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Trifluoroethanol and Halothane Biotransformation in Man

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Trifluoroethanol has been postulated to be an intermediate in the formation of trifluoroacetate during the biotransformation of halothane. As much as 80 per cent of injected ^{14}C-labeled trifluoroethanol could be recovered as trifluoroacetate in the urine of two volunteers. Excretion of radioactivity lasted seven to nine days after administration of trifluoroethanol. It is unlikely that trifluoroethanol is an endproduct of halothane metabolism in man. (Key words: Halothane; Biotransformation; Trifluoroethanol.)

HALOTHANE undergoes considerable biotransformation in man. As much as 25 per cent conversion has been reported.[1, 2] Trifluoroacetate is the only urinary metabolite demonstrated in man by us and others.[1, 2]

Van Dyke and Chenoweth[3] have postulated that trifluoroethanol is an intermediate in the formation of trifluoroacetate during the biotransformation of halothane. However, the presence of trifluoroethanol or trifluoroethanol glucuronide after biotransformation of halothane in man could not be demonstrated.

Since trifluoroethanol is fairly toxic,[4, 5] we thought it important to investigate its fate in man.

Method

One microcurie of trifluoroethanol, ^{14}C-labeled ‡ on the fluorine-carrying carbon, was injected intravenously in a rapidly running infusion into each of the authors. Urine samples were collected every hour for eight hours, followed by total collection for six days. Afterward, 1-ml samples of urine were assayed daily until no radioactivity could be demonstrated.

Immediately after the specimens were obtained, the urine was assayed before and after lyophilization. One-ml samples were shell-frozen and lyophilized in liquid scintillation vials. After lyophilization, 1 ml of water was added to the dry substance in the vial, followed by a sufficient quantity of scintillation fluid. Another 1-ml sample was placed in a vial containing scintillation fluid. All assays were by liquid scintillation counting for 100 minutes per sample. An internal standard was used for the calculation of counting efficiency, and all values are reported as disintegrations per minute (DPM).

The urines were assayed qualitatively by paper chromatography in the following manner:

Urine was spotted on a 46 × 57-cm Whatman #1 paper and developed downwards in a chamber using ammonia:isopropyl alcohol 1:4. The developed papers were sprayed with bromcresol green (0.3 per cent in methanol, water 8:2 and 8 drops of 30 per cent sodium hydroxide). Unsprayed papers were cut into 1 cm strips and assayed by liquid scintillation counting.

Urine also was assayed by liquid–liquid extraction. A volume of about 1,500 ml of iced urine was adjusted to a *p*H less than 1 by the addition of concentrated sulfuric acid, placed into a liquid–liquid extraction apparatus (Ace Glass 6440), and extracted for 24 to 48 hours with diethyl ether. The ether was separated from the water phase and radioactivity back-extracted into 2 ml of concentrated ammonium hyroxide. These concentrations were assayed by paper chromatography as described above.

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TABLE 1. Excretion of Urinary Metabolites after IV Administration of Trifluoroethanol to Two Volunteers (DAB, HFC)

Time after Injection	DAB		HFC	
	Disintegrations per Minute	Per Cent of Dose	Disintegrations per Minute	Per Cent of Dose
1 hour	8,710	0.45	11,224	0.59
2 hours	17,082	0.89	23,850	1.2
3 hours	27,702	1.4	39,285	2.0
4 hours	37,530	2.0	54,058	2.8
5 hours	45,215	2.4	69,122	3.6
6 hours	55,367	2.9	84,797	4.4
7 hours	69,551	3.6	103,585	5.4
8 hours	79,855	4.2	124,969	6.5
24 hours (day 1)	183,496	9.6	616,289	32.2
2 days	430,334	22.5	963,259	48.8
3 days	562,946	29.4	1,163,593	60.7
4 days	758,471	39.6	1,346,542	70.3
5 days	878,646	45.8	1,451,892	75.8
6 days	1,021,811	53.3	1,542,927	85.7
7 days	(No measurement)	—	—	—
8 days	36	—	—	—
9 days	9	—	—	—

Urines were also analyzed by inverse isotope dilution. A 100-ml volume of urine was boiled with 10 ml of concentrated sulfuric acid in a reflux system for an hour. *p*H was then adjusted to about 9 with sodium hydroxide. Three ml of unlabeled trifluoroethanol were added and the mixture distilled at 78–83 C. A sample of distilled trifluoroethanol was counted by liquid scintillation counting. The N-alpha-naphthyl carbamate ester of trifluoroethanol (melting point, 79–80 C) was prepared by heating alpha-naphthyl isocyanate (Eastman Organic Chemical) with the distilled trifluoroethanol in the presence of pyridine and recrystallized from hot ligroin to constant specific activity.

INCUBATION WITH GLUCURONIDASE

Four ml of urine were mixed with 10 ml of Ketodase (brand of beef liver β-glucuronidase, Warner-Chilcott) and 10 ml of acetate buffer at *p*H 5. This mixture was incubated in an open system at 37 C for 24 hours and assayed before and after incubation by liquid scintillation counting.

Results

Table 1 shows the excretion of urinary metabolites after injection of labeled trifluoroethanol. The total amounts recovered after 24 hours were 32.3 and 9.6 per cent of the injected doses. These figures increased to 80.5 and 53.3 per cent six days after injection. No measurable activity appeared after the ninth day in the urine of one volunteer or after the seventh day in that of the other.

Lyophilization of urine did not remove radioactivity, demonstrating that only nonvolatile substance had been excreted.

Liquid–liquid extraction followed by paper chromatography showed that most of the radioactivity had the same characteristics as trifluoroacetate. Inverse isotope dilution with trifluoroethanol after acid hydrolysis indicated that about 15 per cent of the urinary radioactivity was an acid-labile trifluoroethanol conjugate.

Incubation with glucuronidase showed that about 14 per cent of urinary radioactivity could be converted into a volatile substance which was distilled as trifluoroethanol using inverse dilution techniques.

The urinary excretion curves of radioactive metabolite(s) after trifluoroethanol injection are shown in figure 1. The slopes obtained after trifluoroethanol do not differ significantly from those obtained in previous experiments after the injection of halothane in the same volunteers.[2]

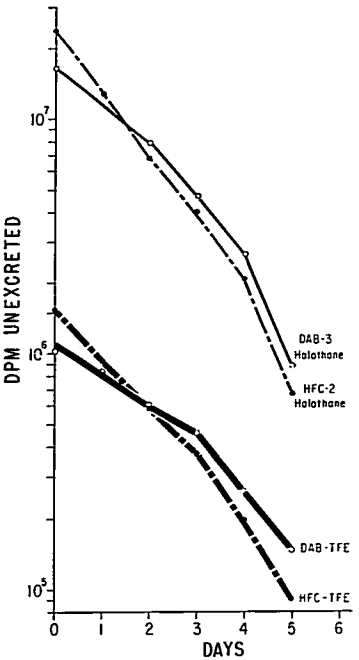

FIG. 1. Urinary excretion of trifluoroethanol metabolite (TFE) and halothane metabolite by two volunteers (HFC and DAB), plotted as unexcreted DPM versus time on a semilogarithmic scale. The tracer doses of TFE and halothane were injected at different times.

Discussion

In the investigation of biotransformation of halothane in man, we are limited to measuring excretion products in the urine or other excreta. The liver, where most of the halogenated anesthetics presumably are metabolized, is not accessible for study in volunteers. Therefore, it is necessary to employ indirect methods to elucidate the metabolic steps in the conversion of halothane to trifluoroacetate. This study demonstrates the conversion of trifluoroethanol to trifluoroacetate and trifluoroethanol glucuronide in man. These results parallel those obtained in similar studies with mice and dogs.[4]

Because trifluoroethanol glucuronide has not been found in urine after administration of halothane in humans,[1, 2] it appears unlikely that trifluoroethanol is a metabolite of this anesthetic. However, this does not exclude trifluoroethanol as an intermediate in the biotransformation of halothane.

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