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ON THE METABOLISM OF [^{14}C]-DIETHYL ETHER IN THE MOUSE

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Abstract—A number of non-volatile *in vivo* metabolites of [^{14}C]-diethyl ether in mouse liver have been identified. The presence of labeled fatty acids and cholesterol was established by thin layer and radio gas chromatography. Additional radioactivity is incorporated into mono-, di- and triglycerides which have been tentatively identified by thin layer chromatographic separations.

FOR SOME time diethyl ether has been considered to be eliminated in an unchanged state from the body. In 1964 Van Dyke *et al.*[1] showed that approximately 4 per cent of an intraperitoneally administered dose of [^{14}C]-diethyl ether was recovered as exhaled $^{14}CO_2$ during a 24-hr period and 2 per cent of the radioactivity was transformed into non-volatile urinary products. These experiments conclusively demonstrated that diethyl ether was metabolized in the body. The distribution of [^{14}C]-diethyl ether in the mouse has been studied recently using a whole body autoradiographic technique.[2] The animals were exposed to [^{14}C]-diethyl ether inhalations for 10 min, transferred to room air and sacrificed after 0, 15 and 120 min. These studies indicated that a number of non-volatile radioactive metabolites of ether had accumulated in the liver and intestine. Approximately 1 per cent of the administered radioactivity was present in the liver as ether soluble non-volatile compounds 2 hr after the inhalation of [^{14}C]-diethyl ether.

The present work describes identification of the major nonvolatile metabolites of [^{14}C]-diethyl ether present in mouse liver.

MATERIALS AND METHODS

Animal experiments

1-[^{14}C]-diethyl ether (35 μc, 1·7 mc/mM, New England Nuclear Corporation, Boston, Mass.) was administered by inhalation for 10 min to N.M.R.I. mice weighing approximately 20 g.[3] Radio gas–liquid chromatography of the diethyl ether demonstrated that the purity was better than 99·7 per cent. The animals were sacrificed 2 hr after anesthesia and the livers ground in solid carbon dioxide. The non-volatile metabolites were extracted with three aliquots of diethyl ether* and the solvent evaporated.

* The water soluble metabolites of [^{14}C]-diethyl ether were not investigated in this study. Approximately equal amounts of radioactivity could be extracted into a water phase, likely representing additional nonvolatile metabolites of [^{14}C-]diethyl ether.

Approximately 1 per cent of the administered radioactivity was recovered in the extract. The extracted metabolites were then separated by thin layer chromatography.

Chromatographic methods

Glass plates (0·4 × 20 × 20 cm) coated by spreading a mixture of 30 g of Silica Gel G and 60 ml of distilled water were used for thin layer chromatography (TLC). The plates were previously heated for 30 min at 110–115°. Two solvent systems were used: solvent system I, petroleum ether (boiling range 40–60°)–diethyl ether–acetic acid (90:10:1), solvent system II, chloroform–methanol (144:15).

A Barber–Colman Gas Chromatograph model 5000 with simultaneous registration of mass and radioactivity was used for the radio gas–liquid chromatography. Column lengths were 1·8 m with an internal diameter of 6 mm. Column packings were 1 per cent SE-30 (F & M Scientific Corporation, Avondale, Pa), 10 per cent EGSS-X (Wilkins Instr. and Research Inc., Walnut Creek, Cal), or 1·4 per cent OV-210 (Pierce Chemical Company, Rockford, Ill) applied on silanized Gas Chrom P (100–120 mesh) as described by Horning *et al.*[4]

Silicic acid chromatography was performed as described earlier.[5] The column was eluted with 0, 20, 40 and 60 per cent ether in hexane (Mallinckrodt, analytical grade) and finally with methanol.

Radioactivity assay

Radioactivity on the thin layer plates was measured using a Berthold LCB 2560 scanner, or alternatively the compounds were detected by immersing the plates for 2 min in a glass jar containing iodine vapor. The spots and areas between them were scraped into test tubes, 1 ml of water was added and the mixture then extracted three times with ether. Aliquots of the combined ether phases were measured in a Frieseke–Hoepfner FH 90A gas flow counter, operating in the proportional range.

Preparation of derivatives for gas–liquid chromatography

Methyl esters were prepared by dissolving individual samples in methanol and treating the mixture with diazomethane in ether. For preparation of trimethylsilyl ethers the material was dissolved in 50 μl of pyridine (Mallinckrodt analytical reagent, distilled from and stored above potassium hydroxide). Trimethylchlorosilane (15 μl) and hexamethyldisilazane (50 μl) (Applied Science Laboratories Inc., State College, Pa) were then added. The reaction mixture was left in a stoppered test tube for 1 hr and the solvent evaporated in vacuum. The compounds were dissolved in carbon disulfide prior to injection into the gas chromatograph.

Mass spectrometry

Mass spectra were obtained on an LKB 9000 combined gas chromatograph–mass spectrometer equipped with a 1·5 per cent SE-30 column. The electron energy was set at 22·5 eV and the trap current was 60 μA.

Reference compounds

The fatty acid standards were obtained from Fluka AG, Buch, Switzerland, and the triglyceride (tristearate), diglyceride (distearate) and monoglyceride (monostearate)

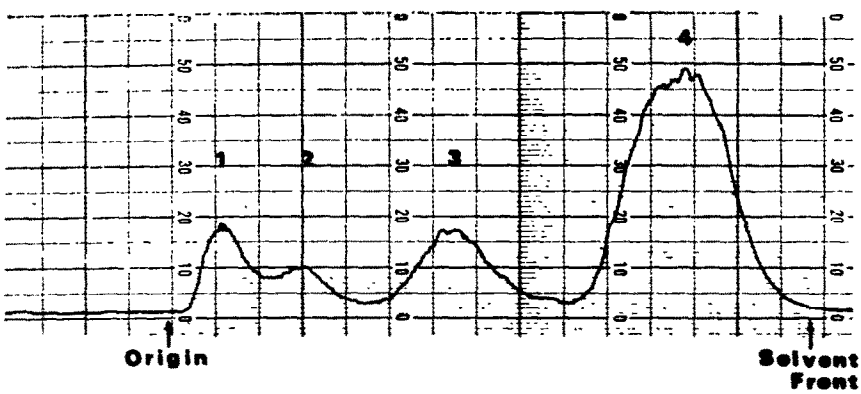

FIG. 1. Thin layer radiochromatogram from liver extract of mouse sacrificed 120 min following 10 min exposure to [^{14}C]-diethyl ether (reprinted from *Anesthesiology* **31**, 61 (1969)).

standards from Sigma Chemical Company, St. Louis, Mo. The cholesterol used as a reference compound was of commercial quality.

RESULTS

The ether soluble metabolites present in mouse liver were originally chromatographed on thin layer plates using solvent system I. The radioactivity measured in the Berthold Scanner indicated four broad peaks (Fig. 1). The R_f values were 0·08 (peak I), 0·21 (peak II), 0·43 (peak III) and 0·8 (peak IV). Peak I represented about 9 per cent, peak II about 4 per cent, peak III about 18 per cent and peak IV about 69 per cent of the extracted radioactivity. Identification of the individual metabolites was accomplished as follows:

Peak III

The material eluted from the TLC plate was subjected to silicic acid chromatography. All radioactivity was eluted with 20% ether in hexane and a portion rechromatographed on TLC with a stearic acid standard using solvent system I. R_f values for stearic acid and the radioactive spot were identical, but the latter showed some tailing suggesting that peak III was composed of fatty acids. Therefore, additional material was treated with diazomethane and subjected to gas–liquid chromatography using a 1 per cent SE-30 column (195°). The chromatogram indicated three radioactive components with retention times corresponding to the methylpalmitate, methyloleate and methylstearate standards. The last two compounds were not completely separated. The metabolites were therefore rechromatographed on a 10 per cent EGSS-X column (Fig. 2). The retention times of the three radioactive peaks and those of methylpalmitate, methyl stearate and methyloleate were identical. The small peak appearing after methylpalmitate has the same retention time as the methyl ester of palmitoleic acid. Furthermore the mass spectra of the radioactive metabolites were identical with those of methylpalmitate, methylstearate and methyloleate.

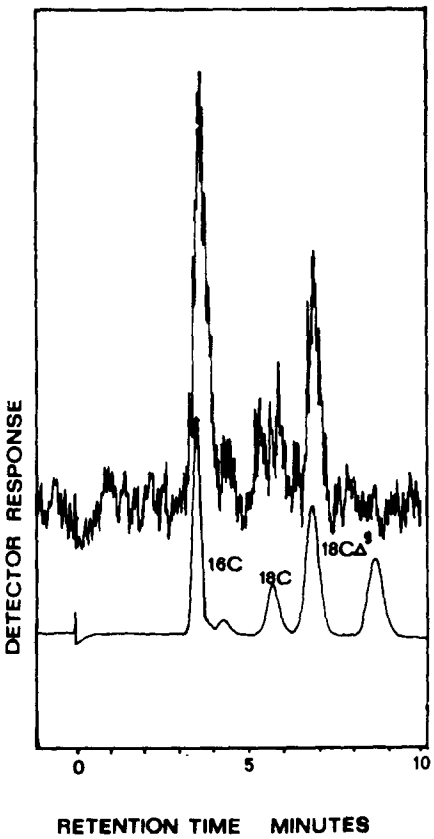

FIG. 2. Gas-liquid chromatogram of the methyl ester derivatives of metabolites in peak III. Separations on a 10% EGSS-X column at 206°. Upper curve: radioactivity registration, lower curve: mass registration. The three radioactive peaks have retention times identical to methyl palmitate (16 C), methylstearate (18 C) and methyloleate (18 C Δ^9).

Peak II

The material in peak II was rechromatographed on TLC using solvent system I with cholesterol and distearate as standards (Fig. 3). After immersion of the plate in iodine vapor two clearly separated radioactive spots were seen having the same R_f values as cholesterol ($R_f = 0{\cdot}23$) and distearate ($R_f = 0{\cdot}18$). The presence of radioactivity in these spots was determined in the flowcounter after extraction from the thin layer plate.

The compound with the same R_f value as cholesterol was silylated as described above. Gas–liquid chromatography of this material on a 1 per cent SE-30 column (245°) showed a single peak of radioactivity with the same retention time as the trimethylsilyl ether of cholesterol. In order to further establish the nature of this radioactive metabolite the silylated derivative was also chromatographed on a 1·4 per cent OV-210 column (210°). Again a single peak of radioactivity appeared, and the retention time was identical with that of the trimethylsilyl ether of cholesterol.

The radioactive material with the same R_f value as distearate was rechromatographed on TLC using solvent system II. The R_f value (0·92) in this system was the

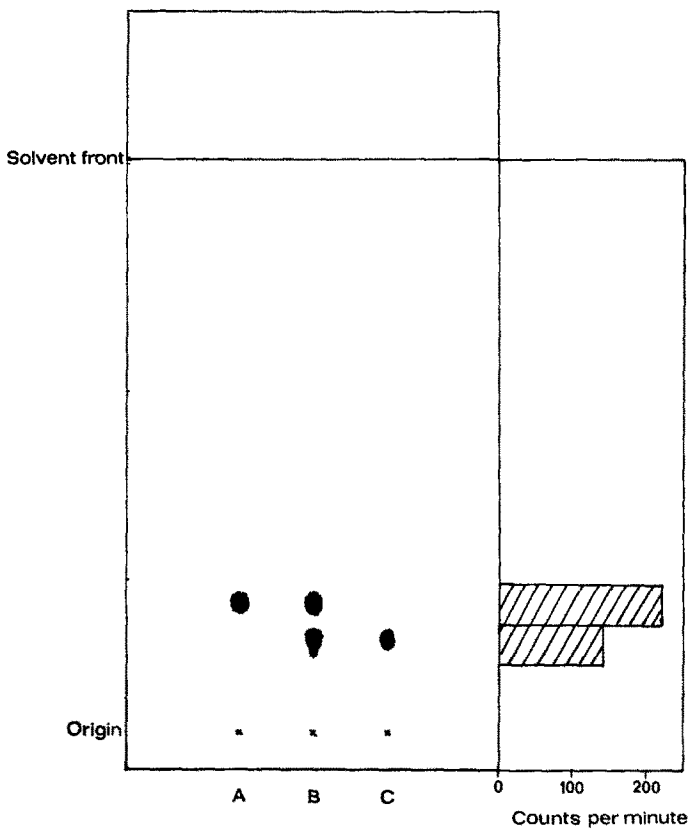

FIG. 3. Thin layer chromatogram of the metabolites in peak I (B), cholesterol standard (A) and distearate standard (C). The spots were detected with iodine vapor. One cm bands were scraped off and the radioactivity determined as described in the text.

same as that of distearate. However the upper and lower limits of the spot due to the ether metabolite were diffuse. These data indicate that this metabolite of [^{14}C]-diethyl ether is composed of diglycerides.

Peak I. This metabolite moved only slightly from the origin with solvent system I (R_f 0·08). A monostearate standard behaved similarly. Therefore, the ether metabolite(s) and monostearate were rechromatographed using solvent system II. The R_f values (0·32) of the two spots were identical although again some tailing of the metabolite could be seen. These data indicate that the material in peak I is comprised of monoglycerides.

Peak IV. The ether extract of peak IV was rechromatographed on a thin layer plate using solvent system I, together with a tristearate standard. The calculated R_f values were identical for the two compounds but the upper and lower limits of the radioactive spot corresponding to the metabolite were somewhat diffuse. It is therefore very likely that the material in peak IV is composed of triglycerides.

DISCUSSION

Previous studies have indicated that 4 per cent of [^{14}C]-diethyl ether injected intraperitoneally into rats is recovered as exhaled carbon dioxide and that small amounts of unidentified non-volatile metabolites are also found in the urine.[1] More recent investigations demonstrate the presence of a number of non-volatile metabolites in mouse liver. Two hr after anesthesia these approximate 2 per cent in the liver, and an additional 1·5 per cent is recovered from the intestinal tract.[2] Metabolites from all sources thus totals 8–10 per cent which is in agreement with studies indicating that 87–90 per cent of the absorbed diethyl ether is eliminated unchanged.[6,7]

The present studies demonstrate that a portion of the 1-[^{14}C]-diethyl ether administered to mice by inhalation is rapidly transformed into fatty acids (palmitic, stearic and oleic acids) and cholesterol which are recovered from an ether extract of liver. Three other non-volatile radioactive metabolites were tentatively identified as mono-, di- and triglycerides (Fig. 4). TLC of the latter compounds showed diffuse upper and lower limits of the spots. This finding supports the proposed nature of these metabolites in that spreading of glycerides on the TLC plates would result from differences in fatty acid composition. In one experiment, the glycerides were subjected to alkaline

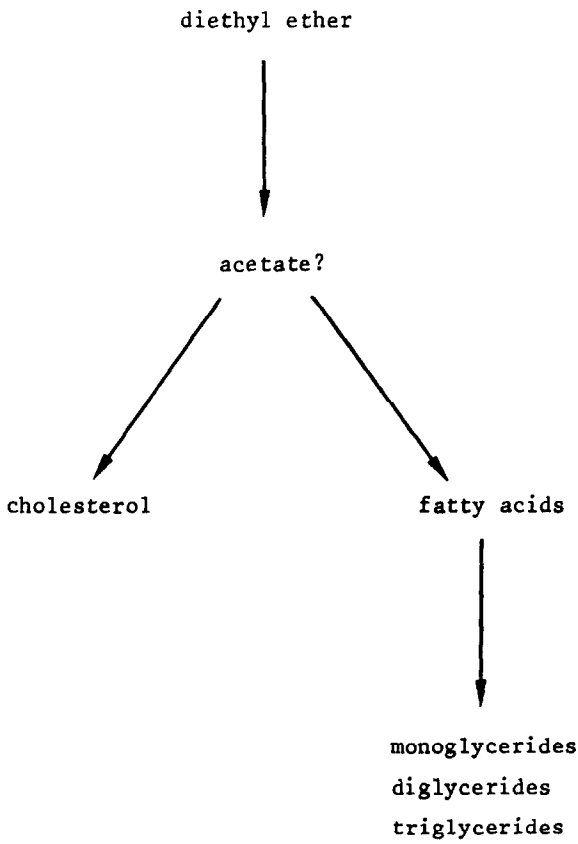

FIG. 4. Suggested route for the incorporation of diethyl ether into non-volatile compounds.

hydrolysis. Unfortunately, the radioactivity of the fatty acids was too low to give a significant response on the gas chromatograph.

From present identification of these metabolites it seems probable that [^{14}C]-diethyl ether is transformed to [^{14}C]-acetate (Fig. 4) which enters the common metabolic pool. This would lead to the formation of a number of different labeled compounds where [^{14}C]-acetate is a precursor. Of these fatty acids, glycerides and cholesterol are prominent. In addition, [^{14}C]-acetate may be incorporated into carbohydrates (water soluble, see above) and it should to a large extent be degraded to CO_2. Due to the low amount of radioactivity in our liver extract, compounds of minor quantitative importance or compounds which are slowly biosynthesized may have escaped detection.

None the less it is clearly demonstrated that [^{14}C]-diethyl ether is transformed into naturally occurring compounds including fatty acids and cholesterol. The presence of such a metabolic pathway is of great importance in the use of diethyl ether as an anesthetic since metabolism and elimination of this drug is accomplished through formation of naturally occurring nontoxic substances. Recent evidence suggests that this may not be true for the halogenated anesthetics. From this point of view, diethyl ether probably can be considered as an unusually safe anesthetic agent.

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