ministered proteolytic enzymes is observed in the absence of any detectable increase in proteolytic activity of the blood(18). This finding suggests an indirect influence of proteolysis on the inflammatory process, although it does not exclude a possible concentration of the enzyme at the inflamed site with a consequent rise in local activity as compared to low systemic levels. The possibility is not excluded, however, that systemically administered proteolytic enzymes act indirectly by activating other systems which in turn counteract inflammation. The problem appears further complicated by the observation that both trypsin and soya bean trypsin inhibitor are effective in suppressing the inflammatory reaction(19).

The nature of the phlogogenic action of the profibrinolysin preparation is obscure. In addition to profibrinolysin the product contains also gamma globulin(20) which may be responsible for this effect. It is likely that the activity of the permeability increasing factor of the preparation is not destroyed by fibrinolysin, since the latter affects in a like manner the reactions induced by histamine. The properties of this factor must await further characterization.

Summary. Application of radioisotope technics to the study of the inflammatory reaction confirmed the antiinflammatory properties of fibrinolysin (plasmin) preparations. The effect was studied in both histamine and profibrinolysin (plasminogen) induced inflammation. In both cases 400 units of fibrinolysin neutralized the response bringing it down to control levels.

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Studies on the Metabolism of Meprobamate. (28069)

J. F. DOUGLAS, B. J. LUDWIG AND NANCY SMITH (Introduced by F. M. Berger)
Wallace Laboratories, Division of Carter Products, Inc., Cranbury, New Jersey

The urinary excretion products obtained after administration of meprobamate (2-methyl-2-propyl-1,3-propanediol dicarbamate) to warm-blooded animals include unchanged drug(1), an oxidized derivative, recently identified as 2-methyl-2-(β-hydroxypropyl)-1,3-propanediol dicarbamate(2), and a glucosyluronide conjugate(1-5). Although the first 2 substances have been well characterized, the literature reveals only 2 references to their quantitative relationship(5,6). We have investigated the excretion pattern of

these 2 metabolites in a number of animal species. This paper summarizes the results of our studies.

Methods. Laboratory animals were administered meprobamate in accordance with the following dosage schedule:

Oral	100 mg/kg
Intraperitoneal	100 mg/kg
Intravenous	40 mg/kg
Subcutaneous	200 mg/kg
Forced feeding	Water equivalent of 5% of body weight followed by oral administration.
Repeated dosage	Daily oral dose for 7 weeks.

The animals were administered meprobamate and their urine collected under toluene for 24 hours. Aliquots were taken at appropriate times and chromatographed directly. Experiments were conducted on fresh specimens from at least 2 of every animal species, with each urine sample being analyzed in duplicate.

Human subjects were divided into 2 categories. One group included patients receiving 1200-1600 mg of meprobamate (approximately 20 mg/kg) over a single 18-hour period, while the other consisted of patients who had received this same daily dose of drug for periods in excess of one month. Aliquots of urine collected from these subjects at appropriate times were chromatographed directly.

Paper partition chromatography, in conjunction with a visual comparison method, was used for estimating the concentration of meprobamate and its hydroxylated derivative. The assay was carried out by spotting 1, 2, 3, and 6 μg of the known compounds in 8 μl of water and comparing this with 2, 5, and 8 μl of urine diluted, when necessary, to a final volume of 8 μl. The descending chromatographic technic was employed using Whatman No. 1 filter paper with the partition solvent consisting of n-butanol:acetic acid:water (4:1:5, v/v/v). The color developing system, which is sensitive to 0.3 μg of either substance measured, is a modification of the method of Rydon and Smith(7), utilizing chlorine gas followed by starch-potassium iodide spray.

Results. The results obtained in 5 different animal species can be effectively evaluated in terms of relative concentration ratios of hydroxymeprobamate to meprobamate. Utilizing this criterion, we found that most of the animals voided these compounds both at 6 hours and at 24 hours after administration of the drug in a ratio in excess of 2:1 and, in some cases, as high as 7:1 (Table I). No substantial difference in this ratio was found using different routes of administration, including oral, intraperitoneal, intravenous, subcutaneous, oral after forced feeding of water, and frequent dosage over long periods.

In human subjects, however, some differences were noted (Table II). Those individuals receiving meprobamate for only one day

TABLE I. Ratio of Hydroxymeprobamate to Meprobamate Excreted in Urine of Animals Receiving Meprobamate.

Species	No.	Admin*	Time, hr	Conc of meprobamate in urine, %	Ratio of excretory products (meprobamate = 1)
G. pig	2	Oral	6	.04	2.4
Mouse	2	"	6	.03	2.7
Rat	4	"	6	.05	4.0
Dog	4	"	6	.06	2.1
G. pig	2	"	24	.03	3.8
Mouse	2	"	24	.02	4.0
Rat	4	"	24	.03	4.4
Dog	3	"	24	.05	4.6
Dog	3	I.P.	6	.01	1.5
G. pig	2	"	6	.03	3.4
Dog	2	"	24	.04	3.3
G. pig	2	"	24	.03	3.9
Rabbit	3	"	24	.04	2.2
Dog	1	I.V.	4	.03	2.1
Mouse	2	"	4	.02	3.7
Dog	3	"	24	.02	5.4
Mouse	2	"	24	.01	3.8
Rabbit	2	"	24	.01	3.0
Dog	1	S.C.	6	.01	2.5
Rat	2	"	6	.04	4.4
G. pig	2	"	6	.07	2.6
Dog	2	"	24	.01	7.0
G. pig	2	"	24	.10	3.3
Rat	2	"	24	.05	3.7
Dog	3	F.F.	6	.02	2.6
Rat	2	"	6	.03	3.3
Mouse	3	"	6	.03	2.3
Dog	2	"	24	.05	3.3
Rat	2	"	24	.03	2.5
Mouse	2	"	24	.01	3.7
Rat	2	R.D.	24	.04	4.0

* I.P., intraperitoneal; I.V., intravenous; S.C., subcutaneous; F.F., forced feeding; R.D., repeated dosage.

TABLE II. Ratio of Hydroxymeprobamate to Meprobamate Excreted in Urine of Human Subjects Receiving Meprobamate Orally.

Period drug admin	No. of subjects	Avg ratio of excreted product (meprobamate = 1)
1 day	5	.9
Daily for over 30 days	5	1.8

had significantly lower ratios (less hydroxymeprobamate) than patients ingesting meprobamate daily for periods exceeding one month. The single-day individuals had a ratio of about unity (0.9:1) while the other group exhibited a corresponding ratio of 1.8:1. In each case these values were substantially lower than those found for the laboratory animals studied. This difference may be due in part to the relatively large doses administered to animals.

The ratio change observed on chronic administration of the drug to humans suggests an adaptive process on the part of these subjects to the metabolism of meprobamate. A similar effect has beeen observed for rats given repeated doses substantially larger than those employed in our studies(6,8,9), on rats given large single doses of meprobamate(10) and for rats whose liver enzyme systems had been prestimulated by other drugs(11-13).

Phenylbutazone, which stimulates the metabolism of meprobamate and aminopyrine by rat liver microsomes(11) has also been shown to accelerate the metabolism of aminopyrine in humans(14). Our observation of the stimulation of meprobamate metabolism by meprobamate itself in humans appears to be analogous to the results obtained with the phenylbutazone-aminopyrine combination and corresponds to the self-induced enhancement of meprobamate metabolism recently reported in laboratory animals(6,8-10).

The shift observed in the ratio of meprobamate urinary excretion products might be due to a stimulation of either the hydroxylation reaction or the glucuronide-forming step. Each produces an inactive water-soluble detoxification product, resulting in removal of meprobamate from the bloodstream.

Summary. Urinary excretion ratios of meprobamate and its major metabolic product, hydroxymeprobamate, were determined in a number of laboratory animal species following administration of meprobamate by several routes. No major differences were apparent. Humans receiving relatively small doses of this drug excreted a higher proportion of unchanged meprobamate, especially after a single administration of the drug. Chronic administration of meprobamate to humans appeared to stimulate its metabolism.

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