Fenfluramine Metabolism

By ROBERT B. BRUCE and WILLIAM R. MAYNARD, Jr.

The metabolism of fenfluramine in man results in the excretion of unchanged fenfluramine, deethylated fenfluramine, and *m*-trifluoromethylhippuric acid. Methods are described for the determinations of these three compounds and the total metabolites in urine by GLC. The *m*-trifluoromethylhippuric acid accounts for 66 to 93 percent of the dose and 79 to 100 percent of the dose may be accounted for as these three metabolites.

FENFLURAMINE [*N*-ethyl-α-methyl-*m*-(trifluoromethyl)-phenethylamine hydrochloride] is an effective anorexigenic agent showing little or no CNS stimulant properties (1). Structurally, this compound is similar to the amphetamines.

Duhault and Fenard (2) have reported a study of the excretion, distribution, and metabolism of fenfluramine using methyl orange complex for its determination. They found that fenfluramine persists in the animal body for considerably longer than amphetamine. The total excretion following oral doses in man and in dogs was 27 to 38% of the dose over a 5- to 6-day period. Relatively large amounts were found in fatty tissues of the dog. These authors found that the drug is deethylated but could find little or no evidence for hydroxylation, as occurs with amphetamine. Beckett and Brookes (3), using gas chromatographic methods, have studied the dependence of the rate of excretion on urinary pH. They found that some 50% of fenfluramine was excreted unchanged or deethylated under acidic urinary conditions, but could account for only 6 to 23% with uncontrolled acidity of the urine.

The relatively low recoveries of the amines, which has been confirmed in these laboratories, led the authors to investigate the fate of the remainder of the drug in the animal body. Extensive use of gas chromatography in these studies was made to determine the amounts of unchanged drug and its metabolities in biological materials. Blood levels in man and the dog have been determined by GLC. The results given below indicate that the major portion of an oral dose of fenfluramine in man is excreted as *m*-trifluoromethylhippuric acid (Scheme I).

EXPERIMENTAL

Human Study—Two normal, healthy males were given a 60-mg. tablet of fenfluramine as a single dose, and urine was collected at intervals for 72 hr. Ammonium chloride was administered the previous day and throughout the period of urine collection, in an attempt to maintain an acidic urine. All urine samples were kept in the refrigerator until analysis. The pH of each sample was determined with a pH meter immediately upon receipt. Blood samples of 20 ml. were collected at intervals during the first 8 hr. for analysis.

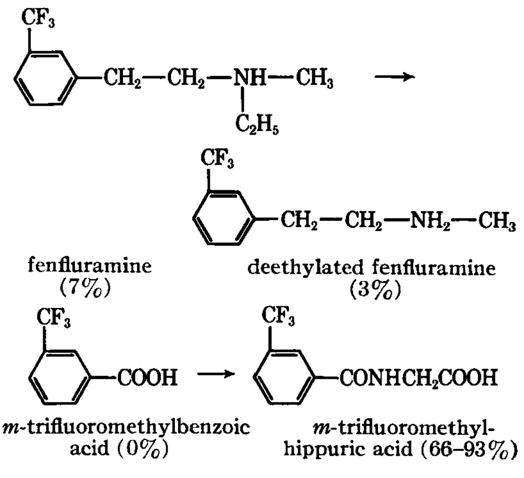
Dog Study—A mongrel male dog weighing 11.7 kg. was given a divided oral dose of 117 mg. of fenfluramine, and urine was collected for 5 days and stored under refrigeration.

Materials—*m*-Trifluoromethylbenzoic acid was purchased from J. T. Baker Chemical Co.

m-Trifluoromethylhippuric acid was prepared by reaction of *m*-trifluoromethylbenzoic acid with thionyl chloride to give *m*-trifluoromethylbenzoyl chloride. The acid chloride was then reacted with glycine to give the hippuric acid.

METHODS OF ANALYSIS

Fenfluramine and Norfenfluramine—These compounds were determined by gas-liquid chromatography according to a modification of a method described by Beckett and Rowland (4). An aliquot of 20.0 ml. of urine or 15.0 ml. of blood was made alkaline with 2 ml. of 2 *N* NaOH and extracted three times with 10-ml. portions of ethyl ether, centrifuging to separate the phases. The combined ether extracts were dried over anhydrous sodium sulfate and evaporated to 0.10 ml. under a gentle stream of nitrogen. Aliquots of 1 to 3 μl. were injected into the gas chromatograph under the follow-



Scheme I—Excretion of fenfluramine and metabolites by man

Received February 27, 1968, from the Research Laboratories, A. H. Robins Company, Inc., Richmond, VA 23220
Accepted for publication April 8, 1968.

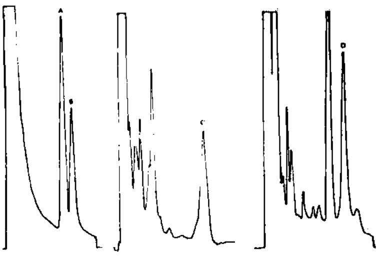


Fig. 1—Gas chromatograms obtained from the analysis of urine samples (Subject A) showing (a) *fenfluramine,* (b) *deethylated fenfluramine,* (c) *the methyl ester of* m-*trifluoromethylhippuric acid, and* (d) *the methyl ester of* m-*trifluoromethylbenzoic acid. The large peak immediately preceding* (d) *is the ester of benzoic acid. See text for conditions.*

ing conditions, and the peak heights compared with those of a standard.

Detector–flame ionization; column—10% polyethylene glycol[1] 20 M and 5% KOH on HMDS treated Chromosorb W, 2.1 m. × 0.31 cm. (7 ft. × 1/8 in.); column temperature—120°.

The retention times of fenfluramine and norfenfluramine under these conditions were 4 and 4.5 min., respectively. The peaks were sharp and well separated (Fig. 1). No interference has been found from control urine samples carried through the procedures. Recoveries varied from 98 to 102% and peak height was proportional to concentration over the range of 10 to 200 ng. (Standard deviation = ±2.2 at the 100-ng. level.)

Total Fenfluramine Metabolities—The following procedure was designed to determine fenfluramine and all metabolities that might be formed. However, any metabolite in which the benzene ring was altered would not be determined.

An aliquot of 2.0 ml. of urine was oxidized with 10 ml. of saturated potassium permanganate and 0.5 ml. of 2 *N* sodium hydroxide by heating for 1 hr. in the steam bath. The cooled solution was then extracted with a total of 20 ml. of ether in three portions and the acids repartitioned into 10 ml. of sodium bicarbonate. The sodium bicarbonate was acidified and the acids returned to ether by extracting three times with a total of 15 ml. The ether solution was treated with diazomethane, washed with 3 × 5-ml. portions of water, dried over anhydrous sodium sulfate, and concentrated to 0.10 ml. An aliquot of 2 µl. was injected into the gas chromatograph.

The major interference that was expected in this determination was benzoic acid, which was formed during the oxidation in relatively large amounts. The methyl esters of benzoic acid and trifluoromethylbenzoic acid are easily separated by GLC under the following conditions:

Column—10% 20 polyethylene glycol M on Gas Chrom Q, 1.2 m. × 0.31 cm. (4 ft. × 1/8 in.) at 75°; flow rate—25 ml./min.; injection port temperature—250°; detector–flame ionization.

The retention time for methyl trifluoromethylbenzoate is 5 min. and that of methyl benzoate is 4 min. (Fig. 1).

m-Trifluoromethylbenzoic Acid—Acidified urine was extracted with ether and carried through the procedure used for total metabolites, omitting the oxidation steps.

m-Trifluoromethylhippuric Acid—The hippuric acid derivative could not easily be extracted from urine without extracting interfering materials. It could be separated from these materials by the use of an ion-exchange column. A column,[2] (200-400 mesh) 12 × 500 mm., was prepared in 95% ethanol. A sample of 4.0 ml. of urine was placed on the column and the column was washed with 330 ml. of 95% ethanol. The trifluoromethylhippuric acid was then eluted with 70 ml. of 0.1 *N* hydrochloric acid in 95% ethanol. The ethanol solution was evaporated to dryness, the residue taken up in 10 ml. of ether, and methylated with diazomethane. After washing with water, the ether solution was evaporated to 0.10 ml. and 2 µl. were injected into the gas chromatograph. Conditions: Column—10% polyethylene glycol 20 M on Gas Chrom Q, 1.2 m. × 1.2 cm. at 190°; flow rate—30 ml./min.; injection port temperature—300°. Both hippuric and trifluoromethylhippuric acids are eluted from the ion-exchange column under these conditions but they are easily separated by GLC. The retention time of methyl trifluoromethylhippurate is 10 min. and that of the methyl hippurate is 14 min. (Fig. 1).

IDENTIFICATION OF METABOLITES

The correspondence of retention times found by GLC was an indication that the metabolites from urine were the same as those of authentic compounds. Beckett and Brookes established the presence of fenfluramine and norfenfluramine.

In addition, the following information as to identity of the other metabolites was found.

m-Trifluoromethylbenzoic Acid—A sample of acidified dog urine was continuously extracted with ethyl ether and the acids were separated from phenols by sodium bicarbonate partitioning. The resulting bicarbonate solution was acidified and the acids reextracted into ether. An aliquot of the ether was concentrated and used for thin-layer chromatography on silica gel. Using a solvent system consisting of benzene, methanol, and acetic acid (90:10:4) two spots were found that corresponded to *m*-trifluoromethylbenzoic acid and its glycine conjugate.

In order to separate a larger quantity of the *m*-trifluoromethylbenzoic acid, a partition column of 0.1 *N* sulfuric acid on silicic acid was prepared. A portion of the ether extract was added to the column and it was eluted with skellysolve B. Fractions were collected and monitored by UV and TLC. Those fractions corresponding to the *m*-trifluoromethylbenzoic acid were combined and evaporated to dryness. The infrared spectrum of the isolated material was identical with that of an authentic sample of *m*-trifluoromethylbenzoic acid. A melting point following sublimation was 101–102°, and a mixed melting point 100–101°.

Anal.—Calcd: C, 50.33; H, 2.64. Found: C, 50.56; H, 2.71.

m-Trifluoromethylhippuric Acid—Although this acid could not be separated in sufficient quantity and purity from urine for infrared identification and elemental analysis, the GLC and TLC results are good evidence for its identification.

---

[1] Carbowax, Union Carbide Corp., New York, N. Y.

[2] Dowex-1X-8($^-$OH), Dow Chemical Corp., Midland, Mich.

Table I—Blood Levels of Fenfluramine in Two Human Subjects Following an Oral Dose of 60 mg. of Drug

Time After Dose, hr.	Fenfluramine, mcg./100 ml. of Blood Subject A	Subject B
Control	0	0
1	2.3	0.9
2	2.6	2.3
3	2.9	2.9
4	1.2	1.5
6	1.2	1.2
8	0.6	0.9

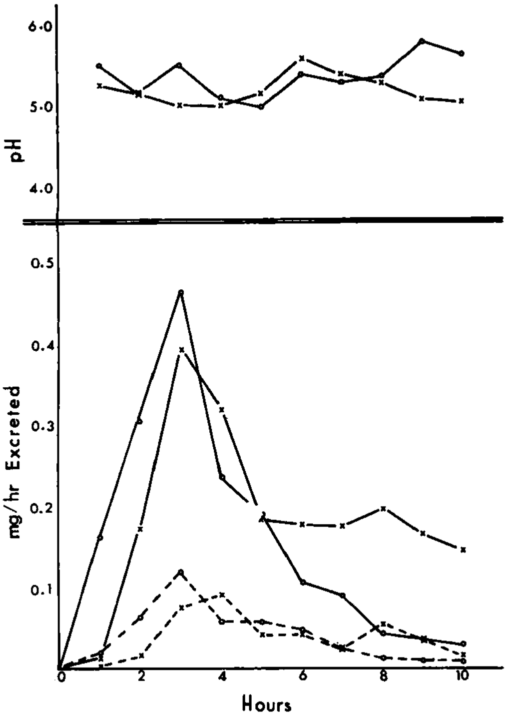


Fig. 2—The urine pH and rates of excretion of fenfluramine (solid line) and deethylated fenfluramine (dashed line) in two normal males.

Hydroxylation—The procedure as described by Axelrod (5) was used to determine whether a hydroxylated product might occur in the urine. No reaction was obtained with 1-nitroso-2-naphthol using either hydrolyzed or unhydrolyzed urine.

RESULTS AND DISCUSSION

The results of the blood level study in man are shown in Table I. These results indicate that fenfluramine is readily absorbed and persists for some time in the blood stream. Even in the final sample 8 hr. after the dose, measurable concentrations are still present. The maximum concentration appears at about the third hour. The persistence of fenfluramine in the body is in agreement with previous studies by Duhault and Fenard (2).

Determination of blood levels in dogs, that received 2 mg./kg. of fenfluramine, showed that both fenfluramine and norfenfluramine were present. However, no norfenfluramine has been found in blood during any of the studies on humans.

Typical results of the urinary excretion of fenfluramine and norfenfluramine are shown in Fig. 2. Although it was attempted to maintain an acidic urine, the pH did not reach levels below 5, as did those reported by Beckett and Brookes. The maximum excretion rate occurred at around 3 hr. which was at the same time as the maximum blood levels. Urine was continued to be collected from these subjects for 72 hr. Determinations of the acidic metabolic products were carried out on samples of urine pooled for 1–10 hr., 10–24 hr., 24–48 hr., and 48–72 hr. These results are shown in Table II. The excellent agreement found between the sum of individual metabolites and the total by oxidation indicates the accuracy of the methods and also that the oxidation procedure results give the desired results.

Although free trifluoromethylbenzoic acid was found in dog urine, none could be detected in human urine. It can be seen from these results that the main excretory product of fenfluramine in man is the trifluoromethylhippuric acid. These two subjects excreted approximately 10% of the dose as fenfluramine and its deethylated metabolite, which corresponds closely with that amount found by others with uncontrolled pH (5). In one subject (A), 93% of the dose was excreted as *m*-trifluoromethylhippuric acid and in the other, 66% was excreted in the 72-hr. period.

Amphetamine and methamphetamine also un-

Table II—Excretion of Fenfluramine, Norfenfluramine, *m*-Trifluoromethylhippuric Acid, and Total Metabolites from Two Subjects Who Received a Single 60-mg. Dose of the Drug

Subject	Sample Time, hr.	Fenfluramine, mg.[a]	Norfenfluramine, mg.[a]	*m*-Trifluoromethylhippuric Acid, mg.[a]	Sum of Individual Metabolites, mg.	Total by Oxidation, mg.[a]
A	1–10	2.17	0.69	12.86	15.72	16.65
	10–24	0.95	0.47	10.33	11.75	12.05
	24–48	0.49	0.40	24.84	25.73	25.03
	48–72	0.32	0.37	7.81	8.50	8.90
Total		3.93	1.93	55.84	61.70	62.63
% of dose		6.55	3.22	93.07	102.9	104.4
B	1–10	1.92	0.51	10.89	13.32	13.11
	10–24	1.68	0.80	8.51	10.99	10.07
	24–48	0.72	0.58	10.11	11.41	11.10
	48–72	0.15	0.17	10.23	10.55	13.02
Total		4.47	2.06	39.74	46.27	47.30
% of Dose		7.45	3.43	66.23	77.12	78.83

[a] All results are calculated in terms of the dose-fenfluramine hydrochloride.

dergo side-chain oxidations. Oxidation occurs through the stages of benzyl methyl ketone and 1-phenyl-propane-2-ol to the acid. A corresponding mechanism would be expected to occur with fenfluramine. The amphetamines also undergo ring oxidation to give the phenol. The presence of the trifluoromethyl group on the benzene ring of fenfluramine appears to prevent such oxidation. The phenolic derivative of fenfluramine was not available to determine whether the reaction given by Axelrod would take place, but the recovery of 77 to 100% of the administered drug as fenfluramine and its two metabolites suggests that if such oxidation does occur, it can be only to a very small extent.

Previous observations (2, 5) have indicated that fenfluramine is rapidly absorbed into the tissues and is then slowly released into the extracellular fluid. This is also indicated by the present studies. The two amines continue to be excreted over at least a 72-hr. period and during the first 48 hr. *m*-trifluromethylhippuric acid is excreted at an almost continuous rate.

REFERENCES

(1) Franko, B. V., Honkomp, L. J., and Ward, J. W., *J. Pharm. Pharmacol.*, **17**, 222(1965).
(2) Duhault, J., and Fenard, S., *Arch. Intern. Pharmacodyn.*, **158**, 251(1965).
(3) Beckett, A. H., and Brookes, L. G., *J. Pharm. Pharmacol.*, **19**, 42S(1967).
(4) Beckett, A. H., and Rowland, M., *ibid.*, **16**, 27T (1954).
(5) Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **110**, 315 (1954).

Keyphrases

Fenfluramine—metabolism
m-Trifluoromethylhippuric acid, de-ethylated fenfluramine—metabolites
Blood levels—fenfluramine
Urinary excretion—fenfluramine and metabolites
GLC—analysis

Structure-Activity Relations in an Imidazoline Series Prepared for Their Analgesic Properties

By W. BROCK NEELY*, H. C. WHITE†, and A. RUDZIK‡

A specific pharmacological property of a series of substituted imidazolines was examined in an effort to determine the structure-activity relationships that might be present. The best measure of the chemical reactivity was based on a charge-transfer complex between the drug and the receptor site. Using the molecular orbital technique for measuring this reactivity index, and combining with the partition coefficient in the manner described by Hansch 94.4 percent correlation was found for the biological activity.

STRUCTURE-ACTIVITY relationships have been an intriguing subject for organic and biological chemists for many years. At the same time, it is an area that has seen many frustrations and disappointments. The reasons for the failures are readily appreciated when the complexity of the biological system is taken into consideration. In attempting any type of rigid structure-activity study all of the the biological parameters such as active and passive transport, metabolism to a more or less active agent, nonspecific binding to proteins, and finally the reaction at the receptor site, must be taken into account. Obviously, the system is much too complicated and too little understood for a detailed analysis. As a starting point, a simple model is required. In such a case, if the model works, *i.e.*, can predict the data, it may give new insights into the mechanism of action. Conversely, if the model fails, then one must look for the reasons; this in itself could provide new concepts in the area under investigation.

The model used in this study is the one that has been developed by Hansch and his co-workers (1) and used extensively in a number of different biological applications (1–5). In effect, the model assumes that the amount of drug found at the receptor site is related to a single substituent constant which is derived from octanol–water partition-coefficient measurements. The reaction of the chemical at the receptor site is related to the rate constant. The model is illustrated by Eq. 1.

$$\log \text{B.R.} = -a\pi^2 + b\pi + c \log k_x + d \qquad \text{(Eq. 1)}$$

where B.R. = biological response; π = log

Received March 13, 1968, from the Dow Chemical Company, Midland, MI 48640
Accepted for publication April 11, 1968.
* The Biochemical Research Laboratory, Zionsville, Ind.
† The E. C. Britton Research Laboratory, Zionsville, Ind.
‡ Present address: The Upjohn Co., Kalamazoo, Mich.
The authors thank R. Bidwell in the Technical Service and Development Group, The Dow Chemical Co., for his help in obtaining the oxidation potential data for the phenols reported in this paper. In addition, the technical assistance of D. V. Wysong and L. E. Begin is gratefully acknowledged for their help in the synthesis program.