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THE METABOLIC FATE OF TYBAMATE IN THE RAT AND DOG*

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Abstract—Studies of the distribution of tybamate (N-*n*-butyl-2-methyl-2-propyl-1,3-propanediol dicarbamate) in the dog and rat show that the drug is readily absorbed, producing a peak serum level within the first hour after oral administration. The serum half-life is about 3 hr. Neither tybamate nor its metabolites are retained by the tissues but are excreted almost entirely in the urine in a 24-hr period. Two hours after administration, however, only about 20 per cent of the dose is excreted, with the remainder present predominantly in the carcass and gastrointestinal tract. Only minor amounts are present in the organs.

After tybamate administration, we have identified four compounds in the urine of both species. These are the unchanged drug; the major metabolite, hydroxytybamate (N-*n*-butyl-2-methyl-2[β-hydroxypropyl]-1,3-propanediol dicarbamate); meprobamate; and hydroxymeprobamate (2-methyl-2[β-hydroxypropyl]-1,3-propanediol dicarbamate).

THE WIDESPREAD use of the tranquilizer meprobamate has led to the examination of a number of related compounds. Among those found to be effective is tybamate, N-*n*-butyl-2-methyl-2-propyl-1,3-propanediol dicarbamate,† which possesses somewhat different pharmacological activity, as recently described by Berger *et al.*[1] Our findings relating to the metabolic fate and distribution of tybamate in the dog and rat are described in this paper.

METHODS

Preparation of tybamate-^{14}C

The substance was prepared as follows: 126 mg of N-*n*-butyl-2-methyl-2-propyl-3-hydroxypropyl carbamate in 5 ml of dry redistilled tetrahydrofuran was added to 68 mg phosgene-^{14}C in 4 ml tetrahydrofuran, and the reaction mixture was stirred continuously at 40° for 4 hr. The mixture was allowed to stand overnight at room temperature, the solvent was removed under vacuum at 40°, and the residue was dried over phosphorus pentoxide. This residue was dissolved in 10 ml anhydrous ethyl ether; 3 ml of liquid anhydrous ammonia was distilled slowly into the reaction flask, and the solution was refluxed for 3 hr in a condenser cooled with dry ice–acetone. After standing for 48 hr at room temperature, the mixture was extracted with water until it was neutral, and the ether layer was concentrated under vacuum. The

* A preliminary report of this paper was presented at the 1964 Spring Meeting of the American Society for Pharmacology and Experimental Therapeutics.

† This compound has also been designated as 2-methyl-2-propyltrimethylene butylcarbamate carbamate by the A.M.A.–U.S.P. Nomenclature Committee.

resultant oil was dissolved in 2 ml of hot trichloroethylene:hexane, 1:2, an additional carrier of 150 mg nonradioactive tybamate was added, and the solution was allowed to stand at room temperature. The crystals which were formed were isolated and dried over phosphorus pentoxide. Recrystallization of this product from trichloroethylene:hexane, 1:2, gave 108 mg tybamate-^{14}C; melting point, 48–49·5°; specific activity, 4·8 $\times$ 10^5 counts/min/mg; and homogeneous as judged by paper chromatography in the solvent systems described below.

Paper chromatography

Paper chromatography was carried out on Whatman 1 paper in the descending technique with three different solvent systems: (a) *n*-butanol:acetic acid:water, 4:1:5 (v/v/v), developed 16 hr at 27°; (b) carbon tetrachloride:acetic acid:water, 1:2:1 (v/v/v), developed for 6 hr at 4°;[2] (c) butanol saturated with water, developed 16 hr at 27°. Unlabeled carbamates were detected by the method of Rydon and Smith.[3]

Absorption, distribution, and serum concentration

Male Sprague–Dawley rats, fed *ad libitum*, received 3 mg tybamate-^{14}C (4 $\times$ 10^5 counts/min) orally in aqueous suspension. Four hours later the animals were sacrificed and the intestinal tract removed and combined with the feces. This material was homogenized with 6 volumes of 1 N potassium hydroxide in a small Waring Blendor, and the suspension was boiled for 5 min. A 3-ml aliquot was diluted to 10 ml with 1 M Hyamine hydroxide in methanol and was assayed in a liquid scintillation counter.*

Unfasted male Charles River rats were given 7 mg (1·5 $\times$ 10^6 counts/min) of tybamate-^{14}C in 1·0 ml propylene glycol, i.p., and were then placed in a metabolism chamber. Urine and feces were collected, and the respired carbon dioxide was absorbed in 2·5 N sodium hydroxide. After 24 hr the animals were sacrificed, and the tissues were excised. The tissues were processed and analyzed for radioactivity as described above for feces. Appropriate volumes of the absorbed carbon dioxide and of the urine were added directly to scintillation counting vials, and the radioactivity was measured. The 2-hr distribution study was carried out in similar fashion except that the rat received 6·5 mg (2·8 $\times$ 10^6 counts/min) of tybamate-^{14}C in 1·0 ml of an aqueous suspension.

Serum levels of tybamate were determined in male mongrel dogs by the method of Hoffman and Ludwig[4] after the administration by capsule of 15 mg tybamate/kg. It should be noted that one of the minor metabolites, meprobamate, also gives a positive response in this assay.

Metabolic fate in dogs

Two mongrel dogs, weighing approximately 14 kg each, were given 100 mg tybamate/kg, in capsule, daily for 5 days. Pooled urine, collected under toluene throughout the experiment, was continuously extracted with carbon tetrachloride for 24 hr. The carbon tetrachloride extract was decolorized with charcoal and was evaporated to dryness under reduced pressure to give a yellow oil which was crystallized from trichloroethylene:hexane, 1:2. The melting point of these crystals was 48–50° (isolate 1).

* The scintillating fluid consisted of 7 g of 2,5-diphenyloxazole, 250 mg of 1,4-bis-2-(4-methyl-5-phenyloxazoyl)-benzene, and 125 g napthalene in 1 l. dioxane.

The aqueous layer remaining after the carbon tetrachloride treatment was then extracted continuously with ether for 24 hr. The ether extract was concentrated to dryness under reduced pressure; the residue was dissolved in water and extracted with 10 volumes of a 1:1 mixture of chloroform–carbon tetrachloride. Concentration of this organic phase produced an oil that could be crystallized from trichloroethylene; the melting point of these crystals was 105–107° (isolate 2).

The aqueous phase remaining after the first two solvent extractions was then continuously extracted with ethyl acetate for 48 hr. Concentration of this extract gave a yellow oil which was purified by paper chromatography; Whatman 3 MM paper and butanol saturated with water as the developing solvent were used. The material having an R_f of 0·75–0·85 was eluted with acetone and was further purified by 50 transfers in a countercurrent distribution apparatus, with mutually saturated butanol and water as the solvent phases. The butanol portions of tubes 29–36 were combined and concentrated to a colorless oil under reduced pressure (isolate 3).

A larger yield of isolate 3 was obtained from another dog which was given 200 mg tybamate/kg, in capsule, daily for 2 days. The urine was collected and was extracted continuously with ether for 24 hr to remove isolates 1 and 2. The ether-extracted urine was then extracted continuously with *n*-butanol for 30 hr, and the butanol extract was concentrated to a small volume. This oily residue was fractionated on a cellulose column 8 cm × 50 cm, with water-saturated butanol as the moving phase. A series of 50-ml fractions was obtained in this way; fractions 11 and 12 were combined and further purified on Whatman 3 MM paper, with butanol-saturated water as the developing solvent. The area of paper with an R_f value of 0·75–0·85 was eluted with acetone, and the organic solvent was removed under reduced pressure. The remaining oily residue, which was shown to be homogeneous by paper chromatographic analysis in three different solvent systems, was refluxed for 3 hr with 1 N potassium hydroxide under a stream of nitrogen, and the effluent gas was bubbled through 6 N hydrochloric acid to trap any amines liberated during hydrolysis. Neutralization of the acid, followed by ether extraction, gave a material which, when subjected to gas liquid chromatographic analysis, proved to be *n*-butylamine. The conditions employed on a F and M model 609 were 10 per cent ethanolamine on Anakrom ABS 70–80 mesh column at 75°; N_2, 60 ml/min; H_2 50 ml/min; O_2 220 ml/min; range 100; attenuation 8.

The remaining hydrolysis reaction mixture was extracted with *n*-butanol, and the solvent was removed *in vacuo*. The residue was taken up in acetone and was found by gas–liquid chromatography to contain 2-methyl-2(β-hydroxypropyl)-1,3-propanediol. Chromatographic specifications: 2 per cent Versamid on Gas-Chrom Q 100–120 mesh column at 300°; N_2, 20 ml/min; H_2, 50 ml/min; O_2, 250 ml/min; range 100; attenuation 16.

Isolate 4 was obtained from fractions 8 through 10 of the cellulose column eluate described above. These fractions were combined and chromatographed on Whatman 3 MM paper, with butanol-saturated water as the developing solvent. The area of paper with an R_f value of 0·65–0·75 was eluted with acetone, and the solvent was removed under reduced pressure to give an oil. This oil was shown to be homogeneous by paper chromatographic behavior in each of the three solvent systems mentioned previously.

Quantitation of metabolic products in the rat

Male Sprague–Dawley rats weighing approximately 150 g were given 7 mg tybamate-^{14}C ($1{\cdot}7 \times 10^6$ counts/min) i.p. in 1·0 ml propylene glycol. The urine was collected under toluene for the next 24 hr and was analyzed for metabolites by radioactive scanning of paper chromatograms developed in both the CCl_4–AcOH–H_2O and the BuOH–H_2O systems. The amounts present were estimated by measuring the area under the recorder trace of each compound.[5]

Glucuronide formation

A male Sprague–Dawley rat weighing approximately 150 g was given 9 mg ($2{\cdot}1 \times 10^6$ counts/min) of tybamate-^{14}C i.p. in 1·0 ml propylene glycol. Urine was collected under toluene for the next 24 hr, and an aliquot containing 5×10^5 counts/min was used to obtain the basic lead glucuronide fraction.[6] Free glucuronides were liberated by treatment with hydrogen sulfide and were assayed for radioactivity by liquid scintilation counting.

RESULTS AND DISCUSSION

Absorption, distribution, and serum concentration

The gastrointestinal absorption of tybamate was investigated in the rat. Four hours after the administration of an oral dose of radioactive drug to the rat, $20{\cdot}7 \pm 3{\cdot}6$ per cent of the radioactivity remained in the gastrointestinal tract, indicating that at least 80 per cent of the dose had been absorbed. Two hours after an oral dose, however, the bulk of the administered radioactivity remained in the gastrointestinal tract, with significant amounts present in the carcass and urine (Table 1).

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN A RAT 2 HOURS AFTER ORAL ADMINISTRATION OF TYBAMATE-^{14}C

Tissue analyzed	Activity recovered (% of dose)
Urine	21·1
Gastrointestinal tract with contents	66·7
Brain	0·004
Spleen	0·01
Heart	0·01
Lung	0·01
Kidney	0·11
Liver	0·30
Carcass, including blood	22·1

A study of the distribution of radioactivity in the rat tissues and urine 24 hr after the intraperitoneal administration of tybamate-^{14}C showed that 87 per cent of the activity was excreted in the urine, with small amounts also present in the feces (4·6 per cent) and intestinal contents (3·4 per cent).

After oral administration to dogs, tybamate was rapidly absorbed, giving a maximal blood serum level of approximately 10 μg/ml within the first hour. Subsequently, the blood concentration of tybamate declined rapidly, with no drug detectable at 24 hr. The half-life of tybamate calculated from these data is approximately 3 hr (Fig. 1).

Identification of urinary metabolites

Paper chromatographic examination of urine extracts obtained from the dogs given 100 mg tybamate/kg daily for 5 days showed the presence of four end products. The three solvent systems utilized and the R_f values of the metabolites are given in

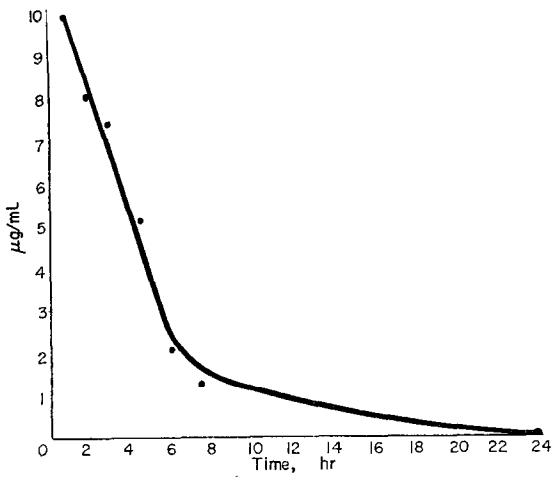


FIG. 1. Serum concentration of tybamate after oral administration of 15 mg drug/kg to dogs (average of six animals).

Table 2. The four compounds have been arbitrarily designated 1 through 4 according to their migration rates in the BuOH–AcOH–H_2O system, with the fastest-moving component assigned the lowest number.

TABLE 2. R_f VALUES OF URINARY METABOLITES

Isolate no.	BuOH:AcOH:H_2O (4:1:5)	BuOH:H_2O (satd.)	CCl_4:AcOH:H_2O (1:2:1)
1	0·94	0·93	0·90
2	0·89	0·89	0·35
3	0·83	0·80	0·02
4	0 78	0·70	0·02

Isolate 1 proved to be unchanged tybamate. This was confirmed by paper chromatography, melting point, and mixed melting point with an authentic specimen of tybamate. The infrared spectrum of the isolate was also identical with that of unchanged tybamate (Fig. 2).

Isolate 2 was shown to be the N-dealkylated derivative of tybamate (meprobamate) by its paper chromatographic behavior in three solvent systems, its melting point,

its mixed melting point with an authentic sample of meprobamate, and a comparison of its infrared spectrum with that of meprobamate (Fig. 3).

Infrared spectral analysis of isolate 3 indicated that it was an N-substituted carbamate, as shown by absorption peaks at 6·5 and 8·0 mμ, and that it probably contained a hydroxyl group (absorption peak at 8·9 mμ; see Fig. 4). Hydrolysis with potassium hydroxide gave the two cleavage products which were identified by gas-liquid chromatography as *n*-butylamine and 2-methyl-2(β-hydroxypropyl)-1,3-propanediol. Hydrolysis of tybamate under similar conditions yields *n*-butylamine

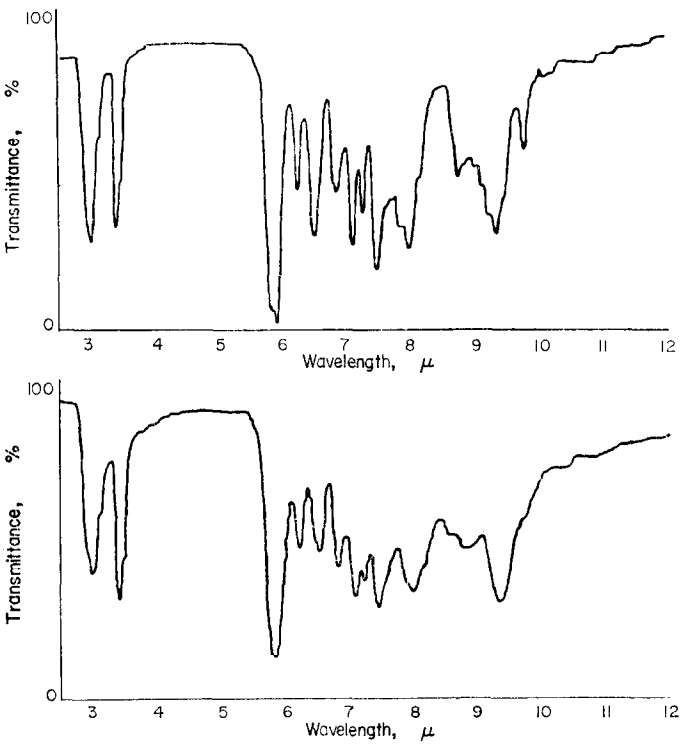


FIG. 2. Infrared absorption spectra of tybamate (upper) and urine isolate 1 (lower).

and 2-methyl-2-propyl-1,3-propanediol. Identity of the two fragments obtained by hydrolysis of isolate 3 confirms its structure as N-*n*-butyl-2-methyl-2(β-hydroxypropyl)-1,3-propanediol dicarbamate (hydroxytybamate). This finding was consistent with the known metabolic fate of the related dicarbamates, meprobamate,[7] mebutamate,[8] and carisoprodol,[9, 10] all of which are hydroxylated at the penultimate carbon of their longer alkyl side chain.

Isolate 4 was identified as hydroxymeprobamate by means of its chromatographic behavior in three different solvent systems and by its i.r. spectrum (Fig. 5), which was identical with that of a synthetic sample of hydroxymeprobamate.

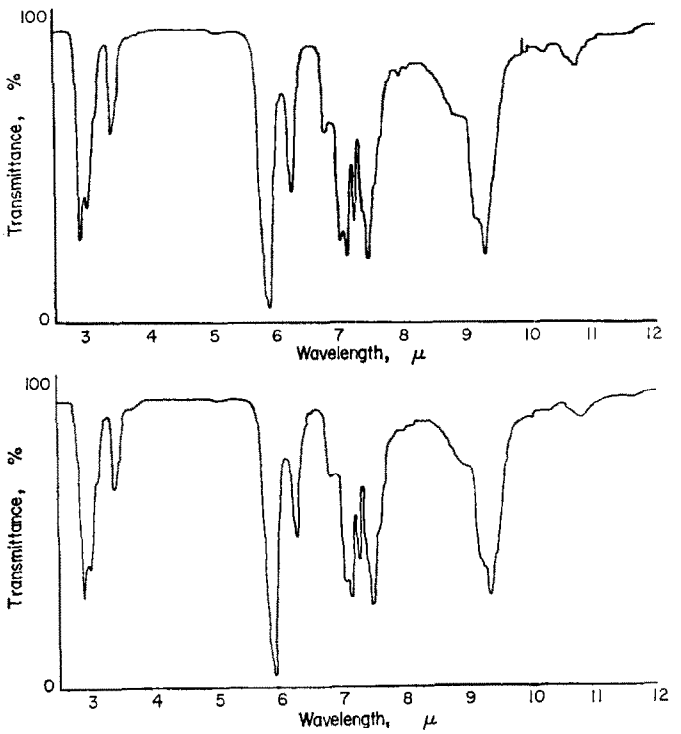


FIG. 3. Infrared absorption spectra of meprobamate (upper) and urine isolate 2 (lower).

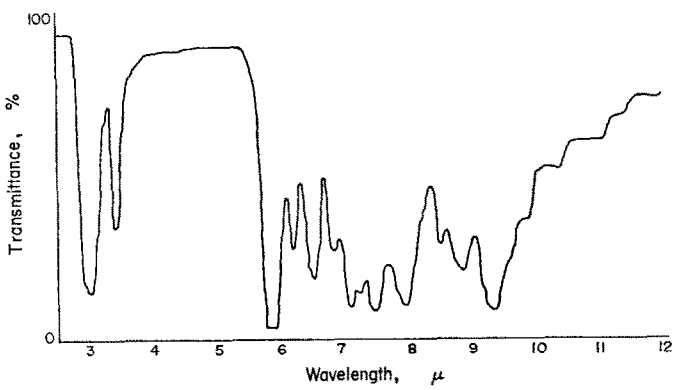


FIG. 4. Infrared absorption spectrum of urine isolate 3.

The structural formulae of the urinary end products are given in Fig. 6.

Quantitation of urinary end products

The relative amounts of the various end products formed were studied in the rat after the administration of tybamate-^{14}C. Urine obtained 24 hr after i.p. administration of the radioactive drug was chromatographed on paper and the resultant chromatograms scanned quantitatively for radioactivity. The results, which are averages of

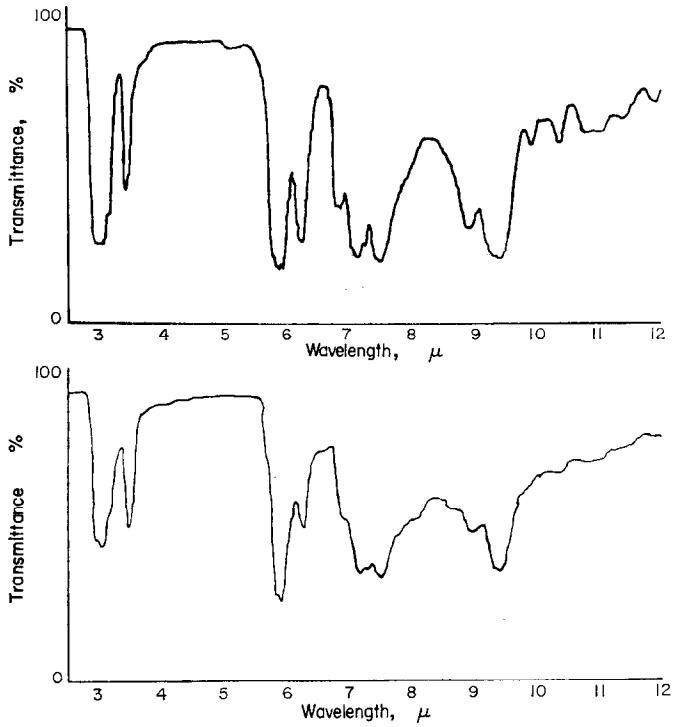
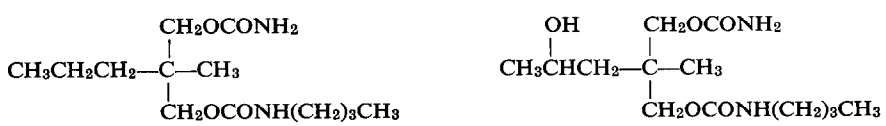
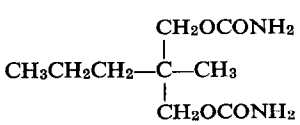


FIG. 5. Infrared absorption spectra of hydroxymeprobamate (upper) and urine isolate 4 (lower).

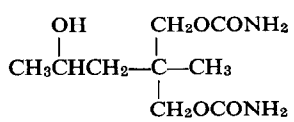


TYBAMATE

HYDROXYTYBAMATE



MEPROBAMATE



HYDROXYMEPROBAMATE

FIG. 6. Structural formulae of tybamate and three metabolic products.

three separate experiments, are shown in Table 3. Hydroxytybamate was the primary metabolite, with hydroxymeprobamate present in urine in lesser amounts. Unchanged tybamate and its dealkylated product (meprobamate) were excreted in only minor quantities.

TABLE 3. QUANTITATION OF URINARY END PRODUCTS AFTER INTRAPERITONEAL ADMINISTRATION OF TYBAMATE TO RATS*

	Per cent of radioactivity	
	Average	Range
Tybamate	7·5	0–22·5
Hydroxytybamate	60·5	50·8–69·8
Hydroxymeprobamate	30·3	21·8–38·7
Meprobamate	2·2	1·6–3·2

* Average of three animals.

It is unlikely that the biological effects of tybamate are due to its degradation to meprobamate. This view is supported by the observation that tybamate itself has a much more powerful biological effect than meprobamate. Thus, for example, the mean paralyzing dose of tybamate in mice is about 96 $\pm$ 2 mg/kg, as compared with 235 $\pm$ 7 mg/kg for meprobamate. This indicates that tybamate must exert its biological effects prior to metabolic transformation. This view is also supported by the observation that tybamate has several biological properties that meprobamate does not possess. For example, tybamate has the ability to reverse the electroencephalographic effects of LSD-25 in rabbits and to antagonize the serotonin-produced blood pressure rise in dogs.[1]

Glucuronide metabolites

Since other carbamates such as meprobamate[7] are known to be excreted in part as glucuronide conjugates, the glucuronide fraction, obtained as the basic lead salt, was examined for radioactivity. Between 1 and 2 per cent of the dose was present in this fraction, indicating that, although tybamate or one of its metabolites may be excreted in conjugated form, the amount voided is very small.

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