Quantitation of Methadone Enantiomers in Humans Using Stable Isotope-Labeled [$^2H_3$]-, [$^2H_5$]-, and [$^2H_8$]Methadone

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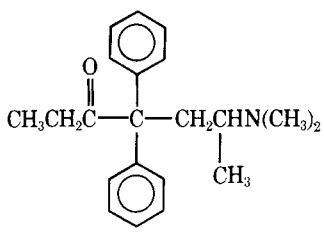
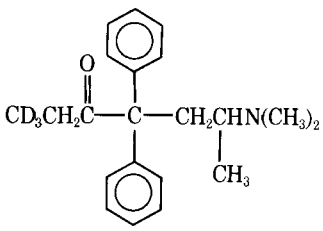
Abstract □ A new technique for simultaneous stereoselective kinetic studies of methadone enantiomers was developed using three deuterium-labeled forms of methadone and GLC–chemical-ionization mass spectrometry. A racemic mixture (1:1) of (*R*)-(−)-[$^2H_5$]methadone (*l*-form) and (*S*)-(+)-[$^2H_3$]methadone (*d*-form) was administered orally in place of a single daily dose of unlabeled (±)-[$^2H_0$]methadone in long-term maintenance patients. Racemic (±)-[$^2H_8$]methadone was used as an internal standard for the simultaneous quantitation of [$^2H_0$]-, [$^2H_3$]-, and [$^2H_5$]methadone in plasma and urine. A newly developed extraction procedure, using a short, disposable C_{18} reversed-phase cartridge and improved chemical-ionization procedures employing ammonia gas, resulted in significant reduction of the background impurities contributing to the ions used for isotopic abundance measurements. These improvements enabled the measurement of labeled plasma methadone levels for 120 hr following a single dose. This methodology was applied to the study of methadone kinetics in two patients; in both patients, the analgesically active *l*-enantiomer of the drug had a longer plasma elimination half-life and a smaller area under the plasma disappearance curve than did the inactive *d*-form.

Keyphrases □ Methadone—enantiomers, quantitation in humans using stable isotope labeling □ Enantiomers—of methadone, quantitation in humans using stable isotope labeling □ Narcotics—methadone, quantitation of enantiomers in humans using stable isotope labeling

Racemic methadone is used in maintenance therapy of heroin addicts (1, 2). The (*R*)-(−)-enantiomer (*l*-form) is the more pharmacologically active isomer in experimental animals and humans (3, 4). In addition, small amounts of the (*S*)-(+)-enantiomer (*d*-form) may be converted to an active enantiomer of dimepheptanol by hepatic microsomal drug-metabolizing enzymes (5). It has been shown that the binding of methadone and other narcotics to specific opiate receptors is stereospecific (6–12). It was also shown that several aspects of narcotic disposition, including metabolism, may be stereoselective (13, 14).



(±)-methadone



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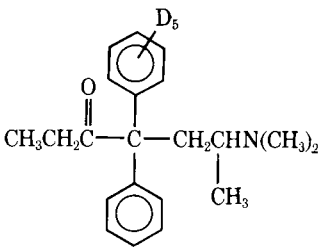

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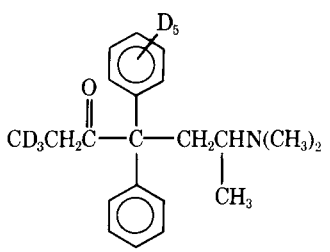

(±)-[$^2H_8$]methadone internal standard

BACKGROUND

Little work has been devoted to clarifying the disposition and pharmacokinetic differences between active and inactive enantiomers of methadone (4, 13). Such studies have been hindered by rapid and extensive drug distribution into tissues, with resultant low plasma levels, and by ethical constraints on the use of large amounts of radioisotopes in research patients.

Stable isotope-labeled compounds are now used often as tracers for the quantitation of drugs and drug metabolites in biological fluids (15, 16). Racemic [$^2H_3$]methadone (17) and [$^2H_5$]methadone (18) have been used as internal standards or tracers in the determination of methadone in plasma by GLC–chemical-ionization mass spectrometry. The pharmacokinetics of (*R*)-(−)- and (*S*)-(+)-methadone in maintenance patients using the separate enantiomers of [$^2H_5$]methadone as tracers were reported (13). In that study, kinetic differences were found between the two enantiomers in three clinically well-stabilized methadone maintenance patients; in each case, (*R*)-(−)-methadone had a significantly longer elimination half-life than the inactive enantiomer.

Exploration of these differences in normal patients as well as in patients whose drug disposition may be altered by liver disease, alcoholism, multiple drug abuse, or anticonvulsant therapy, etc., requires the performance of three sequential studies in each patient. Multiple studies are more difficult and expensive; the status of the patient may also change. Ideally, one would like to quantitate and trace both enantiomers simultaneously in the same patient using stable isotope tracers.

Three forms of deuterium-labeled methadone were synthesized: the two enantiomers, (*R*)-(−)-[$^2H_5$]methadone and (*S*)-(+)-[$^2H_3$]methadone, and racemic (±)-[$^2H_8$]methadone. Technology for the simultaneous measurement of all three labeled forms as well as unlabeled racemic (±)-methadone was developed using GLC–chemical-ionization mass spectrometry. In addition, clinical studies were carried out using these stable isotopic tracers; significant differences in the disposition and pharmacokinetics of the two enantiomers were observed in each patient.

EXPERIMENTAL

Synthesis—(*S*)-(+)-[$^2H_3$]Methadone was prepared from ethyl-2,2,2-[$^2H_3$]magnesium bromide and (*S*)-(+)-4-dimethylamino-2,2-diphenyl-valeronitrile according to known procedures (17). (*R*)-(−)-[$^2H_5$]Methadone was prepared from ethyl bromide and (*R*)-(−)-4-dimethylamino-2-phenyl-2-[$^2H_5$]phenyl-valeronitrile (18). Racemic (±)-[$^2H_8$]methadone was prepared by coupling ethyl-2,2,2-[$^2H_3$]magnesium bromide with racemic 4-dimethylamino-2-phenyl-2-[$^2H_5$]phenyl-valeronitrile by analogy to known procedures (18). Hydrochloride salts were prepared by dissolving the free bases in 25 ml of anhydrous ethanol and passing dry hydrogen chloride gas into the ice-cold solution. The ethanolic hydrogen chloride solution was then treated with 100 ml of ether and chilled at −10° until crystallization was complete. The salts were recrystallized twice from ethanol–ether (1:10).

Standard Solutions—Aqueous stock solutions, 1 mg/ml of (±)-[$^2H_0$]methadone, (*S*)-(+)-[$^2H_3$]methadone, (*R*)-(−)-[$^2H_5$]methadone, and (±)-[$^2H_8$]methadone hydrochloride, were prepared separately. Aliquots of these standard solutions were added to methadone-free plasma and urine obtained from healthy adult donors to establish standard curves. The plasma solution of combined enantiomers of unlabeled methadone and [$^2H_3$]- and [$^2H_5$]methadone was prepared gravimetrically. Concentrations ranged from 25 to 800 ng/g of plasma for methadone and

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Table I—Fractional Ion Abundances of the Deuterated Methadone Isotopic Species

Number of ^2H Atoms	$[^2H_0]$	$[^2H_3]$	$[^3H_5]$	$[^2H_8]$
0	78.98	0.12	0.12	
1	18.49	0.20		
2	2.32	2.04	0.58	
3	0.18	77.32	2.93	
4		17.94	13.39	
5		2.21	56.37	0.07
6		0.17	16.58	0.40
7			5.80	6.04
8			3.96	74.55
9				16.92
10				2.00

from 5 to 200 ng/g of plasma for $[^2H_3]$- and $[^2H_5]$methadone. Urine concentrations ranged from 0.1 to 10 μg/ml of urine for each methadone species.

Sample Preparation—*Plasma Analysis*—Plasma (1 g) was mixed with 1 ml of the internal standard, $[^2H_8]$methadone (200 ng/ml), and allowed to equilibrate at room temperature for 45 min. The sample solution was poured into a 10-ml syringe barrel fitted with a C_{18} silica gel cartridge[1]. Prior to use, the cartridge was conditioned by washing with 2 ml of methanol followed by 4 ml of water. The sample solution was forced through the cartridge with the syringe plunger and was followed by 2 ml of water and 3 ml of 0.01 *M* NH_4OH. The methadone was eluted with 6 ml of ethyl acetate and an additional 3 ml of 0.01 *M* NH_4OH (19).

The aqueous layer was discarded, and the organic phase was evaporated under nitrogen. The residue was dissolved in 3 ml of 0.3 *N* HCl and was shaken with 4 ml of *n*-heptane to remove residual lipids. After two extractions with *n*-heptane, the methadone-containing aqueous layer was separated and adjusted to pH 9–10 with 3 ml of 0.6 *M* NH_4OH. The aqueous layer was then extracted with 6 ml of ethyl acetate, and the organic layer was washed with an additional 3 ml of 0.6 *M* NH_4OH and evaporated under nitrogen. The residue was reconstituted with 10 μl of methanol, and the entire sample was analyzed. Recovery of methadone by this procedure was 40–70%.

Urine Analysis—Urine (1 ml) was mixed with 1 ml of internal standard (10 μg/ml). The sample was adjusted to pH 9–10 with 3 ml of 0.6 *M* NH_4OH, and the solution was extracted with 6 ml of ethyl acetate. The aqueous layer was discarded, and the organic phase was evaporated under nitrogen. The residue was reconstituted with 50 μl of methanol. A 2–3-μl aliquot was used for analysis. Recovery was 85–90%.

Mass Spectrometric Analysis—Analyses were performed using a quadrupole mass spectrometer[2] operating at an ion source temperature of 160° with a 0.5-torr ion source pressure of ammonia reagent gas (20). Samples were introduced through the GLC inlet system and were analyzed on a 1.8-m × 1.0- mm i.d. glass column packed with 10% OV-17 on Chromosorb W-HP (100–120 mesh) at a column temperature of 250° and a helium flow rate of 9 ml/min. Under these conditions, the retention time of methadone was 3.5 min. Isotopic ratios were measured by monitoring the intensity of the protonated molecular ions at *m/z* 310, 313, 315, and 318 for $[^2H_0]$-, $[^2H_3]$-, $[^2H_5]$-, and $[^2H_8]$methadone, respectively, using a microprocessor-based data system[3].

Standard isotope dilution curves were prepared for each methadone series in plasma and urine by plotting the observed, corrected isotopic ratio against the mole ratio of $[^2H_0]$methadone/$[^2H_8]$methadone, $[^2H_3]$methadone/$[^2H_8]$methadone, and $[^2H_5]$methadone/$[^2H_8]$methadone. In all cases, plasma and urine blank values were determined daily with each set of samples. The isotopic ratio data were mathematically treated to correct the values for the small cross-contributions to the ion intensities by the individual deuterated methadone species using the following methods.

Patients and Clinical Procedures—*Patient 1*—Patient 1, a 24-year-old male, began to use alcohol at age 11 and heroin at age 13. He began methadone maintenance treatment at age 18 with no subsequent problems with illicit narcotics or other drug abuse. He had a history of chronic alcoholism since age 21 but was detoxified and remained alcohol free for 9 months prior to the present study. He was stabilized on 80 mg of methadone/day and received no other medications. Physical examination revealed no significant abnormalities. Laboratory findings included: Hgb 15.0, Hct 44, WBC 6.2 with normal differential, normal urinalysis, hepatitis B antigen negative, SGOT 15, alkaline phosphatase 59, bilirubin 0.3, 5′-nucleotidase 6, total protein 6.7, albumin 4.0, and BUN 16, all within normal limits.

Patient 2—Patient 2, a 37-year-old male, began to use illicit narcotics at age 17. He entered a methadone maintenance treatment program at age 30 and subsequently remained free of heroin abuse. Over the past 10 years, he consumed no alcohol nor used other drugs. At age 33, he was told of abnormal liver function test results and subsequently had a liver biopsy, which showed chronic active hepatitis. At the time of these studies, he was receiving 100 mg of methadone/day with no other medications. Positive findings on physical examination revealed spider angiomata on the face and thorax and enlarged firm, nontender liver but an otherwise normal physical examination. Laboratory results included: Hgb 16.6, Hct 49.3, WBC 6.7 with normal differential, normal urinalysis, and hepatitis B antigen negative. His SGOT was 67 (upper normal 25), alkaline phosphatase was 127 (upper normal 82), bilirubin was 0.5, 5′-nucleotidase was 18 (upper normal 14), total protein was 7.4, and BUN was 18.

Both patients were admitted[4] for metabolic studies, and oral and written informed consent was obtained. The oral daily dose of methadone was substituted by a single dose of the same amount of stable isotopic methadone (containing equal amounts of *d*-$[^2H_3]$- and *l*-$[^2H_5]$methadone) for each patient on 1 study day. On the following day, oral dosing with unlabeled methadone was resumed. Multiple blood specimens were obtained on the study day and once daily thereafter. On several days following the initial study day, multiple blood samples also were obtained and analyzed for protocols unrelated to the results reported here. Six-hour urine collections were made on the 1st study day, and 24-hr collections were made daily thereafter.

Table II—Summary of Plasma Disappearance Half-Life and Area under the Curve (*AUC*)

Patient	Enantiomer	$T_{1/2\beta}$, hr	$(AUC)_0^{119}$, ng hr	Plasma Compartment, %
1	(*S*)-(+)-	28.1 ± 0.7	5296	1.58
	(*R*)-(−)-	37.9 ± 4.4	4002	1.19
2	(*S*)-(+)-	34.8 ± 2.4	4795	1.14
	(*R*)-(−)-	58.9 ± 4.8	2596	0.62

Data Analysis—Each isotopic species of methadone contributes a known, but variable, fraction to the ion intensity measured at a given mass. The ion intensity at a given mass (I_x), can be described as the sum of the individual fractional ion intensities according to:

$$I_x = A_1X_1 + A_2X_2 + \ldots A_nX_n \qquad \text{(Eq. 1)}$$

where *A* is the fractional ion abundance listed in Table I, X_n is the unknown fractional contribution to be determined, and I_x is the measured ion intensity at a given mass. The set of four linear equations, which consists of one equation for each ion measured, can be solved by linear least-squares techniques described previously (21, 22). The corrected ion intensity ($I_{x'}$) was normalized to the corrected ion intensity ($I_{318'}$) of the internal standard to give a set of three corrected isotope ratio values (R_{310}, R_{313}, and R_{315}) for each sample. The actual mole ratio for each component in a sample was obtained from linear calibration curves as described[5].

Plasma drug concentration *versus* time data were fitted to a three-exponential decay function using the SAAM 27 computer program (23). Areas under the curves were calculated using computer routines that employ the trapezoidal rule to perform the integration. The kinetic data for both patients are summarized in Table II.

RESULTS AND DISCUSSION

The isotopic composition of the various deuterated methadone species was determined by ammonia GLC–chemical-ionization mass spectrometry. The data, expressed as the fractional ion abundances of the various species, are listed in Table I. Multiple isotopic tracer studies employed in the present work were complicated by isotopic overlap of the various deuterated compounds. The problem was minimized, but not

[1] Sep-Pak cartridge, Waters Associates, Milford, Mass. Different lots of cartridges had different abilities to remove undesired compounds from plasma samples.

[2] Biospect quadrupole mass spectrometer, Chemetron Medical Products, St. Louis, Mo.

[3] Spectral analysis microprocessor, Chemetron Medical Products, St. Louis, Mo.

[4] Rockefeller University Hospital Clinical Research Center.

[5] A general purpose computer program written in the SPEAKEZ language to perform the calculations for any number of isotopic species is available from the authors

Table III—Contributions of Plasma and Urine to the Ion Species Used to Quantitate [$^2H_0$]-, [$^2H_3$]-, and [$^2H_5$]Methadone Using [$^2H_8$]-Methadone as an Internal Standard

Sample	Extraction Method	Chemical-Ionization gas	n	Isotope Ratio, % m/z 310/318	m/z 313/318	m/z 315/318
Buffer[a]	—	Isobutane	3	0.13 ± 0.10	0.46 ± 0.08	0.90 ± 0.29
Plasma[b]	Solvent[c]	Isobutane	3	4.66 ± 3.04	8.18 ± 4.04	22.14 ± 6.74
Plasma	Cartridge[d]	Isobutane	12	1.06 ± 0.32	6.27 ± 1.17	3.32 ± 0.94
Buffer	—	Ammonia	9	0.16 ± 0.14	0.18 ± 0.14	0.21 ± 0.13
Plasma	Cartridge	Ammonia	17	1.65 ± 0.95	1.24 ± 0.49	0.65 ± 0.47
Urine	Solvent[e]	Ammonia	4	0.16 ± 0.14	0.14 ± 0.14	0.10 ± 0.06

[a] Internal standard solution in buffer ([$^2H_8$]methadone, 100 ng/μl), 2 μl/injection. [b] Control plasma, 1 g + internal standard ([$^2H_8$]methadone, 200 ng/ml), 1 ml. [c] Solvent extraction including back-extraction procedure. [d] Sep-Pak C_{18} cartridge cleanup. [e] Solvent extraction alone.

eliminated completely, by using isotopic tracers separated by two or more mass units. The worst case of isotopic overlap occurred between [$^2H_3$]-methadone and [$^2H_5$]methadone, where the latter contributed 5.19% of its relative ion intensity to the ion intensity of [$^2H_3$]methadone measured at m/z 313.

The problem can be viewed as a special case of mixture analysis, where each component of the mixture contributes a small portion to the measured abundance of the other components. Mathematically, ion intensity can be expressed as a set of simultaneous linear equations describing all isotopic contributions to the ions being measured. These equations can be solved by simple matrix algebraic techniques (21, 22, 24). The ratio of (*S*)-(+)-[$^2H_3$]methadone to (*R*)-(−)-[$^2H_5$]methadone in plasma varies only between 2:1 and 1:2, even though the actual total drug concentration in plasma may range over a 40-fold span during a single clinical study. The relative isotopic cross-contribution of one enantiomer to the other is <10% between the two isotopic species. Both [$^2H_0$]methadone and [$^2H_8$]methadone are present in relatively constant amounts (200 ng/g) in plasma; consequently, there is very little interference (typically 0.1–0.2%) between the latter two species and the two *in vivo* kinetic tracers.

In plasma studies, the intensity of the background spectrum, often referred to as "matrix interference," depends strongly on the isolation procedure used to recover methadone from biological samples and on the reagent gas used for chemical-ionization mass spectrometry. It is imperative in these studies to minimize matrix interference to quantitate the low levels of isotopically labeled methadone expected in plasma.

Data presented in Table III show that simple solvent extraction of buffered control plasma containing only [$^2H_8$]methadone had an ion intensity ~20–30 times that of the background level obtained with the pure standard determined with isobutane as the reagent gas. This interference effectively precludes measurement of drug levels below 45 ng/g. Using a short C_{18} silica gel cartridge to recover methadone reduces ion contamination, especially for (*R*)-(−)-[$^2H_5$]methadone, so that detection limits fall to 6–12 ng/g. However, use of the highly selective ammonia reagent gas further decreases the background for masses m/z 313 and 315 so that the detection limits fall to 3–5 ng/g (20).

A simple solvent extraction is sufficient to recover methadone from urine and gives results identical to those of the pure standard obtained using ammonia reagent gas. The lower detection limit in the calibration curve and in the individual patient studies is usually ~1–5 ng/g for [$^2H_3$]- and [$^2H_5$]methadone or approximately twice the blank plasma background level. Two advantages from using the C_{18} silica gel cartridges for extracting methadone from plasma are that the GLC column lifetime is improved markedly and analysis time is shortened to ~5 min/sample since no interfering peaks elute from the column after methadone.

The present data illustrate the practicality of using multiple stable isotopic tracers in a clinical research protocol. Previous studies, using only a single isotopic tracer, required three separate stable isotopic tracer experiments to explore the behavior of the separate enantiomers. Such studies were exceedingly tedious, both with respect to performance of the clinical study and the time required for the analyses. During such lengthy studies, the patient's status is subject to change. Thus, the multiple, simultaneous stable isotopic tracer technique described here offers significant advantages in studying complex drug systems such as methadone in a steady-state, multiple-dose protocol.

Data from Patient 1 (Table II and Fig. 1) reconfirm earlier observations that (*R*)-(−)-methadone is metabolized ~1.35 times slower than (*S*)-(+)-methadone (13). Plasma drug level data *versus* time were fitted to a three-exponential decay function, where the half-life is that of the terminal exponential component. In both cases, (*S*)-(+) methadone reaches and maintains a higher peak plasma level over the first 48 hr of the study than does the (*R*)-(−)-methadone. Thereafter, the (*R*)-(−)-methadone curve crosses the (*S*)-(+)-methadone concentration curve and remains higher for the rest of the study. The area under the plasma disappearance curve was integrated over the first 119 hr. These data indicate that (*S*)-(+)-methadone has a higher plasma level and, therefore, higher relative bioavailability following an oral dose than does (*R*)-(−)-methadone. The total plasma compartment for methadone (both enantiomers) accounts for only 1.8–2.8% of the administered dose.

The second patient (Fig. 2) displays similar pharmacokinetic behavior but with two differences: (*a*) the plasma disappearance half-life of *R*-(−)-methadone is 1.69 times as long as that of the (*S*)-(+)-enantiomer, compared with the more normal 1.35-fold difference observed in the first patient; and (*b*) the integrated area under the plasma disappearance curves of *S*-(+)-methadone is 1.85 times greater than that of the (*R*)-(−)-enantiomer. The significance of these observations is not yet clear; Patient 1 had a history of chronic alcohol misuse, and Patient 2 had a history of chronic acute hepatitis.

The described methodology for plasma drug level measurements can also be applied to measure urinary excretion of unmetabolized methadone

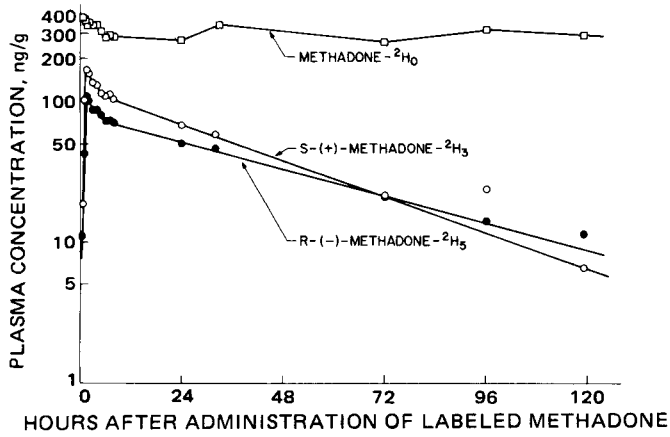

Figure 1—*Plasma methadone disappearance curves for methadone, (R)-(−)-[$^2H_5$]methadone, and (S)-(+)-[$^2H_3$]methadone determined in Patient 1.*

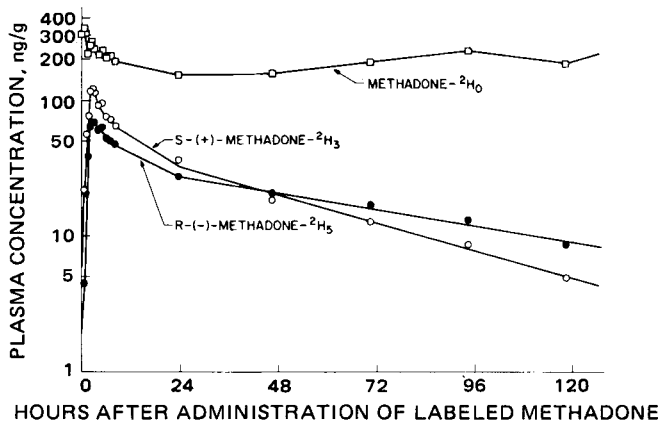

Figure 2—*Plasma methadone disappearance curves for methadone, (R)-(−)-[$^2H_5$]methadone, and (S)-(+)-[$^2H_3$]methadone determined in Patient 2.*

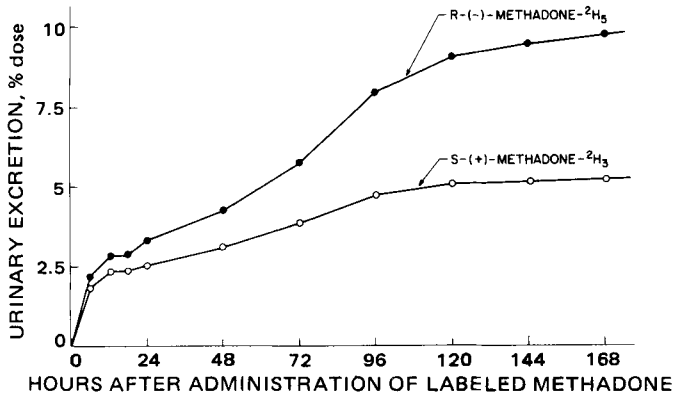

Figure 3—*Cumulative urinary excretion of (R)-(−)-[$^2H_5$]methadone and (S)-(+)-[$^2H_3$]methadone determined in Patient 1.*

enantiomers. Data presented in Fig. 3 illustrate the cumulative urinary excretion of the two methadone enantiomers in Patient 1. (*S*)-(+)-methadone reaches a cumulative excretion plateau value of 5.2% by 120 hr. In contrast, the (*R*)-(−)-methadone continues to be excreted throughout the study; by 168 hr, 9.8% of the dose was excreted as the unmetabolized drug. The urinary data, together with the plasma drug level data, suggest a differential renal clearance mechanism for the excretion of the unchanged methadone enantiomers. Full assessment of each step of the disposition of the separate enantiomers of methadone and its metabolites, including hepatic uptake metabolism, biliary excretion, fecal excretion, and urinary clearance, is needed before the mechanisms underlying the observed differences in overall disposition can be evaluated (25).

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Effect of Surface Charge and Particle Size on Gel Structure of Aluminum Hydroxycarbonate Gel

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Abstract □ The effect of surface charge and particle size on the gel structure of aluminum hydroxycarbonate gel was studied through the use of a specially designed tension cell. Surface charge has a major effect on the coefficient of bulk compressibility. The charged state is more compressible at lower tensions while the neutral gel is more compressible at higher tensions. In addition, physical properties of gels having a small particle size are more profoundly influenced by interparticle forces than are gels consisting of larger particles. The effect of surface charge and particle size on gel structure is applied to physical properties such as viscosity and dewatering.

Keyphrases □ Aluminum hydroxycarbonate gel—gel structure, effect of surface charge and particle size □ Gels—aluminum hydroxycarbonate, effect of surface charge and particle size on structure □ Physicochemistry—effect of surface charge and particle size on gel structure of aluminum hydroxycarbonate gel

Investigation of the structure of aluminum hydroxide gel at an atomic level has led to improved understanding of the arrangement of atoms and the types of bonds present in the various materials collectively known as aluminum hydroxide (1–4). An equally important type of structure, gel structure, which deals with the manner in

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