of Mn^{2+}. When sodium L-thyroxine was incorporated into the ergot culture medium, an appreciable decrease in alkaloid production resulted but an increase in growth was obtained. Thyroxine is known to inhibit cholesterol synthesis (22), a process which may be related to the effect of L-thyroxine on oxygen consumption. Evans (23), in discussing the mechanism of microbial hydroxylations, points to the involvement of oxygenases in these phenomena. One of the hydroxylase inhibitors is *p*-chlorophenylalanine. When it was incorporated into the medium, a marked decrease (52%) in alkaloid production resulted.

The results show, on one hand, that the incorporation of some oxygenase inducers into the ergot culture produces considerable increase in alkaloid production, while, on the other hand, reduction in alkaloid production is obtained from substances which have inhibitory effects on oxygenases. The findings, therefore, lend strong support to the concept that oxygenases are involved in the biogenesis of the ergot alkaloid.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 22, 1968, from *Purdue University School of Pharmacy and Pharmacal Sciences, Lafayette, IN 47907*

Accepted for publication October 11, 1968.

Acknowledgment is given to the African-American Institute for Fellowship (E.O.O.).

Gas Chromatographic Determination of Mebutamate, Carisoprodol, and Tybamate in Plasma and Urine

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Abstract □ A gas chromatographic method for the determination of mebutamate, carisoprodol, and tybamate in plasma and urine is described. The procedure permits the measurement of these drugs individually or in combination with meprobamate.

Keyphrases □ Carbamates—determination, biological fluids □ Plasma—mebutamate, carisoprodol, tybamate determination □ Urine—mebutamate, carisoprodol, tybamate determination □ GLC—analysis

A previous paper (1) described the gas chromatographic determination of meprobamate in plasma and urine. These observations have been extended to the analysis in biological fluids of three related carbamate compounds—mebutamate, carisoprodol, and tybamate.

EXPERIMENTAL

Equipment and Reagents—A gas chromatograph, equipped with a flame-ionization detector,[1] and a recorder[2] were employed. The chromatographic columns used were 121.9-cm. (4-ft.) glass tubes packed with 3.8% UC-W98 methyl silicone on 80–100-mesh Diataport S (Hewlett Packard). The instrument settings were as follows:

Temperature—Column, 180°; injection port, 275°; detector block, 225°.

Gas Flow Rates—Hydrogen, 20 ml./min.; helium (carrier gas), 65 ml./min.; oxygen, 50 ml./min.

Sensitivity settings were range 10 with an attenuation of 2×. Redistilled chloroform and dibutyl phthalate[3] were used.

[1] F and M model 402 dual-column.
[2] 1-mv. Minneapolis-Honeywell.
[3] Supplied by Eastman Chemical Products, New York, N. Y.

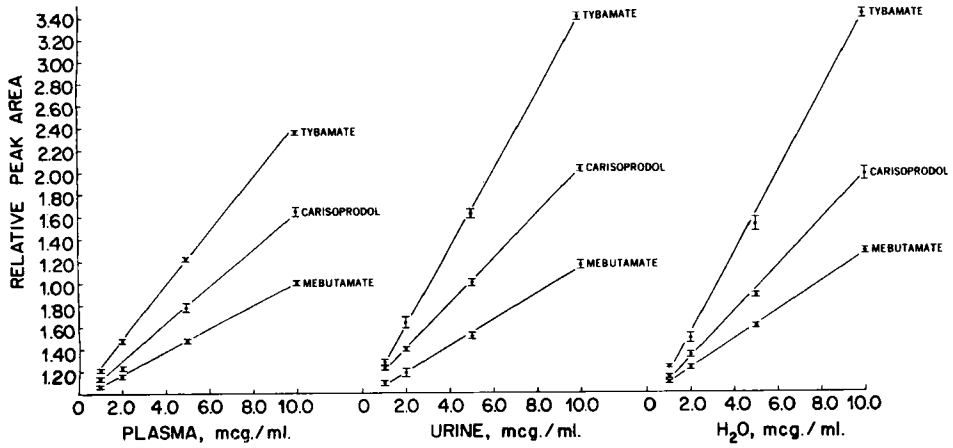

Figure 1—*Relationship between relative peak area and tybamate, carisoprodol, and mebutamate concentration in three fluids. (Averages of quintuplicate determinations are given with their standard errors.)*

Procedure—Chloroform, 0.2 ml., containing 2.0 mcg. of dibutyl phthalate was added to 1.0 ml. of biological fluid, and the mixture was thoroughly shaken and centrifuged. The aqueous layer was removed by aspiration and 2.6 μl. of the chloroform extract was injected into the gas chromatograph. Carbamate concentration was determined by the relative peak area method, using dibutyl phthalate as internal standard.

RESULTS AND DISCUSSION

Earlier work has shown that each of the four pharmacologically active carbamates—meprobamate, mebutamate, carisoprodol, and tybamate—can be extracted from biological fluids with chloroform (2, 3), and that these compounds can be separated in the gas chromatograph (1). These techniques have been extended to individual quantitation of the latter three carbamates and the simultaneous determination of each in biological fluids containing one or more of the four carbamate compounds. With this procedure, the carbamates can be effectively separated from plasma constituents normally occurring in man, dog, rabbit, and monkey, and from interfering materials normally present in human urine.

A linear relationship exists between relative peak area and mebutamate concentration in the range of 1–10 mcg./ml. of plasma, urine, or water. Similar findings between relative peak area and drug concentration were obtained for tybamate and for carisoprodol (Fig. 1). The reproducibility of each of these procedures is indicated by the standard errors presented in the figure.

Any one of the four carbamates, including meprobamate, can be determined in the presence of any other carbamates present in a single specimen. Table I shows the results obtained with various mixtures of the four drug compounds in plasma.

A number of drugs were investigated as possible interfering substances. Of those studied, only caffeine and secobarbital had a retention time close to that of meprobamate, whereas none interfered with the determination of any of the other carbamates (Table II). Confirmatory evidence for the identification of meprobamate can be obtained by the addition of either caffeine or secobarbital to the analytical specimen prior to chromatography. If meprobamate is present, a split peak will result with each of the interfering compounds. Another somewhat more involved procedure for detecting the presence of caffeine and secobarbital is the use of a 3% QF-1 column under the same experimental conditions. This column separates the three compounds with the following retention times: secobarbital, 1.0 min.; caffeine, 1.2 min.; and meprobamate, 1.7 min.

Collection and subsequent IR analysis of the volatile products corresponding to each of the carbamate peaks obtained in the gas chromatograph did not indicate any evidence for the degradation of the compounds under the conditions employed.

Table I—Values Obtained from the Simultaneous Gas Chromatographic Determination of Four Carbamates

Run	Meprobamate, mcg./ml. Found	Theor.	Carisoprodol, mcg./ml. Found	Theor.	Mebutamate, mcg./ml. Found	Theor.	Tybamate, mcg./ml. Found	Theor.
A	11.5	12.0	4.7	4.5	15.9	16.0	3.0	3.0
A	11.2	12.0	4.7	4.5	15.0	16.0	3.0	3.0
B	11.6	12.0	2.0	2.0	15.6	16.0	11.0	10.0
B	11.6	12.0	2.0	2.0	15.8	16.0	10.3	10.0
C	5.3	5.0	4.5	4.5	15.9	16.0	10.9	10.0
C	5.1	5.0	4.5	4.5	15.7	16.0	10.9	10.0
D	11.7	12.0	4.4	4.5	7.2	7.0	10.9	10.0
D	11.7	12.0	4.4	4.5	7.2	7.0	11.4	10.0

Table II—Retention Times of Various Pharmacological Agents on a 3.8% UC-W98 Methyl Silicone Column[a]

Compd.	Retention Time, min.
Barbital	0.6
Amobarbital	1.4
Emylcamate	1.0
Pentobarbital	1.5
Hydroxyphenamate	1.5
Secobarbital	1.9
Caffeine	1.95
Meprobamate	2.0
Glutethimide	2.2
Theobromine	2.2
Hexobarbital	2.4
Carisoprodol	2.6
Mebutamate	2.9
Dibutyl phthalate	3.1
Phenobarbital	3.8
Theophylline	4.1
Tybamate	5.0

[a] The instrument settings are described in *Experimental.*

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 2, 1968, from *Wallace Laboratories Division of Carter-Wallace, Inc., Cranbury, NJ 08512*

Accepted for publication September 19, 1968.

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