Properties and metabolism of 2-alkylalkanoates. III: Absorption of methyl and ethyl 2-methylpalmitate

THOMAS A. SALADIN* and EDWARD A. NAPIER, JR.

Gastroenterology Research Laboratories, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan

ABSTRACT The recovery from rat and rabbit tissues of fed methyl-^{14}C and ethyl-2-^{14}C 2-methylpalmitate with unaltered specific activity has demonstrated the existence of mechanisms for the absorption and deposition of both methyl and ethyl esters of fatty acids, at least for 2-methylpalmitate. In thoracic duct-cannulated rats, approximately 9% of the fed compounds was recovered from the lymph during the first 24 hr, the rate of recovery reaching a maximum between 6 and 8 hr. In the rabbit, the fed, unaltered esters in plasma were transported principally by means of the low density lipoproteins.

Only trace amounts of the unaltered esters were subsequently detected in the blood and tissue lipids after feeding, however, even during the period of maximal absorption; moreover, in contrast to at least one report by others, further analyses for methyl or ethyl esters of other fatty acids has shown that such esters of short-chain alcohols constitute no more than a trace amount (0.004–1.03%) of the lipids extracted from a wide variety of mammalian tissues. The possibility remains that even these trace amounts of esters arose as artifacts of autolysis, extraction, or assay.

KEY WORDS methyl · ethyl · esters · 2-methylpalmitate · absorption · occurrence · transport · lipoproteins · gas–liquid chromatography

UNEQUIVOCAL EVIDENCE for the absorption and deposition of either the methyl or ethyl esters of fatty acids has not been previously reported. Recent studies with methyl esters of elaidate (2, 3) and oleate (4) fed to the guinea pig and rat, respectively, have suggested that some esters of fatty acids with short-chain alcohols can be transported unaltered; but the possibility that the fed esters were diluted with endogenous methyl esters, which have been reported to occur in mammalian tissues (2–6) has made it difficult to evaluate these studies.

Esters of 2-methylalkanoates, readily identified in complex lipid mixtures by GLC (7, 8) and known to be resistant to hydrolysis by pancreatic enzymes (9–11), may serve as favorable models to explore pathways for the biological transport of unaltered lipids. In the present report, evidence is provided that both methyl-^{14}C and ethyl-^{14}C 2-methylpalmitate are absorbed unaltered in the rat and rabbit; as measured in the rat, this absorption was significant (about 10%). Only trace amounts of these esters, however, were subsequently deposited in tissues. Moreover, further analyses have shown that if the methyl (2–4, 6) or ethyl (5) esters of *other* fatty acids reported by others in tissues did not arise as artifacts of extraction or assay (12, 13), then they comprise no more than a trace amount (0.004–1.03%) of the lipids extracted from various mammalian tissues.

The present study thus provides support for the existence of pathways for the absorption and transport of both methyl and ethyl esters of fatty acids, at least for 2-methylpalmitate, even though such esters of fatty acids with short-chain alcohols are apparently deposited in tissues in only negligible amounts.

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; MML, methyl 2-methyllaurate; MMP, methyl 2-methylpalmitate; MMM, methyl 2-methylmyristate; EMP, ethyl 2-methylpalmitate; ^{14}C-MMP and ^{14}C-EMP, methyl-^{14}C and ethyl-^{14}C esters of 2-methylpalmitic acid; d_4^{25}, density at 25 °C, corrected to 4 °C.

Enquiries relative to this paper should be directed to Dr. Napier. A preliminary abstract has appeared (1).

* Advanced Clinical Fellow of the American Cancer Society (ACF-64B). Present address: Captain, M.C., U.S. Army Hospital, Ft. Campbell, Ky.

MATERIALS AND METHODS

Synthesis

The 2-methylalkanoates were synthesized by the diethyl methyl malonate procedure (14), and assayed for purity by GLC (7) and IR spectra as previously described (8). Esters of 2-methylpalmitic acid were prepared from the acyl chloride and either methanol-^{14}C or ethanol-2-^{14}C, (radioactive compounds from New England Nuclear Corp., Boston, Mass.) and fractionally distilled to remove residual impurities. TLC of the distillates resulted in the recovery of 99.0 and 98.1%, respectively, of the applied ^{14}C-MMP and ^{14}C-EMP in one spot. The specific activities were 261,000 and 46,000 dpm/mg, respectively, for ^{14}C-MMP and ^{14}C-EMP.

Preparation of Animals

Sprague-Dawley male rats, weighing 250–300 g, were fed 2 ml of olive oil by orogastric intubation under light ether anesthesia, and thoracic duct fistulas were prepared 90 min after feeding essentially according to the procedure of Bollman, Cain, and Grindlay (15). Confined to restraining cages, the animals were fed physiological saline for 24 hr prior to receiving the radioactive compounds by orogastric intubation. The animals were then fed rat chow ad lib. and lymph was collected at hourly intervals. At the end of the experimental period, the animals were exsanguinated at the bifurcation of the aorta under ether anesthesia.

Tissues were excised from dogs, rabbits, and monkeys under anesthesia and extracted promptly so that autolysis was minimized. Blood was obtained from the antecubital vein of fasting adult male human volunteers.

Extraction and Analysis of Lipids

Tissues and diet samples were homogenized in 0.9% NaCl solution and extracted with at least 20 volumes of chloroform-methanol 2:1 according to the procedure of Folch, Lees, and Sloane Stanley (16). The solutions were filtered through solvent-washed, sintered-glass funnels to remove insoluble residues. The extracts were then washed with 0.2 volume of water and at least twice with chloroform–methanol–water 3:48:47 as recommended (16). The washed extracts were dried in vacuo, weighed, and stored at −12°C when necessary, prior to assay. All solvents were distilled and all operations were conducted at or below ambient temperature.

Chromatography. When it was desirable to separate the neutral lipids from the phospholipids prior to TLC, the extracted lipids were applied on silicic acid (325 mesh) columns and eluted with petroleum ether–diethyl ether mixtures as described by Hirsch and Ahrens (17). Neutral lipids were separated by TLC (18) on Silica Gel G with petroleum ether–diethyl ether–acetic acid 90:10:1, and phospholipids with chloroform-methanol–water–acetic acid 65:25:8:4 (19).

Quantitative gas–liquid chromatography of the methyl and ethyl ester content of lipids was carried out with added internal standards of MML or MMM as previously described (7). Lipid extracts diluted to volume with hexane solutions containing MML or MMM were injected directly into the chromatograph, and the concentration of esters was measured by triangulation of peak responses as compared with those of the internal standard. The specific activity of the GLC-resolved esters was determined by collecting the appropriate ester, diluting it with hexane solutions that contained an internal standard, and repeating GLC and radioassay of the solution. In this manner, the need for quantitative injection and collection of GLC eluates, which are difficult to achieve, was obviated, and the specific activity of as little as 5–10 μg of ester could be determined.

Radioassay

Lipid fractions were dried in vacuo in counting vials and diluted with 10 ml of toluene containing 4 mg of 2,5-diphenyloxazole and 0.1 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene. The efficiency of the scintillation system was 86.5% when recorded with a Packard 314 EX instrument. Lipids separated by TLC were washed from the plates with the scintillation fluid directly into counting vials. The insoluble silica gel in the bottom of the vials gave rise to no significant quenching, as determined by the recovery of applied radioactive standards such as ^{14}C-MMP and cholesteryl-4-^{14}C palmitate (8).

Ultracentrifugation

Serum was ultracentrifuged for the separation of low density lipoproteins ($d_4^{25} < 1.063$) from the high density lipoproteins ($d_4^{25} = 1.063$–1.21) in a Spinco model L centrifuge with a 30.2 rotor (14° angle) at 4°C and at 30,000 rpm (median force 79,420 *g*). Serum (5 ml) was diluted with NaCl solution (4 ml, $d_4^{25} = 1.1315$) and centrifuged at 30,000 rpm for 16 hr, and the top 1 ml was then removed to yield the low density lipoproteins (20). The subnatant solution was adjusted to a density of 1.21 by the addition of solid KBr and recentrifuged for 16 hr to give the high density lipoproteins. After aspiration of the top layer (1 ml per tube), the subnatant solution was divided by means of a tube slicer into three successive fractions subadjacent to the top layer and designated subnatants A (2.0 ml), B (3.0 ml), and C (the residue, 2.0 ml), respectively. The volume of each fraction was measured, the protein content was assayed (21), and the lipid was extracted by the Folch procedure.

RESULTS

Absorption of Unaltered Methyl-^{14}C and Ethyl-2-^{14}C 2-Methylpalmitate

As illustrated in Fig. 1, GLC assay of lipids extracted from the lymph of thoracic duct-cannulated rats revealed the presence of MMP in animals previously fed ^{14}C-MMP. Furthermore, the specific activity (260 dpm/μg) of the MMP collected after elution by GLC from the lymph lipids was essentially the same as that of the fed compound (262 dpm/μg). Feeding ^{14}C-EMP to thoracic duct-cannulated rats also resulted in the recovery of unaltered ^{14}C-EMP in the lymph of the animals (Fig. 2). Thus the absorption of both ethyl and methyl 2-methylpalmitate as the unaltered esters was demonstrated.

Radioactivity appeared in the lipids of the lymph at a maximum rate between 4 and 6 hr after ^{14}C-MMP had been fed (Fig. 3). After 12 hr, the radioactivity decreased to less than 0.2% appearing each hour in lymph samples collected up to 5 days. TLC separation of the lymph lipids collected up to 24 hr after the ^{14}C-MMP had been fed demonstrated that more than 90% of the radioactivity was present in the methyl ester fraction, 98% of which was found to be MMP by GLC analyses. The total radioactivity that accumulated in the lymph lipids of the six animals studied during the first 24 hr after feeding represented 9.04% ± 1.57 (SEM) of the 25 mg of ^{14}C-MMP fed.

The minimal amount of lipid extracted from the lymph of the six animals studied was found to be 480 mg/24 hr. Even if the 9% of the fed radioactivity recovered in the lymph lipids in 24 hr had consisted entirely of ^{14}C-MMP, then even during the period of maximal absorption the methyl ester would have represented less than 0.5% of the total lymph lipids present.

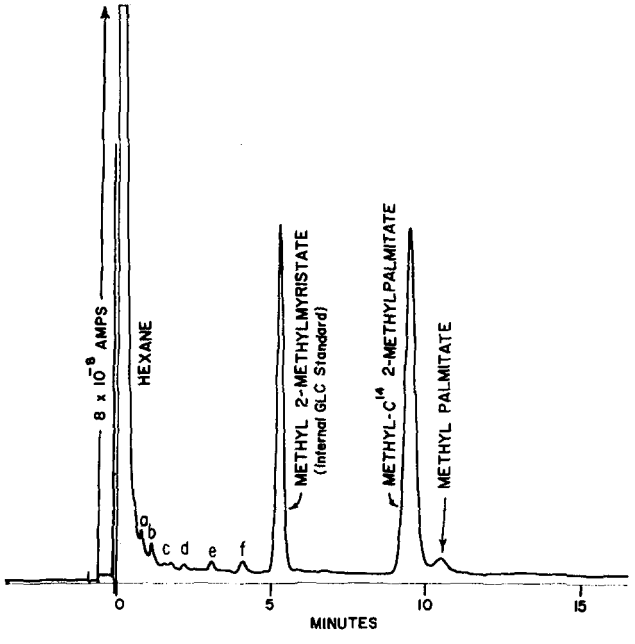

FIG. 1. Recovery of unaltered methyl-^{14}C 2-methylpalmitate in rat lymph. A 370 g rat was fed 25 mg of ^{14}C-MMP (262 dpm/μg) in 1 ml of olive oil by orogastric intubation 24 hr after cannulation of the thoracic duct, and the lymph was collected at hourly intervals for 12 hr. Lipid extracts of the lymph were injected into the gas chromotograph after dilution with internal standard. On the basis of radioassay of the MMP collected (11,960 dpm) on elution from GLC, and the GLC weight response (46 μg) determined by the use of internal standard (MMM), the specific activity of the ^{14}C-MMP recovered from lymph was 260 dpm/μg. Trace components which may be methyl esters were eluted (peaks *a* through *f*), but their total weight response represented less than 0.08% of the lymph lipids. GLC conditions: 6 ft × 0.25 inch column, 15% ethylene glycol succinate polyester on 80–100 mesh Chromosorb W, 187°C, argon ionization detector.

Recovery of Fed Radioactivity in Serum Lipids

A total of 70 fasting rats were each fed 25 mg of ^{14}C-MMP in 1 ml of olive oil by orogastric intubation under light ether anesthesia, and then exsanguinated in groups of 5–7 at hourly intervals up to 10 hr, and at 12 and 24 hr. From the radioactivity of the serum lipids and on the assumptions of a blood volume of 7% of the weight for each rat and a hematocrit value of 50%, the percentage of the fed radioactivity in the total serum of each animal was calculated. On the basis of these calculations, the maximal percentage at any one time was 0.61% of the fed radioactivity, and this was in only one animal. The mean recovery of fed radioactivity for all the animals at any time up to 10 hr was 0.14% ± 0.26 SEM. The low recovery of fed radioactivity and the wide variation between rats sacrificed at the same time interval did not permit an accurate determination of the time of maximal appearance of the radioactivity in the serum. Nevertheless, it was evident that the peak accumulation of radioactivity occurred between 2 and 6 hr after feeding and diminished to less than 0.03% after 8 hr; it was also evident from GLC analyses of pooled serum lipids that unaltered ^{14}C-MMP was present in the rat serum.

Accumulation of Radioactivity in Lipids of Liver

The low level of accumulated radioactivity in the serum lipids suggested that the methyl esters were rapidly removed from the serum. Radioassay of lipids extracted from 74 rat livers at various time intervals after 25 mg of ^{14}C-MMP had been fed to each animal showed a maximal accumulation of the fed radioactivity at 8 hr (1.01% ± 0.06 SEM), which diminished to about 0.75% after 12 hr (Fig. 4). Thus, the peak appearance of radioactivity in the lymph lipids preceded the time of maximal accumulation of radioactivity in the liver lipids, the expected result of the transport of MMP from one compartment to another in which the compound was metabolized or stored.

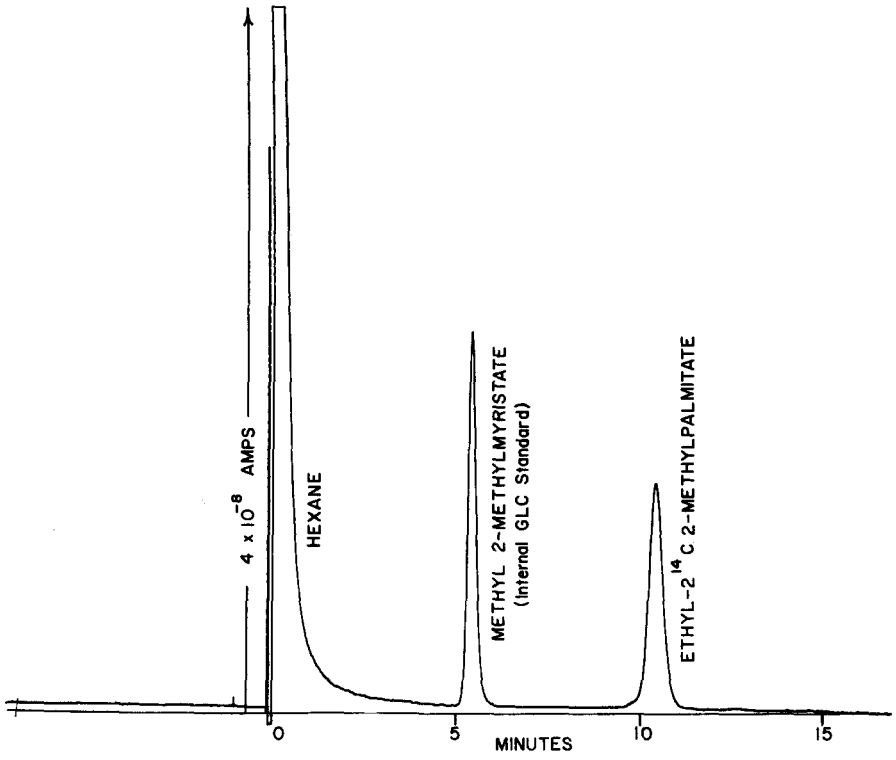

FIG. 2. GLC of ethyl-2-^{14}C 2-methylpalmitate recovered from rat lymph. A 225 g rat was fed 30 mg of ^{14}C-EMP (specific activity 46.0 dpm/μg), and the lipids were then extracted from pooled lymph collected for a period of 24 hr. The ethyl esters were initially separated from the lymph lipids by TLC with petroleum ether–ether–acetic acid 90:10:1, eluted from the silica gel with diethyl ether, and then subsequently purified twice more by TLC with hexane–benzene 65:35 as the developing solvent. The specific activity of the ^{14}C-EMP thus recovered from lymph was 46.3 dpm/μg, assayed as noted in Fig. 1.

Silicic acid column chromatography of pooled lipids from the rat livers revealed that 57% of the recovered radioactivity was in the neutral lipids eluted with petroleum ether–diethyl ether mixtures, and the remainder in the phospholipid fractions subsequently eluted with chloroform–methanol 1:4. TLC of the eluates[1] showed that 77% of the radioactivity was in the neutral lipids and GLC showed that it was all contained in unaltered ^{14}C-MMP. Thus, less than 0.5% (or 0.125 mg) of the fed radioactivity recovered in the lipids of rat livers was identified as the unaltered ^{14}C-MMP. It is of further interest to note that if one assumed that the *minimal* lipid content of the liver were 250 mg, and that as much as 1% of the fed radioactivity were recovered as unaltered ^{14}C-MMP in the liver, then the *maximal* amount of accumulated methyl 2-methylpalmitate would be less than 0.25% of the total liver lipids.

Distribution of Radioactivity among Rabbit Serum Lipoproteins

To explore the mode of transport of the methyl and ethyl ester of 2-methylpalmitate in serum, we fed 2–3-kg New Zealand white male rabbits approximately 100 mg of the ^{14}C-MMP (or EMP) in 5 ml of olive oil by orogastric intubation under sodium pentobarbital anesthesia (30 mg/kg body weight), and sacrificed them 2.5–3.5 hr after feeding. The serum from the animals was separated by ultracentrifugation into the low and high density lipoproteins and residual fractions, which were then assayed for distribution of radioactive lipids. In these experiments, the radioactivity in the serum lipids was principally in the low density lipoprotein fraction (Table 1). TLC of the lipids extracted from both serum and the low density lipoproteins showed that less than 75% of the radioactivity was in the methyl (or ethyl) fatty acid ester fractions; GLC showed it to be present as the unaltered fed compound.

Occurrence of Methyl and Ethyl Fatty Acid Esters in Mammalian Tissues

The trace amounts of either ^{14}C-MMP or ^{14}C-EMP detected unaltered in tissues after they had been fed suggested a quantitative reexamination of the occurrence of other fatty esters of short-chain alcohols, particularly

[1] Preliminary TLC studies of the chloroform–methanol eluates showed that radioactive phosphatidyl choline can be isolated, which suggests one-carbon transfer of the methanol-^{14}C from the methyl ester.

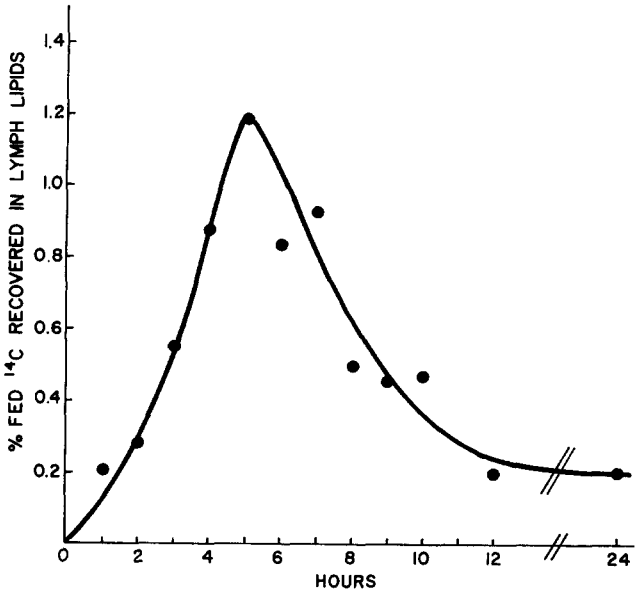

FIG. 3. Rate of appearance of radioactivity in lymph lipids after the feeding of ^{14}C-MMP to rats. Rats were fed 25 mg of ^{14}C-MMP in 1 ml of olive oil and lymph was collected at hourly intervals for 12 hr, and for the period of 12–24 hr. Each point is the mean value for six animals. The 24 hr point was obtained by division of the 12–24 hr value by 12.

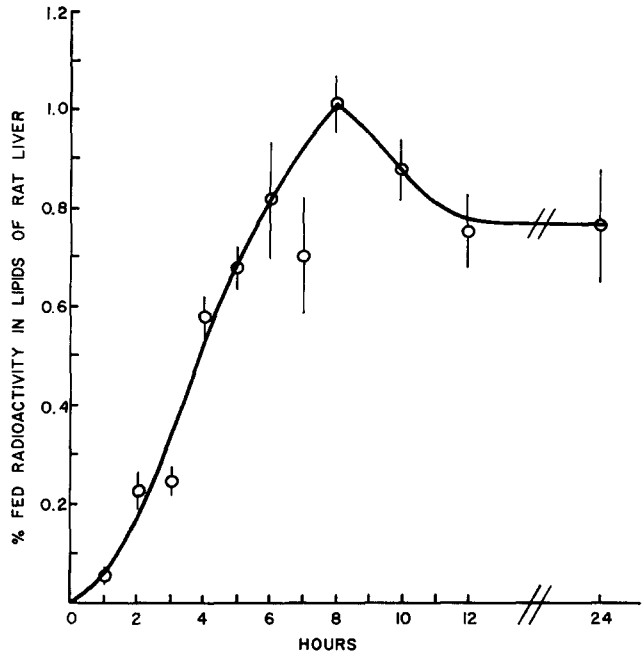

FIG. 4. Accumulation of radioactivity in whole liver lipids after the feeding of ^{14}C-MMP. 74 fasting rats were each fed 25 mg of ^{14}C-MMP in 1 ml of olive oil and then sacrificed at hourly intervals for 8 hr, and after 10, 12, and 24 hr. Each value represents the mean ± SEM for 5–10 animals.

in view of one report (6) that fatty acid methyl esters are a major constituent (up to 18%) of the lipids extracted from pancreas with chloroform–methanol 2:1 (the solvent being subsequently removed at 60°C).

As Table 2 shows, the total quantity of compounds eluted from the gas chromatograph after direct injection of lipids extracted by the Folch technique did not exceed 1% of the total lipids in any of the tissues and diets studied. These values varied among different tissues from 0.004% (rat adipose tissue) to 1.03% (pooled organs in rats), and by as much as 10-fold from one experiment to another on the same tissue (e.g., 0.004 to 0.043% in rat adipose tissue and 0.006 to 0.240% in human serum). Furthermore, delay in the extraction of tissues, or storage of lipid extracts in chloroform–methanol 2:1 at room temperature for extended periods resulted in at least two to three fold increase in the quantity of fatty acid esters in the rat. These esters increased, for example, to

TABLE 1 DISTRIBUTION OF RADIOACTIVE LIPIDS AMONG RABBIT SERUM LIPOPROTEINS*

	Volume†	Protein	% of Radioactivity Recovered	
			^{14}C-MMP Fed	^{14}C-EMP Fed
	ml	%	%	%
Serum	5	(100)	(100)	(100)
I. Low density lipoproteins ($d_4^{25} < 1.063$)	1	1.4	98.2	84.4
II. High density lipoproteins ($d_4^{25} = 1.063$–1.21)	1	1.9	0.4	15.6
III. Subnatant ($d_4^{25} > 1.21$)	7.0	96.7	1.3	tr.
A	2.0	13.2	0.2	—
B	3.0	33.3	0.8	—
C	2.0	50.2	0.3	—

* New Zealand, white male rabbits, 2.62 and 2.26 kg, were fed 117.7 and 105.1 mg of ^{14}C-MMP and ^{14}C-EMP, respectively, in 5 ml of olive oil, and sacrificed 2.5 hr later.

† The volumes represent the initial mixture of 5 ml of serum and 4 ml of NaCl solution for the first centrifugation, and are corrected for the increase in volume when KBr was added to a density of 1.21.

TABLE 2 PROPORTION OF FATTY ESTERS OF SHORT-CHAIN ALCOHOLS SEPARATED BY GLC FROM VARIOUS TISSUE AND DIETARY LIPIDS

	Mean Lipid Content	% Fatty Acid Esters in Lipids*		
		Expt 1	Expt 2	Expt 3
	g/100 g wet wt			
Rat				
Adipose tissue	60.8	0.004	0.020	0.043
Liver	4.5	0.035	0.131	0.328
Lymph	4.9	0.254	0.360	0.440
Pancreas	10.2	0.186	—	—
Red cells	0.9	0.057	0.279	—
Serum	0.3	0.504	0.602	—
Pooled organs†	11.2	0.075	0.604	1.03
Dog				
Pancreas	5.0	0.097	0.293	0.578
Red cells	0.7	0.264	—	—
Serum	0.8	0.902	—	—
Monkey pancreas	4.2	0.526	—	—
Human serum	0.8	0.006	0.009	0.046
		0.052	0.240	—
Rat chow	6.2	0.054	0.112	0.167
Corn oil	99.5	0.250	0.260	0.300
Olive oil	96.9	0.160	0.230	0.260

Animals were fasted for 12–16 hr before specimens were collected. Aliquots of the weighed lipids, extracted by the Folch technique, were diluted to volume with hexane containing either MML or MMM as internal standard and injected directly into the GLC apparatus. The total area of the peaks eluted, compared to the internal standard, was used to calculate the percentage of fatty esters of short-chain alcohols in the lipids.

* Each experimental value for the rat represents pooled tissues from 10–12 animals; all other data are single assays.

† Pooled organs included heart, kidney, lung, testes, thymus, and spleen.

1.3 and 1.7% after the total lipids from serum and red cells, respectively, had stood for 3 days in chloroform–methanol 2:1, and to 1.8 and 2.4% when lipids of serum and mixed organs, respectively, had stood for 8 days. Reextraction of the protein residues with chloroform–methanol 2:1 did not release any additional lipid, as previously shown (16), and the data thus represent the maximal amount of fatty esters of short-chain alcohols in lipids extracted by the Folch technique, (a technique which could, in theory, bring about methanolysis).

Extraction of dog pancreas with ethanol–diethyl ether 3:1 by the procedure of Bloor (22) yielded the same amount of lipid and quantity of esters, as assayed by GLC, as did the Folch technique. But the extraction of rat liver or pooled rat tissues (kidneys, adipose tissue, pancreas, heart, lungs, spleen, thymus, testes, and adrenals) with either hexane or diethyl ether gave markedly lower results for esters detectable by GLC (less than 0.03% of the extractable lipid); and similar extractions of rat serum or adipose tissue yielded no detectable fatty esters. The total lipid extracted with hexane or diethyl ether was lower, however, as compared with the Folch or Bloor techniques, and these results do not permit one to exclude the presence of methyl esters in tissues.

On the basis of the retention time of standards (23), the principal peaks eluted by GLC were identified as methyl esters of the commonly occurring acids (e.g., palmitate, stearate, oleate, and linoleate). In no case were ethyl esters detected; but without stringent identification and quantification of each of the many trace components eluted by GLC (e.g., peaks *a* through *f*, Fig. 1), the question of the occurrence of specific fatty acid esters of short-chain alcohols must rest with the validity of the methods employed in their detection.

DISCUSSION

Reports supporting the presence of pathways for the absorption and deposition of fatty esters of short-chain alcohols have been difficult to assess, primarily because of: (*a*) dilution of the recovered, fed ester with "endogenous" esters; (*b*) conflicting reports on whether or not a particular methyl or ethyl ester is absorbed; and (*c*) the absence of quantitative data relative to either the amount absorbed or the limits of detection in tissues.

In their initial report (2) on the metabolism of methyl elaidate in the guinea pig, Dhopeshwarkar and Mead reported the separation of methyl esters of a specific activity of 708 dps/mg 4 hr after feeding methyl elaidate-1-^{14}C with a specific activity of 69,890 dps/mg. The presence of unaltered elaidate would have thus required 100-fold dilution with methyl esters other than elaidate, and suggested both the absorption of methyl elaidate, as such, and the occurrence of other fatty acid methyl esters. In a confirming report (3), the specific activity was found to be diluted only 6-fold in the fatty acid methyl esters of guinea pig lipids, one-third of which were estimated to be elaidate and (or) oleate by GLC assay. Blank and Privett (24), however, reported no evidence of methyl esters in the livers of rats fed geometric isomers of methyl linoleate or linolenate but the limits of detection were not reported by these workers.

Insensitivity of detection may have been a factor in earlier studies of the absorption of ethyl esters of palmitate (25, 26) and oleate (27). Frank (25), for example, found that lipid extracts of dog chyle could not have contained more than 1% of the fed ethyl palmitate and concluded that such esters were "completely hydrolyzed in the intestine." Similarly, from the data presented by Borgström (27), one may calculate that up to 2.6% of the lipids collected within 24 hr in rat lymph contained fed ethyl oleate, on the basis of ethoxyl determinations carried out on the chromatographed lipids. This careful worker concluded, however, that there was no definite evidence for the occurrence of ethyl esters in the lymph, since significant amounts of ethoxyl compounds were

also present in the lymph of rats fed corn oil. Since our studies have demonstrated that mechanisms do exist for the absorption of ethyl esters of *specific* fatty acids, as illustrated with the nonnaturally occurring 2-methylpalmitate, these earlier studies may warrant repetition with doubly-labeled compounds and current isolation techniques.

Recent studies demonstrating in vitro synthesis of methyl or ethyl esters of fatty acids by mammalian systems (28–32), mediated by direct acylation (29, 31, 32) or possibly through transacylases (30), are consistent with the reported occurrence in tissues (2–6, 33, 34) of such esters of short-chain alcohols. Conclusions as to whether specific fatty acid esters found in tissues arose from unaltered absorption, biosynthesis, or artifacts, may thus depend on different metabolic routes, as suggested by Blank and Privett (24), and on the validity of the methods used in their detection. Patton and McCarthy (34), for example, could not detect ethyl esters in human adipose tissue, or in the milk or blood of goats, although Kaufmann and Viswanathan (5) reported "traces" of both methyl and ethyl esters in lipids extracted from human liver. After feeding ethanol, however, Patton and McCarthy did detect ethyl esters of palmitate, stearate, oleate, and linoleate in the milk of goats; but these esters were estimated to represent no more than 0.001 and 0.01% of the milk lipids in the two animals studied. The latter study would thus be compatible with a report of the presence of ethyl esters in the lipids of rat carcass 4 hr after the intravenous injection of ethanol (33), and further illustrates the need for quantitative expression of the absorption, occurrence, and biosynthesis of *each* fatty ester of short-chain alcohols.

Our studies with MMP and EMP showed that only trace levels of the fed unaltered ester could be subsequently detected in tissues. It was evident, however, that if methyl or ethyl esters of *other* fatty acids were present in tissues, that such esters of short-chain alcohols also constituted only a trace of the extractable lipids. In marked contrast, Leikola, Nieminen, and Salomaa (6) reported that methyl esters were a major component of the total lipids extracted from the pancreas of dogs and humans. From 305 mg of human pancreatic lipids, extracted with chloroform–methanol 2:1, the latter workers separated a fraction weighing 55.6 mg which was identified as principally methyl esters of fatty acids. This fraction thus represented about 18% of the total lipids, and was exceeded only by one other weighing 109.6 mg (or about 36% of the lipids) and labeled as principally free fatty acids. Although further quantitative data were not given, these workers also extracted methyl esters from pancreas by means of a wide variety of organic solvents and suggested that methyl esters were not artifacts of extraction.

In view of the general acceptance of the technique of Folch, Lees, and Sloane Stanley (16) for the quantitative extraction and characterization of tissue lipids, the latter study was of particular relevance to our investigations of the transport mechanisms of fatty acid esters in mammals. It was therefore pertinent to establish, under GLC conditions routinely employed for the assay of fatty acid esters, quantitative measures of the *maximum amount of total GLC eluates that may arise from mammalian lipids* carefully extracted by the procedure of Folch et al. Since control extractions of large volumes of solvents (up to several liters) yielded no residues eluted on GLC, it was apparent that esters of short-chain alcohols did arise as a result of extracting pancreatic tissue, but constituted no more than a trace (less than 0.6%) of the lipids extracted from the pancreas of the dog, rat, or monkey, and indeed, no more than a trace (0.004–1.03%) of the lipids in any of the mammalian tissues assayed. The marked discrepancy between our results and those of Leikola et al. (6) may have resulted from autolysis, as indicated by the preponderantly high content of free fatty acids which were apparently the major lipid component in their extracts of pancreas.

It should be emphasized that the presence of mechanisms that permit the transport and deposition of unaltered 2-methylpalmitate esters need not be valid for the naturally occurring fatty acids; but in the absence of quantitative studies of the absorption and deposition of each fatty acid methyl or ethyl ester, our studies do invoke caution in the interpretation of metabolic interrelationships of fatty acids fed as the methyl or ethyl esters. This is further illustrated by the early studies of Frank (25) who showed that ethyl stearate was not as effectively utilized by the dog as was ethyl palmitate, and by recent studies in the rat (35) which suggest that the absorption of methyl ricinoleate is different from that of the free acid. On the other hand, our data would also be consistent with the thesis that the "particulate" (or pinocytotic) pathway is *not* a major mechanism for the absorption of fatty acid esters; indeed, this contention is supported by the recovery from lymph of only about 10% of an ester that would presumably, because of its resistance to hydrolytic enzymes, be more likely than others to be absorbed.

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