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Characterization of Fluorinated Metabolites by a Gas Chromatographic-Helium Microwave Plasma Detector—The Biotransformation of 1*H*,1*H*,2*H*,2*H*-Perfluorodecanol to Perfluorooctanoate

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A gas chromatographic technique utilizing a microwave-sustained helium plasma detector (GC/MPD) was developed to study the biotransformation of 1*H*,1*H*,2*H*,2*H*-perfluorodecanol ($C_8F_{17}CH_2CH_2OH$) in adult male rats. The metabolic products resulting from a single oral dose of the alcohol were isolated from the blood plasma by an extraction technique, derivatized, and characterized by the GC/MPD system with confirmation by fluorine NMR. Four fluorine-containing metabolites were detected by the fluorine-specific channel of the element-selective MPD and one of these was shown to be perfluorooctanoate ($C_7F_{15}COO^-$). Appearance of perfluorooctanoate as a metabolite and an additional observation of concomitant elevation in plasma and urinary inorganic fluoride suggests that the biotransformation of the above alcohol to the perfluorooctanoate involves defluorination of the CF_2 group adjacent to the CH_2 group in the parent compound.

In recent years, a variety of analytical techniques for differentiating and quantitating the inorganic fluoride and organic fluorine in biological samples has been described by Taves (1,2), Venkateswarlu *et al.* (3–5), and Belisle and Hagen (6). However, characterization and determination of the specific organic compound(s) in the samples is more informative than determination of total organic fluorine. Based on fluorine NMR data, Guy *et al.* (7,8) concluded that perfluorooctanoic acid or a similar compound was present in a fraction prepared from a large pool of human plasma samples. A quantitative microanalytical method, based on GC[1] with electron-capture detector, was reported by Belisle and Hagen (9) specifically for perfluorooctanoate in blood plasma though the analysis gave no evidence for the presence of perfluorooctanoate. It should be emphasized that this research (9) describes an analytical method for the determination of perfluorooctanoate and is not a study of perfluorooctanoate levels in normal human plasma since only a few public donor samples were analyzed.

Gas chromatography is well suited for the complex matrices of biological extracts and the electron-capture detector, which gives a measure of specificity for the halogenated species present, is perhaps the most sensitive and widely used detector for compounds of this type. This detector, however, lacks sufficient specificity when large quantities of interfering coextractants are present. We are studying the metabolism of various fluorine-containing compounds, and this paper will present our study of the biotransformation of 1*H*,1*H*,2*H*,2*H*-perfluorodecanol in male rats using the element-specific gas chromatography/microwave plasma detector (GC/MPD) system. Specific characterization of fluorinated compounds will be described in this work.

The detector plasma (not to be confused

[1] Abbreviations used: GC, gas chromatography; MPD, microwave plasma detector; FID, flame ionization detector.

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with blood plasma) is an extremely energetic ionization source of metastable atoms, molecules, ions, and electrons generated in a microwave cavity. The plasma generates atomic line emission spectra for the elements present in the compounds eluted from the GC column.

Each element (C, H, Cl, F, S, etc.) has a discrete emission spectrum and, using the described detector, one can monitor a representative line. For example, channel A can record chlorine, channel B can record fluorine, channel C can record sulfur, all simultaneously from the same Cl-, F-, and S-containing compound. This specific detection for each element simplifies the interpretation of a complex, multicomponent GC chromatogram.

EXPERIMENTAL

Gas chromatographic/microwave plasma detector analyses. Derivatized and nonderivatized reference compounds and samples were analyzed on the following chromatographic systems. Hewlett-Packard Models 7620 and 5830 gas chromatographs equipped with a flame ionization detector (FID) and the MPD were used with a 40:60 splitter at the column exit between the FID:MPD. The carrier gas was helium at 25 ml/min with an injection port temperature of 150°C. A 12 ft (1/8 in. o.d.) stainless-steel column packed with 20% DC-200 (methyl silicone) + 10% Bentone 34 (diatomaceous earth) on 80/90-mesh C-22A support was used for the GC/MPD analyses. The column was fitted for on-column injection of sample and programmed from 60 to 200°C at 15°C/min.

The microwave-sustained helium plasma detector is the Model MPD-850 from Applied Chromatography Systems Limited, Luton, England. A Hewlett-Packard 3354 computer is interfaced to the amplifier array (Fig. 1). Helium from the column is split between the FID detector and a transfer line to the microwave cavity. Makeup helium at 25 ml/min is utilized after the splitter to optimize flow velocity through the transfer line. A microwave generator at 2450 MHz supplies the energy (approximately 100 W) to the cavity. A vacuum system maintains the pressure of the cavity at 4–5 mm Hg and oxygen or nitrogen at 0.2 ml/min is introduced into the He carrier gas at the inlet of the cavity to prevent carbon buildup on the optical walls of the plasma tube. The plasma tube is a 0.1-mm i.d. 1/4-in. o.d. quartz tube 15.2 cm in length. The highly energetic helium plasma is initiated with a Tesla coil and is normally maintained without extinguishing throughout the day. Microliter quantities of solvent would completely extinguish the plasma so the cavity is fitted with a bypass-valve arrangement to shunt solvent around the quartz cell. The helium plasma is of sufficient energy to completely ionize the eluting component from the column, that is, metastable helium and energetic electrons in this plasma ionize each compound in the GC eluant to its respective elements transforming them to excited states; atomic line spectra result and the elemental response is independent of molecular configuration. The light emitted from a selected area of the plasma plume is monitored by the spectrometer where separate secondary slits and photomultiplier detectors are situated at the appropriate spectral positions for individual and simultaneous element monitoring. Fluorine is monitored at 685.60 nm. Large amounts of carbon generate a carbon continuum which can give an interference signal on noncarbon elemental channels. A "ghost" correction is electronically applied via an amplifier which constantly monitors the carbon emission and supplies a negative correction signal to the individual elemental channels. This limits the dynamic range of the detector for trace analysis if interfering components are not adequately separated from the peak of interest. The sensitivity ranges from 0.01 to 1 ng/s for various elements.

The improved separation offered by GC capillary columns, coupled to the MPD,

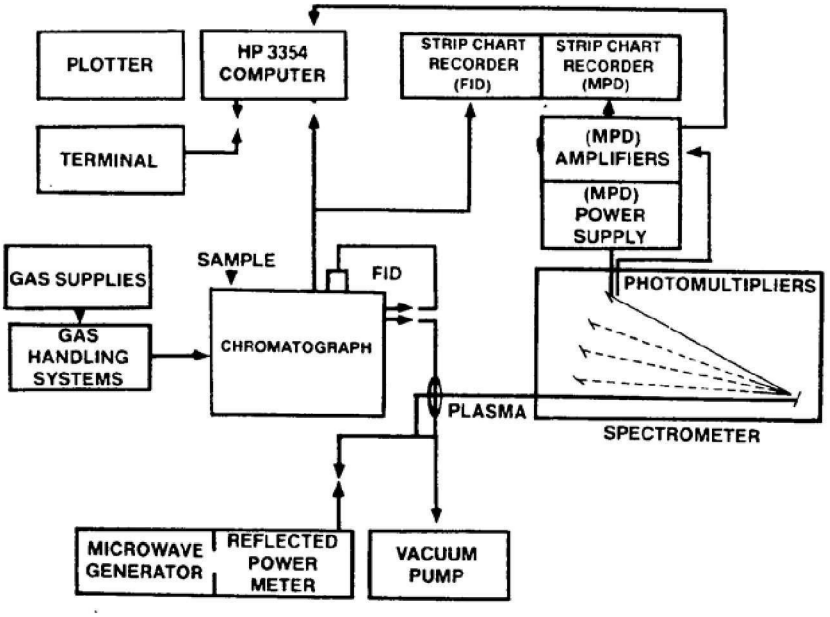
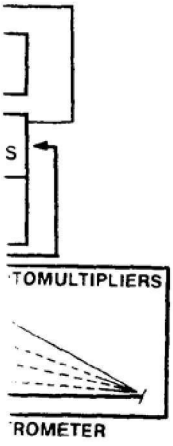


FIG. 1. Schematic diagram of the GC/MPD-850 data system. FID, flame ionization detector; and MPD, microwave plasma detector. Spectral lines of up to eight different elements can be monitored simultaneously via the data system.

would be preferred since it would permit calculating elemental ratios (C/F, C/H) in addition to specific element detection. A capillary system was briefly evaluated for the characterization of the plasma extracts. In this case, a Hewlett-Packard 5840 chromatograph equipped with a 30-m fused silica methyl silicone capillary column (J and W Scientific, Rancho Cordova, Calif.) with an electron-capture detector was employed. The temperature program was 50°C for 1 min, then 2°C/min for 15 min, and 20°C/min to 280°C. A 2-μl sample injection was utilized with a 70/1 split ratio.

Animal dosing and sample collection. Thirty male Charles River CD rats (Charles River Breeding Lab., Wilmington, Mass.), 9 weeks old, were divided into groups of three (nine test groups and one control group). The rats were conditioned for 24 h to individual metal metabolism cages with free access to water and fasted overnight prior to dosing. The rats were allowed free access to Purina Ground Chow (Ralston Purina Company, St. Louis, Mo.) and water immediately after dosing with a single oral dose. The 1*H*,1*H*,2*H*,2*H*-perfluorodecanol (Hoechst, Frankfurt, W. Germany) was analyzed to ensure its suitability for biotransformation studies and found to contain less than 1 ppm perfluorooctanoate and less than 5 ppm inorganic fluoride. Eight grams of the alcohol was suspended in 100 ml of pure Mazola corn oil. The mixture was resuspended with a tissue homogenizer before each dose to assure homogeneity.

The rats were weighed immediately before dosing. The volume of the alcohol–corn oil suspension used was calculated to give an alcohol dosage of 400 mg/kg and that volume was administered to each rat with a 2-ml glass syringe fitted with a stainless-steel intubation tube. Groups of three rats were sacrificed by exsanguination at 1, 2, 6, 12, 24, 48, 96, 144, and 480 h postdosage. The rats were anesthetized with diethyl ether and



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blood was drawn from the descending aorta and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. Urine was collected and stored frozen.

Inorganic and organic fluorine analyses. Inorganic fluoride in rat plasma and urine samples was determined by a microprocedure using the hanging-drop fluoride electrode (10). Total fluorine was also measured with the electrode, following reductive cleavage of the organic halogen with sodium biphenyl reagent (11,12). This method, as applicable to microdetermination of fluorine in biological samples, will be the subject of a separate publication by one of the authors (P. V.). Organic fluorine present in the sample was calculated by subtracting inorganic fluoride from the total fluorine.

Extraction procedure. The extraction technique was similar to the one previously used to extract perfluorooctanoate from plasma (9); however, the 80% hexane/20% diethyl ether extractant was replaced with ether, a more polar solvent, to ensure a more complete extraction of metabolites. In several cases, it was necessary to pool plasma to obtain sufficient quantities for the GC/MPD analysis.

One milliliter of rat plasma was pipetted into a 50-ml polypropylene tube (DuPont 3284) followed by 5 ml of water and 1 ml reagent grade hydrochloric acid. The contents were extracted with 7 ml of ether (Baker, anhydrous grade) and centrifuged 3 min (11,000*g*) with transfer of the ether phase using a polyethylene dropper (Nalgene 6219) to a 10-ml polyallomer centrifuge tube (Nalgene 3119). The extract was concentrated under N_2 at 50°C to about 1 ml. The extraction was repeated, this time using 6 ml of ether, and a third time using 5 ml of ether. The total extract was then concentrated to about 1 ml and divided into two portions by splitting the sample between two 10-ml Nalgene tubes (tubes A and B).

About 0.3 ml diazomethane in diethyl ether (toxic) was added to tube A. Appropriate precautions must be taken in its usage as described in previous work (9). The method of preparation from Diazald is supplied in literature from the Aldrich Chemical Company. After intermittent swirling of the tube (5 min), the contents were transferred with the above plastic dropper to a 1-ml volumetric flask and brought to volume with one ether rinse of the 10-ml tube. This sample was analyzed via GC/electron capture.

The contents of tube B were concentrated under N_2 to about 50 μl, and 50 μl diazomethane reagent added. After 5 min, the contents were transferred with the plastic dropper to a 1-ml Reacti-Vial (Pierce 13221) and brought to 100 μl final volume. This sample was analyzed with the GC/MPD system.

RESULTS AND DISCUSSION

The inorganic fluoride and organic fluorine levels in plasma of rats sacrificed at different intervals are shown in Table 1. Inorganic fluoride levels in plasma and in selected urinary samples were significantly higher in the experimental animals than in the controls. This observation suggested a defluorination step in the biotransformation of the above perfluorodecanol. Selected samples of the rat plasma were then chosen for further characterization of the organofluoro compounds present.

The effect of methylation is shown in Fig. 2 where the same sample (Rat 17, 24 h postdosage) was run on the GC/MPD system before and after derivatization with diazomethane. As a result of methylation, at least three additional fluorine-containing components were observed in the chromatogram.

The single peak in the nonderivatized extract eluting at 10.8 min was shown to be the original alcohol ($C_8F_{17}CH_2CH_2OH$) by retention-time matching. Since one can expect the biotransformation of an alcohol functional group into a carboxylic acid, the new peaks appearing only after derivatiza-

tion with diazomethane were assumed to be acids. Two possible metabolites of the alcohol were suggested, namely, $C_8F_{17}COOH$ and $C_8F_{17}CH_2COOH$. Perfluorononanoic acid ($C_8F_{17}COOH$) was purchased from Riedel-de Haen (via the American Hoechst Corp., Somerville, N. J.). 2*H*,2*H*-Perfluorodecanoic acid ($C_8F_{17}CH_2COOH$) was kindly supplied by the American Hoechst Corporation. These two reference acids were analyzed after diazomethane derivatization separately and also were used to "spike" rat plasma isolates for retention-time matching. Figure 3 illustrates the chromatograms obtained. The new peak appearing at 7.5 min is the methyl ester of perfluorononanoic acid and the peak at 8.9 min is the methyl ester of 2*H*,2*H*-perfluorodecanoic acid. These data, therefore, indicate that perfluorononanoic acid was *not* present as a metabolite while the peak at 8.9 min matches the retention time of methyl 2*H*,2*H*-perfluorodecanoate.

A German patent (13) indicated that the methyl ester of 2*H*,2*H*-perfluorodecanoic acid is readily (as least chemically) converted to the corresponding unsaturate, $C_7F_{15}CF{=}CHCOOCH_3$ via defluorination. It was speculated that this unsaturate might also be a metabolite of the alcohol and therefore was synthesized according to the above patent and shown by fluorine/NMR to be

TABLE 1

INORGANIC FLUORIDE AND ORGANIC FLUORINE CONCENTRATIONS IN RAT PLASMA

Time, postdosage (h)	Rat No.	Rat plasma concentration (μM)[a]	
		Organic fluorine	Inorganic fluoride
Control	1	<50	<5
	2	<50	<5
	3	<50	<5
1	4	80	25
	5	140	35
	6	140	40
2	10	350	40
	11	110	30
	12	250	30
6	7	410	80
	8	660	70
	9	470	35
12	13	360	20
	14	250	30
	15	430	30
24	16	890	65
	17	960	20
	18	600	30
48	19	500	10
	20	280	5
	21	590	5
96	22	320	<5
	23	320	5
	24	250	5
144	25	250	<5
	26	390	5
	27	250	5
20 days	29	60	<5
	30	130	<5
	31	90	<5

[a] 1 μM = 0.019 ppm.

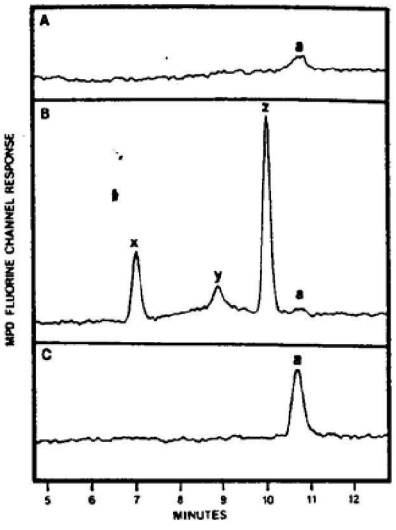
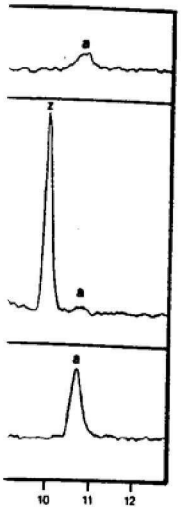


FIG. 2. Effect of esterification. (A) Rat 17 (24 h postdosage) plasma extract nonesterified; (B) A after esterification; (C) 1*H*,1*H*,2*H*,2*H*-perfluorodecanol reference. (a) 1*H*,1*H*,2*H*,2*H*-perfluorodecanol ($C_8F_{17}CH_2CH_2OH$); (x) perfluorooctanoate ($C_7F_{15}COO^-$); (y) 2*H*,2*H*-perfluorodecanoate ($C_8F_{17}CH_2COO^-$); (z) unidentified metabolite.



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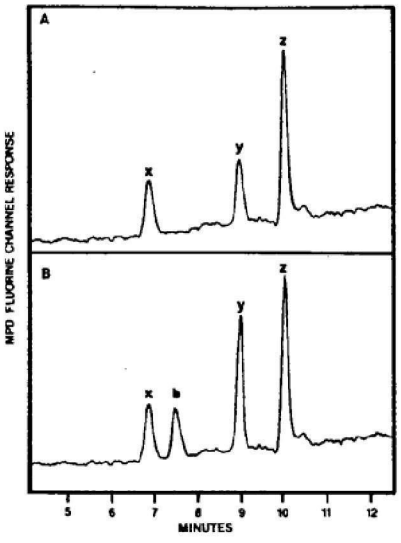

FIG. 3. Rat plasma extracts before and after the addition of two known reference compounds. (A) Rats 8 and 17, pooled (6 h and 24 h postdosage, respectively); (B) A spiked with perfluorononanoic acid ($C_8F_{17}COOH$) and 2*H*,2*H*-perfluorodecanoic acid ($C_8F_{17}CH_2COOH$). (b) Perfluorononanoate ($C_8F_{17}COO^-$); (x) perfluorooctanoate ($C_7F_{15}COO^-$); (y) 2*H*,2*H*-perfluorodecanoate ($C_8F_{17}CH_2COO^-$); (z) unidentified metabolite.

greater than 90% in purity. This unsaturate was not well resolved from the 2*H*,2*H*-perfluorodecanoic acid ester on the packed column. Figure 4 illustrates the chromatograms obtained for derivatized rat plasma extract before and after spiking with this unsaturated reference. As shown, the unsaturate elutes slightly ahead of methyl dihydroperfluorodecanoate. In related work, the capillary column (described earlier) provided the expected greater resolution and indicated a component whose retention time matched that of the reference unsaturate (Fig. 5). Thus, the peak eluting at 10.0 min on the packed column (Fig. 3) is not the unsaturate but perhaps a derivative thereof.

The first eluting fluorine-containing compound at 6.8 min appeared to be an ester since it required diazomethane derivatization to render it GC volatile. The column utilized in this case separates components principally by volatility. The 6.8-min peak was therefore lower boiling than the reference sample of methyl perfluorononanoate and matched the retention time of the methyl ester of perfluoroctanoic acid (PCR, Incorporated, Gainesville, Fla.). Fluorine/NMR data confirmed the presence of perfluorooctanoic acid and 2*H*,2*H*-perfluorodecanoic acid in the rat plasma extract.

Ophaug and Singer (14) have studied the metabolism of perfluorooctanoic acid in female rats and concluded that perfluorooctanoate is not further metabolized. In the Griffith and Long (15) study of ammonium perfluorooctanoate, vastly different levels (about 100-fold) of organic fluorine in the serum of male vs female rats were reported.

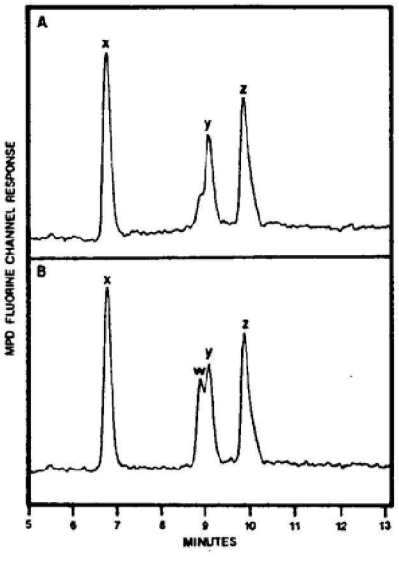

FIG. 4. The identification of the unsaturate ($C_7F_{15}CF{=}CHCOO^-$) in addition to 2*H*,2*H*-perfluorodecanoate ($C_8F_{17}CH_2COO^-$) in rat plasma extracts. This represents a separate experiment where six rats were given a single dose of 1*H*,1*H*2*H*,2*H*-perfluorodecanol and sacrificed 6 h later as described in the experimental section; the plasma from the six rats was pooled. (A) Rat plasma, 6 h postdosage; (B) A spiked with the unsaturate ($C_7F_{15}CF{=}CHCOO^-$). (w) 2-Hydroperfluoro-2-decenate ($C_7F_{15}CF{=}CHCOO^-$); (x) perfluorooctanoate ($C_7F_{15}COO^-$); (y) 2*H*,2*H*-perfluorodecanoate ($C_8F_{17}CH_2COO^-$); (z) unidentified metabolite.

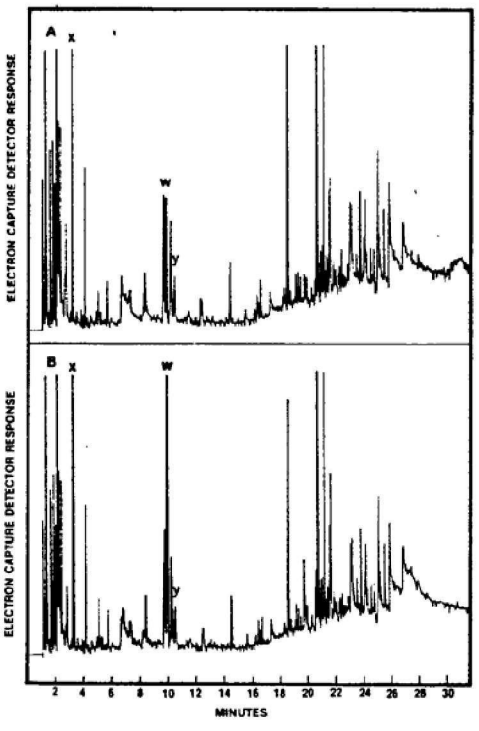

FIG. 5. GC/electron-capture detector of the pooled plasma (6 h postdosage); see Fig. 4A for the *same* sample on MPD and note the simplified chromatogram using the MPD-specific fluorine detector. (A) Rat plasma, 6 h postdosage; (B) A spiked with the unsaturate ($C_7F_{15}CF{=}CHCOO^-$). (w) 2-Hydroperfluoro-2-decenate ($C_7F_{15}CF{=}CHCOO^-$); (x) perfluorooctanoate ($C_7F_{15}COO^-$); (y) 2*H*,2*H*-perfluorodecanoate ($C_8F_{17}CH_2COO^-$). Note the unidentified fluorine containing metabolite (z) is not apparent with the electron-capture detector. Furthermore, while the MPD responds to the total fluorine content of a compound per the empirical formula, the electron-capture detector has different sensitivities for different compounds. In this series, the sensitivity is $C_7F_{15}CF{=}CHCOOCH_3 > C_7F_{15}CF_2CH_2COOCH_3 > C_7F_{15}CF_2CH_2CH_2OH$.

The fluorine-containing biotransformation product of the alcohol eluting at 10.0 min has not as yet been identified but it is speculated that it contains a carboxyl group since diazomethane renders it volatile for GC. The unsaturate has been identified (by GC retention data) and an additional metabolic derivative is possible in that the smallest molecular metabolite ($C_7F_{15}COO^-$) observed in this work has one less CF_2 group than the starting alcohol.

The metabolism of fluorine-containing and perfluoro long-chain acids has not been studied in great detail. It is known that the perfluoroheptyl chain (specifically perfluorooctanoate) is metabolically stable (14–16) while ω-fluorocarboxylic acids (17) undergo β oxidation. The high inorganic fluoride level in the plasma (Table 1) and formation of perfluorooctanoate suggest the overall re-

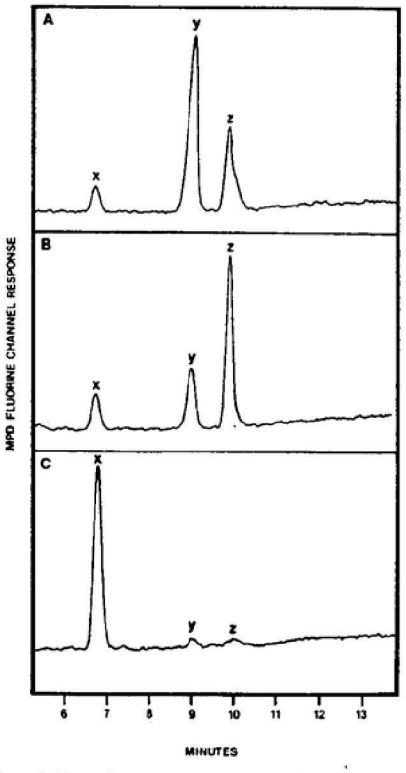

FIG. 6. Rat plasma extracts at various times, postdosage. (A) Rat 10 (2 h postdosage; (B) Rat 9 (6 h postdosage); (C) Rat 20 (48 h postdosage). (x) Perfluorooctanoate ($C_7F_{15}COO^-$); (y) 2*H*,2*H*-perfluorodecanoate ($C_8F_{17}CH_2COO^-$); (z) unidentified metabolite.

action,

$$C_7F_{15}CF_2CH_2CH_2OH \rightarrow C_7F_{15}CO_2^- + 2\,HF.$$

1H,1H,2H,2H-perfluorodecanol — perfluorooctanoate

Figure 5 illustrates the electron capture response obtained for the esterified plasma extract on the capillary column system and shows the complex mixture which is simplified by monitoring only the fluorine content on the GC/MPD. It is obvious that an ideal system would combine the selectivity of the microwave-sustained helium plasma detector with the improved separation capabilities of a capillary system and this is under development at this time.

MPD plots of the fluorine channel response are shown for various rats (Fig. 6). Note that the 2*H*,2*H*-perfluorodecanoic acid ester is the predominant component in the 2-h-postdosage rat. The chromatograms in Fig. 6 illustrate the progressive biotransformation of the alcohol to perfluorooctanoate. Differences in the rate of biotransformation of the alcohol between rats were observed.

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