Laboratory Report

Biotransformation and Elimination of $^{14}$C-Trichlorofluoromethane (FC-11) and $^{14}$C-Dichlorodifluoromethane (FC-12) in Man

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Radioisotopically labeled trichlorofluoromethane (FC-11; $^{14}$CCL$_3$F) and dichlorodifluoromethane (FC-12; $^{14}$CCl$_2$F$_2$) were separately inhaled by a female subject and a male subject. A predetermined volume of fluorocarbon (1000 ppm; 108 μCi) in air was delivered through a nonrebreathing system and a tight-fitting face mask for 7–17 minutes. Total expired gases were collected during fluorocarbon exposure and afterward until no radioactivity was detectable. Expired $^{14}$CO$_2$ and $^{14}$C-fluorocarbon were assayed. Urine was collected for 72 hours and assayed for nonvolatile radioactivity. Total recoveries of FC-11 were 99.3 and 79.4 per cent in the woman and the man, respectively. Total recoveries of FC-12 were 95.4 and 103.2 per cent. Traces of radioactivity were found in urine (FC-11, 0.07 and 0.09 per cent; FC-12, 0.02 and 0.03 per cent) and in exhaled carbon dioxide (FC-11, 0.13 and 0.10 per cent; FC-12, 0.08 per cent in both subjects). Total metabolites were equal to or less than 0.2 per cent of the administered dose. The amount of radioactivity in urine was insufficient to permit identification of possible fluorocarbon metabolites. The trace levels of metabolites could be products of radiolabeled impurities. (Key words: Gases, non-anesthetic, dichlorodifluoromethane (Freon 12); Gases, non-anesthetic, trichlorofluoromethane (Freon 11); Pharmacology, fluorocarbons.)

The fluorinated hydrocarbons dichlorodifluoromethane (FC-12) and trichlorofluoromethane (FC-11) are in widespread use as aerosol propellants for nonmedicinal (e.g., hair spray) and medicinal (e.g., bronchodilators) purposes. FC-12 is a gas at room temperature (boiling point −28 C) and FC-11, a volatile liquid (boiling point 24.9 C). Both fluorocarbons share certain properties, including production of reversible unconsciousness1 and cardiac sensitization,2,3,4 with some of the halogenated anesthetics.

Fluorinated hydrocarbons and ethers used as anesthetics (e.g., halothane, methoxyflurane, fluroxene) have been shown to be metabolized in significant amounts.5 To date, the metabolism of FC-11 and FC-12 in man has not been reported. We previously reported results of studies of the biotransformation of FC-11 and FC-12 in dogs.6 Since very little of the inhaled dose appeared to be metabolized in dogs, a confirmation of the results in man seemed warranted.

This investigation has examined the biotransformation of FC-11 and FC-12 in man following short-term inhalational exposure by analyzing nonvolatile metabolites in urine and a possible volatile metabolite, namely CO$_2$, in exhaled air, employing radioactively labeled compounds.
Methods

Unlabeled FC-11 or FC-12, 0.1 per cent (v/v) in air, was metered from an anesthesia machine into a large Teflon bag. The final volume was 80–100 l. To this were added approximately 100 μCi of 14C-labeled FC-11 or FC-12 at a specific activity of 5 mCi/mmole. The total gaseous volume of the labeled compound was less than 1 ml. The radioactive fluorocarbons were prepared by New England Nuclear Company, Boston, Mass. 5 and stored in metal cylinders at 4 C. The anesthesia machine (Ohio, Model 2000) was calibrated by gas chromatography according to the method of Shargel and Koss. 7 The gas volume was cumulatively measured with a dry gas meter (Thomas Glover Co.), which was calibrated with a Tissot spirometer.

Total radioactivity in the bag was determined by assay of 5-ml gas samples (n = 5) withdrawn by syringe and immediately in-

5 14C-FC-11, lot no. 613-247; 14C-FC-12, lot no. 728-007.
6 The study was approved by the Human Volunteers Research Committee of the University of Maryland School of Medicine.

Fig. 1. Procedure for inhalational administration and collection of fluorocarbons.

INHALATION TECHNIQUE

One female volunteer (Subject 1) and one male volunteer (Subject 2) were exposed to FC-11. Three weeks later the same subjects were exposed to FC-12. 5 Both subjects had unremarkable medical histories and had not been exposed occupationally to anesthetics. Hemoglobin, hematocrit, and urine were normal. Both subjects were smokers. They were instructed not to take any drugs for two weeks prior to the experiment. The day before the experiment, a practice run was done using air only. The next day the subject was placed, without premedication, in the supine position, and a face mask with an airtight fit applied. The subject inhaled the air–fluorocarbon mixture from a Teflon bag,
and exhaled air was collected in a separate Teflon bag. Rebreathing was prevented by a directional nonrebreathing valve (fig. 1).

Blood was withdrawn intermittently during and after exposure from an intravenous catheter passed from the antecubital space into a central vein, but not into the right heart. Lead 2 of the EKG was recorded at the time each blood sample was obtained.

Inhalation times to complete evacuation of the bag ranged from 7 to 17 minutes, depending on minute volume. During exposure, exhaled gases were collected. Following inhalation, expired air was collected continuously for 17 to 44 minutes.

**RADIOCHEMICAL PURITY OF ¹⁴C-FLUOROCARBONS**

Although the manufacturers alleged the radiochemical purity to be more than 99 per cent (determined by radiochromatography), further analysis by gas chromatography–mass spectrometry** showed that ¹⁴C-FC-11 was 89.6 per cent pure and ¹⁴C-FC-12 was 96 per cent pure. The labeled impurities in FC-11 were 1.4 per cent CHCl₃ and 9 per cent CCl₄, and in FC-12, 4 per cent CCl₃ and/or CF₃. However, after a more than a thousandfold dilution, the chemical purity of the administered fluorocarbon was assumed to be that of the non-radioactive carrier, namely 99.9 per cent.

**DETERMINATION OF URINARY RADIOACTIVITY**

Total voided urine was collected at 8-hour intervals for 72 hours in polyethylene containers and kept refrigerated at 4°C until assayed. One-milliliter urine samples were added to 15 ml of scintillation solution and counted to less than 1 per cent SD. Some of the urine samples were lyophilized and reconstituted with 1 ml distilled water and recounted. There was no loss of radioactivity during lyophilization, and it was therefore assumed to be associated with nonvolatile compounds.

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ASSAY OF EXHALED GASES

Exhaled CO₂ was removed from the bags by absorption in 4 N sodium hydroxide and then precipitated with 2 N barium chloride. The precipitate was dried under a heat lamp. The CO₂ was volatilized in a closed system with 4 N lactic acid and absorbed in 10 N hyamine hydroxide. This procedure of precipitation and revolatilization was used to avoid contamination by unmetabolized fluorocarbon.⁹ The method of determination of fluorocarbon in blood and a detailed description of the assay of exhaled gases have been reported.⁶

Results

Dosages and recovery data for FC-11 and FC-12 are given in table 1. Each volunteer was exposed to FC-11 first and three weeks later to FC-12. Since only two subjects were studied, only certain trends can be pointed out. Recovery of administered radioactivity in exhaled air was essentially complete, 99 per cent and 79 per cent for FC-11 and 95 and 103 per cent for FC-12. Errors in collection of these rapidly eliminated gases may account for some of the differences from 100 per cent. Only a very small fraction of the administered labeled fluorocarbon was exhaled as ^14CO₂ or excreted as nonvolatile urinary radioactivity. There was slightly more ^14CO₂ than nonvolatile urinary radioactivity following FC-11 and FC-12 inhalation. ^14C in urine and ^14CO₂ exhaled together amounted to less than 0.2 per cent of administered radioactivity. Figure 2 shows the accumulative excretion of nonvolatile radioactivity in urine. The curves reflect the greater excretion of nonvolatile metabolites following inhalation of FC-11 in both subjects. The male subject had greater excretion of both compounds.

Blood levels of these fluorocarbons increased and decreased very rapidly (Figs. 3 and 4). Blood-gas partition coefficients have been reported as 0.2 for FC-12 and 1.4 for FC-11.⁸ Our method of measuring radioactivity in blood and converting dpm into μg was checked once by gas chromatography and found to give comparable results. No changes were evident on the EKG, and neither subject noticed any effect during inhalation of 0.1 per cent FC-11 or FC-12. They both stated that there was no difference between the practice run and the exposure.

Discussion

There are no published studies to date concerning possible biotransformation of the commonly used fluorocarbon aerosol propellants. Substitution of fluorine for hydrogen or other halogens produces a chemically more stable compound and reduces toxicity.¹⁰ The presence of fluorine on the same carbon appears to stabilize other halogens.¹¹ Sherman¹² exposed rats and dogs for approximately three months to either high or low doses of FC-11 in corn oil by intragastric administration. He found no abnormality in results of tests of hematologic, hepatic, or
renal values. A slight increase in urinary fluorides was statistically not significant.

In early studies of anesthetic agents, the extent of biodegradation was described as percentage of the administered dose, whether the agent was administered by the intravenous, inhalational, or oral route. This method tends to underestimate the biodegradability of a gaseous compound when a large fraction of the inhaled dose either does not enter pulmonary circulation or is exhaled rapidly. More recently, the fraction of an inhalational anesthetic metabolized has been calculated from the apparent dose absorbed.

Van Dyke (1964) studied the metabolism of methoxyflurane in rats and found 3–5 per cent of the intraperitoneally administered dose in urine as metabolite and 1–2 per cent as CO₂. Holaday (1972) later described the metabolism of methoxyflurane as percentage of absorbed dose, the absorbed dose taken as the difference between the inhaled dose and the exhaled dose, collected in separate systems. He found that nearly 50 per cent of the “absorbed” dose was metabolized.

![Fig. 3. Venous blood levels during uptake and elimination of 14C-trichlorofluoromethane (FC-11) in the female subject.](image)

![Fig. 4. Venous blood levels during uptake and elimination of 14C-dichlorodifluoromethane (FC-12) in the female subject.](image)
When exhaled gases are collected during exposure to a gaseous drug, some of the molecules collected have never entered pulmonary blood, some have entered but have been exhaled immediately, and some have been absorbed and entered various organs, either to be exhaled later or to be biotransformed. How many molecules actually enter the blood and enter various tissues cannot be measured experimentally in man. This has been attempted by autoradiography in animals.\textsuperscript{19} By using computer-assisted compartmental kinetic analysis of the data in this study, the actual fraction of the administered dose that was absorbed can be estimated.\textsuperscript{20}

Accurate collection of gases is somewhat difficult, and small differences during collection would greatly amplify any error in calculating the “absorbed dose,” i.e., the small difference between the amount administered and the amount exhaled during exposure. For example, Subject 1 “absorbed” only 0.72 per cent of FC-11 and 4.73 per cent of FC-12. This would indicate that 36 per cent of the absorbed dose of FC-11 was metabolized and 2.3 per cent of FC-12. However, the computer-predicted absorbed fractions were 92.2 and 34.7 per cent of the administered doses, respectively. It appears prudent, therefore, to express our data as a fraction of the administered dose, since this amount could be measured directly and accurately.

Using the same procedure, we previously found\textsuperscript{6} similar results in four male and two female beagle dogs, i.e., < 0.2 per cent of the administered dose was excreted as nonvolatile metabolites and \(^{14}\)CO\(_2\). Neither phenobarbital pretreatment (60 mg daily for three days) nor prolonged exposure (50–90 min) produced any alteration of these results. Less than 1 per cent of the administered radioactivity was detected in the major tissues 24 hours after exposure.

The impurities in FC-11, namely chloroform and carbon tetrachloride, are known to be partially metabolized.\textsuperscript{21} Assuming that only 2 per cent of the CHCl\(_3\) impurity is metabolized, this amount of radioactivity could account for all the radioactivity found in urine and exhaled CO\(_2\) after exposure to FC-11.

Radioactive impurities are present in all radiolabeled preparations. They do not present a problem unless trace amounts of radioactivity are measured. In the majority of metabolic studies with volatile anesthetics, where only a small fraction of the administered dose is metabolized, the significance of radioactive impurities is not known.\textsuperscript{22} Methods of minimizing the problem of impurities have been advocated.\textsuperscript{23}

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References
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Obstetrics

DIAZEPAM AND LABOR The effects of diazepam on mother and fetus during labor were studied in 31 patients, using sophisticated biophysical and biochemical techniques. A total dose of 5 to 10 mg diazepam caused tranquility of the mother. Diazepam caused mild transient tachycardia and a marked decrease in the beat-to-beat heart rate interval in both mother and fetus. There were no adverse effects on pH, PaO₂, PaCO₂, or base deficit of the mother, fetus, or newborn following drug administration. The effect of diazepam on uterine activity could not be determined from this study. No adverse effect of diazepam on the neonatal outcome was found. (Yeh, S. Y., and others: A Study of Diazepam during Labor. Obstet Gynecol 43:363–373, 1974.)

MATERNAL ASPIRATION Three cases of aspiration of liquid vomitus as a cause of maternal mortality are presented. The etiology and pathology of this catastrophe are discussed from an experimental and pathophysiologic point of view. Logical steps to allow for a rapid, accurate early diagnosis are detailed. The use of regional versus general anesthesia and the use of prophylactic antacids in labor are suggested as preventative measures. A treatment procedure based on the physiologic alterations produced by the action of gastric acid on the pulmonary parenchyma is outlined, with special emphasis on massive, therapeutic doses of adrenal corticosteroids. (Baggish, M. S., and Hooper, S.: Aspiration as a Cause of Maternal Death. Obstet Gynecol 43:327–336, 1974.)

FLUORIDE CONCENTRATIONS Fluoride concentrations in maternal and cord blood were measured for the first time with a method specific for inorganic fluoride. The concentrations averaged 0.88 µM in blood from 16 mothers and 0.68 µM in the cord blood, with a correlation of 0.86. These results are consistent with the hypothesis that fluoride diffuses passively across the placenta. The term placenta, as previously reported, can have much higher concentrations (average 50 µM), but the acid-stable organic fluoride averaged only 4 µM, which is similar to the values found in human sera. (Shen, Y-W., and Taves, D. R.: Fluoride Concentrations in the Human Placenta and Maternal and Cord Blood. Am J Obstet Gynecol 119:205–207, 1974.)