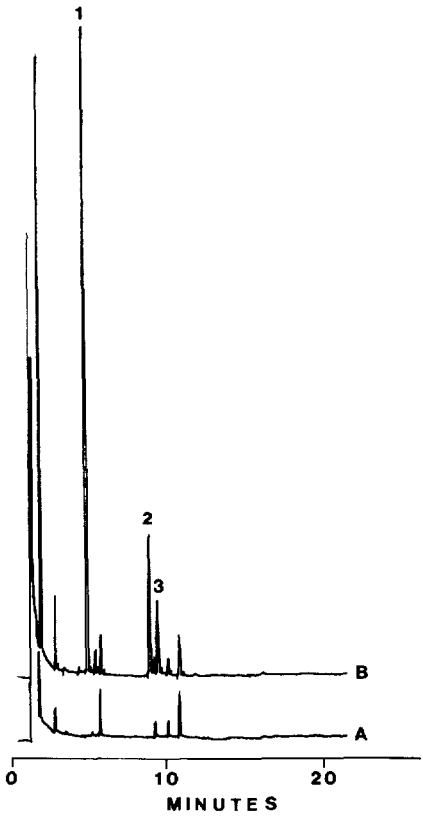

Fig. 1. B = Chromatogram of the heptafluorobutyryl derivatives of tocainide enantiomers extracted from plasma 24 h after oral administration of a racemic mixture. Peaks: 1 = internal standard; 2 = *S*-(+)-tocainide; 3 = *R*-(—)-tocainide. A = Blank plasma chromatogram.

TABLE II

PLASMA CONCENTRATION—TIME DATA

Concentrations are expressed in μg/ml.

Time (h)	Subject 1		Subject 2			
	Chiral column		Chiral column			Carbowax
	S(+)	*R*(—)	*S*(+)	*R*(—)	Total	*R,S*
0.25	0.585	0.536	0.080	0.083	0.163	0.281
0.50	0.842	0.741	0.287	0.220	0.507	0.565
0.75	0.802	0.728	0.372	0.317	0.689	0.613
1.00	0.793	0.708	0.308	0.248	0.556	0.563
1.50	0.768	0.670	0.425	0.345	0.770	0.897
1.75	0.781	0.696	0.355	0.301	0.656	0.657
2	0.844	0.742	0.403	0.352	0.755	0.519
3	0.629	0.538	0.368	0.325	0.693	0.545
5	0.618	0.508	0.300	0.206	0.506	0.545
7	0.481	0.375	0.289	0.252	0.541	0.533
24	0.234	0.125	0.176	0.118	0.294	0.270
48	0.071	0.028	0.113	0.076	0.189	0.137
72	—	—	0.049	0.032	0.081	0.086

The application of the method in human studies was shown by analysis of plasma and urine samples collected at intervals from two subjects given oral solutions of *R,S*-tocainide hydrochloride at a dose of 3 mg/kg. The chromatogram shown in Fig. 1 is representative of the isomer separation on the chiral phase and shows the differing enantiomer ratios seen in plasma at 24 h after the initial dose.

The data given in Table II for plasma concentrations, at each sample time, show the more rapid disappearance of the *R*-(—)-enantiomer in both subjects. In order to compare the values obtained on the chiral phase for the individual enantiomers to those obtained by analysis of the total racemate, samples were also analysed by the use of a Carbowax 20M capillary column. This procedure had earlier been found [13] to be suitable for the analysis of racemic tocainide in rat plasma and urine. It is also found equally suitable for human studies. Accordingly, the same plasma samples from one subject

TABLE III

APPARENT PLASMA HALF-LIFE (h) OF *S*-(+)-, *R*-(—)- AND *R,S*-(±)-TOCAINIDE IN TWO VOLUNTEERS

	Subject 1	Subject 2
R,S-(±)-Tocainide	22.1	9.2
S-(+)-Tocainide	25.6	11.1
R-(—)-Tocainide	20.5	9.0

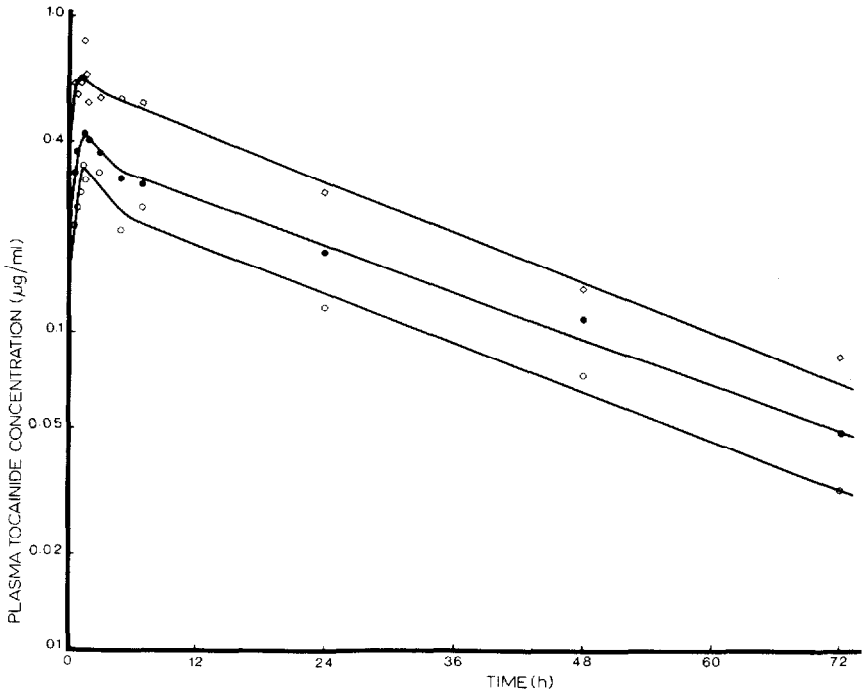

Fig. 2. Log plasma concentration—time curves for tocainide enantiomers and racemic mixture. (□—□) racemate; (●—●) *S*-(+)-tocainide; (○—○) *R*-(—)-tocainide.

TABLE IV

QUANTITIES OF *S*-(+)- AND *R*-(—)-TOCAINIDE EXCRETED IN URINE

Quantities are given in μg/ml of urine.

Time (h)	Subject 1			Subject 2		
	(*S*)-(+)	(*R*)-(—)	Ratio *S*/*R*	(*S*)-(+)	(*R*)-(—)	Ratio *S*/*R*
1	6.29	6.30	0.99	5.72	5.83	0.98
2	6.74	6.53	1.06	16.35	14.77	1.12
3	3.73	3.61	1.09	11.48	10.62	1.14
5	6.63	6.17	1.10	73.72	63.6	1.15
7	7.93	7.33	1.11	60.89	53.5	1.17
14			—	28.76	23.23	1.27
15	4.70	4.27	1.11			—
22	14.36	11.8	1.24			—
24	—	—	—	10.66	7.75	1.45
30	—	—	—	63.53	32.66	1.94
31	5.22	3.68	1.50			—
38	—	—	—	10.28	6.34	1.62
42	1.90	1.22	1.88			—
48	18.16	9.61	1.88	10.21	4.62	2.36
54	4.56	2.60	1.91	14.48	4.96	3.03

were assayed by the two procedures. The data for the analysis using Carbowax 20M are included in Table II. It can be observed that the totals for the individual *R*- and *S*-enantiomers observed on the chiral phase are in close agreement with racemate of tocainide eluting from the Carbowax phase. The apparent plasma half-lives ($t_{1/2}$) for the terminal elimination phase of the two enantiomers were calculated by an iterative non-linear least squares regression computer program [14] and are summarized in Table III along with the apparent $t_{1/2}$ of the total racemate. The two subjects were noted to exhibit significantly different $t_{1/2}$ values for the two enantiomers, however, in both cases the *R*-(—)-enantiomer of tocainide is eliminated from plasma at a faster rate. The log plasma concentration—time curves obtained from one subject for the individual enantiomers as well as the racemate are presented graphically in Fig. 2. The values for the apparent $t_{1/2}$ for the racemate in both subjects lies between that observed for the individual enantiomers. This would be anticipated since the apparent $t_{1/2}$ for the racemate is made up by contributions for each enantiomer at every data point.

The urinary excretion of *R*- and *S*-tocainide was also studied and as shown in Table IV, the two subjects evidenced more rapid excretion of the *S*-(+)-enantiomer. The higher levels of *S*-(+)-tocainide (up to 3.03 times the *R*-(—)-enantiomer at 54 h in one subject) are likely due to a more rapid metabolism of the *R*-(—)-enantiomer. This is consistent with the plasma data, which showed a more rapid disappearance of the *R*-(—)-enantiomer.

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