

Increased Endobiotic Fatty Acid Methyl Esters Following Exposure to Methanol

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Human exposure to methanol is likely to increase in the future due to its proposed use as an alternate automobile fuel. Since alcohols are known to esterify the fatty acids in the body and some of those esterified esters are toxic, we studied the formation of fatty acid esters of methanol in Long-Evans male rats given a single oral dose of 3.5 g/kg body weight of methanol in saline. Animals given an equal volume of saline served as control. Three rats were euthanized at 1, 3, 6, 12, and 24 hr following the treatment. Fatty acid methyl esters, extracted from whole blood, liver, pancreas, and brown fat were separated by thin-layer chromatography and quantitated by gas chromatography (GC). Their identity was then confirmed by GC-mass spectrometry. Average levels as high as 596, 5293, 2239, 1106, 9665, 7728, 562, and 2792 $\mu\text{g/g}$ (wet weight basis) of 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, and 20:4 fatty acid methyl esters, respectively, were found in the pancreas of methanol-treated rats. The average concentration of total fatty acid methyl esters was computed to be 4513, 29594, 22871, 18956, 17014, and 9702 $\mu\text{g/g}$ in the pancreas compared to 1.9, 25.4, 36.8, 18.5, 18.9, and 14.2 $\mu\text{g/g}$ in the liver at 0, 1, 3, 6, 12, and 24 hr, respectively, following methanol exposure. On dry lipid weight basis, the levels were significantly higher again in pancreas followed by brown fat and liver. In whole blood, only low levels of 16:0, 18:0, and 20:4 fatty acid methyl esters could be detected at all time points. The highest concentration of total fatty acid methyl esters in the pancreas, liver, and brown fat was detected at 1, 3, and 24 hr, respectively. Most of the fatty acid methyl esters found in the liver and pancreas decreased after 6 hr of methanol exposure. The fatty acid methyl esters of higher concentrations were 16:0 in the whole blood, 18:0, 18:1, 18:2, and 20:4 in liver, 18:1, and 18:2 in pancreas and 16:0, 18:1, and 18:2 in brown fat. These fatty acid methyl esters were also detected in the tissues of control rats indicating their endogenous formation. Significant increase in methylation of the fatty acids during methanol exposure, as found in this study, may serve as a defense mechanism for preventing available methanol from oxidative metabolism to render toxicity. However, the biological significance of these fatty acid methyl esters is yet to be understood.

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posed as future alternative automobile fuel (Reed and Lerner, 1973; Anderson, 1984). The anticipated use of methanol for meeting the demand of gasoline formulation and the Clean Air Act (EPA, 1992) will increase environmental as well as occupational human exposure to methanol.

Methanol is a natural component of body tissues, yet it is known to cause acute and chronic toxicity in humans and primates (Tephly, 1991; Von Burg, 1994). Methanol abuse as a substitute for ethyl alcohol in alcoholic beverages and susceptibility of humans to methanol poisoning have long been recognized. Severe metabolic acidosis and ocular damage are the major acute toxicities observed in humans and primates as a result of oxidative metabolism of methanol (Tephly, 1991). Gastric irritation, marked hyperesthesia in arms and hands, and mild ptosis of eyelids are the main symptoms of chronic methanol poisoning (NIOSH, 1976). In view of the acute and chronic toxicity, increased exposure of methanol could pose a risk to human health.

A number of aliphatic and halogenated alcohols are known to form esters with fatty acids *in vivo* as well as *in vitro* (Ansari *et al.*, 1995). Various fatty acid ethyl esters found in parenchymal organs had a linear correlation with blood alcohol concentration in persons acutely intoxicated with ethanol at the time of death (Laposata and Lange, 1986). Formation and accumulation of these esters in the body are also suggested to be involved in pathogenesis of myocardial and pancreatic diseases in alcoholics (Laposata and Lange, 1986; Hamamoto *et al.*, 1990) and in fetal alcohol syndrome (Bearer *et al.*, 1992). About 72% of the intracellularly synthesized fatty acid ethyl esters bind to mitochondria isolated from intact tissue incubated with ethanol and reduce the respiratory control index (Lange and Sobel, 1983). Previously, we reported the pancreatic and hepatic toxicity of palmitic acid ester of pentachlorophenol and linoleic acid ester of 2-chloroethanol, respectively, in rats (Ansari *et al.*, 1987; Kaphalia *et al.*, 1992a,b). A number of studies have shown the presence of fatty acid methyl esters in the tissues of humans and laboratory animals with no known exposure to methanol (Dhopeswarkar and Mead, 1962; Leikola *et al.*, 1965; Lough and Garton, 1968; Mueller and Kabara, 1970). However, very little is known about the biological

Methanol is widely used as an organic solvent, in the synthesis of several industrial chemicals, and has been pro-

significance, metabolism, or toxicity of these esters. Some of the fatty acid methyl esters decrease microviscosity of the synaptosomal membrane core of rat and monkey brain cortex (Lazor and Medzihradsky, 1992), activate protein kinase C in intact human platelets (Fan *et al.*, 1990), and can penetrate living cells of the epidermis resulting into significant changes in lipid metabolism (Wertz and Downing, 1990). Because they are lipophilic in nature, fatty acid methyl esters could be retained in the body and cause chronic toxic manifestations.

Whether exogenous exposure to methanol results in elevation of fatty acid methyl esters in the blood and in body tissues and the persistence of those esters in the body have not been studied. Since the concentration of methanol in the blood or formic acid in the serum lacks a dose-dependent response (d'Alessandro *et al.*, 1994), any correlation established between the levels of fatty acid methyl esters in the whole blood and other tissues could be useful in assessing methanol exposure and eventually in risk assessment. This study was undertaken to determine the formation of fatty acid methyl esters and their persistence in various tissues of rats following methanol exposure.

MATERIALS AND METHODS

Chemicals

Mixed standards of methyl esters of myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and arachidonic (20:4) acids and methyl ester of heptadecenoic (17:0) fatty acid (internal standard) were procured from Metreya Inc. (Pleasant Gap, PA). Pesticide and HPLC-grade solvents and high-purity-grade chemicals were purchased from Fisher Scientific (Fair Lawn, NJ).

Animals

Long-Evans (LE) male rats VHS/Plus (~200 g) were obtained from Charles River Laboratories (Wilmington, MA). The animals were maintained in temperature- and humidity-controlled animal room set at a 12-hr light/dark cycle and acclimatized for 7 days. Purina Rat Chow and drinking water were provided *ad libitum*. On the 8th day, 15 rats were given a single dose of 3.5 g/kg methanol in sterilized saline (30% methanol-saline solution) via gavage. This dose was chosen because toxicity similar to that in humans could be mimicked in LE rats (Lee *et al.*, 1994a,b). Three rats given an equal volume of the saline served as control. Three animals were killed under ether anesthesia at 1, 3, 6, 12, and 24 hr following the methanol treatment. Control animals were killed immediately after dosing. Blood withdrawn from inferior vena cava was stored at 4°C in tubes containing heparin as an anticoagulant. Brown fat (found between the scapulae, in the ventral neck and axillary region, in the thoracic inlet, and inguinal region), liver, and pancreas were excised and stored at -20°C until analysis.

Extraction and Analysis of Fatty Acid Methyl Esters

Whole blood. Whole blood (5 ml) from each animal was incubated with a known concentration of internal standard at 37°C. After 15 min, the incubation mixture was extracted thrice with 5 vol of chloroform:methanol (2:1, v/v). The extracts were pooled, filtered through glass fiber filter paper, and evaporated using rotatory flash evaporator. The dry residue was redissolved in a minimum volume of chloroform and subjected to preparative

thin-layer chromatography (TLC) on 500- μ m-thick silica gel-coated glass plates (Analtech, Delaware, PA) using petroleum ether:diethyl ether:acetic acid (75:10:1, v/v) as the solvent. Silica gel from the region corresponding to the relative flow of fatty acid methyl esters was scraped, eluted with chloroform, filtered, and dried under nitrogen. The dried residue was redissolved in 200 μ l pesticide grade hexane and analyzed by gas chromatography (Hewlett Packard 5890 gas chromatograph) using a flame ionization detector (FID) and DB 225 fused silica capillary column (J & W Scientific, Folsom, CA; 20 m long, film thickness 0.2 μ m) under split mode. Ultrahigh purity grade nitrogen, used as a carrier gas (flow rate 0.5 ml/min), was passed through molecular sieve and silica gel to remove the impurities of oxygen and moisture, respectively. The initial column temperature was set at 150°C with an increase of 10°C/min to a final temperature of 225°C. The temperatures of the injector port and detector were set at 200 and 300°C, respectively. The peaks, corresponding to the retention time of standard fatty acid methyl esters, were quantitated using Hewlett Packard 3396A integrator. The data were corrected using the percentage of recovery of the internal standard.

Tissues. A known amount of liver (6.03–6.94 g), pancreas (0.43–0.76 g), or brown fat (0.15–0.54 g) was homogenized in 5 vol of 50 mM sodium phosphate buffer (pH 7.4) and the resulting homogenate was incubated with a known concentration of internal standard at 37°C for 15 min. The incubation mixture was extracted with chloroform:methanol (2:1, v/v) and the fatty acid methyl esters were separated by preparative TLC and analyzed as described above for whole blood. The total dry weight of the extracted lipids was also determined. The lipid extracts of pancreas and brown fat were further diluted for GC analysis.

Gas Chromatography–Mass Spectrometry (GC–MS)

The ester fraction obtained from the lipid extracts of whole blood and tissues by preparative TLC was dissolved in hexane and analyzed by GC–MS using a Varian 3400 gas chromatograph interfaced with a Fennigan Incos-50 at the Analytical Chemistry Center (University of Texas Health Science Center, Houston, TX). The esters were separated on a DB 23 column (30 m long, 0.32 mm i.d.) at a temperature range from 100 to 250°C with an increase of 10°C/min and the peaks corresponding to the retention time of standard fatty acid methyl esters were subjected to electron impact (EI) (70 eV) and chemical ionization (methane as reagent gas) mass spectrometry.

RESULTS

In general, 16:0, 18:0, 18:1, 18:2, and 20:4 fatty acid methyl esters were found in all tissues while in the whole blood only 16:0, 18:0, and 20:4 fatty acid methyl esters could be detected (Tables 1–4). In addition to the above-mentioned esters, 14:0, 16:1, and 18:3 fatty acid methyl esters were also detected in the pancreas and brown fat of methanol-treated rats (Tables 3 and 4). Many of the fatty acid methyl esters detected in the methanol-treated animals were also found in the control animals, but the levels were significantly lower than those in the respective tissues of methanol treated rats. Gas chromatographic separation of various fatty acid methyl esters was based upon the carbon chain length as well as the degree of unsaturation (Fig. 1). Significantly higher levels of the fatty acid methyl esters were found in the pancreas followed by brown fat, liver, and whole blood. Preference of various fatty acids to form methyl esters, in term of their concentration, was found for 16:0 in

TABLE 1
Fatty Acid Methyl Esters ($\mu\text{g/ml}$) in Whole Blood

Time (hr)	Fatty acid methyl esters						Total
	16:0	18:0	18:1	18:2	18:3	20:4	
0 (Control)	0.43 $\pm$ 0.04	0.06 (1) ^a	—	—	—	—	0.49 $\pm$ 0.02
1	1.24 $\pm$ 0.30	0.77 $\pm$ 0.19	—	0.24 (1) ^a	—	0.32 $\pm$ 0.20 (2) ^a	2.57 $\pm$ 0.84
3	0.79 $\pm$ 0.17	0.57 $\pm$ 0.04	—	—	—	0.22 (1) ^a	1.58 $\pm$ 0.30
6	1.12 $\pm$ 0.14	0.62 $\pm$ 0.39	—	—	—	0.25 $\pm$ 0.12 (2) ^a	1.99 $\pm$ 0.21
12	0.87 $\pm$ 0.47	0.42 $\pm$ 0.21	—	—	—	0.15 (1) ^a	1.44 $\pm$ 0.72
24	1.34 $\pm$ 0.05	0.73 $\pm$ 0.01	—	—	—	0.30 $\pm$ 0.13	2.37 $\pm$ 0.08

Note. Values are means $\pm$ SE of three animals.

^a Number of sample(s) of three in which the esters were detected.

whole blood (Table 1), 18:0, 18:1, 18:2, and 20:4 in liver (Table 2), 18:1 and 18:2 in pancreas (Table 3), and 16:0, 18:1, and 18:2 in brown fat (Table 4).

Fatty acid methyl esters (16:0, 18:0, and 20:4) were detected in the whole blood of methanol-treated animals at all time points; 20:4 fatty acid methyl ester was detected only in 9 of 15 animals. Fatty acid methyl ester (18:2) was also detected at 1 hr in whole blood sample of one methanol-treated animal. Only 16:0 and 18:0 fatty acid methyl esters were detected in the whole blood of control rats. Total fatty acid methyl esters were found to be comparable at different time points in methanol-treated group and the mean values were approximately three- to fivefold higher than the control values (Table 1).

Methyl esters of the 16:0, 18:0, 18:1, and 18:2 fatty acids were detected in the liver of all the methanol-treated rats; 20:4 fatty acid methyl ester was detected in 8 of 15 treated animals (Table 2). Fatty acid methyl ester (18:3) could not be detected in the livers of any treated or control animals at all time points examined. The maximum concentration of all

the methyl esters was found at 3 hr following the methanol treatment (except for 18:2 fatty acid methyl ester at 1 hr) which declines slowly from 6 to 24 hr. At 24 hr the average concentration was approximately one-third of the maximum levels detected at 3 hr. However, the background levels of different fatty acid methyl esters were either very low (16:0, 18:1, and 18:2) or undetectable (18:0, 18:3, and 20:4) in the control livers. The mean values of all the fatty acid methyl esters were found to be 7.5- to 19.5-fold higher (on wet weight basis) in methanol-treated groups compared to the controls (Table 2). This trend of fatty acid methyl esters at different time points was comparable with the levels computed on dry lipid weight basis (data is not shown).

The fatty acid methyl esters estimated in the pancreas of both treated and control rats were found to be severalfold higher on wet weight basis when compared to those found in the whole blood and liver thus indicating a higher rate of fatty acid methyl ester biosynthesis and/or accumulation in this tissue. The mean values of 596, 2239, 9665, 7728, 562, and 2792 $\mu\text{g/g}$ for 14:0, 16:1, 18:1, 18:2, 18:3, and 20:4

TABLE 2
Fatty Acid Methyl Esters ($\mu\text{g/g}$ Wet Weight) in the Liver

Time (hr)	Fatty acid methyl esters						Total
	16:0	18:0	18:1	18:2	18:3	20:4	
0 (Control)	0.09 $\pm$ 0.03	—	0.97 $\pm$ 0.49	0.83 (1) ^a	—	—	1.89 $\pm$ 1.13
1	0.58 $\pm$ 0.27	5.53 $\pm$ 3.03	5.92 $\pm$ 3.03	10.18 $\pm$ 4.65	—	3.22 $\pm$ 0.85	25.44 $\pm$ 11.81
3	1.31 $\pm$ 0.44	11.69 $\pm$ 3.39	9.50 $\pm$ 4.78	7.87 $\pm$ 5.73	—	6.47 $\pm$ 3.39 (2) ^a	36.84 $\pm$ 13.93
6	0.42 $\pm$ 0.06	6.30 $\pm$ 0.53	4.07 $\pm$ 0.74	6.07 $\pm$ 1.44	—	1.66 (1) ^a	18.52 $\pm$ 5.48
12	0.55 $\pm$ 0.20	5.43 $\pm$ 1.24	4.31 $\pm$ 1.24	6.99 $\pm$ 4.13	—	1.67 (1) ^a	18.95 $\pm$ 10.69
24	0.34 $\pm$ 0.10	6.93 $\pm$ 1.41	2.62 $\pm$ 0.65	4.01 $\pm$ 0.83	—	0.29 (1) ^a	14.20 $\pm$ 1.64

Note. Values are means $\pm$ SE of three animals.

^a Number of sample(s) of three in which the esters were detected.

TABLE 3
Fatty Acid Methyl Esters ($\mu\text{g/g}$ Wet Weight) in Pancreas

Time (hr)	Fatty acid methyl esters								Total
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	
0 (Control)	131 $\pm$ 115	1563 $\pm$ 1099	639 $\pm$ 320	94 $\pm$ 53	315 $\pm$ 172	1301 $\pm$ 935	86 $\pm$ 72	384 $\pm$ 36	4513 $\pm$ 2460
1	596 $\pm$ 69	4944 $\pm$ 191	2239 $\pm$ 410	1069 $\pm$ 6	9665 $\pm$ 302	7728 $\pm$ 638	562 $\pm$ 46	2792 $\pm$ 258	29594 $\pm$ 1380
3	500 $\pm$ 85	5293 $\pm$ 934	1604 $\pm$ 255	1106 $\pm$ 128	6667 $\pm$ 1072	5325 $\pm$ 79	199 $\pm$ 103	2177 $\pm$ 1027	22871 $\pm$ 3022
6	539 $\pm$ 122	4378 $\pm$ 943	1917 $\pm$ 373	1087 $\pm$ 9	5783 $\pm$ 203	4634 $\pm$ 261	219 $\pm$ 112	399 $\pm$ 123	18956 $\pm$ 810
12	545 $\pm$ 40	4822 $\pm$ 246	1088 $\pm$ 134	1000 $\pm$ 28	5083 $\pm$ 396	3926 $\pm$ 348	281 $\pm$ 47	269 $\pm$ 80	17014 $\pm$ 1290
24	421 $\pm$ 47	2672 $\pm$ 1196	687 $\pm$ 279	627 $\pm$ 313	2638 $\pm$ 1360	2198 $\pm$ 1142	314 $\pm$ 82	146 $\pm$ 82	9702 $\pm$ 3340

Note. Values are means $\pm$ SE of three animals.

fatty acid methyl esters, respectively, at 1 hr and 5293 and 1106 $\mu\text{g/g}$ for 16:0 and 18:0 fatty acid methyl esters, respectively, at 3 hr were maximum in the pancreas of methanol-treated rats (Table 3). The decreasing pattern of different fatty acid methyl esters with increasing exposure time after 6 hr was found to be similar to that of liver except for 20:4 fatty acid methyl ester. The average levels of 20:4 fatty acid methyl esters were found to be 2797 and 2177 $\mu\text{g/g}$ at 1 and 3 hr, respectively, and rapidly decreased to the control levels over the remaining time points examined in the present study. Levels as high as 3760 and 3168 $\mu\text{g/g}$ of 16:0 and 18:2 fatty acid methyl fatty acid esters, respectively, were detected in the pancreas of one control rat. The average of the total fatty acid methyl esters at 1, 3, 6, 12, and 24 hr following treatment was computed to be 29,594, 22,871, 18,956, 17,014, and 9702 $\mu\text{g/g}$ wet weight of pancreas, respectively, compared to 4513 $\mu\text{g/g}$ in control rats (Table 3). This pattern of the total fatty acid methyl esters was found to be similar when expressed on dry lipid weight basis.

The fatty acid methyl esters estimated in brown fat are expressed only on dry lipid weight basis (Table 4). The fatty acid methyl esters detected in the pancreas were also found

in the brown fat of all the methanol-treated rats. However, 14:0, 16:1, 18:3, and 20:4 fatty acid methyl esters could not be detected in the brown fat of control rats. Low levels of other fatty acid methyl esters (16:0, 18:0, 18:1, and 18:2) were detected in brown fat of all the control animals compared to that of methanol-treated rats. Unlike liver and pancreas, the mean values of total fatty acid methyl esters at 1, 3, 6, 12, and 24 hr following methanol treatment was found to be 9.31, 16.46, 23.34, 12.74, and 27.77 $\mu\text{g/mg}$ lipids, respectively, compared to 1.12 $\mu\text{g/mg}$ in control rats (Table 4). On the dry lipid weight basis, these values are 2- to 15-fold higher than those found in the liver and significantly lower than those in the pancreas of methanol-treated rats (data not shown). Although 14:0, 16:0, 16:1, and 18:0 fatty acid methyl esters were found to be highest at 6 hr, the remaining fatty acid methyl esters peaked at 24 hr following the methanol treatment. The total fatty acid methyl esters computed for the brown fat again showed highest concentration at 24 hr following the methanol treatment.

Representative mass spectra of the TLC purified ester fraction obtained from the lipid extracts of whole blood, liver, pancreas, and brown fat of control and methanol-treated rats

TABLE 4
Fatty Acid Methyl Esters ($\mu\text{g/mg}$ Dry Lipid Weight) in Brown Fat

Time (hr)	Fatty acid methyl esters								Total
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	
0 (Control)	—	0.45 $\pm$ 0.08	—	0.09 $\pm$ 0.05	0.31 $\pm$ 0.18	0.26 $\pm$ 0.16	—	—	1.12 $\pm$ 0.43
1	0.19 $\pm$ 0.05	1.44 $\pm$ 0.35	0.63 $\pm$ 0.20	0.35 $\pm$ 0.10	3.15 $\pm$ 1.09	3.01 $\pm$ 1.20	0.19 $\pm$ 0.06	0.34 $\pm$ 0.16	9.31 $\pm$ 3.16
3	0.31 $\pm$ 0.13	2.35 $\pm$ 1.01	0.85 $\pm$ 0.45	0.60 $\pm$ 0.25	5.69 $\pm$ 2.55	5.29 $\pm$ 2.36	0.33 $\pm$ 0.19	1.03 $\pm$ 0.63	16.46 $\pm$ 1.41
6	0.57 $\pm$ 0.66	6.24 $\pm$ 5.54	2.18 $\pm$ 1.69	1.12 $\pm$ 0.92	7.98 $\pm$ 5.63	4.87 $\pm$ 2.86	0.09 $\pm$ 0.05	0.28 $\pm$ 0.09	23.34 $\pm$ 15.08
12	0.31 $\pm$ 0.02	2.06 $\pm$ 0.42	0.92 $\pm$ 0.22	0.48 $\pm$ 0.13	4.69 $\pm$ 0.81	3.64 $\pm$ 0.96	0.23 $\pm$ 0.04	0.40 $\pm$ 0.15	12.74 $\pm$ 3.01
24	0.53 $\pm$ 0.07	3.42 $\pm$ 0.51	1.62 $\pm$ 0.13	0.94 $\pm$ 0.18	9.20 $\pm$ 0.40	10.17 $\pm$ 0.88	0.62 $\pm$ 0.12	1.27 $\pm$ 0.05	27.77 $\pm$ 3.82

Note. Values are means $\pm$ SE of three animals.

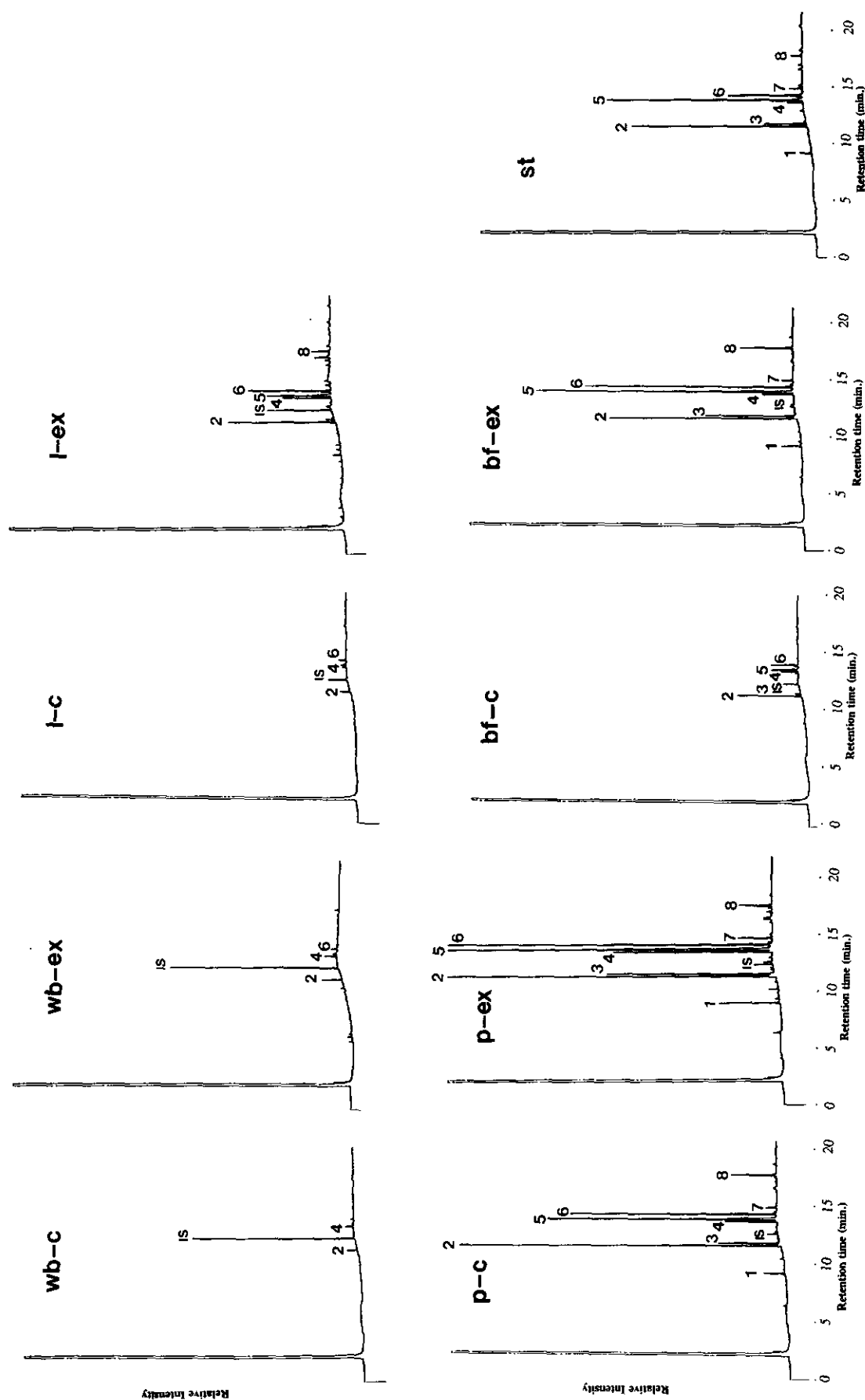


FIG. 1. Gas chromatographic analysis of the ester fraction of lipids extracted from whole blood (wb), liver (l), pancreas (p), and brown fat (bf). Peaks 1, 2, 3, 4, 5, 6, 7, and 8 represent 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, and 20:4 fatty acid methyl esters, respectively, for standards (st) and those detected in the tissues of methanol-treated (ex; at 1 hr) and control (c) rats. The peak of the internal standard, shown by IS, was eluted at 12.7 min. Detailed operating conditions for gas chromatographic analysis are described under Materials and Methods.

by GC-MS analysis are shown in Fig. 2. The mass spectra of different peaks eluted at the retention time corresponding to the standard fatty acid methyl esters showed respective molecular ion (M^+) with characteristic fragmentation pattern (M-31 and McLafferty ion, m/z 74) (Murphy, 1993) of the fatty acid methyl esters detected in different tissues (Fig. 2). The presence of these esters in the extracts of tissues of control and methanol-treated rats was also confirmed by the chemical ionization mass spectrometric analysis using methane as a reagent gas. $M + 1$, $M + 16$, $M + 29$ ions and the fragmentation pattern (data not shown) (Murphy, 1993) confirm the formation and presence of the fatty acid methyl esters in methanol-treated and control rats, respectively.

DISCUSSION

Formation of xenobiotic esters of lipids, particularly of fatty acids, and characterization of the enzymes catalyzing such reactions have been of much interest in view of their lipophilic nature and toxicity (Ansari *et al.*, 1995). Oxidative metabolism of methanol is known to be associated with ocular toxicity and metabolic acidosis (Tephly, 1991; Von Burg, 1994). On the other hand, formation of fatty acid esters of several other aliphatic and halogenated alcohols via nonoxidative metabolism and their toxicity are well documented (Kaphalia and Ansari, 1987, 1989; Hungund *et al.*, 1988; Bhat and Ansari, 1990; Hamamoto *et al.*, 1990; Manautou and Carlson, 1991; Manautou *et al.*, 1992; Carlson, 1993, 1994; Ansari *et al.*, 1995). Based on the concentration of the fatty acid methyl esters found in human pancreas (Leikola *et al.*, 1965; Lough and Garton, 1968) in the tissues of guinea pigs (Dhopeswarkar and Mead, 1962), mouse liver (Mueller and Kabara, 1970), and rat gastric mucosa (Yurkowski and Walker, 1969), nonoxidative metabolism appears to be a major pathway for endogenous methanol disposition. The pattern of fatty acid methyl esters formation in various tissues, observed in the present study, was found similar to that reported *in vivo* and/or *in vitro* for fatty acid ethyl esters by us and others (Laposata and Lange, 1986; Hungund *et al.*, 1988; Kaphalia *et al.*, 1994). Our results in this study also indicate that pancreas is the major organ responsible for the biosynthesis of fatty acid methyl esters via nonoxidative metabolism. As mentioned earlier, the occurrence of fatty acid methyl esters in human pancreas and tissues of laboratory animals with no known exposure to methanol supports our findings on the presence of fatty acid methyl esters in the tissues of control rats. Tissues of human subjects used as control with alcoholic subjects also contained fatty acid ethyl esters (Laposata and Lange, 1986). Therefore occurrence of fatty acid methyl esters in the control animals appears to be a natural phenomenon.

Methanol is generated metabolically from demethylation reaction involving chemotaxis receptor protein by methyl

esterase (Alberts *et al.*, 1989). Vegetables, fresh fruits, juices, and aspartame (artificial sweetener for carbonated beverages) could be the major source of methanol in human body (Kavet and Nauss, 1990; HEI, 1993). Another potential source of methanol could be *S*-adenosylmethionine (Ado-Met), an endogenous substrate and methyl group donor (Usdin *et al.*, 1979), also known to be present in the mammalian cells (Stramentinoli and Pezzoli, 1979). The artifactual formation of fatty acid methyl esters due to methanol in extracting solvent has been ruled out by using propanol instead of methanol and also by extracting the tissues with ethyl ether (Dhopeswarkar and Mead, 1962). Although the possibility of ingestion of such fatty acid methyl esters via feed in control animals cannot be ruled out, the enzyme-catalyzed methylation of free fatty acids by methanol available from the diet and/or endogenous sources could contribute to the background levels of fatty acid methyl esters found in the tissues of control rats. We propose the fatty acid methyl ester formation in control rats by two mechanisms as shown in Fig. 3. One mechanism is related to methylation and demethylation of macromolecules catalyzed by methyl transferases and methyl esterase, respectively. The former reaction uses AdoMet as a methyl group donor and the methanol generated in the later reaction from methylated macromolecules may esterify free fatty acids via an enzyme (fatty acid methyl ester synthase)-catalyzed reaction (unpublished results). On the other hand, the methyl group donor AdoMet may directly esterify the free fatty acid moieties (Zatz *et al.*, 1981).

Methylation of phospholipids and several proteins, nucleic acids, peptides, hormones, and carbohydrates (Akamatsu and Law, 1970; Usdin *et al.*, 1979; Hirata and Axelrod, 1980) with AdoMet is well documented. Fatty acid methyl esters have been found to be the major lipid products following incubation of rat lung membranes (Zatz *et al.*, 1981) or soluble enzyme fraction from *Mycobacterium phlei* (Akamatsu and Law, 1970) with AdoMet. Many cell types methylate phospholipids by two types of methyltransferases that are asymmetrically distributed in the cell membranes. Methylation of phospholipid is coupled with Ca^{2+} influx and release of arachidonic acid, lysophosphatidylcholine, and prostaglandins. This process is closely associated with transduction of cell signaling, regulation of β -adrenergic receptors, release of histamine in mast cells, and chemotaxis in microphages (Hirata and Axelrod, 1980). In contrast to the biological functions associated with methylation of phospholipids mentioned above, some fatty acid methyl esters decrease the microviscosity of the synaptosomal membrane core in rat and monkey brain cortex (Lazor and Medzihradsky, 1992) and cause changes in lipid metabolism in epidermal cells (Wertz and Downing, 1990). In the case of ethanol, organs other than liver which lack or possess minimal oxidative metabolic capacity for ethanol (leading to formation of acet-

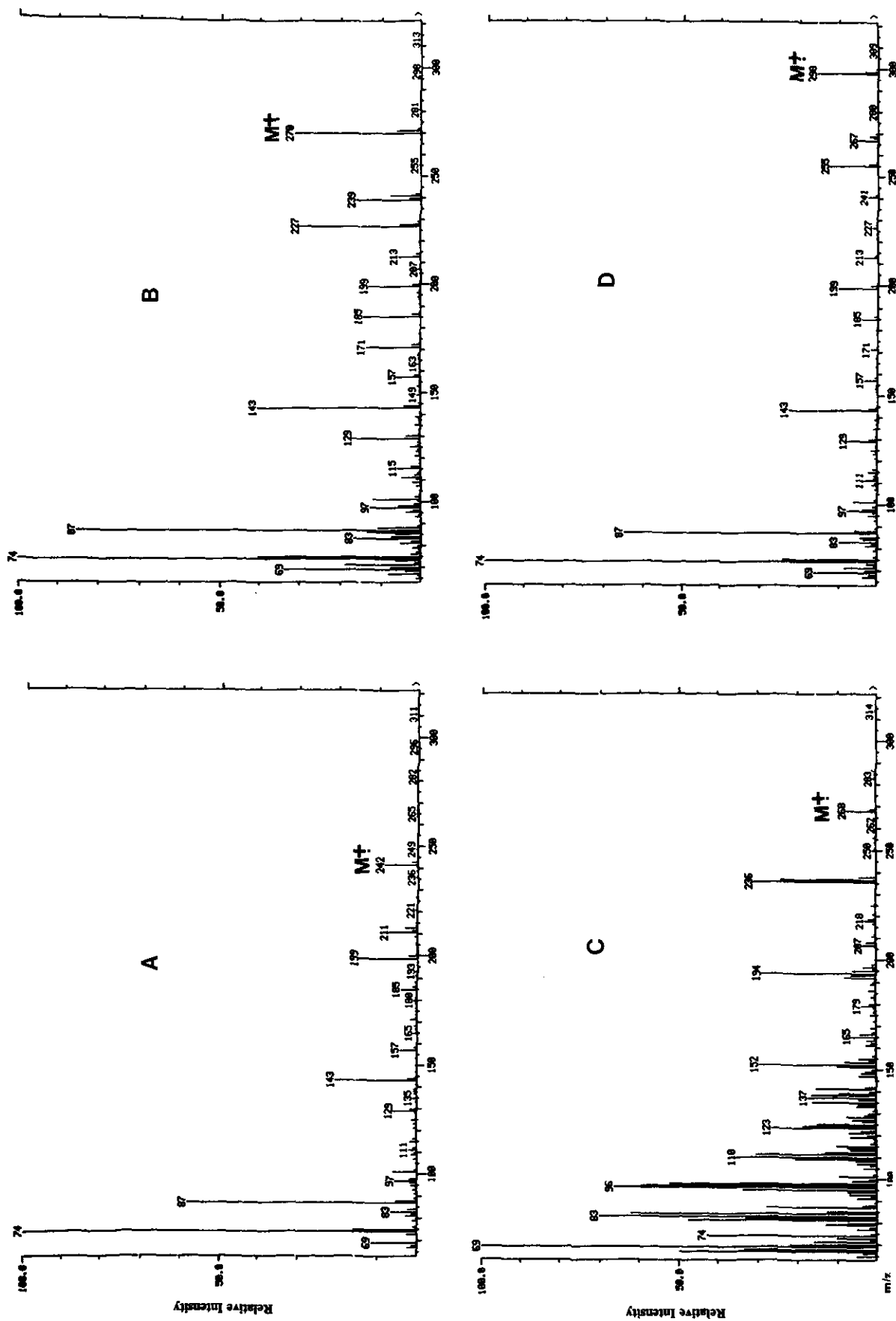


FIG. 2. Representative EI mass spectra of 14:0 (A), 16:0 (B), 16:1 (C), 18:0 (D), 18:1 (E), 18:2 (F), 18:3 (G), and 20:4 (H) fatty acid methyl esters isolated from whole blood, liver, pancreas, and brown fat obtained by GC-MS analysis.

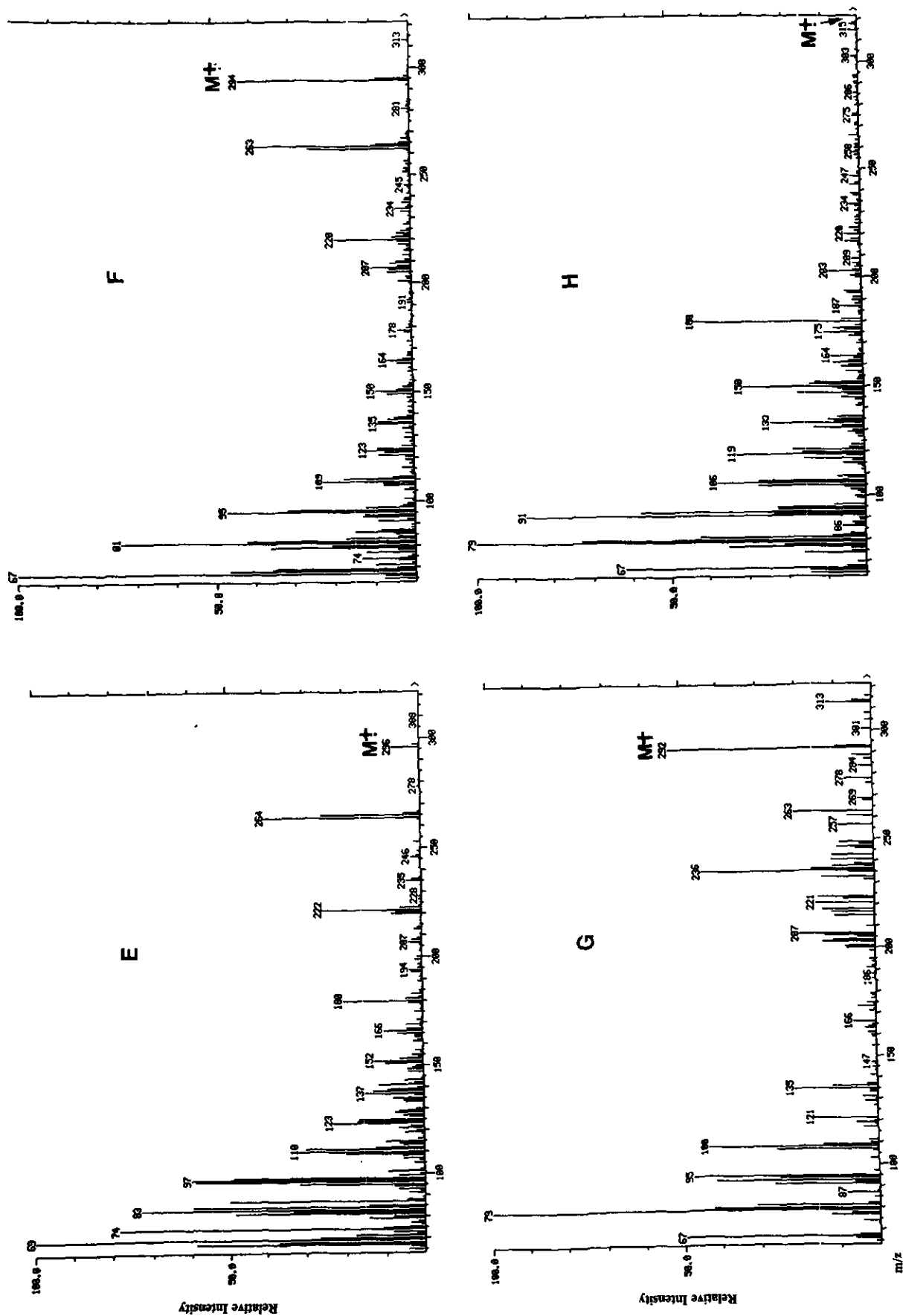


FIG. 2-Continued

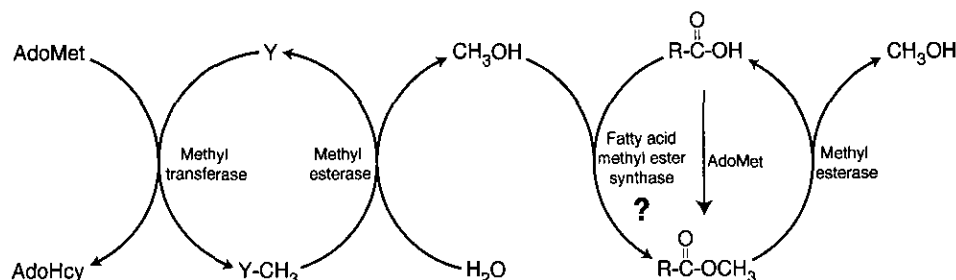


FIG. 3. Schematic presentation of fatty acid methyl ester formation. AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; Y, macromolecule, and R, alkyl fatty acid chain.

aldehyde), especially pancreas, heart, and brain, develop alcohol-induced damage (Lieber and DeCarli, 1968; Lochner *et al.*, 1969; Raskin and Sokoloff, 1972; Laposata and Lange, 1986). Alcohol-induced toxicity to the pancreas could be correlated with its fatty acid ester-synthesizing activity. Therefore, determining the biological functions associated with methylation of biomolecules including fatty acids and toxicity of corresponding esters is of great importance in understanding the mechanism(s) of endobiotic methylation and demethylation reactions.

The absorption of methanol and the formation of fatty acid methyl esters appears to be rapid in the pancreas and liver because a maximum concentration of different esters was detected at 1 and 3 hr, respectively, following the methanol exposure. On the other hand, the highest concentration of the methyl esters in brown fat found at 24 hr suggests their accumulation in tissues rich in lipid, indicating that brown fat is a potential candidate for determining the methanol exposure. However, comparable levels of different fatty acid methyl esters detected at various time points in the whole blood need further studies exploring their persistence and the relationship with those present in other tissues for a better evaluation of methanol exposure. A significant depletion of these esters at 12 and 24 hr in liver and pancreas could be related to their distribution from the site of synthesis to other organs or their elimination via enzyme-catalyzed hydrolysis. In fact, presence of a long chain fatty acid methyl ester hydrolase which hydrolyzes these esters has been demonstrated previously in rat liver and heart, with the maximum activity being in the microsomal fraction (Spector and Soboroff, 1972). The role of fatty acid methyl ester hydrolase, in preserving the homeostasis of long chain fatty acid methyl esters, as well as other esters in the body, is of further research interest. The greater preference of unsaturated fatty acids for methylation over saturated fatty acids observed in this study and *in vitro* (unpublished data) is comparable to our previous results on ethanol and haloethanols (Bhat and Ansari, 1990). Once formed, these fatty acid methyl esters can be incorporated into cell membranes and/or affect their fluidity and structural and functional integrity and alter the

lipid metabolism. The fatty acid methyl esters, particularly arachidonic acid methyl ester, can also serve as an alternative source for the eicosanoids, once hydrolyzed by methyl esterase (Fan *et al.*, 1990). It is possible that methyl ester formation may represent a defense mechanism and methanol thus trapped could not be oxidatively metabolized to render toxicity. The biological role of fatty acid methyl esters is purely speculative at this point. Further studies are needed to establish the significance of the endobiotic synthesis of the fatty acid methyl esters.

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