Evidence for Occurrence of Methyl Esters in Body and Blood Lipids.* (27226)

G. A. DHOPESHWARKAR AND JAMES F. MEAD
Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles

Recognition that a fraction of the dietary triglycerides may be absorbed from the intestine in an unhydrolyzed or partially hydrolyzed state bears the implication that other lipids may be absorbed similarly. In most cases, however, either sufficient evidence bearing on this point is not available or, as in the case of the sterol esters(1) points in the opposite direction. Since the methyl esters are commonly employed as compounds of choice for administering experimental fatty acids, it might be supposed that their digestion and absorption would have been clarified. However, the scanty evidence available gives only an equivocal answer favoring complete digestion, or at least absorption in some other form (2,3).

The present experiments, originally undertaken to throw light on the metabolism of the *trans* acids,† not only bear on this subject but also pose a more difficult and potentially more interesting problem concerning the natural occurrence of methyl esters in the tissue lipids.

Experimental. Methyl elaidate-1-C^{14}, (3 g, sp. act. 4100 dpm/mg) prepared according to the method of Nevenzel and Howton(4), was fed to 4 fat-deficient‡ guinea pigs. After 4 hours the animals were sacrificed and the liver, kidney, spleen, heart, lung and adipose tissue were excised. Total lipids were extracted with chloroform-methanol (2:1) and chromatographed (Table I) on a silicic acid column(5). A suitable aliquot of each fraction was further purified by thin-layer chromatography(6). A good separation of methyl from cholesteryl esters of fatty acids was obtained by development with 1.5% ethyl ether in hexane (Fig. 1). Ether (16%) and acetic acid (1%) in hexane gave clean separation of triglycerides and free fatty acids. After a short exposure of the plates to iodine to visualize the spots, the iodine vapors were blown off and the separated fractions from the plates were extracted with chloroform. No irreversible reaction of the methyl esters with iodine vapor was noticed as the gas chromatographic curves before and after exposure to iodine vapors were identical.

Pure fractions in amount ranging from 7 to 12 mg, obtained from several thin-layer silicic acid plates, were weighed into vials and counted in a liquid scintillation counter.§ Table I gives the activity of the various pure components. The methyl ester fraction was extracted and analyzed by gas-liquid chromatography‖ giving peaks not only for elaidate (and/or oleate) but also major peaks for palmitate and stearate and minor peaks for myristate, palmitoleate and linoleate (Fig. 2). Estimation of the per cent composition of this mixture from the graph showed that it contained 33.6% elaidate (and/or oleate). Assuming that the other components contributed very small amounts of activity, the activity attributed to the mixture of oleate and elaidate was estimated (Table I).

To determine whether the methyl esters could have been artifacts from fatty acids of triglycerides (major component of total body

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† Results of the study of the metabolism of methyl elaidate-1-C^{14} will be reported later.

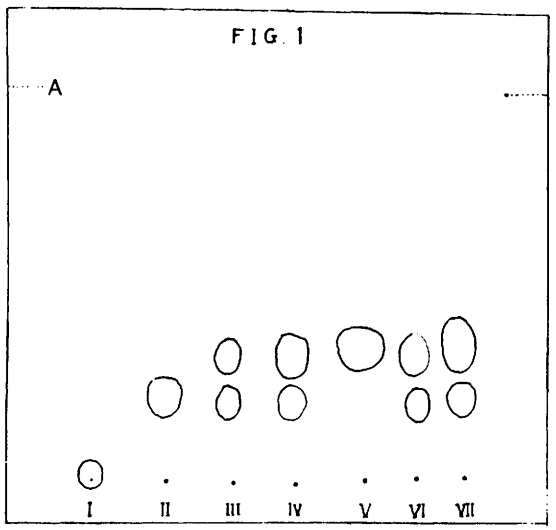
‡ Fat deficiency was indicited by scaly paws and loss of hair on the abdomen.

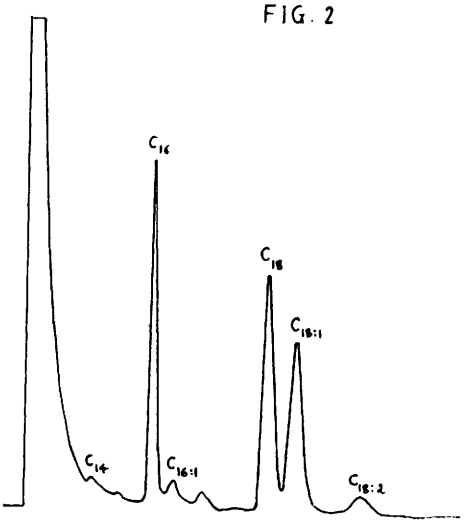
§ All counting was performed with a single-channel scintillation counter of the type described by Hodgson, Gordon and Ackerman (7) at 1150 volts and 77 ± 0.2% efficiency. Samples were weighed into 3-dram vials and dissolved in 5 ml of toluene containing 5 g of phenyl biphenyl oxadiazole and 0.1 g of "POPOP" per liter.

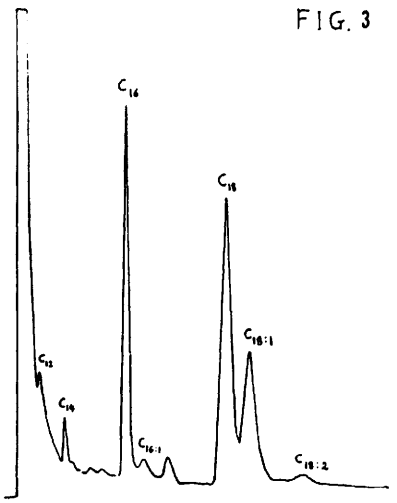
‖ Gas liquid chromatographic analysis was performed using a Wheelco Model 10 (Barber Coleman Co.) apparatus with a 6-foot, 6-mm. I.D. column of ethylene glycol succinate polyester on 80-100 mesh siliconized Chromosorb at 185-200° C, or a 3-foot column of 0.29% silicone rubber gum SE 30 (General Electric Co.) on 80-100 mesh glass beads at 185-200° C.

TABLE I. Silicic Acid Chromatography of Total Body Lipids of Guinea Pigs Following Ingestion of Methyl Elaidate-1-C^{14}.

Eluent	Pentane	2% ether in pentane		5% ether	12% ether	20% ether	100% ether	Methanol
Wt (g)	.054	.220		1.134	.114	.022	.151	.871
Component identified and separated by thin-layer chromatography	Hydrocarbon	Cholesteryl esters	Methyl esters	Triglyceride	—	Free fatty acids	Cholesterol	Phospholipids
Sp. act. (dpm/mg)	13.2	5.2	708	1.8	—	79.2	5.4	1.5
Estimated sp. act. (dpm/mg) of mixture of oleate and elaidate			2112					







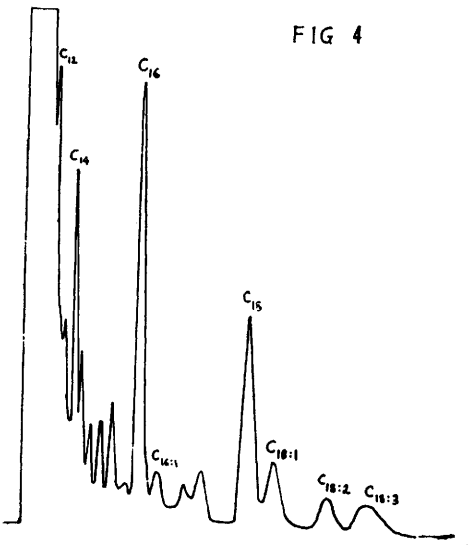


FIG. 1. Separation of methyl and cholesteryl esters of fatty acids. Solvent: 1.5% ethyl ether in hexane. A: Solvent front. I: Standard sample of triglyceride (triolein). II: Standard sample of methyl oleate. III: Fraction from normal animals; lipids extracted with propanol and chloroform instead of usual mixture of methanol-chloroform. IV: Fraction from fat-deficient guinea pig lipids. V: Standard sample of cholesteryl palmitate. VI: Fraction from methyl elaidate-fed fat-deficient guinea pig lipid. VII: Fraction from normal guinea pig blood lipid.

FIG. 2. GLC of methyl esters from total lipids of methyl elaidate-fed animals. Temp.: Col. 198, cell 240, F.H. 265. Gas flow 76.3 ml/min. Rel. gain 30, cell volt 750.

FIG. 3. GLC of methyl esters from total lipids of fat-deficient guinea pigs. Temp.: Col. 200, cell 243, F.H. 265. Gas flow 93.7 ml/min. Rel. gain 30, cell volt 750.

FIG. 4. GLC of methyl esters from total lipids of normal animals. Lipids extracted using propanol-chloroform. Temp.: Col. 200, cell 242, F.H. 265. Gas flow 84.9 ml/min. Rel. gain 30, cell volt 750.

lipids) or other components such as phospholipids or cholesterol esters, total lipids from 6 animals killed 4 hours after injection of sodium acetate-1-C^{14} (total activity 1 mc) were examined. The total lipids were extracted in the usual manner and separated into major components on the silicic acid column. Further separation and purification by thin-layer chromatography gave pure fractions of methyl esters, cholesterol esters, triglycerides, free fatty acids and phospholipids. All the fractions, except the methyl esters, were transmethylated(5) and the fatty acid methyl esters were isolated and analyzed by gas-liquid chromatography. The composition and specific activity are shown in Table II.

In order to check the possibility that the methyl esters in the body lipids originated from the fed methyl elaidate, total lipids extracted from fat-deficient guinea pigs that had not been fed any methyl ester, were examined by similar methods. The methyl plus cholesteryl ester fraction, eluted with 2% ether in pentane from the silicic acid column, gave major peaks for palmitate, stearate and oleate when analyzed by gas-liquid chromatography (Fig. 3). The cholesteryl esters have considerably longer retention times on a polyester column compared to the methyl esters and do not interfere when a mixture is analyzed by gas-liquid chromatography. All the methyl ester peaks were identified by comparing the retention times with those of a known mixture. The methyl plus cholesteryl ester fraction, when analyzed by thin-layer chromatography, gave 2 distinct spots which were identified by comparison with known compounds as methyl and cholesteryl esters.

In all previous experiments methanol was used in the extraction mixture. It was thus necessary to ascertain whether methyl esters might be formed when large amounts of methanol are used during homogenization and extraction. Two guinea pigs raised on the usual pellet diet were sacrificed and the blood as well as the body lipids extracted using propanol (instead of methanol) and chloroform. Repeated extractions were made to ensure complete extraction of lipids. By use of the same chromatographic technics it was shown that this fraction also contained methyl esters (Fig. 4). Further confirmation that the methyl esters are not artifacts was obtained from the finding that normal guinea pig liver and adipose tissue lipids, prepared merely by soaking in ethyl ether followed by chromatographic separation and purification, gave major peaks for methyl myristate, palmitate and stearate when examined by gas-liquid chromatography on both the non-polar silicone and polar polyester columns.

Discussion. Previous studies(8,9,10) have shown that regardless of the form fed, labeled fatty acids appear in the lymph, blood and tissues almost entirely as triglycerides and phospholipids and, to a smaller extent, sterol esters. No evidence for absorption of unhydrolyzed esters of monohydric alcohols has been reported(1,2,3).

The present experiments indicate first that methyl esters of certain acids (*e.g.*, elaidic) may be absorbed if they are not readily hydrolyzed in the intestine, and second, that another source of methyl esters may exist.

Although the first finding was not predicted, it is readily understandable. Failure of intestinal enzymes to hydrolyze methyl elaidate was shown by the high activity of this fraction in the intestinal contents sev-

TABLE II. Specific Activity and % Composition of Body Lipid Components.

	Sp. act., dps/mg	%											
		C12	C14	C16	C16:1	C17	C18	C18:1	C18:2	C18:3	C20:1	C20:3	C20:4
Total fatty acids	11.2	—	2.4	28.5	3.0	Trace	12.5	45.6	5.1	.4	.2	.6	1.7
Methyl esters	14.6	.1	.4	27.8	.8	2.2	57.8	7.3	1.6	—	—	—	—
Cholesterol ester fatty acids	22.2	—	3.9	12.8	.9	—	3.2	9.5	.6	.9	—	—	66.3
Triglyceride fatty acids	12.8	—	1.3	33.4	2.11	.7	5.3	55.5	1.7	—	—	—	—
Free fatty acids	21.7	.7	1.6	32.1	4.7	1.4	22.1	29.3	4.36	—	—	—	—
Phospholipid fatty acids	13.2	—	.3	10.0	2.0	.6	33.5	30.6	3.9	.4	.4	2.2	6.1

eral hours after administration.† Moreover, the finding of highly active methyl ester by thin-layer chromatography leaves little doubt that this ester was indeed absorbed and deposited unchanged. It could be postulated that any ester for which hydrolysis is similarly slow could be handled in like manner.

The second finding, the occurrence of methyl esters of the common fatty acids in lipids of animals to which no methyl esters had been given, is not so readily explained. There can be little doubt, from thin-layer and gas-liquid chromatographic evidence, that methyl esters actually exist in the extracts of the tissue lipids. Thin-layer chromatography cleanly separates methyl and cholesterol esters and offers good evidence that the former were present in column chromatographic fractions of the latter. These esters were also identified by gas-liquid chromatography.

The composition of the methyl esters (Table II) is quite different from that of the total fatty acids or triglyceride fatty acids particularly with respect to the ratio of stearate to oleate. If the methyl esters were artifacts formed during extraction or isolation one would expect some similarities in the composition either with total fatty acids or triglyceride fatty acids (major component of total lipids). In fact the composition of methyl esters is different from the fatty acid spectrum of any other lipid component. The specific activity data further support the postulation that the methyl esters could not be artifacts. That they were not artifacts of the isolation technics was further indicated by use of 3 different solvents and methods of extraction. If propyl esters had been formed during propanol extraction, these would have been recognized on gas-liquid chromatography. Moreover, the ether extraction appears to have eliminated this source of alcohols for esterification. No methanol is used during the separation of the cholesterol ester fraction by silicic acid column chromatography.

The knowledge that methyl esters may be absorbed and deposited unchanged permits the possibility of a dietary source of the esters, but a change of diet and the inability to find appreciable quantities of the common

methyl esters in either diet leave this source open to question.

There has been no evidence that methyl esters may be formed in animal tissues. However, McManus, Contag and Olson(11) have reported the presence of endogenous ethanol (but not other alcohols) in various tissues and Sato, Byerrum and Ball(12) have investigated the formation of pectinic acid methyl esters in plant tissues by transmethylation with methionine. It is possible that a similar reaction could occur in the animal body.

These studies show that more work is required to explain the source of the methyl esters reported here. However, they also point to a potentially interesting pathway and show that in experiments in which labeled fatty acids are fed as methyl esters, the separated cholesteryl ester fraction should be examined with care.

Summary. Methyl elaidate-1-C^{14} was fed to fat-deficient guinea pigs. Analysis of the body lipids by gas-liquid and by thin-layer silicic acid chromatography gave conclusive proof of the occurrence of methyl esters. The estimated activity attributed to recovered methyl elaidate compared well with the activity of the fed material. Occurrence of methyl esters in body as well as in blood lipids was conclusively demonstrated in normal pellet-fed animals. By using propanol instead of methanol and also by merely soaking the whole liver or adipose tissue in ethyl ether for extracting lipids it was further shown that the methyl esters are not formed during homogenization and extraction. Distribution of the activity in the various purified lipid components was determined.

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Effect of Lipopolysaccharides of Gram Negative Bacilli in the Rat Litter *in utero.** (27227)

J. B. THIERSCH
Department of Pharmacology, University of Washington School of Medicine, Seattle

Bang(1) described abortions in cattle due to infection with gram negative micro-organisms; Zahl and Bjerknes(9) observed abortions in mice injected with endotoxins of gram negative organisms. The present paper reports systematic studies of the effect of lipopolysaccharides (LS) of gram neg. organisms on the litter of rats. The LS isolated and purified according to Boivin(2), purchased from Difco, included the LS of a gram positive streptococcus. The compounds investigated were isolated from cultures of: *E. typhosa* 901, (Difco #107832) (Table II); *S. marcescens* (Difco #902153 and USPH #Rx-B12555) (Table IV); *B. abortus* Bang (Difco #108539) (Table V); *E. coli*

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