sults with MT and nMT. The antifibromatogenic activity of 15 μg/day of nMT approaches that of 255 μg/day of MT (Fig. 2). With 11 μg/day of MT no antifibromatogenic action was obtained; 15 μg/day of nMT were sufficient to produce a very pronounced action, with a significant difference of 4.0 compared to 11 μg/day of MT. The antihysterotrophic activity of nMT, compared to that of MT, is even more remarkable; the small quantities of nMT used were more effective than the large quantity of MT. The antifibromatogenic activity of nMT is about 10 times that of MT.

Table I summarizes also the comparative results with the two Δ^1-cpds. The antifibromatogenic activity of the 2 cpds. decreases considerably in comparison to T and A (significant difference between Δ^1-T and T: 2.7). A decrease of the antihysterotrophic activity also is very probable.

Summary. The antifibromatogenic and antihysterotrophic activities of 19-nor-testosterone phenylpropionate and 19-nor-methyltestosterone are 6 to 10 times greater than those of testosterone propionate and methyltestosterone respectively. The activity of testosterone is decreased by Δ^1. No activity is obtained even with very considerable quantities of Δ^1-testosterone or Δ^1-androstenedione.

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Determination and Physiologic Disposition of Meprobamate.* (23449)

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Although the use of the tranquilizing agent 2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate (meprobamate) has become widespread, relatively little is known about its physiologic disposition and metabolic fate. This report includes a specific method for determination of meprobamate in urine and the results of metabolic studies *in vivo* and *in vitro*.

Methods. Estimation of Meprobamate. Meprobamate is isolated from biological material by extraction into ether at an alkaline pH. After evaporation of the ether the amount of drug is determined spectrophotometrically at 450 mμ, at which wavelength the drug exhibits a pronounced peak when heated with sulfuric acid (Fig. 1). *Procedure.* To 3 ml of urine in a 15 ml glass-stoppered centrifuge tube are added 0.2 ml 1 N sodium hydroxide and 5 ml of ether.† Shake 5 minutes and centrifuge the tube. Two ml of the ether phase are evaporated to dryness under a stream of air in a heavy-walled test tube, and

* A preliminary report was presented at Am. Chem. Soc., Miami, April, 1957.

† Ether was Mallinckrodt anhydrous analytical reagent.

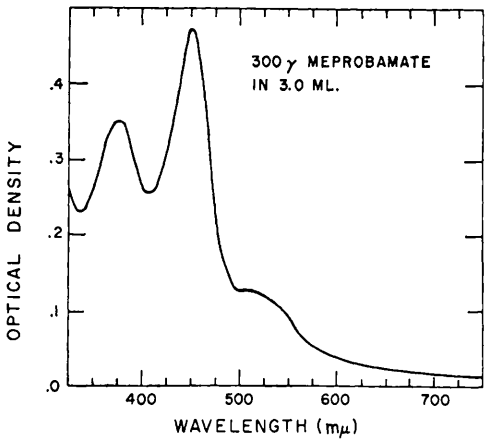

FIG. 1. Absorption spectrum of meprobamate after heating with sulfuric acid.

1 ml of concentrated sulfuric acid is added. The tube is heated at 100°C 30 minutes. After the solution is cooled, 2 ml of water are cautiously added to intensify the color. The mixture is allowed to stand for 15 minutes and the optical densities are measured in a Beckman DU spectrophotometer at 450 and 500 mμ. Since the ether extraction results in the recovery of only 85 ± 5% of the meprobamate, a standard solution containing 60 μg of meprobamate/ml is run at the same time as the unknown. The difference between the optical densities at 450 mμ and 500 mμ is used for meprobamate estimation in biological materials rather than the 450 mμ absorption alone in order to eliminate the interference of non-specific absorption by naturally occurring substances. The linearity of the color reaction is shown in Fig. 2. The sensitivity of the

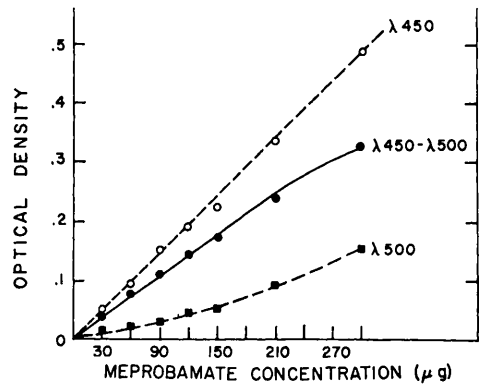

FIG. 2. Linearity of color developed in sulfuric acid reaction.

TABLE I. Molar Extinction Coefficient of Meprobamate and Related Compounds.

Compound	E_M*
2-Methyl-2-*n*-propyl-1,3-propanediol dicarbamate	9.2×10^4
2-Methyl-2-*n*-propyl-1,3-propanediol	1.1×10^4†
Methyl-*n*-propyl malonic acid	0
2-Methyl-2-*n*-propyl-3-hydroxypropyl carbamate	5.0×10^4
α-Methyl-α-*n*-propyl hydracrylic acid	0
α-Methyl-α-*n*-propyl-β-carbamoxy propionic acid	0

* Molar extinction coefficient based on difference between optical densities at 450 and 500 mμ for 1 cm path length.

† This material was not of high purity.

method can be increased by using smaller amounts of sulfuric acid and water and reading the optical density in a 1 ml cuvette. As little as 2 μg of meprobamate can be detected by this procedure.

Results. Few compounds related to meprobamate reacted‡ with sulfuric acid (Table I). However, alcohols having 4 or more carbon atoms and no carboxyl groups showed the 450 mμ maximum when heated with sulfuric acid. For example, n-butyl and sec-butyl alcohols gave the reaction, but α, β, or γ hydroxybutyric acid did not.

Identification of meprobamate in urine. A test for meprobamate in the ether extract of urine of subjects who took the drug was positive. To establish the identity of the urinary product as meprobamate, an aliquot of the dried ether extract was subjected to the technic of comparative distribution ratios between a number of organic solvents and an equal volume of 0.1 N sodium hydroxide. It can be seen (Table II) that the apparent meprobamate isolated from urine and measured spectrophotometrically after sulfuric acid treatment had the same distribution ratios as the authentic compound, and was therefore presumably the same.

Urinary excretion. Four human subjects were each given a single dose of 1600 mg of meprobamate orally. Urine samples were collected from 0 to 24 hours and 24 to 48

‡ The authors are indebted to Dr. B. Ludwig, Wallace Laboratories for samples of meprobamate and analogues.

TABLE II. Distribution Ratios of Apparent and Authentic Meprobamate.

Solvent	Authentic meprobamate	Apparent meprobamate isolated from urine
Ether	.94	.88
Ethylene dichloride	.82	.79
Benzene	.56	.58
n-Heptane	.31	.34

hours and examined for free meprobamate (Table III). It can be seen that meprobamate is excreted relatively slowly for at least 2 days following a single dose. About 12 to 20% of the administered meprobamate was excreted unchanged in the urine. The cumulative urinary excretion in one of the subjects is shown in Fig. 3.

Metabolites of meprobamate. Berger(1) reported an increase in urinary glucuronides following ingestion of meprobamate. In order to obtain more direct evidence of glucuronide formation, urine from a subject given meprobamate was extracted with ether to remove free meprobamate and then incubated at 37°C with β-glucuronidase.§ Under these conditions, an ether-soluble substance was released that gave the characteristic meprobamate reaction with sulfuric acid. The urine incubated without glucuronidase or with boiled β-glucuronidase did not liberate any meprobamate-like material. The nature of the glucuronide formed has not been established, but the formation of an N-glucuronide appears unlikely, since this class of substances

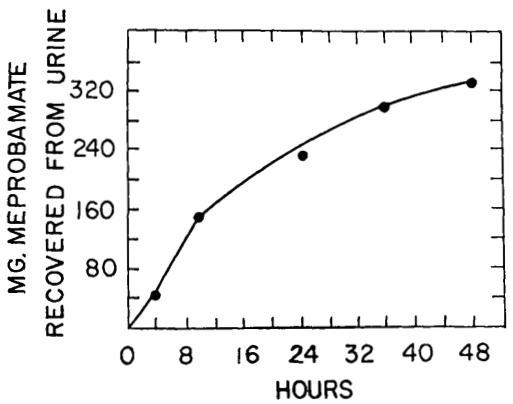

FIG. 3. Cumulative urinary excretion of meprobamate following oral administration of 1600 mg.

§ Ketodase, Warner-Chilcott Laboratories, N. Y.

TABLE III. Urinary Excretion of Meprobamate in 4 Adult Human Subjects.*

Meprobamate excreted, mg		Total meprobamate excreted, % of dose
0-24 hr	24-48 hr	
159	33	12.0
148	43	11.9
234	96	20.6
145	49	12.1

* Each subject received 1600 mg of meprobamate orally.

is more unstable than the urinary product obtained and is not hydrolyzed by β-glucuronidase(2). It is possible that β-hydroxylation occurs and that an O-glucuronide is formed. There is no evidence as yet that the carbamate linkage is altered metabolically. After treatment with β-glucuronidase, the meprobamate-like material was removed from the urine by ether extraction. Heating the residual urine with hydrochloric acid or sodium hydroxide resulted in the liberation of additional ether-soluble material that gave the characteristic meprobamate spectrum with sulfuric acid. The total amount of meprobamate-like material recovered, including substances released by the action of the β-glucuronidase and acid hydrolysis, account for about one-half of the administered dose, assuming that these products, if not identical with meprobamate, possess extinction coefficients in the color reaction of the same order of magnitude as meprobamate.

Metabolic studies with radioactive meprobamate. Meprobamate was tritiated by the technic of Wilzbach(3). The resultant product was diluted with nonisotopic meprobamate and recrystallized to constant radioactivity. The site of metabolic alteration of meprobamate was studied by incubating radioactive meprobamate with rat tissue slices as follows: 250 mg of tissue slice were incubated in an atmosphere of 95% O_2 - 5% CO_2

TABLE IV. Disappearance of H^3-Meprobamate after Incubation with Tissue Slices.

Tissue	Meprobamate disappeared, μM/g/hr
Brain	.205
Kidney	.302
Liver	.906
Hemidiaphragm	.330

in 2.5 ml Krebs-Ringer bicarbonate buffer containing 10 μM of glucose and 1.38 μM of radioactive meprobamate. At the end of 2 hours, the incubation mixture was homogenized, made alkaline, and the meprobamate was extracted with ether and counted in a liquid scintillation counter. The results shown in Table IV indicate that liver metabolizes the drug to a greater extent than do the other tissues examined. Following intravenous injection of 4 mg/kg of H^3 meprobamate significant levels of radioactivity were found in all tissue examined (liver, kidney, spleen, brain, testes, muscle, fat, spinal fluid, and blood).

Summary. (1) A specific method for the determination of meprobamate in urine is described. (2) Metabolic studies indicate that about 12 to 20 percent of the unchanged drug is slowly excreted in the urine in 48 hours and that additional products are formed, including several conjugates at least one of which is a glucuronide. (3) Radioactive meprobamate has been prepared and metabolic studies have been performed. The liver is the principal site of metabolic alteration.

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Effect of Pyrazinamide and Pyrazinoic Acid on Urate Clearance and Other Discrete Renal Functions.*† (23450)

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Cullen *et al.*(1) and Gleason *et al.*(2) recently reported the occurrence of marked hyperuricemia, associated with decreased urinary urate excretion, after administration of the antituberculous agent pyrazinamide (pyrazinoic acid amide). The present report summarizes the results of clearance studies intended to determine more precisely the effect of the drug on the renal mechanisms regulating urate excretion. When it became apparent in the course of the study that the free acid (pyrazinoic acid) might be the active agent, appropriate studies with this compound also were initiated.

Methods. Fifteen male adults were available for study. Ten of these were gouty, in the intercritical phase of the disorder, and afforded opportunity to determine whether the drug could exert its effects in the face of already established hyperuricemia and, in some instances, excessive urinary urate excretion. To ascertain whether the renal effects occurred soon enough after oral administration of the drug to permit application of conventional renal clearance technics, preliminary experiments were performed in 5 subjects who received single oral doses of 3 g or 1 g. Determinations of the urate excreted in urine specimens spontaneously voided at hourly or 2-hourly intervals indicated a sufficiently prompt response, even with 1.0 g dosage. Standard renal clearance technics (3) were then employed in 10 subjects. After appropriate control periods, in which clearances of inulin, p-aminohippurate and urate were measured, the drug in 1 g or 3 g dosage was given by mouth; pyrazinamide in 4 experiments, pyrazinoic acid in 6 including 2 with 50 and 100 mg dosage. Post-medication urine collections, in 15-20 minute periods,

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