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ACKNOWLEDGMENTS

The authors wish to express their appreciation to the U.S. Environmental Protection Agency (Contract CR 807566) for support of this work and to the University of Minnesota, Duluth Computer Center for partial financial assistance.

Pharmacokinetics and Anesthetic Potency of a Thiopental Isomer

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Abstract □ In developing a high-performance liquid chromatographic assay for thiopental [5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid], a thiopental isomer [5-ethyl-5-(1-ethylpropyl)-2-thiobarbituric acid] was found. This isomer occurs (6–7%) in supposedly pure thiopental and in the commercially available thiopental sodium administered to patients for induction of anesthesia. A similar type of isomer also occurs in pentobarbital, the oxybarbiturate analogue of thiopental. Because the disposition and anesthetic potency of the isomer is unknown, its pharmacokinetic properties were determined in humans and its anesthetic potency in mice. In five surgical patients, the terminal elimination half-life, clearance, and volume of distribution at steady state of the isomer were not statistically different from those of thiopental. In mice, the isomer proved to be as effective as thiopental for induction of anesthesia. The LD_{50} and sleep time at one-half the LD_{50} did not statistically differ between the two compounds in mice. The close structural similarity of thiopental and the isomer results in similar pharmacokinetic and anesthetic properties. It does not appear critical that the isomer be separated from thiopental in subsequent pharmacological research.

Keyphrases □ Thiopental—isomer determination in serum by high-performance liquid chromatography, pharmacokinetics in humans, anesthetic potency in mice □ Pharmacokinetics—of a thiopental isomer in humans, comparison with thiopental □ Anesthetic agents—potency of a thiopental isomer in mice, comparison with thiopental

Previous high-performance liquid chromatographic (HPLC) assays for thiopental apparently lacked the chromatographic resolution to separate thiopental from any side-chain isomer (1–4). This paper describes an HPLC assay that separates thiopental, 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid, from this isomeric material, 5-ethyl-5-(1-ethylpropyl)-2-thiobarbituric acid (see Fig. 1). The isomer was found both in standard thiopental obtained from the manufacturer and the commercially available thiopental sodium used for the induction of anesthesia in patients. It apparently is formed during the manufacture of thiopental[1]. The presence of a similar isomer has been described for pentobarbital [5-ethyl-5-(1-methylbutyl) barbituric acid], the oxybarbiturate analogue of thiopental (5).

EXPERIMENTAL

Apparatus and Reagents—A liquid chromatograph[2] was equipped with a variable-wavelength detector[3] and column heater. The phosphoric acid, monobasic potassium phosphate, sodium hydroxide, and sodium carbonate were certified ACS grade[4]; anhydrous ethyl ether was reagent grade[5]; acetonitrile was HPLC grade[6]. The sodium salt of thiopental[7] and the thiopental isomer[8] were used for preparation of the standard curves. The thiopental contained 9.0% of the isomer, based on peak heights from a sample separated on the HPLC assay. The isomer did not contain thiopental; only a single peak was seen when the isomer was chromatographed on the HPLC assay. Thiamylal acid[9] was used as an internal standard for measurement of thiopental and the isomer.

Thiopental Protein Precipitation and Chromatography—The HPLC assay described by Kabra *et al.* (6) was modified. For thiopental concentrations >200 ng/ml, an equal volume of acetonitrile containing the internal standard (2.5 or 25 μg/ml) was added to human serum (200 μl) and mixed on a vortex mixer. Following two sequential centrifugations, the acetonitrile supernatant was injected into the chromatograph.

[1] Personal communication, Abbott Laboratories, North Chicago, Ill.
[2] Model 5020, Varian, Palo Alto, Calif.
[3] Model UV-50, Varian, Palo Alto, Calif.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] J. T. Baker Chemical Co., Phillipsburg, N.J.
[6] Distilled in glass; Burdick & Jackson Laboratories, Muskegon, Mich.
[7] Lot 845-7283, Abbott Laboratories, North Chicago, Ill.
[8] Abbott 13750, Lot No. 16-859-AX, Abbott Laboratories, North Chicago, Ill.
[9] Lot 7274 x 24-6, Parke-Davis & Co., Detroit, Mich.

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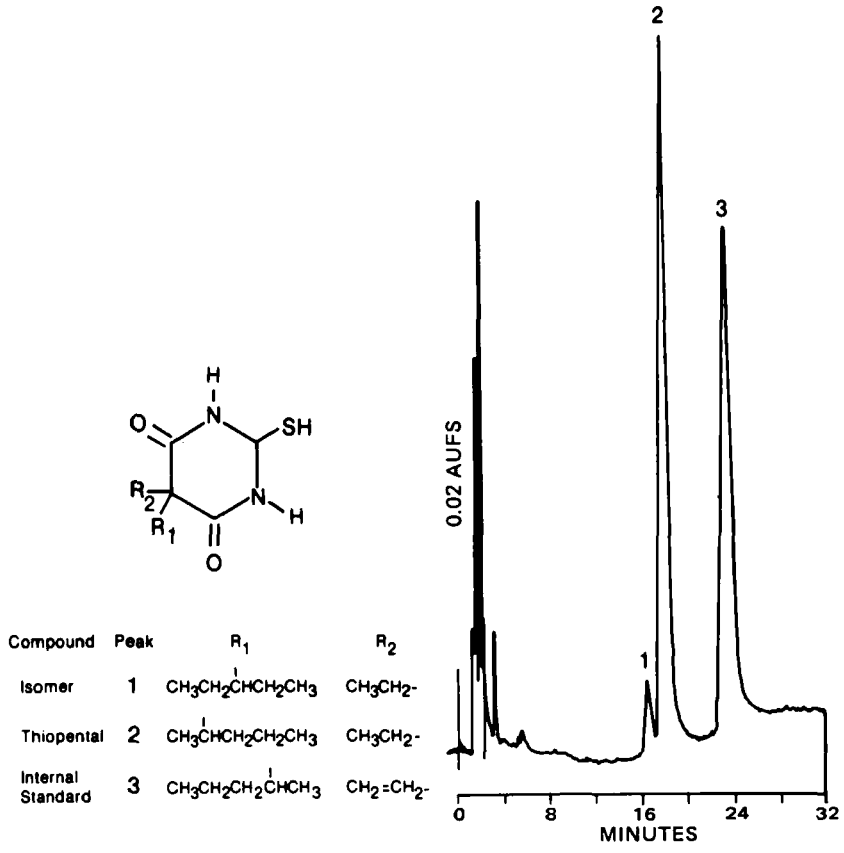

Figure 1—*A chromatogram demonstrating the presence of the isomer in a sample of supposedly pure thiopental. The structures of thiopental, the isomer, and the internal standard (thiamylal) are as indicated.*

Appropriate concentrations of the internal standard were added before the protein precipitation. To increase the sensitivity of the assay to 25 ng/ml, the ether extraction procedure described for the thiopental isomer can be used. Because the thiopental concentrations were >200 ng/ml for the duration of the study, it was not necessary to use the extraction.

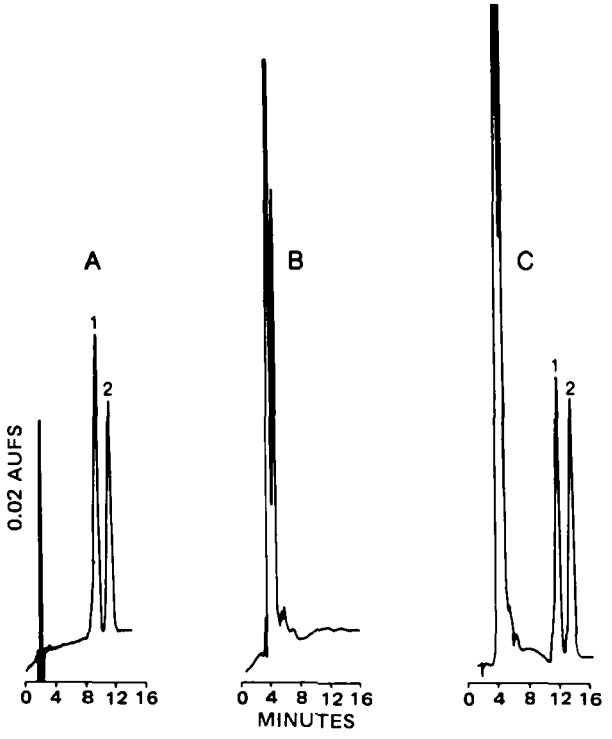

Figure 2—*Typical chromatograms obtained using the assay described for measurement of thiopental. Key: (A) separation of thiopental (peak 1) and the internal standard (peak 2) when both are injected directly onto the chromatograph; (B) a human serum blank; (C) separation of 1.25 μg of thiopental and 2.5 μg of the internal standard from 1.0 ml of human serum.*

The HPLC mobile phase was acetonitrile–phosphate buffer (38:62). The buffer was prepared by adding 175 μl of 1 *M* KH_2PO_4 and 50 μl of 1 *M* H_3PO_4 to 1 liter of glass-distilled and filtered water. The pH of the phosphate buffer was 4.5, and the molarity was 4.5×10^{-4} *M* acid and 1.75×10^{-4} *M* salt. The flow rate was 1.2 ml/min with a C_{18} reverse-phase column[10] (4-mm i.d. × 30 cm) at 50°. The detector wavelength was 290 nm and ranged from 0.01 to 0.05 AUFS.

To prepare standard curves, aliquots of thiopental in methanol were evaporated to dryness and diluted to volume with serum. The standard was then handled as described above. Standard curves were linear at all concentrations from 200 ng/ml to 50 μg/ml. The retention times of thiopental and the internal standard were 9.2 and 11.2 min, respectively (Fig. 2). The assay does not separate the isomer from thiopental and therefore measures both compounds.

Isomer Extraction and Chromatography—For analysis of isomer concentrations between 25 and 500 ng/ml, variable volumes of serum (0.1–1.0 ml) were extracted with 200 ng of thiamylal as the internal standard. Following acidification with 1 *M* phosphoric acid two-thirds saturated with potassium phosphate, an extraction with ether (3 ml) was performed. The ether phase was removed and evaporated to dryness. The residue was reconstituted in 100 μl of the HPLC assay eluant and injected into the chromatograph. The HPLC mobile phase was acetonitrile–dilute phosphate buffer (33:67). The flow rate was 1.2 ml/min on a C_{18} reverse-phase column[11] (4-mm i.d. × 30 cm) at 50°. The detector wavelength was 290 nm and 0.01 or 0.02 AUFS.

Standard curves for the isomer assay were prepared by evaporating to dryness aliquots of the pure isomer in methanol and diluting to volume with serum. The standard was then prepared using the aforementioned acidification and ether extraction. Standard curves were linear from 25 to 500 ng/ml of the pure isomer. Retention times were: isomer, 16.7 min; thiopental, 18.1 min; and the internal standard, 23.6 min (Fig. 3).

Isomer Identification—The isomer was identified by addition of pure 5-ethyl-5-(1-ethylpropyl)-2-thiobarbituric acid to a sample of thiopental. When chromatographed using the aforementioned assay that separated the two compounds, addition of the isomer resulted in an increase of the isomer peak height in the thiopental sample, with an identical retention time. The UV spectra of thiopental and the isomer were identical, showing a maximum absorption at 290 nm. The structure of the isomer

[10] Varian MCH-10, Varian, Palo Alto, Calif.
[11] μ-Bondpack C_{18}, Waters Associates Inc., Milford, Mass.

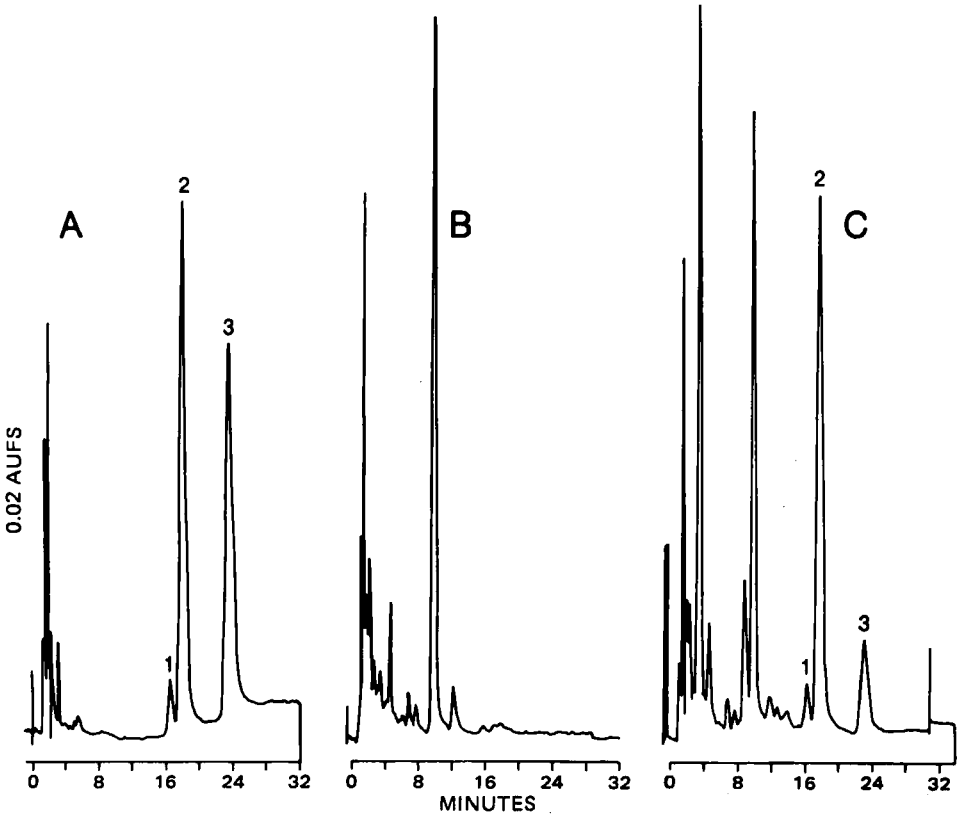

Figure 3—*Typical chromatograms obtained using the assay for measurement of the isomer. Key: (A) separation of the isomer (peak 1) from thiopental (peak 2) and the internal standard (peak 3) when thiopental and internal standard are injected directly onto the chromatograph; (B) a human serum blank; (C) separation of the compounds from human serum. Peak 1 in chromatogram C represents 56 ng of the isomer, peak 2 is 781 ng of thiopental, and peak 3 is 200 ng of internal standard extracted from 0.5 ml of serum.*

also was characterized using ^{13}C- and ^{1}H-NMR[12]. No evidence of interconversion of the two compounds was found during the extraction and HPLC chromatography.

Pharmacokinetics in the Human—One female and four male patients undergoing elective surgical procedures were studied. An institution review board approval was obtained along with consent from each patient. Subject age, weight, and thiopental dose are indicated in Table I. Commercially available thiopental sodium was used to induce anesthesia; therefore, both thiopental and the isomer were administered to the patients. A sample of the thiopental sodium administered was analyzed for the amount of isomer present (Table I).

Following rapid intravenous injection of the thiopental sodium for induction of anesthesia, blood samples were obtained from a catheter inserted into a radial artery or an antecubital vein. Arterial blood samples were obtained at 1, 2, 3, 5, 10, 15, 30, 45, 60, 90, and 120 min; venous blood samples were obtained at 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hr. This protocol allows accurate characterization of the distribution and elimination phases of thiopental and the isomer. Serum samples were frozen and subsequently analyzed using two assays: one that collectively measured the thiopental and isomer, and one that measured only the isomer. Following the administration of thiopental sodium, anesthesia was maintained with nitrous oxide–oxygen (60:40) and enflurane (1–2%) for 1–3 hr.

The pharmacokinetic data analysis used only the serum concentration *versus* time data. Linear regression of the postdistribution log serum concentration *versus* time data was used to determine the terminal elimination half-life. The dose divided by the area under the curve (linear trapezoid rule) was used to determine total body clearance. The area under the first-moment curve, as described by Benet and Galleazzi (7), was used to determine the volume of distribution at steady state. A two-tailed paired t test at a significance level of $p < 0.05$ was used to statistically compare the pharmacokinetic parameters for thiopental and the isomer.

Anesthetic Potency in Mice—Thiopental and the isomer were compared for anesthetic potency in male white Swiss mice[13] weighing 18–24 g. Thiopental was used as the commercially available sodium salt containing 8.3% sodium carbonate. The free acid isomer was converted to the sodium salt by adding 1 meq of NaOH and 8.3% Na_2CO_3 to 1 meq of the free acid. Both compounds were used as a 0.4% solution.

Because the anesthetic effect of these compounds is influenced by the peak blood level achieved, the speed of intravenous injection into the tail vein was rigidly controlled at 0.01 ml/5 sec. Sleep occurred immediately, during, or at the end of the injection. The anesthetized animals were placed on their backs and left undisturbed until they righted themselves.

In the fatal-dose range, death was due to respiratory arrest a few minutes after the drug injection. After the initial dose-ranging studies,

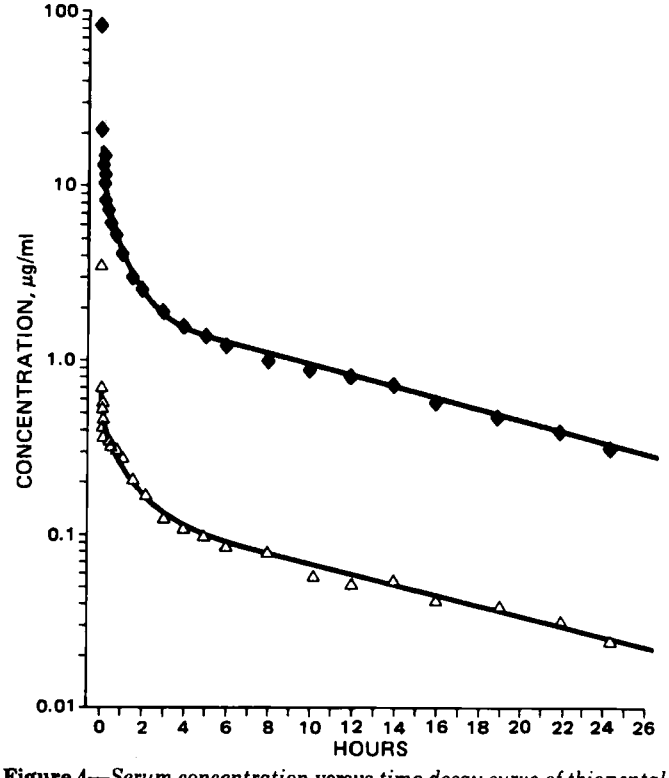

Figure 4—*Serum concentration* versus *time decay curve of thiopental (◆) and the isomer (△) for subject 5 in Table I.*

[12] Varian XL100, Varian, Palo Alto, Calif.

[13] Simson, Gilroy, Calif.

Table I—Demographic and Pharmacokinetic Data

Patient	Age, year	Weight, kg	Isomer, %	Dose, mg/kg	Elimination Half-Life, min		Clearance, ml/kg/min		Volume of Distribution at Steady State, liter/kg	
					Thiopental	Isomer	Thiopental	Isomer	Thiopental	Isomer
1	30	55	6.1	8.2	409	291	3.3	3.4	1.52	1.30
2	23	79	6.1	5.9	932	835	3.1	4.9	2.82	4.32
3	30	81	6.2	5.4	566	636	2.6	2.4	1.66	1.85
4	28	67	6.1	6.6	378	752	4.4	3.9	2.01	2.6
5	35	82	6.0	5.6	465	396	3.1	2.7	1.4	1.4
Mean	29.2	72.8	6.10	6.3	550	582	3.3	3.8	1.88	2.29
±*SD*	4.3	11.6	0.07	1.1	225	231	0.6	1.0	0.57	1.24

the LD_{50} was determined for both drugs using groups of eight mice at three dosage levels for each drug. The LD_{50} and 95% confidence limits were determined using the method of Litchfield and Wilcoxon (8). In addition to the LD_{50}, the sleep time at one-half the LD_{50} was also determined using eight mice per drug. A third study was performed with an additional eight mice per drug to determine if cumulative properties of these two barbiturates were the same. One-half of the LD_{50} dose was given to a mouse and a second injection $\frac{1}{8}$ of the LD_{50} dose was given when the animal righted itself. The sleep times from the first and second doses were compared using a two-tailed unpaired t test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Assay Sensitivity, Reproducibility, and Chromatography—With the acetonitrile protein precipitation, the assay sensitivity for thiopental was 200 ng/ml. Analysis of the same serum sample 6–8 times on the same day gave the following coefficients of variation: 300 ng/ml, 4.2%; 1.1 μg/ml, 3.7%; and 15.4 μg/ml, 1.8%. The between-day coefficient of variation at a thiopental concentration of 2.5 μg/ml was 5.0%. The isomer assay was sensitive to 25 ng/ml. Analysis of the same serum sample for the isomer 6–8 times on the same day gave the following coefficients of variation: 25 ng/ml, 7.9%; 125 ng/ml, 7.8%, and 500 ng/ml, 5.4%. The between-day coefficient of variation at an isomer concentration of 125 ng/ml was 5.1%. Figure 1 indicates the chemical structures of thiopental and the isomer, along with a chromatogram showing the presence of the isomer peak in a sample of thiopental obtained from the manufacturer[7]. Analysis of 10 different lots of commercially available thiopental sodium showed that the isomer comprised 6–7% of the total drug content. Figure 2 is a representative chromatogram obtained with the thiopental assay where thiopental and the isomer are not separated. Figure 3 demonstrates a representative chromatogram of the isomer assay where thiopental and the isomer are effectively separated.

Pharmacokinetics in the Human—Table I summarizes the derived pharmacokinetic parameters for thiopental and the isomer in each patient. Figure 4 displays the serum concentration *versus* time data for patient 5. Simultaneous arterial and venous samples in three patients demonstrated minimal thiopental concentration differences due to sample site, which had no effect on the derived pharmacokinetic variables. There was no statistical difference between the terminal elimination half-life, clearance, or volume of distribution at steady state when thiopental was compared with the isomer. The variability occurs because the isomer serum concentrations are 10–15 times lower than the thiopental concentrations and measurement varies more at the low isomer concentrations. While not formally characterized in this analysis, there was no visible difference in the initial distribution phases of thiopental relative to the isomer. Thus, in humans, the distribution and elimination of thiopental and the isomer are similar. This would be expected given the close structural similarity and (presumably) physicochemical properties of thiopental and the isomer.

Table II—LD_{50} and Anesthetic Potency in Mice

Drug	LD_{50}, mg/kg	Sleep Time[a] min	Cumulative Sleep Times, min	
			1st Dose[a]	2nd Dose[b]
Thiopental	90 (84.9–95.4)[c]	25.4 ± 17.3	25.3 ± 15.5	32.3 ± 20.9
Isomer	90 (84.9–85.4)	38.0 ± 18.3	31.3 ± 33.0	52.7 ± 22.1

[a] Dosed at 45 mg/kg; mean ± *SD*. [b] Dosed at 11.5 mg/kg; mean ± *SD*. [c] Range in parentheses.

Anesthetic Potency in Mice—Thiopental and the isomer had an identical fatal dose as judged by the LD_{50} studies (Table II). With one-half of the LD_{50}, the duration of sleep (as judged by return of the righting reflex) was not statistically different. Additionally, there was no evidence of a statistical difference in the cumulative duration of sleep when the anesthetic was administered a second time. Thus, it appears that the thiopental isomer is an anesthetic of potency comparable with that of thiopental in mice.

In summary, there is a 6–7% level of an isomer form present in thiopental. In the human, this isomer has distribution and elimination characteristics similar to thiopental. It also has, in mice, a degree of anesthetic potency similar to that of thiopental. It is therefore not critical that subsequent analytical technology used for thiopental pharmacokinetic or pharmacodynamic research quantitate or separate this isomer from thiopental.

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ACKNOWLEDGMENTS

Supported in part by National Institutes of Health Grant 1R23-GM28032, the Parker B. Francis Foundation, and the Veterans Administration Research Fund.

The authors are obliged to Mr. D. E. Williamson and Dr. O. Geisler of Abbott Laboratories, North Chicago, Ill. for the supply of the thiopental isomer and to Ms M. Schuler, Institute of Pharmacology, Syntex Research, Palo Alto, Calif. for assistance in the animal experiments.