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ANESTHESIA LXXIV: BIOTRANSFORMATION OF FLUROXENE—I. METABOLISM IN MICE AND DOGS *IN VIVO**

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Abstract—Fluroxene has been found to undergo biotransformation in the mouse and dog after i.p. administration of subanesthetic doses. Trifluoroethanol glucuronide, trifluoroacetic acid, and $^{14}CO_2$ (from vinyl carbons only) have been identified as metabolites. The extent of metabolism was increased in mice by pretreatment with phenobarbital sodium, 3-methylcholanthrene, and 3,4-benzpyrene, and these effects could be blocked by preadministration of actinomycin D. Phenobarbital pretreatment also enhanced the metabolism of fluroxene in the dog. Carbon tetrachloride-induced hepatotoxicity greatly reduced the metabolism of fluroxene in the mouse. SKF 525-A and Lilly 18947 had no inhibitory effect in mice but rather caused a slight enhancement of the metabolic activity. Repeated pretreatments with ethyl ether and nitrous oxide had a slight stimulatory action on the biotransformation of fluroxene in mice, whereas fluroxene and methoxyflurane pretreatments had no significant effect.

RECENT investigations have indicated that several of the volatile anesthetics undergo some biotransformation.[1–3] Previously these compounds were assumed to be refractory to the enzymatic destruction encountered by other drugs, primarily because so much of an anesthetic dose could be recovered unchanged in the expired air.[4] In animals receiving radioactively labeled volatile anesthetics, however, it is evident that certain of these agents are biodegradable.

This report presents evidence that fluroxene¶ undergoes biotransformation in the mouse and dog and that trifluoroethanol glucuronide, trifluoroacetic acid, and $^{14}CO_2$ are metabolites. Various compounds known to affect the metabolic pathways of other drugs also affect the biotransformation of fluroxene.

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¶ 2,2,2 Trifluoroethyl vinyl ether (Fluoromar).

MATERIALS AND METHODS

Radioactive materials. Radioactivity labeled compounds, obtained from New England Nuclear Corp., had the following formulae: fluroxene, $^{14}CF_3CH_2—O—CH=CH_2$ (FM—F) and $CF_3CH_2—O—^{14}CH=^{14}CH_2$ (FM—V); trifluoroethanol, $^{14}CF_3CH_2OH$ (TFE-F). The synthesis of the latter compound has recently been published.[5] All three compounds were assayed by gas chromatography and were shown to have a purity exceeding 99·5 per cent. Labeled fluroxene was stabilized with 0·2 mg of *N*-phenyl-α-naphthylamine/ml and all radioactive materials were stored at − 20°. The specific activity of each compound was adjusted to 0·1 μc/μl with the non-radioactive compound as diluent.

Isotope administration and metabolite collection. Male albino Swiss-Webster ICR mice (20–35 g), maintained on a stock diet, were injected i.p. with the labeled compound and immediately placed in a Delmar glass metabolism cage[6] for the collection of urine, faeces, and expired vapors. Room air was vacuum-drawn through Drierite and soda lime before entering the animal chamber. The expired air was first drawn through a conc. H_2SO_4 scrubbing tower to remove the expired unchanged anesthetic prior to entering a 2 N NaOH trap for the collection of CO_2. Control experiments indicated that conc. H_2SO_4 was completely effective in removing fluroxene from the expired air and that none of the anesthetic decomposed to $^{14}CO_2$. The expired gases were collected for 6 hr, and urines were collected for either 24 or 48 hr.

A female mongrel dog weighing 10 kg was trained to remain in a restraint harness for periods of up to 48 hr without any visible signs of stress. Urine samples were collected by urethral catheterization for 24–30 hr after isotope administration.

Radioassay procedures. Because of the difficulty encountered in radioassay of conc. H_2SO_4, a toluene trap (chilled in dry ice–methyl Cellosolve) was used to collect the anesthetic when the expiration of fluroxene was studied. Samples of the toluene trap were withdrawn at various intervals and mixed with 15 ml of a liquid scintillation fluid made as follows: 3 g PPO (2,5-diphenyloxazole), 100 mg POPOP [1,4-bis-2-(5-phenyloxazole) benzene], and 1000 ml spectral grade toluene.[7]

NaOH traps were assayed for $^{14}CO_2$ by modifying the method of Passman *et al.*[8] with a diffusion apparatus described by Towne *et al.*[9] $BaCO_3$ precipitates were dried and weighed prior to diffusion. Radioassay of the resultant Hyamine carbonate was performed in the toluene liquid scintillation fluid.

To be certain that no unchanged anesthetic was present in urine samples, they were initially evaporated to dryness *in vacuo* and resuspended in 100 ml water. Two-ml samples of diluted urine were mixed with 15 ml of a liquid scintillation fluid made as follows: 12 g PPO, 300 mg POPOP, 76·8 g naphthalene, and 960 ml spectral grade *p*-dioxane.[10] In control studies, flash evaporation removed all radioactivity from mouse urine to which 1 μl of either FM-F or FM-V had been added and which was allowed to stand in a sealed flask for 24 hr at room temperature.

Radioassays were performed on a Nuclear Chicago model 720 liquid scintillation spectrometer with the internal standard technique for the determination of counting efficiency.

Pretreatment with modifiers of drug metabolism. Unless otherwise indicated, all injections were made by the i.p. route. Phenobarbital sodium (PB) in saline was given twice daily for 3 days at a dose of 40 mg/kg to mice and 60 mg three times a day for 3 days as oral tablets to the dog. Methylcholanthrene (3-MC) and

3,4-benzpyrene (3,4-BP) were dissolved in olive oil and administered at a dosage of 50 mg/kg once, 24 hr before the injection of fluoxene-^{14}C. SKF 525-A and Lilly 18947 in saline were given at a dose of 50 mg/kg at either 1 hr, 24 hr, or at both 48 and 24 hr before the administration of the radioactive anesthetic. Actinomycin D (ACT D) was given at a dose of 1 mg/kg 1 hr before the first injection of PB or the injection of 3-MC or 3,4-BP. The anesthetic vapors of fluroxene* (7·5 per cent), methoxyflurane (Penthrane) (1·25 per cent), ethyl ether (7·5 per cent), and nitrous oxide (80 per cent) were mixed with oxygen in a 3·8-l. jar. A mouse was quickly introduced into the jar and allowed to remain for 10–15 min twice daily for 2 days. CCl_4 was given subcutaneously as a 20% solution in olive oil at a dose of 0·2 ml/mouse and preceded fluroxene-^{14}C by either 15 min or 24 hr. Saline and olive oil control injections were made at a dose and time comparable to those when they were used as drug solvents.

Identification of urinary metabolites. Derivatives of trifluoroethanol (TFE) and trifluoroacetic acid (TFAA) were used for inverse isotope dilution. Recrystallization was repeated to a constant sp. act. which was used to calculate the quantity of metabolite in urine. The *N*-α-naphthylcarbamate ester of TFE (m.p. 79–80°) was prepared according to Cheronis and Entrikin[11] and recrystallized from hot ligroin. Carrier TFE was mixed with diluted urine samples and removed by distillation prior to formation of the derivative. The benzyl thiouronium salt of TFAA (m.p. 179–180°) was prepared by adding a saturated solution of 2-benzyl-2-thiopseudourea HCl to urine samples after the addition of carrier TFAA. Upon standing, a white crystalline material separated which was filtered, decolorized with charcoal, and recrystallized from hot water. The derivatives were identified by elemental analyses and infrared spectra and were radioassayed in the dioxane liquid scintillation fluid. Determination of conjugated metabolites was performed by incubation of urine samples with β-glucuronidase (Ketodase; 100,000 U at pH 5 for 24 hr at 37°) or by refluxing with N HCl for 4 hr prior to the isotope dilution procedures.

Ion-exchange chromatography was used to partially purify the metabolites prior to thin-layer chromatography. Suitable urine samples were applied to glass columns (2·5 × 30 cm) filled with Dowex-50 cation-exchange resin (Biorad AG-50W-X8, 50–100 mesh) in the hydrogen form. All the radioactivity was elutable with water. These eluates were applied to the same size columns filled with Dowex-1 anion-exchange resin (Biorad AG-1-X8, 50–100 mesh) in the hydroxide form. The columns were eluted first with 0·1 NH_4OH and then with water; these fractions were discarded since they contained no radioactivity. The radioactive metabolites were eluted with 0·1 N formic acid and then 5% KBr. These eluates were collected in small fractions in a Vanguard fraction collector. One-ml samples of the fractions were placed on stainless-steel planchets, dried in an oven, and counted in a Nuclear Chicago model 4306 gas-flow counter. The fractions corresponding to each eluted peak were combined, evaporated *in vacuo* at 50°, and resuspended in a small volume of water. These samples were applied to glass plates coated with layers of silica gel G (0·25 mm), activated for 30 min at 100°, and ruled by scratching lines 1 cm apart. The plates were

* SKF 525-A (β-diethylaminoethyldiphenyl-n-propyl acetate) was a gift of Smith Kline & French. Lilly 18947 (2,4-dichloro-6-phenylphenoxyethyldiethylamine HCl) was donated by Eli Lilly. Actinomycin D (Cosmagen) was kindly supplied by Merck, Sharp and Dohme. Fluroxene (Fluoromar) was a gift of the Ohio Chemical and Surgical Equipment Co.

developed for a distance of 15 cm in an ascending solvent system of isopropanol and NH_4OH (4:1). After air drying, the plates were covered with Saran Wrap and Kodak No-Screen X-ray film for exposure of 1–4 weeks. The films were developed, fixed, and either passed through a recording densitometer or printed. The Saran Wrap was removed from the desired areas of the plate, and non-radioactive TFAA was detected by spraying with bromphenol blue in *n*-butanol, which formed a yellow spot on a blue background. The rest of the covering was then removed and the plate sprayed with 50% H_2SO_4 followed by a 1 per cent alcoholic solution of naphthoresorcinol. Heating the plate for 15 min at 100° produced a dark violet color with compounds containing glucuronic acid.

RESULTS AND DISCUSSION

Mice, not pretreated

The time course for the excretion of unchanged FM-F after i.p. administration to five mice (10 μl/mouse) is shown in Fig. 1. A similar curve was obtained with FM-V. It can be seen that the expiration of the anesthetic is rapid over the first hour with

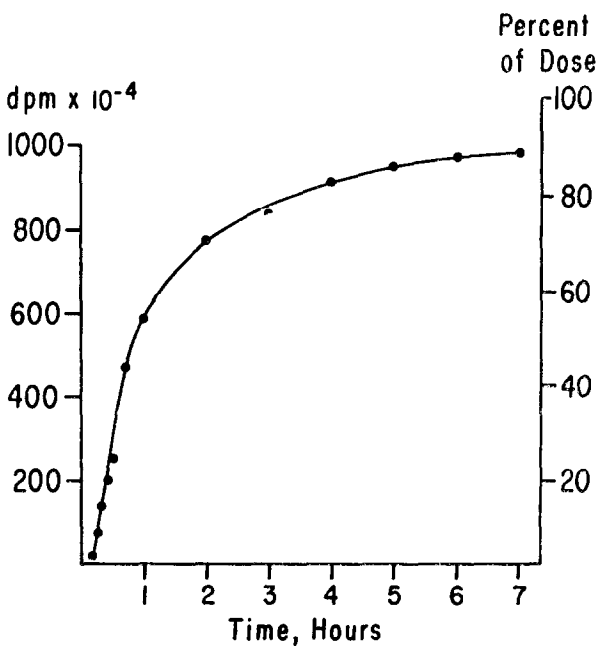

FIG. 1. Excretion of unchanged fluroxene by mice after i.p. injection of $^{14}CF_3CH_2—O—CH=CH_2$ (FM-F). Group of 5 mice received 10 μl (1 μc) each and were placed together in a large metabolism cage. Values represent cumulative excretion by the group.

approximately 50 per cent of the dose being eliminated. During the next 5 hr another 40 per cent is excreted, and only traces were obtained after 7 hr. Figure 2 shows the average time course for excretion of $^{14}CO_2$ from six mice after i.p. injection of FM-V (10 μl/mouse). It should be noted that measurable quantities of $^{14}CO_2$ were collected within 5 min of injection (first point on graph), and only small amounts were excreted after 6 hr.

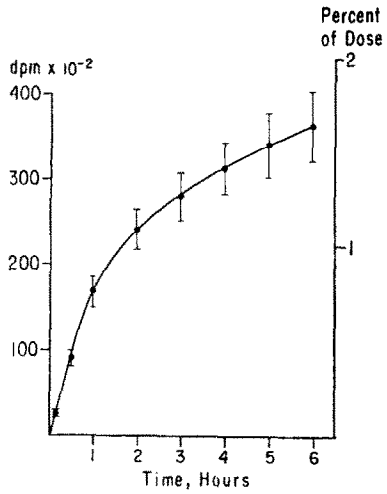

FIG. 2. Excretion of $^{14}CO_2$ by mice after i.p. injection of CF_3CH_2–O–^{14}CH=$^{14}CH_2$ (FM–V). Each mouse received 10 μl (1 μc). Values denote mean ± S.E. of six experiments.

Table 1 summarizes the results obtained on the conversion of FM-V and FM-F to $^{14}CO_2$ and urinary metabolites by mice. No significant radioactivity was detected in faeces. In the case of FM-V, approximately 11 per cent of the dose was excreted as $^{14}CO_2$ and urinary metabolites in a ratio of 1:5 respectively. A similar percentage of

TABLE 1. CONVERSION OF CF_3CH_2—O—^{14}CH=$^{14}CH_2$ (FM–V) AND $^{14}CF_3CH_2$—O—CH=CH_2 (FM–F) TO $^{14}CO_2$ AND URINARY METABOLITES IN UNPRETREATED MICE

Experiment		Body weight (g)	Percent of dose metabolized		
			$^{14}CO_2$	Urinary	Total
FM-V	1	23	2·25	9·65	11·90
	2	21	1·52	6·52	8·04
	3	24	2·04	11·70	13·74
	4	23	1·53	8·95	10·48
	5	27	1·53	8·26	9·79
	6	23	3·43	13·48	16·91
	7	27	2·43	9·47	11·90
	8	23	1·70	7·63	9·33
	9	34	2·42	10·52	12·94
	10	23	1·13	6·68	7·81
	11	27	2·84	9·07	11·91
	12	24	1·86	8·16	10·02
Mean		24·9	2·06	9·17	11·23
S.D.			0·66	2·01	2·56
FM-F	1	25	0·06	7·13	7·19
	2	20		12·12	12·12
	3	31		10·82	10·82
	4	23*	0·05	4·78	4·83
	5	22*	0·05	8·28	8·33
	6	19	0·08	12·54	12·62
Mean		23·3	0·06	9·28	9·32
S.D.				3·07	

Each mouse received 10 μl i.p., and CO_2 was collected for 6 hr, urine for 48 hr.

* Average weight of 5 mice which were run simultaneously.

a dose of FM-F (10 μl i.p.) was metabolized; however, as expected, only negligible amounts appeared as $^{14}CO_2$, attesting to the biochemical stability of the CF_3 bonds. Consequently, urine was essentially the only route of FM-F ^{14}C-metabolite excretion.

It was of interest to determine what effect altering the i.p. dose would have on the percentage of the dose metabolized. Fig. 3 presents the results of experiments in which doses of FM-V ranging from 5 to 100 μl were injected into mice of similar weight. As the dose was increased, the percentage metabolized fell off linearly until

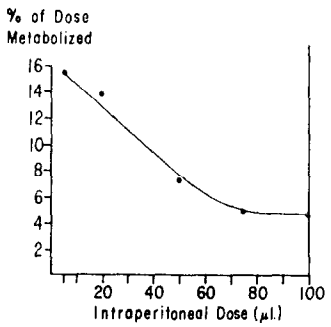

FIG. 3. Effect of varying the i.p. dose of FM-V on the per cent of the dose metabolized by mice. Values are the sum of $^{14}CO_2$ and urinary metabolites.

an apparent minimum was reached at 75 μl, the approximate minimal anesthetic dose. Since mice receiving this dose had depressed respiration, it is possible that expiration of unchanged anesthetic was slower, thus allowing more extensive metabolism before excretion by the lungs.

Mice, pretreated

Fig. 4 shows the effects of three well-known stimulators: phenobarbital, methylcholanthrene and 3,4-benzpyrene. All these compounds were found to increase the

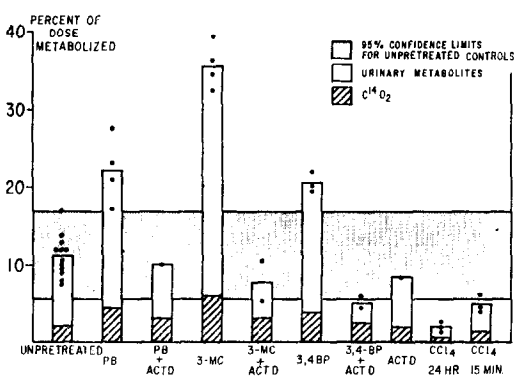

FIG. 4. Effect of stimulators of drug metabolism and CCl_4 hepatotoxicity on the biotransformation of FM-V by mice. Pretreatments were performed as described in Materials and Methods. Mice received 10 μl (1 μc) of FM-V i.p., and each dot represents the sum of $^{14}CO_2$ and urinary metabolites for a single experiment. Columns are averages.

percentage metabolized of a 10-μl dose of FM-V (i.p.), with 3-MC being the most active. Saline and olive oil control results were within the limits for unpretreated controls (saline: 9·4 per cent; olive oil: 11·9 per cent). The stimulatory effects could be blocked

in each case by administering actinomycin D 1 hr before the first stimulator injection; actinomycin D pretreatment had no apparent effect on the biotransformation of FM-V. Since this antibiotic is known to interfere with DNA-dependent RNA synthesis,[12] the stimulatory effects are assumed to be caused by induction of fluroxene-metabolizing enzyme synthesis.

The effect of a hepatotoxic dose of CCl_4 is also shown in Fig. 4. When given 24 hr before FM-V, CCl_4 markedly decreased the metabolism of fluroxene. A 15-min pretreatment with this hepatotoxin also appeared to have an inhibitory effect. These results suggest that the liver is the site of fluroxene metabolism, since chemically induced hepatotoxicity is known to prolong the duration of action of drugs whose inactivation is mediated by metabolism in the liver.[13]

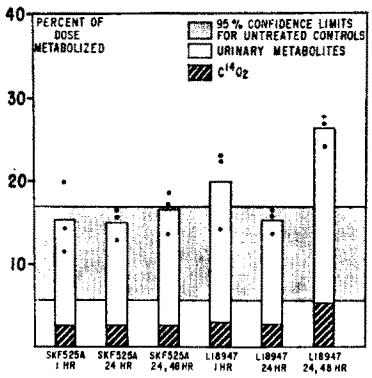

FIG. 5. Effect of pretreatment with SKF 525-A and Lilly 18947 on the biotransformation of FM-V in mice. Mice received 10 μl (1 μc) i.p., and each dot represents the sum of $^{14}CO_2$ and urinary metabolites for a single experiment. Columns are averages.

Fig. 5 is a similar graph showing the effects of two so-called "prolonging agents": SKF 525-A and Lilly 18947. Mice were pretreated at various times with these compounds, but at no time was any inhibitory effect demonstrable. Rather a tendency toward increased metabolism occurred. A 2-day pretreatment with Lilly 18947 caused a definite stimulation which could be blocked by prior pretreatment with ACT D. SKF 525-A also does not inhibit the dechlorination of methoxyflurane[14] or the *o*-dealkylation of *p*-ethoxyacetanilid,[15] although these reactions occur in liver microsomes. Stimulation of some metabolic pathways by SKF 525-A and Lilly 18947 has been observed to occur after the initial inhibitory phase.[16, 17] Thus the action of these inhibitors on fluroxene metabolism is apparently monophasic, owing to an insensitivity to the inhibitory phase.

Fig. 6 shows the effects of repeated short exposures of mice to anesthetic doses of fluroxene, methoxylflurane, diethyl ether, and nitrous oxide. Of the four anesthetics, only diethyl ether and nitrous oxide pretreatments were noted to have any effect; a moderate stimulation was observed, especially with nitrous oxide. Remmer reported that these two anesthetics could stimulate the metabolism of other drugs.[18]

Dog

Fig, 7 summarizes the results of experiments in a dog, designed to elucidate some of the relationships of dose and route of administration to the biotransformation

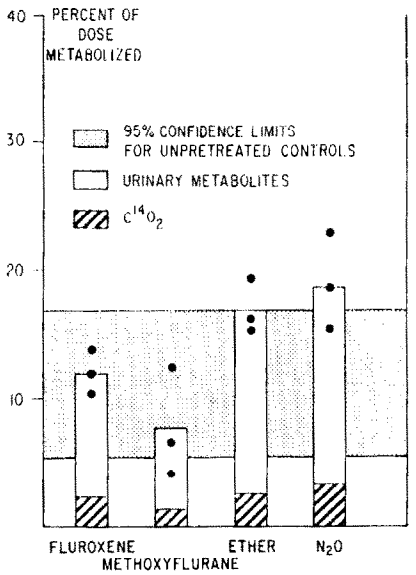

FIG. 6. Effect of pretreatment with various anesthetics on the biotransformation of FM–V in mice. Mice received 10 μl (1 μc) i.p., and each dot represents the sum of $^{14}CO_2$ and urinary metabolites for a single experiment. Columns are averages.

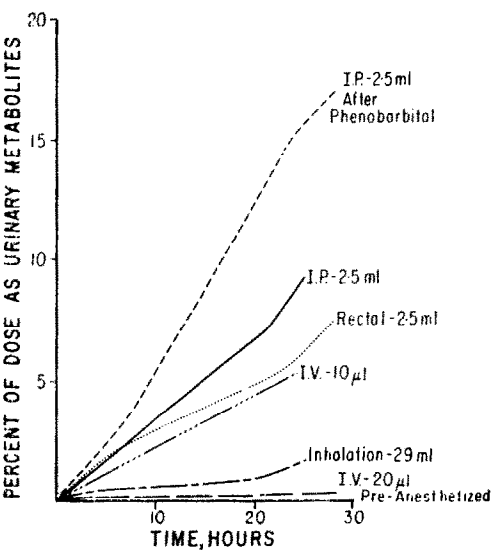

FIG. 7. Metabolism of FM–F by a dog under various conditions of administration and phenobarbital pretreatment. Rectal dose given as a 10% solution in mineral oil. Phenobarbital given as oral tablets 60 mg three times daily for 3 days. Inhalation anesthesia supplemented with 65% N_2O during induction. In the "pre-anesthetized" experiment, the dog was induced with nonlabeled fluroxene and N_2O (65%) and maintained for 30 min with fluroxene (7·5%) prior to i.v. injection of FM–F.

of fluroxene. The results of these studies indicate that the dog's capability for metabolizing a sub-anesthetic i.p. dog of FM-F is essentially the same as that of the mouse; i.e. approximately 10 per cent of the dose appears in the urine within 24 hr as non-volatile metabolites. A 2·5-ml dose was chosen for the 10-kg dog since this dose is comparable on a weight basis to that used in mice. Rectal administration of 2·5 ml in mineral oil did not appreciably alter the results. After oral pretreatment with phenobarbital, the metabolized percentage of a 2·5-ml i.p. dose of FM-F nearly doubled. No attempt was made to determine the sensitivity of this effect to actinomycin D. When FM-F was administered by inhalation in anesthetic concentrations, only 1·3 per cent of the administered radioactivity was excreted in the urine as non-volatile metabolites in a 24-hr postanesthetic period. Approximately 5 per cent of a small (10 μl) i.v. dose was metabolized; however, when the dog was preanesthetized with non-labeled fluroxene and then given a small i.v. dose of FM-F (20 μl), there was a marked reduction in the biotransformation of the labeled molecules. The inability to demonstrate greater metabolism of a small i.v. dose might be attributed to binding to serum proteins and rapid excretion by the lungs prior to transport to the liver. It therefore appears that the metabolic pathways can be saturated with anesthetic doses of fluroxene, and consequently a smaller percentage of the dose is metabolized.

Urinary metabolites of FM-F and TFE-F

FM-F was administered i.p. to mice (10 μl) and the dog (2·5 ml) and urine collected for 48 hr. By inverse isotope dilution, trifluoroacetic acid was detected in mouse and dog urine in amounts representing 10–20 per cent of the urinary radioactivity, and no increase was seen after refluxing with N HCl, indicating the absence of acid-labile conjugates of trifluoroacetic acid. Only traces of free TFE-F (0·5 per cent) were detected in untreated urine samples; however, after reflux with N HCl or incubation with β-glucuronidase, the alcohol derivative accounted for 20–50 per cent of the urinary radioactivity. Qualitatively, similar results were obtained for urinary metabolites of TFE-F after i.p. administration of 1 μl to mice, but the entire dose was metabolized. No $^{14}CO_2$ was excreted by these mice.

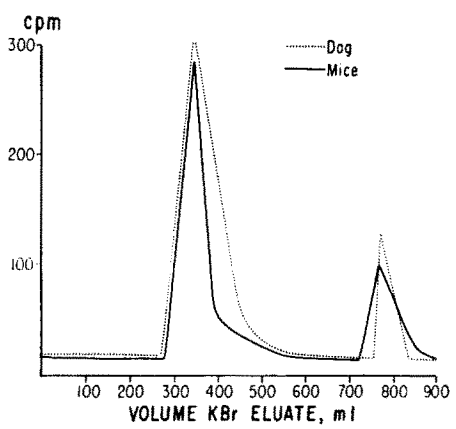

FIG. 8. Column chromatographic elution patterns of dog and mouse FM-F urinary metabolites from Dowex-1 resin. The urines had been previously passed through Dowex-50 resin and the metabolites eluted with water.

Fig. 8 illustrates the elution patterns of mouse and dog urinary FM-F metabolites from Dowex-1 with 5% KBr. The first peak to be eluted contained approximately six times more radioactivity than the second. Both of these metabolites appeared to be anions since they were elutable with water from Dowex-50 and were eluted from Dowex-1 with KBr.

Fig. 9 is a thin-layer chromatogram of various ion-exchange resin column eluates of urinary TFE-F mouse urine metabolites. Radioassay of the eluates that were applied indicated that the naphthoresorcinol-positive glucuronide metabolite (lane 2) contained approximately six times more radioactivity than the faster moving metabolite (lane 4), which had the same mobility as potassium trifluoroacetate (lane 5). Reflux of the radioactive glucuronide eluate in N HCl liberated 30 per cent of the radioactivity as a volatile compound. Incubation of another aliquot with β-glucuronidase resulted in the transformation of 70 per cent of the radioactivity to a volatile material. The faster moving radioactive spot in lane 3 which appeared after incomplete acid hydrolysis of the glucuronide metabolite cannot be free TFE-F,

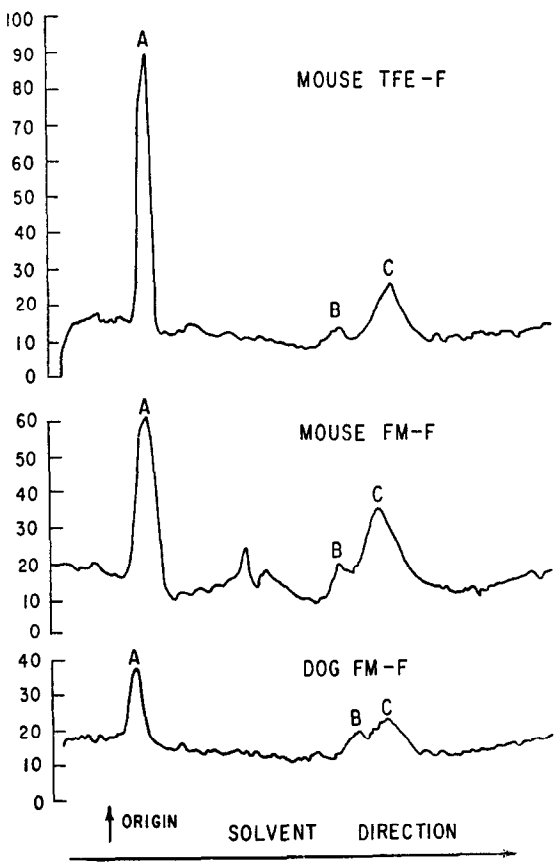

FIG. 10. Comparison of urinary metabolites of TFE–F from mice with those of FM–F from mice and dog on thin-layer chromatography. Densitometer tracings of autoradiograms were obtained as described.

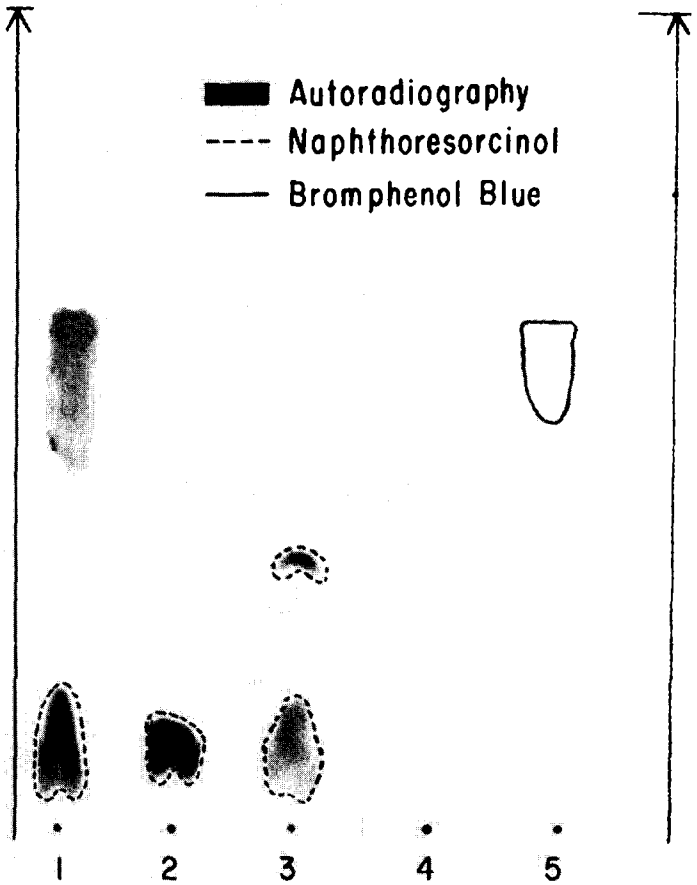

FIG. 9. Thin-layer chromatogram of TFE–F mouse urine metabolites. The metabolite fractions had been purified as described. 1. Water eluate from Dowex–50. 2. Formic acid elutate (0·1 N) of lane 1 from Dowex–1. 3. Lane 2 after reflux with N HCl. 4. KBr eluate (5%) of lane 1 after 0·1 N formic acid elution from Dowex–1. 5. $CF_3COO^- K^+$.

facing page 1246 B.P.

since this alcohol readily evaporates from the plate during sample application. Also, the spot gave the characteristic glucuronic acid reaction with naphthoresorcinol. It is possible that this spot represents a different salt of the unhydrolyzed glucuronide.

Figure 10 is a comparison of the radioactive urinary metabolites of FM-F, collected from mice and dog, with those of TFE-F from mice, separated by thin-layer chromatography. An autoradiogram was obtained from the thin-layer plate which was developed as described. Tracings of the darkened areas of the film were obtained by passing strips of the developed X-ray film through a recording densitometer. These recordings indicate a similarity for the mouse urine metabolites of TFE-F (peaks A, B, and C) and the metabolites of FM-F from either mice or dog. The mouse TFE-F urine sample was an aliquot of the sample on lane 1 of Fig. 9. Apparently the faster moving metabolite which has an R_f similar to trifluoroacetic acid has been resolved into two metabolites (B, C) with the use of densitometry, although it had appeared as a single spot with tailing on the autoradiogram (Fig. 9). The peaks which are located between metabolite A and B in the FM-F mouse tracing have the same location as the unidentified product of acid hydrolysis of the glucuronide from TFE-F (Fig. 9, lane 3). It is possible that during passage of the urine through Dowex-50, some alteration of the glucuronide occurs, since these spots were not obtained from urine which had not been applied to the resin.

These results suggest that trifluoroethanol is an initial metabolite of fluroxene and a precursor to the other urinary metabolites of the trifluoroethyl group. The major metabolite was identified as trifluoroethanol-glucuronide by the following criteria: it gave a positive test for glucuronic acid with naphthoresorcinol, and it was hydrolyzed by either acid reflux or β-glucuronidase to yield a labeled volatile compound which was identified as trifluoroethanol by isotope dilution. One of the minor metabolites was identified as trifluoroacetic acid by isotope dilution and a comparison of its mobility on thin-layer chromatography to the nonradioactive compound. In addition, this metabolite exhibited the chemical properties of a volatile acid. No attempt was made to identify the other minor metabolite, since only traces were detected and the identified metabolites accounted for more than 80 per cent of the urinary radioactivity; much of the balance can probably be ascribed to an artifactual loss of volatile trifluoroethanol liberated from its glucuronide during storage and handling.

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