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NONOXIDATIVE METABOLISM OF 2-BUTOXYETHANOL VIA FATTY ACID CONJUGATION IN FISCHER 344 RATS

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Nonoxidative metabolism of ethylene glycol monobutyl ether (2-butoxyethanol or BE) via fatty acid conjugation was studied in the liver of Fischer 344 male rats following a single oral administration of 500 mg/kg body weight [ethyl-1,2-^{14}C]BE (70 μCi/kg). Animals were killed 2 h after the treatment, hepatic lipids extracted, and the neutral lipids were separated using solid-phase extraction. The neutral lipid fraction was subjected to preparative thin-layer chromatography, and the esters corresponding to the relative flow of authentic fatty acid 2-butoxyethyl esters were recovered and analyzed by reversed-phase high-performance liquid chromatography (HPLC) using methanol–water (37 : 3, v/v) as solvent. Approximately 85% of the ^{14}C label present in the ester fraction was coeluted at retention times corresponding to the different fatty acid 2-butoxyethyl ester standards. The radioactive fractions were analyzed by electron impact mass spectrometry. Molecular ion peaks and fragmentation patterns similar to that of 16 : 0, 18 : 0, 18 : 1, 18 : 2, and 20 : 4 fatty acid 2-butoxyethyl ester standards were detected in the corresponding radioactive HPLC fractions. Fatty acid ethyl ester synthase (FAEES), purified from the rat liver microsomal fraction, was also found to catalyze the formation of 18 : 1 fatty acid 2-butoxyethyl ester. These studies demonstrate that BE is metabolized nonoxidatively via conjugation with long-chain fatty acids, and the formation of these esters appears to be catalyzed by the enzyme(s) involved in fatty acid conjugation of xenobiotic alcohols. However, the biological significance of BE conjugation with fatty acids remains to be investigated.

Ethylene glycol monobutyl ether (2-butoxyethanol or BE) has been extensively used in hard-surface cleaners and paints for the last 60 yr (Smith, 1984). Unlike the short chain ethylene glycol ethers (2-methoxyethanol and 2-ethoxyethanol), BE is devoid of teratogenic and testicular toxicity (Hardin et al., 1984; Nelson et al., 1984; Tyl et al.,

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1984). Currently, BE is the major ethylene glycol ether of commerce and widely used in household cleaning products (Chemical Manufacturers Association, 1993).

As for other xenobiotic alcohols, the oxidative metabolism of ethylene glycol monoalkyl ethers to corresponding acids is well known (Ghanayem, 1995). Butoxyacetic acid (BAA) is the major metabolite of BE in animals and humans (Carpenter et al., 1956; Ghanayem et al., 1987a; Ghanayem, 1995) and has been detected in the urine of workers (Sakai et al., 1994) and rats (Johanson, 1994) exposed to BE. Urinary excretion of BE as the glucuronic acid conjugate and as BAA has been reported in rats (Ghanayem et al., 1987b; Corley et al., 1994; Ghanayem, 1995); BAA is excreted both free and as a glutamine conjugate in humans (Rettenmeier et al., 1993). A case of severe poisoning with BE after massive ingestion of window cleaner containing 12% BE caused moderate hemoglobinuria, including a progressive erythropenia (Rambourg-Schepens et al., 1988). BAA has been shown to cause intravascular hemolysis and subsequent anemia in several laboratory animals (Carpenter et al., 1956; Ghanayem et al., 1987a; Ghanayem, 1995). When BAA was incubated with red blood cells in vitro, human were found to be less sensitive than those of rats and mice (Ghanayem et al., 1987a; Ghanayem & Sullivan, 1993; Udden & Patton, 1994; Ghanayem, 1995). Increased liver and kidney weights without any histopathological changes, and extensive hemolysis were the major effects of BE in rats (Tyler, 1984). Similar observations were also reported in the rats and mice following short- and long-term exposure to BE (Carpenter et al., 1956; Dodd et al., 1983; Krasavage, 1986). In a recent study, rats administered BE for 13 wk in drinking water at concentrations up to 600 ppm exhibited only minimal hepatocellular degeneration. However, no chemical-related lesions were reported in the mice similarly exposed (NTP, 1993). In rats, the liver was found to contain maximum radioactivity following the administration of [ethyl-1,2-^{14}C]BE (Ghanayem et al., 1987b). The nature of this radioactivity and the possible presence of BE and BAA as conjugates with lipids have not previously been investigated.

Fatty acid conjugation is a rapid pathway for disposition of those xenobiotics and/or their metabolites having hydroxyl or amino groups (Ansari et al., 1995; Kaphalia et al., 1995). Several such conjugates were found to be toxic in laboratory animals and cell lines (Ansari et al., 1995). We have previously isolated and characterized several fatty acid esters of halogenated ethanols and methanol (Kaphalia & Ansari, 1987, 1989; Bhat & Ansari, 1990; Ansari et al., 1995; Kaphalia et al., 1995) and studied the hepatic toxicity of 2-chloroethyl linoleate in rats (Kaphalia et al., 1992). In this report we have examined the potential for BE to form lipid conjugates with fatty acids in the liver of Fischer 344 male rats treated with [ethyl-1,2-^{14}C]BE and in vitro

using fatty acid ethyl ester synthase (FAEES), purified from rat liver microsomal fraction. However, not investigated at this time is the ability of BAA to form lipid conjugates in red blood cell membranes in order to understand the mechanism(s) of hemolytic effect of BE in rats.

MATERIALS AND METHODS

Chemicals

2-Butoxyethanol (BE, purity 99+%) from Aldrich Chemical Co., Milwaukee, WI, [ethyl-1,2-^{14}C]BE (50 mCi/mmol) from Amersham (Buckinghamshire, England), and [1-^{14}C]oleic acid (53 mCi/mmol) from Dupont NEN, Wilmington, DE, were used. High-purity grade solvents for extraction and high-performance liquid chromatography (HPLC) and other chemicals were obtained from Fisher Scientific, Fairlawn, NJ. Palmitic (16 : 0), stearic (18 : 0), oleic (18 : 1), linoleic (18 : 2), linolenic (18 : 3), and arachidonic (20 : 4) acids were procured from Sigma Chemical Co., St. Louis, MO.

Synthesis, Purification, and Characterization of Fatty Acid 2-Butoxyethyl Esters

Fatty acid (16 : 0, 18 : 0, 18 : 1, 18 : 2, 18 : 3, and 20 : 4) esters of BE were synthesized by an acid-catalyzed reaction. Equimolar concentrations of BE and fatty acid containing a trace amount of hydrochloric acid (HCl) were heated to boil for 6 h in Teflon-lined screw-capped vials as described previously (Kaphalia & Ansari, 1987, 1989; Bhat & Ansari, 1990). The reaction mixture was extracted with diethyl ether and washed with 10% sodium carbonate followed by 0.1 *N* HCl and water. The solvent layer was passed through an anhydrous sodium sulfate column and dried under a stream of nitrogen. The esters of 16 : 0 and 18 : 0 fatty acids were crystallized using hexane–methanol. Esters of other fatty acids were purified over a silica-gel column (25 × 1.25 cm ID) using chloroform–hexane (1 : 9, v/v) at a flow rate of 2–3 ml/min. Each fraction was collected and analyzed by thin-layer chromatography (TLC) using hexane–diethyl ether–methanol–acetic acid (70 : 20 : 5 : 2, v/v) as mobile phase, and the fractions containing ester were pooled and dried under nitrogen. The purity of each ester was evaluated by TLC, reversed-phase HPLC, and gas chromatography (GC).

Reversed-Phase High-Performance Liquid Chromatography

Separation of all the 6 fatty acid esters of BE was achieved on a reversed-phase column (C18, 25 × 0.46 cm ID, 5 μm pore size) using a 334 Beckman liquid chromatograph connected with 165 variable wavelength detector set at 215 nm and methanol–water (37 : 3, v/v) as solvent (flow rate 1 ml/min).

Gas Chromatography

A known concentration of each synthesized fatty acid ester of BE and their mixture was dissolved in hexane and subjected to gas chromatographic analysis using a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector. The esters were separated on a DB225 fused silica capillary column (J & W Scientific, Folsom, CA; 20 m long, film thickness 0.2 μm) under split mode (split ratio 1 : 33) using ultra-high-purity grade nitrogen as carrier gas (flow rate 0.5 ml/min). The initial column temperature was set at 180°C with an increase of 10°C/min to a final temperature of 225°C. The temperatures for detector and injector port were set at 300 and 200°C, respectively.

The conditions used for the reversed-phase HPLC and GC provided a baseline separation of all the six fatty acid 2-butoxyethyl esters.

Nuclear Magnetic Resonance Spectroscopy

High-resolution proton nuclear magnetic resonance (^{1}H-NMR) spectrum of each purified ester was acquired by using JEOL GX 270 WB Fourier-transform NMR spectrometer (6.3 T) (Kaphalia & Ansari, 1987, 1989; Bhat & Ansari, 1990). The purified authentic standards were dissolved in anhydrous deuteriochloroform, and tetramethylsilane was used as an internal reference. The ^{1}H-NMR spectral data of all the synthesized fatty acid esters of BE were found to be consistent with the assigned structures. All the ^{1}H-NMR spectra showed triplets (integrated for two protons) at δ 4.22, 3.62, and 3.47 corresponding to -$\underset{\underset{O}{\|}}{C}$-O-$CH_2$-$C\underline{H}_2$-O-$C\underline{H}_2$-$C\underline{H}_2$-, which are present in all the fatty acid esters of BE and conform with the structure (Figure 1).

Mass Spectrometry

Electron impact (70 eV) mass spectrometric analysis using a Fennigan Incos-50 mass spectrometer was performed at the Analytical Chemistry Center, University of Texas Health Science Center, Houston, TX. Electron impact mass spectra of all the fatty acid 2-butoxyethyl esters are shown in Figure 2. The $M^{\ddagger}$ ions for all the esters except

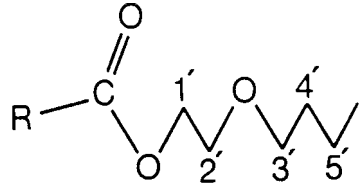

FIGURE 1. Assigned structure of the fatty acid 2-butoxyethyl esters. R = 16 : 0 (palmitic), 18 : 0 (stearic), 18 : 1 (oleic), 18 : 2 (linoleic), 18 : 3 (linolenic), or 20 : 4 (arachidonic) fatty acid carbon chain, 1′ and 2′, and 3′, 4′, and 5′ are ethyl and butyl carbons of BE, respectively.

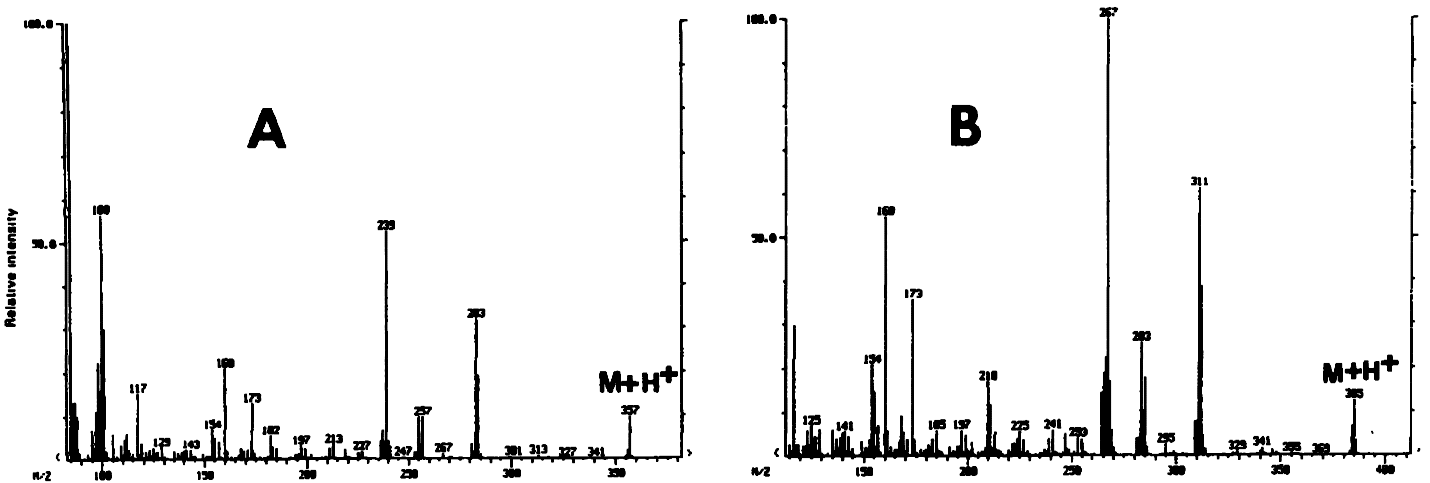

FIGURE 2. Electron impact mass spectra of authentic 16 : 0 (A) and 18 : 0 (B) fatty acid 2-butoxyethyl esters, respectively.

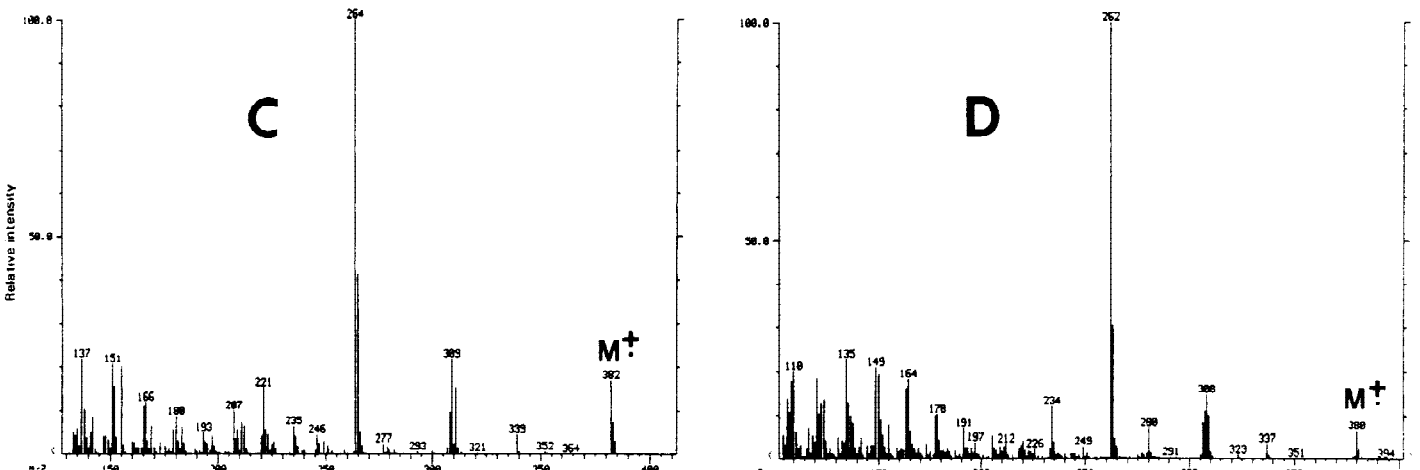

FIGURE 2. (*Continued*) Electron impact mass spectra of authentic 18 : 1 (C) and 18 : 2 (D) fatty acid 2-butoxyethyl esters, respectively.

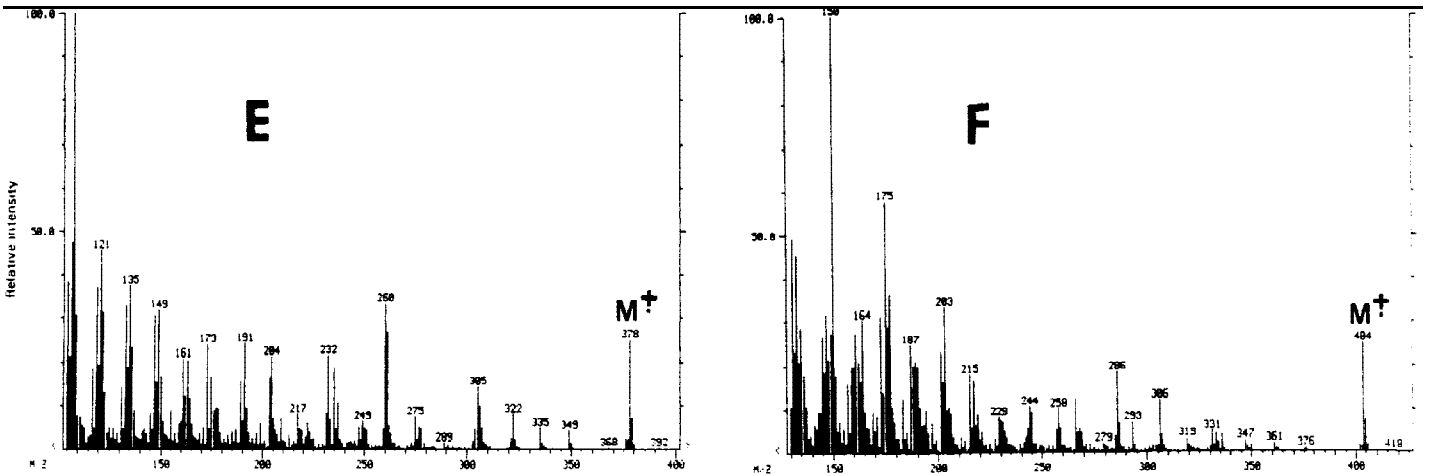

FIGURE 2. (*Continued*) Electron impact mass spectra of authentic 18 : 3 (E) and 20 : 4 (F) fatty acid 2-butoxyethyl esters, respectively.

$M + H^+$ (*m/z* 357 and 385) and $M^{\ddagger}$ (*m/z* 356 and 384) ion peaks for 16 : 0 and 18 : 0 fatty acid esters, respectively, were detected. Spectra of both esters (16 : 0 and 18 : 0) showed loss of $^{\bullet}CH_3$ (M-15), $^{\bullet}CH_2CH_3$ (M-29), $^{\bullet}CH_2CH_2CH_3$ (M-43), $^{\bullet}OCH_2CH_2CH_2CH_3$ (M-73), and $^{\bullet}OCH_2CH_2OCH_2CH_2CH_2CH_3$ (M-117).

Other major ions were $^{+}CH_2CH_2\text{-}\overset{\overset{O}{\|}}{C}\text{-}OCH_2CH_2OCH_2CH_2CH_2CH_3$ (*m/z* 173), the McLafferty ion (*m/z* 160), and *m/z* 117 (160 – $^{\bullet}CH_2CH_2CH_3$). Mass spectra of 2-butoxyethyl esters of 18 : 1, 18 : 2, and 18 : 3 fatty acids showed molecular ion peaks at *m/z* 382, 380, and 378, respectively, and major ions corresponding to the loss of $^{\bullet}CH_2CH_3$, $^{\bullet}CH_2CH_2CH_3$, $^{\bullet}OCH_2CH_2CH_2CH_3$, and $HOCH_2CH_2OCH_2CH_2CH_2CH_3$ fragments. Similarly, the mass spectrum of the 20 : 4 fatty acid ester of BE showed a molecular ion (*m/z* 404) and a fragmentation pattern similar to that of other fatty acid 2-butoxyethyl esters (Figure 2).

Animal Treatment

Six Fischer 344 male rats (~200 g) from Charles River Laboratories (Raleigh, NC) were maintained on NIH 31 diet and water ad libitum. The animals were allowed to acclimatize for 1 wk in the animal quarters (12-h light/dark cycle, 21–23°C ambient temperature, and 40–60% relative humidity). The Fischer 344 rat strain is sensitive and has been extensively used for studying BE-induced hemolytic toxicity (Ghanayem, 1995). Three animals received 500 mg/kg body weight [ethyl-1,2-^{14}C]BE (70 µCi/kg) in water via gavage. Control animals were given an equivalent amount of water only. Since the nonoxidative metabolism of alcohols is known to be a rapid process (Deykin & Goodman, 1962; Kaphalia et al., 1995), the animals were killed 2 h after the exposure. The livers were excised and stored at –80°C.

Extraction and Analysis of Liver Lipids

After thawing, livers of control and [ethyl-1,2-^{14}C]BE-treated rats were homogenized in 0.1 *M* phosphate buffer (pH 7.4) and the lipids were extracted with chloroform–methanol (2 : 1, v/v; 20 ml/g tissue). The solvent layer was separated and dried using a rotatory flash evaporator. The dried residue was redissolved in 200 µl chloroform and subjected to a solid-phase extraction using a Sep-Pak silica cartridge (Juaneda & Rocquelin, 1985). The neutral lipid fraction was dried again as mentioned earlier and subjected to preparative TLC on 500-µm-thick silicagel coated glass plates using the solvent described earlier. Silica gel corresponding to the relative flow of authentic fatty acid 2-butoxyethyl ester standards was scraped and eluted with chloroform. The chloroform layer was filtered through a glass-fiber filter and dried under nitrogen. The dried residue was redissolved in 100 µl methanol and analyzed by reversed-phase HPLC as described earlier. Fractions were collected at 1-min intervals and the radioactivity was measured

in each fraction using a Packard 1900 CA liquid scintillation analyzer. The radioactive fractions coeluted corresponding to the retention times of various authentic standards of fatty acid 2-butoxyethyl esters were analyzed by electron impact mass spectrometry as described earlier. The ester fractions, obtained from control rat livers, corresponding to different fatty acid 2-butoxyethyl esters were also analyzed by electron impact mass spectrometry.

In Vitro Formation of 18 : 1 Fatty Acid Ester of BE

We have purified and characterized fatty acid ethyl synthase (FAEES) or haloethyl ester synthase from rat liver microsomes to homogeneity and found that this enzyme is most probably a high-molecular-weight carboxylesterase (p*I* 6.1) (Kaphalia et al., 1994, unpublished). In order to examine the role of this enzyme in catalyzing the formation of fatty acid 2-butoxyethyl esters, 2 μmol of [1-^{14}C]oleic acid was incubated with 750 μmol BE and different quantities of the enzyme purified from rat liver microsomal fraction in 0.1 *M* phosphate buffer (pH 7.4) at 37°C for 2 h (Bhat & Ansari, 1990; Kaphalia et al., 1994). A control reaction mixture was also incubated without enzyme. The incubation mixture was extracted with 2 ml chloroform three times, and the extracts were pooled and dried under the stream of nitrogen. The residue was dissolved in a minimum quantity of chloroform and separated by TLC using the solvent system mentioned earlier. The silica gel corresponding to the relative flow of 18 : 1 fatty acid 2-butoxyethyl ester was scraped and eluted in chloroform. Radioactivity was measured in a small known amount of the ester fraction as described earlier. The remaining ester fraction was analyzed by reversed-phase HPLC and GC as described earlier.

RESULTS

The distribution of radioactivity in total lipids and in the phospholipid and neutral lipid fractions of liver of Fischer 344 rats treated with [ethyl-1,2-^{14}C]BE is summarized in Table 1. Approximately 16%

TABLE 1. Distribution of ^{14}C label in the lipid fractions of the liver homogenate of Fischer 344 male rats given [ethyl-1,2-^{14}C]BE

Fraction	Total dpm/g tissue
Homogenate	122,167 ± 8883
Total lipids	19,429 ± 8778
Phospholipids	16,465 ± 7784
Neutral lipids	1994 ± 648
Esters	598 (pooled)

Note. Values are mean ± SD of three animals.

of the total radioactivity detected in the liver was associated with lipids, mostly in the phospholipid fraction (~85% of the total lipids). Only about 3% radioactivity of the total lipids was detected in the ester fraction. Approximately 85% of the radioactivity of the ester fraction was recovered as fatty acid 2-butoxyethyl esters by reversed-phase HPLC analysis (Figure 3). The reversed-phase HPLC method developed in this study for the analysis of different fatty acid esters of BE provides a minimum detectable concentration of 0.8, 0.5, and 0.3 μg for 18 : 2, 18 : 3, and 20 : 4 fatty acid 2-butoxyethyl esters, respectively, and in the range of 10–12 μg for 16 : 0, 18 : 0, and 18 : 1 at 0.1 absorbance unit full scale. The gas chromatographic (GC) separation of these fatty acid 2-butoxyethyl esters was found to be based upon carbon chain length and degree of unsaturation with a detection range of 6–10 ng. The retention times were found to be 14, 16.6, 16.9, 17.5, 18.5, and 22 min for 16 : 0, 18 : 0, 18 : 1, 18 : 2, 18 : 3, and 20 : 4 fatty acid esters of BE, respectively (data not shown). Electron impact mass spectrometric analysis of the HPLC fractions of the ester fraction obtained from the liver of rats administered [1,2-^{14}C]BE corresponding to the retention time of 18 : 1, 18 : 2, and 20 : 4 fatty acid 2-butoxyethyl esters showed $M^{\ddagger}$ ions at *m/z* 382, *m/z* 380, and *m/z* 404, respectively. The fragmentation pattern of these ester ions was found similar to that of authentic standards of 18 : 1, 18 : 2, and 20 : 4 fatty acid 2-butoxyethyl esters, respectively. Molecular ions for 16 : 0 (*m/z* 356) and 18 : 0 (*m/z* 384) fatty acid 2-butoxyethyl esters were also detected; the molecular ion (*m/z* 356) detected in the spectrum of oleic acid 2-butoxyethyl ester and also evidenced by the loss of $^{\bullet}CH_2CH_3$ (M-29) and $^{\bullet}OCH_2CH_2OCH_2CH_2CH_2CH_3$ (M-117). Mass spectra of the fraction corresponding to the retention time of 18 : 0 fatty acid 2-butoxyethyl ester showed the presence of the molecular ion (*m/z* 384) followed by a fragmentation pattern corresponding to the structure of 18 : 0 fatty acid 2-butoxyethyl ester (Figure 4). However, similar fatty acid esters of BE were not detected in the ester fraction of neutral lipids of the control Fischer 344 rat liver.

The present study also demonstrates that the formation of fatty acid 2-butoxyethyl ester is catalyzed by the rat liver microsomal FAEES responsible for esterification of xenobiotic alcohols with long-chain fatty acids. Incubation of [1-^{14}C]oleic acid and BE in the presence of the FAEES results in the formation of 18 : 1 fatty acid 2-butoxyethyl ester. This was shown by the coelution of radioactivity corresponding to the retention time of 18 : 1 fatty acid ester of BE by reversed-phase HPLC of the extracted material from the incubation mixture (Figure 5). This observation is also supported by the results of GC analysis. However, the nonenzymatic formation of 18 : 1 fatty acid ester of BE was not detected in the mixture incubated without enzyme.

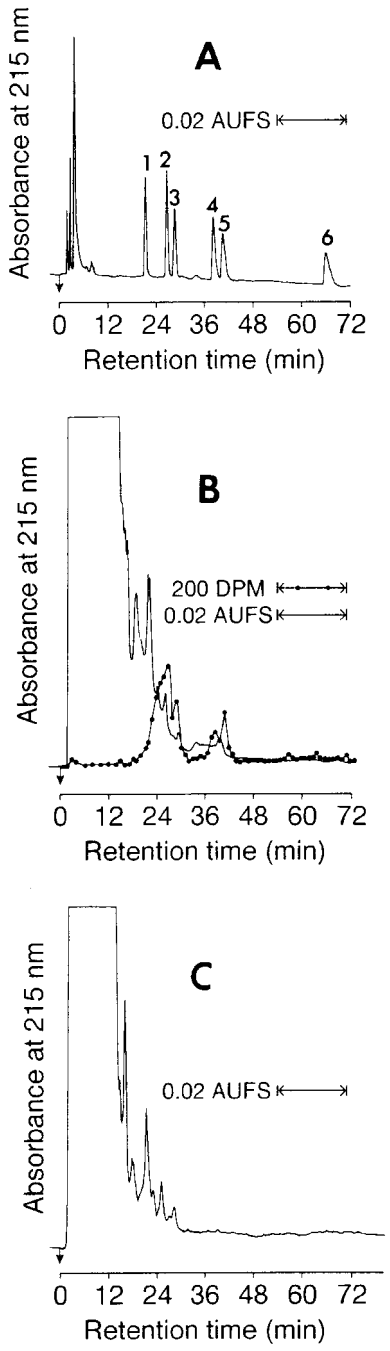

FIGURE 3. Reversed-phase HPLC elution profile of authentic fatty acid 2-butoxyethyl ester standards (A), the fatty acid ester fraction of hepatic neutral lipids of Fischer 344 rats treated with [ethyl-1,2-^{14}C]BE (B), and controls (C). Peaks 1–6 in chromatogram A represent 18 : 3, 20 : 4, 18 : 2, 16 : 0, 18 : 1, and 18 : 0 fatty acid 2-butoxyethyl esters, respectively.

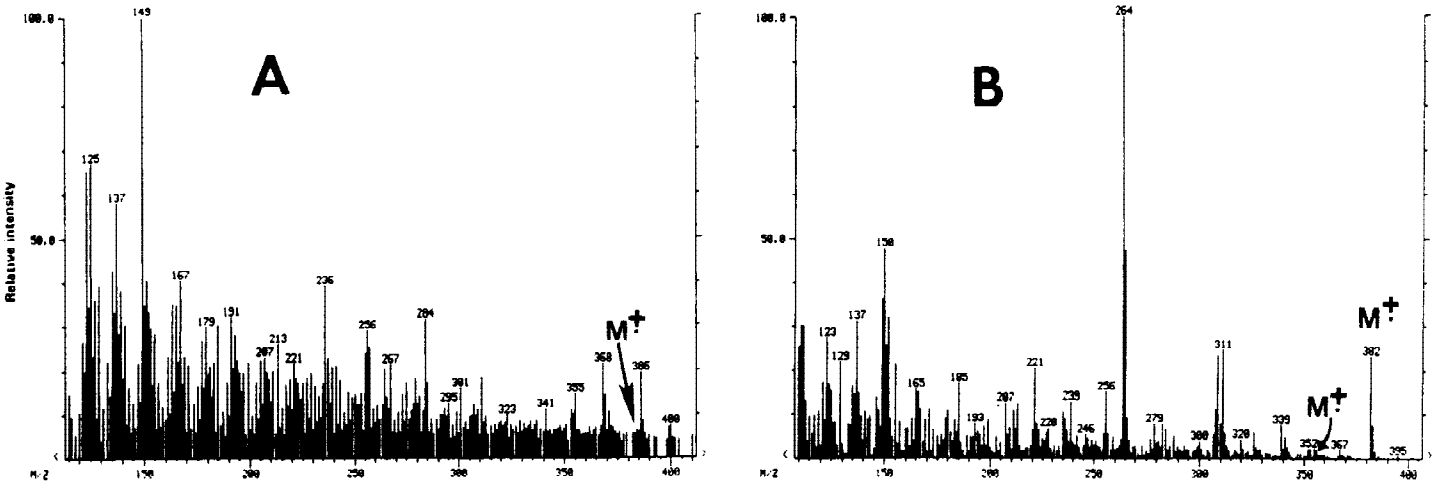

FIGURE 4. Electron impact mass spectra of different radioactive HPLC fractions obtained from ester fractions of hepatic neutral lipids of Fischer 344 rats treated with [ethyl-1,2-^{14}C]BE. Spectrum A shows the $M^{+\cdot}$ ion of the 18 : 0 fatty acid 2-butoxyethyl ester. Spectrum B shows $M^{+\cdot}$ ions for the 16 : 0 and 18 : 1 fatty acid 2-butoxyethyl esters.

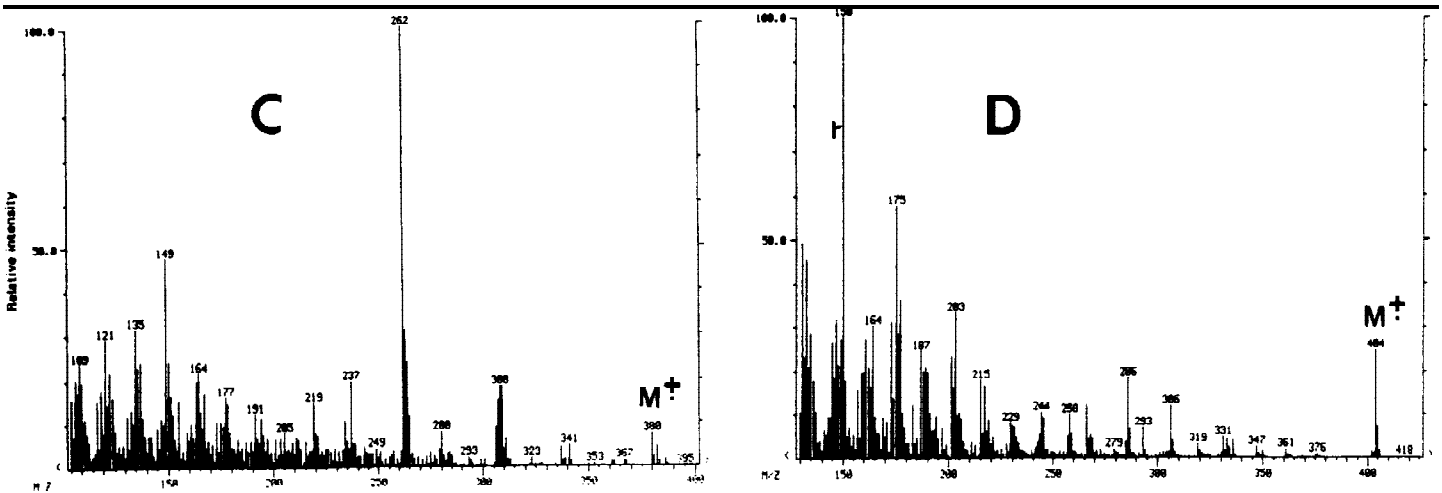

FIGURE 4. (*Continued*) Electron impact mass spectra of different radioactive HPLC fractions obtained from ester fractions of hepatic neutral lipids of Fischer 344 rats treated with [ethyl-1,2-^{14}C]BE. Spectra C and D show $M^{+\cdot}$ ions and fragmentation pattern of the 18 : 2 and 20 : 4 fatty acid 2-butoxyethyl esters, respectively.

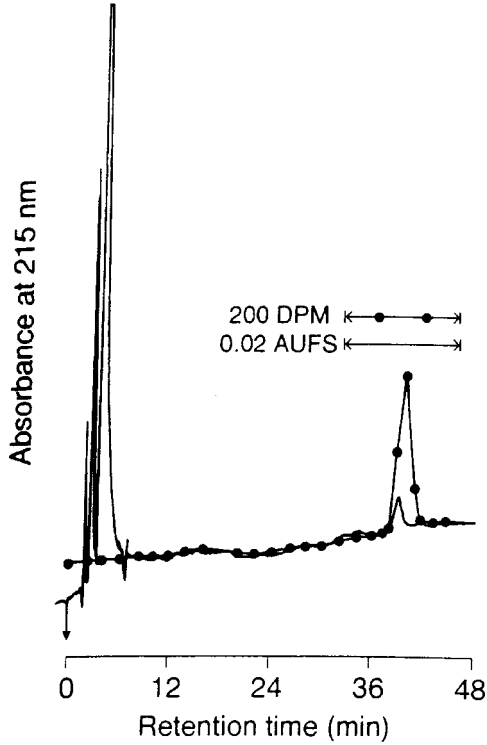

FIGURE 5. Coelution of ^{14}C-label of the chloroform extract of the incubation mixture of BE and [1-^{14}C]oleic acid in the presence of FAEES by reversed-phase HPLC analysis.

DISCUSSION

BE is an important industrial chemical that is used in several household cleaning products and paints. The usage of BE in consumer products suggests that there is a significant potential for human exposure. The major toxic effects of BE appear to be mediated by its metabolite BAA, that is, intravascular hemolysis in several animal species including rats, and metabolic acidosis after large oral doses as reported in some human cases of intentional ingestion of products containing BE (Carpenter et al., 1956; Rambourg-Schepens et al., 1988; Ghanayem & Sullivan, 1993; Ghanayem, 1995). One of the potential mechanisms for the disposition of BE and/or its metabolite BAA could be via their lipid conjugation. Our central hypothesis is that xenobiotic alcohols and acids form conjugates with endogenous lipids, which render them lipophilic so they accumulate in the body. Therefore, the results of this study on formation of fatty acid conjugates of BE and their possible role in increased relative liver weights in the rats and mice treated with BE (Carpenter et al., 1956; Dodd et al., 1983) could be important for the safety evaluation of BE and other ethylene glycol ethers.

The elution profile of the fatty acid 2-butoxyethyl esters by reversed-phase HPLC and GC also supports the synthesis as well as in vivo formation of these esters in the liver of rats administered with [1,2-^{14}C]BE and in vitro. The majority of ^{14}C-label present in the liver phospholipid fraction (Table 1) isolated 2 h after exposure to [ethyl-1,2-^{14}C]BE could be due to their conjugation with BAA. The reversed-phase HPLC (Figure 3) and mass spectrometric (Figure 4) analyses of the ester fraction of the neutral lipids, obtained from livers of Fischer 344 rats treated with [ethyl-1,2-^{14}C]BE, showed the presence of 16 : 0, 18 : 0, 18 : 1, 18 : 2, and 20 : 4 fatty acid 2-butoxyethyl esters. Two broad radioactive peaks eluted corresponding to the retention times of authentic standards of BE esters of 18 : 2, 18 : 3, and 20 : 4 fatty acids and 16 : 0 and 18 : 1 fatty acids, respectively, are in agreement with the mass spectrometric data. However, the molecular ion peak, as well as the fragmentation pattern for the 18 : 3 fatty acid 2-butoxyethyl ester, was not detected in the former peak. The presence of several unidentified peaks in the mass spectra of 16 : 0 and 18 : 0 fatty acid 2-butoxyethyl esters could be due to impurities coeluted during reversed-phase HPLC separation. Although a small fraction of BE was found to conjugate with fatty acids in relation to the administered dose, the present results clearly demonstrate that BE is metabolized nonoxidatively as reported for several xenobiotic alcohols including methanol and ethanol (Ansari et al., 1995; Kaphalia et al., 1995). In vitro study regarding the formation of 18 : 1 fatty acid 2-butoxyethyl ester catalyzed by FAEES, previously purified from rat liver (Kaphalia et al., 1994, unpublished), confirms that nonoxidative metabolism of BE and probably other ethylene glycol ethers is catalyzed by FAEES. Not investigated at this time is the ability of BE and BAA to form lipid conjugates in red blood cell membranes, which could be important for understanding the mechanism(s) of hemolytic effect of BE in laboratory animals.

Conventional conjugation reactions are generally thought to increase the polarity of xenobiotic compounds and expedite their excretion. However, the lipid conjugation of xenobiotics and/or their metabolites results in the formation of lipophilic conjugates, which may delay their metabolism and elimination, and could contribute to systemic toxicity. It is apparent that BE and most probably other ethylene glycol ethers are metabolized nonoxidatively and generate their fatty acid esters. The persistence and biological half-lives of the fatty acid esters of BE and their biological significance need to be investigated. One possibility is that this metabolic pathway competes with oxidative metabolism and acts as a limiting factor for the metabolic formation of BAA. These fatty acid esters of BE could also be hydrolyzed slowly, yielding BE to be available for the oxidative metabolism resulting in the prolongation of the $t_{1/2}$ of BAA in the body. Therefore, an interplay between these

two metabolic pathways could be important in determining the biological half-life of BAA. Determination of the correlation between the concentration of fatty acid esters of BE in circulating blood and body organs in laboratory animals could also be useful in evaluating the chronic and occupational human exposure to BE.

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