Paramethadione and Metabolite Serum Levels in Humans after a Single Oral Paramethadione Dose

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Abstract □ A GLC method was developed to determine quantitatively paramethadione and its major metabolite, 5-ethyl-5-methyl-2,4-oxazolidinedione, in serum. The method was reproducible and sensitive to 0.2 μg/ml. After administering a single 300-mg oral dose to human subjects, the average paramethadione serum levels of 6.0 μg/ml occurred at 1 hr and decreased to 0.3 μg/ml after 48 hr. Metabolite serum levels gradually increased to 8.4 μg/ml at 32 hr and were still at this level at 48 hr, which was the last sampling point.

Keyphrases □ Paramethadione and metabolite—serum levels, single oral dose, humans, GLC analysis □ 5-Ethyl-5-methyl-2,4-oxazolidinedione—paramethadione metabolite, GLC determination of serum levels, single oral dose, humans □ GLC—analysis, paramethadione and metabolite serum levels, humans

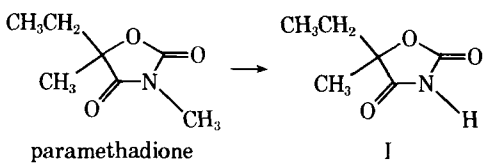
Several GLC methods (1, 2) are available for the quantitative determination of anticonvulsants in biological fluids. However, these methods lack the sensitivity needed to determine serum levels after a single oral dose. Butler (3) studied the metabolism of paramethadione in humans given multiple doses of paramethadione. A UV spectrometric method (4) was used to determine metabolite, 5-ethyl-5-methyl-2,4-oxazolidinedione (I), serum levels. Paramethadione levels were not determined by this method.

The metabolism of paramethadione to I is shown in Scheme I. A method is presented that can quantitatively determine paramethadione and its metabolite in serum after administration of a single oral 300-mg dose of paramethadione to humans.

EXPERIMENTAL

Subjects—Three normal, healthy, adult male subjects were fasted 8 hr prior to dosing (only water was allowed). Paramethadione capsules[1] were administered with 120 ml (4 oz) of water. A standard breakfast was provided to the subjects after the 2-hr blood sample was withdrawn. Twenty milliliters of blood was taken before dosing and at 0.5, 1, 2, 4, 8, 24, 32, and 48 hr after dosing. The serum was separated and frozen until assayed.

GLC—Chromatographic separations were performed with a gas chromatograph[2] equipped with an electronic integrator[3], automatic sampler, and dual flame-ionization detectors. Two 1.85-m × 2-mm (i.d.) glass columns were treated overnight with a 5% dimethylchlorosilane solution in toluene and then packed with 3% OV-17 coated on 100–120-mesh Gas Chrom Q[4]. All glass wool was also treated with the 5% dimethylchlorosilane solution. Before the columns were connected to the detectors, they were further silated by injecting 5 μl of *N,O*-bis(trimethylsilyl)acetamide three times at 10-min intervals and were conditioned overnight at 260°.



paramethadione I

Scheme I

The flow of helium carrier gas was 45 ml/min. The hydrogen flow rate was 30 ml/min, and the air flow rate was 250 ml/min. The temperature of the injection port was 172° and that of the detector was 190°. The oven temperature was programmed as follows: 2 min at 90°, then to 130° at 8°/min, and hold at 130° for 2 min. The electrometer range was 10 with an attenuation of ×4.

Isolation and Assay Procedures (1)—To 4 ml of serum in a 15-ml conical centrifuge tube was added 3 ml of 1 *N* H_2SO_4 and 3

Table I—Precision of Serum Paramethadione and Metabolite Assay at Various Concentrations

Parameth-adione Concentration, μg/ml	Coefficient of Variation, %[a]	Metabolite I Concentration, μg/ml	Coefficient of Variation, %[a]
0.5	10.2	0.5	8.4
1.0	8.4	1.0	6.1
2.0	6.2	2.0	5.8
4.0	5.1	4.0	4.2
8.0	5.5	8.0	3.7

[a] Three samples at each concentration.

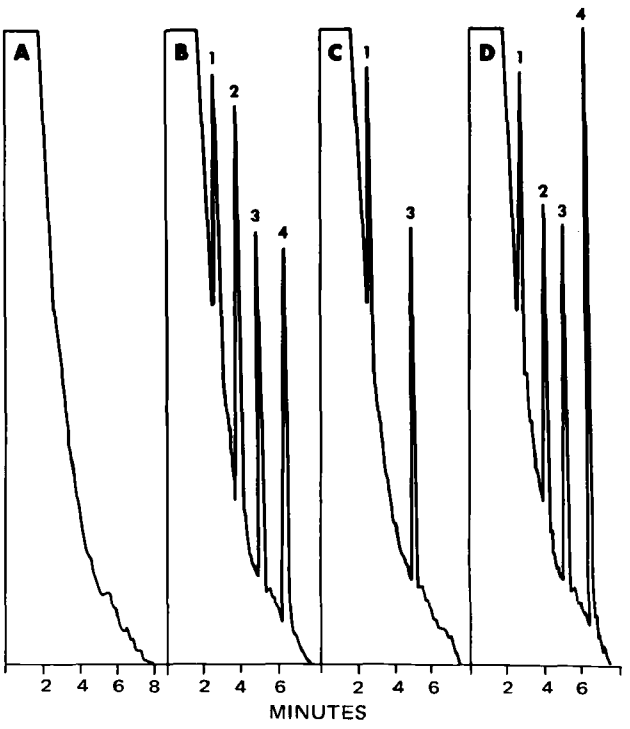


Figure 1—*Gas-liquid chromatograms of serum extracts. Key: A, serum blank without internal standards; B, serum standard containing paramethadione and I at 2.5 μg/ml; C, serum sample from Subject H.J. before dosing with paramethadione; D, serum sample from Subject H.J. 4 hr after a 300-mg oral dose of paramethadione; 1, trimethadione (internal standard); 2, paramethadione; 3, 5-dimethyl-2,4-oxazolidinedione (internal standard); and 4, Metabolite I.*

[1] Paradione Capsules, 300 mg, List 3868, Lot 08-078-AF, Abbott Laboratories, North Chicago, IL 60064
[2] Hewlett Packard model 7620A.
[3] Hewlett Packard model 3370A.
[4] Applied Science Laboratories.

Table II—Serum Paramethadione and Metabolite Levels after Oral Administration of 300 mg of Paramethadione

Subject	Age	Height, m	Weight, kg	Compound	Serum Concentration, μg/ml 0.5 hr	1 hr	2 hr	4 hr	8 hr	24 hr	32 hr	48 hr
H.J.	30	1.78 (5 ft 10 in.)	73.9 (163 lb)	Paramethadione	7.6	6.8	3.7	1.1	1.6	0.6	1.0	0
				Metabolite I	0.8	1.6	2.4	3.8	5.3	7.4	8.1	9.2
K.L.	30	1.75 (5 ft 9 in.)	74.8 (165 lb)	Paramethadione	0	5.4	2 3	2.7	2.0	—[a]	1.2	0.5
				Metabolite I	0	1.7	2.5	3.2	4.9	—[a]	8.6	7.4
J.M.	41	1.85 (6 ft 1 in.)	85.7 (189 lb)	Paramethadione	0.5	5.8	2.6	2.4	2.0	0.9	0.8	0.3
				Metabolite I	0	1.1	2.6	3.5	5.3	7.7	8.6	8.7
			Mean	Paramethadione	2.7	6.0	2.9	2.1	1.9	0.8	1.0	0.3
			Mean	Metabolite I	0.3	1.5	2.5	3.5	5.2	7.6	8.4	8.4

[a] Sample lost.

ml of 10% sodium tungstate dropwise, and the solution was mixed. After standing for 15 min, the samples were centrifuged at 3000 rpm for 15 min. The supernate (6 ml) was transferred to a 20-ml screw-capped tube containing 0.2 ml of 1 *N* H_2SO_4. Three grams each of anhydrous sodium sulfate and crystalline magnesium sulfate (0.7 μg/ml) was added with mixing. Chloroform (7 ml) containing the internal standards trimethadione (0.3 μg/ml) and 5-dimethyl-2,4-oxazolidinedione (0.7 μg/ml) was added, and the sample was shaken for 10 min and centrifuged at 2500 rpm for 10 min.

The aqueous layer was discarded by aspiration, and 5 ml of the chloroform layer was evaporated to about 0.1 ml at 30° with a gentle stream of air. Chloroform (0.1 ml) was added with mixing and then transferred to a microvial[5] and capped. The sample (10 μl) was automatically injected into the gas chromatograph, and the temperature program was initiated.

Standard serum solutions of both paramethadione and I were prepared in duplicate at concentrations of 0, 0.5, 1.0, 2.0, 4.0, and 8.0 μg/ml. Standard curves were constructed by plotting concentration *versus* paramethadione–trimethadione and I–5-dimethyl-2,4-oxazolidinedione peak area ratios.

RESULTS AND DISCUSSION

This GLC method has sufficient sensitivity to determine quantitatively paramethadione and its metabolite in serum after a single oral dose of paramethadione. The sensitivity of the method is 0.2 μg/ml. This value is based on a sample signal equivalent to about 2% of full-scale deflection. The precision of the assay is shown in Table I. Variance, as expected, was greater for the low concentrations. Coefficients of variation for paramethadione ranged from 5.5% at 8 μg/ml to 10.2% at 0.5 μg/ml. For I, they ranged from 3.7 to 8.4% at the same concentrations. Standard curves of serum paramethadione or metabolite concentrations *versus* peak area ratios were linear to at least 8 μg/ml. As shown in Fig. 1, GLC tracings were free of interfering peaks.

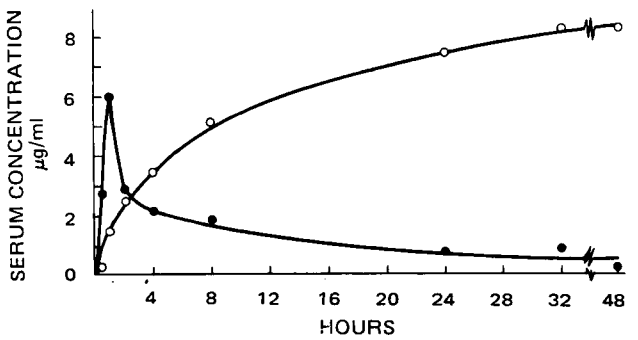


Figure 2—*Mean serum paramethadione and metabolite levels after oral administration of 300 mg of paramethadione in humans. Key: ●, paramethadione; and ○, Metabolite I.*

Individual paramethadione and metabolite serum levels are shown in Table II. The mean serum levels are plotted in Fig. 2. Paramethadione is rapidly absorbed with peak serum levels of 5.4–7.6 μg/ml occurring between 0.5 and 1 hr after a single 300-mg oral dose of paramethadione. A biphasic decline of serum paramethadione was observed, with serum levels decreasing to 0.3 μg/ml at 48 hr.

There appeared to be a slight rise in levels at 32 hr. The average serum half-life of the β-phase determined graphically was estimated to be 16 hr for the three subjects. This value is comparable to a serum half-life of 17 hr for trimethadione[6].

Metabolite serum levels were detectable within 1 hr after drug administration and gradually increased to 8.4 μg/ml in 32 hr; they remained at this level up to the last sampling time (48 hr) (Fig. 2). These results suggest that paramethadione is rapidly metabolized to I and that this metabolite has a long serum half-life, as evidenced by the apparent plateau of serum levels after 48 hr. This view is in accord with the data of Butler (3), from which a serum half-life of 14 days was estimated.

5-Dimethyl-2,4-oxazolidinedione, the metabolite of trimethadione, has a half-life of about 13 days in humans (4) and appears to play a considerable role in the anticonvulsant effect of trimethadione (5). The long serum half-life of 5-dimethyl-2,4-oxazolidinedione is attributed to its extremely slow, pH-dependent, renal excretion (6). The structural similarity of 5-dimethyl-2,4-oxazolidinedione and I and the apparently long serum half-life suggest that the latter metabolite also has a slow renal excretion rate.

REFERENCES

(1) H. E. Booker and B. Darcey, *Clin. Chem.*, **17**, 607(1971).

(2) W. C. Griffiths, S. K. Oleksyk, P. Dextraze, and I. Diamond, *Ann. Clin. Lab. Sci.*, **3**, 369(1973).

(3) T. C. Butler, *J. Pharmacol. Exp. Ther.*, **113**, 178(1955).

(4) *Ibid.*, **108**, 11(1953).

(5) H. Frey, *Acta Pharmacol. Toxicol.*, **27**, 295(1969).

(6) W. J. Waddel and T. C. Butler, *Proc. Soc. Exp. Biol. Med.*, **96**, 563(1957).

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[5] Hewlett Packard, 200-μl volume microvial.

[6] Dr. H. E. Booker, University of Wisconsin Medical Center, Madison, Wis., personal communication.