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Studies on the metabolism of *cis,trans* isomers of methyl linoleate and linolenate*

M. L. Blank and O. S. Privett

The Hormel Institute, University of Minnesota, Austin, Minnesota

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SUMMARY

Studies were made of the group composition of the lipids and of the fatty acid components in each class of lipids in the livers of rats fed a fat-free diet or diets supplemented with methyl linoleate (*cis*-9, *cis*-12-octadecadienoate); methyl *cis*-9, *trans*-12-octadecadienoate; and methyl linolenate, which contained 30% of the double bonds in the *trans* configuration (designated as "*cis,trans*"-methyl linolenate because it consisted of a mixture of all-*cis* and *cis,trans* isomers).

The conversion of methyl *cis*-9, *trans*-12-octadecadienoate to an isomer of arachidonic acid, believed to be *cis*-5,*cis*-8,*cis*-11,*trans*-14-eicosatetraenoic acid, and of "*cis,trans*"-methyl linolenate to polyenoic acids containing *trans* unsaturation in rats is reported. The metabolism of *trans* acids in relation to the nutritional requirements of the rat for essential fatty acids is discussed.

4,7,10,13-Eicosatetraenoic acid was found in the liver phospholipids of rats fed a fat-free diet and, to a lesser extent, in the liver phospholipids of a group of rats receiving a supplement of methyl linoleate.

Although *trans* acids are devoid of essential fatty acid activity (1–3), little is known of their fate and influence in the animal body. Holman and Aaes-Jørgensen (2) observed no adverse effect of *trans* acids in the diet of rats, but their studies suggested that these acids might increase the requirement for essential fatty acids (EFA) (4). Mattson (5) concluded, on the basis of nutritional experiments in which EFA-deficient rats were fed mixtures of *cis* and *trans* acids, that *trans* acids exhibited no anti-EFA activity. Dhopeshwarkar and Mead (6) observed that, in contrast to elaidic acid, oleic acid increased the guinea pig's requirement for EFA and concluded that the decline in growth rate was due to competition of oleic acid with linoleic acid as a substrate for the enzymes involved in the conversion of the latter to arachidonic acid. An unusual finding by Dhopeshwarkar and Mead (7) was that methyl elaidate was deposited as such in the tissues of guinea pigs.

We have studied the metabolism of methyl *cis*-9,-*trans*-12-octadecadienoate (designated in what follows as *cis,trans*-linoleate) and methyl linolenate containing *cis,trans* isomers in the EFA-deficient rat.

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EXPERIMENTAL METHODS

Materials. Highly purified methyl linoleate (*cis*-9,*cis*-12) and methyl linolenate were obtained from The Hormel Foundation, Austin, Minn. The methyl linoleate was a natural product obtained from safflower seed oil by physical methods and was >99% pure. Methyl linolenate was prepared via the bromination procedure from linseed oil. Infrared analysis (8) of this preparation of methyl linolenate showed that 30% of the double bonds had the *trans* configuration. Theoretically, the *trans* double bonds could exist in the form of mono-, di-, and tri-*trans* isomers. Thin-layer chromatography (TLC) analysis on silver nitrate-coated plates (9, 10) indicated that this preparation consisted primarily of the all-*cis* isomer, and that the *trans* unsaturation was present primarily in the form of *cis-trans* isomers. The presence of a small amount of the all-*trans* isomer could not, however, be precluded. The mixture is designated as methyl "*cis, trans*"-linolenate.

Methyl *cis,trans*-linoleate was prepared from dehydrated castor oil esters by a combination of fractional distillation and low-temperature fractional crystallization (11). The final product contained less than 0.3% conjugation and was free of methyl *cis,cis*-

linoleate and elaidate as determined chromatographically by the silver nitrate–TLC technique (9, 10). Infrared analysis (8) of this preparation, using pure methyl elaidate as a reference standard, indicated that it consisted of 86% *cis,trans*-linoleate. The assignment of the *trans* double bond to the 12-position is based (11) on the assumption that the *cis,trans* acid is produced by the dehydration of ricinoleic (12-hydroxy-*cis*-9-octadecenoic) acid, the chief constituent of castor oil fatty acids. The major impurities in this preparation were methyl oleate and lesser amounts of saturated fatty esters.

The silicic acid used as adsorbent for TLC in this study was prepared in our laboratory. Commercial products, even those marked "specifically for TLC," invariably contained contaminant organic matter that made them unsatisfactory for preparative TLC. There are many procedures for making silicic acid; the one used here is based on the study of the characteristics of these adsorbents by Wren (12).

Four liters of 40% sodium silicate solution (Sargent Co.) was diluted with 12 liters of distilled water, and the silicic acid was precipitated by the addition of 2 liters of concentrated HCl. The excess acid was removed by washing the precipitate with distilled water. When the washings were neutral to methyl orange, the silicic acid (about 1400 g) was washed three times with about 10 liters of acetone, then three times with about 10 liters of a 1:1 mixture of acetone and diethyl ether, and, finally, twice more with about 5 liters of diethyl ether. The silicic acid was then dried in vacuo, ground with glass marbles (1 cm diam) in a small laboratory ball mill, and sieved through a No. 200 mesh screen. The yield of final product was 1100 g. Reagent grade calcium sulfate (10% by weight) was then mixed with it to serve as a binder.

Methods. Quantitative analysis of the lipid classes was carried out by TLC, followed by densitometry of the charred spots using the general procedure previously described (13, 14). This method is being reported more fully elsewhere (15). Briefly, the lipid classes were separated using 15% diethyl ether and 1.0% acetic acid in petroleum ether (bp 35–60°). The size and density of the charred spots, determined with a Photovolt densitometer (Photovolt Corp., N.Y.), were compared with those of a standard mixture of lipids obtained from The Hormel Foundation. The standard mixture consisted of equal weights of cholesterol, cholesteryl oleate, oleic acid, triolein, and hydrogenated lecithin. The crude phospholipid fraction did not migrate from the origin in these analyses and was estimated by difference.

The fatty acid analysis was carried out on a small amount of each lipid class obtained by preparative TLC. The conditions employed for preparative TLC were the same as those used for analytical separations. Approximately 100 mg of lipid was applied to a 20 x 20-cm silicic acid chromatoplate. The phospholipids remained at the origin; the sterols, free fatty acids, triglycerides, and sterol esters had R_F values of approximately 0.1, 0.3, 0.5, and 0.8, respectively. The phospholipids, triglycerides, and sterol esters were scraped from the chromatoplate in a band of adsorbent and converted to methyl esters by methanolysis in refluxing dry 1 N HCl–methanol for about 2 hr. The crude esters were extracted into petroleum ether, washed with distilled water, dried, and recovered by evaporation of the solvent.

The fatty acids of the phospholipid fractions were analyzed further. The pooled lipid extract from each group of animals was dissolved in a minimum volume of chloroform, and the phospholipids were precipitated with about 20 volumes of acetone. Three precipitations were carried out on each sample. The yield of final product was about 85% on the basis of the lipid class analysis; there was no selective loss of fatty acids, as indicated by a comparison of the analyses of these preparations with those isolated by TLC.

Each isolated phospholipid fraction was heated in refluxing dry 1 N HCl–methanol for about 2 hr. The course of the esterification was followed by TLC. The methyl esters obtained from each group of animals were then fractionated into classes, determined by the degree of unsaturation and geometric isomer composition, by means of $AgNO_3$–TLC (9, 10). In this procedure, the adsorbent was prepared by slurrying 1 part of silver nitrate and 4 parts of silicic acid (containing 10% calcium sulfate) with 10 parts of water. The plates were sprayed with 2,7-dichlorofluorescein and viewed under ultraviolet light. Small amounts of each class of esters from each group of animals were isolated by preparative TLC by this technique for further analysis as illustrated in Fig. 1.

The R_F values of the components of a mixture of methyl esters in this technique depends, to some extent, on their concentrations and explains the reason that the reference compounds in Fig. 1 do not line up exactly with their counterparts in the isolated samples. Nevertheless, the monoenes, dienes, trienes, tetraenes, and other polyenes were separated, as evidenced by analyses of individual spots by gas-liquid chromatography (GLC). Note that D1 refers only to the *trans*-tetraene spot, which is separated from the esters of the *cis,trans*-linoleate group (II, Fig. 1). This spot was identifiable and was scraped from the plate, but

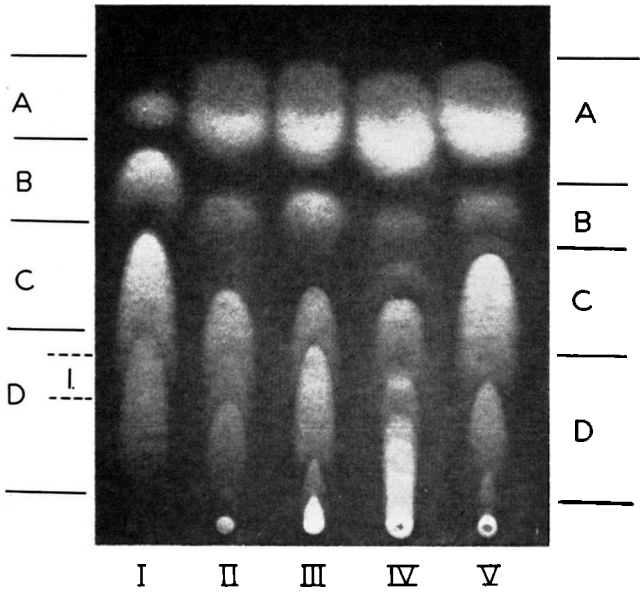

FIG. 1. Silver nitrate–TLC chromatoplate of methyl esters derived from the phospholipids: I, standard mixture of A, methyl oleate; B, methyl linoleate; C, methyl 5,8,11-eicosatrienoate; D, methyl arachidonate; II, group supplemented with methyl *cis*-9,*trans*-12-linoleate; III, group supplemented with methyl linoleate; IV, group supplemented with methyl "*cis,trans*"-linolenate; V, fat-free group. A = saturated and monoenes; B = dienes; C = trienes; D = polyenes; D1 = *trans*-tetraenes in group II.

it could not be obtained free of methyl 5,8,11-eicosatrienoate.

The isolation of the methyl esters was carried out by chromatographing about 150 mg of sample applied in a continuous row of spots on 20 x 20-cm chromatoplates. Each row of spots (detected by 2,7-dichlorofluorescein) was scraped in toto from the plate and recovered by extraction from the adsorbent with freshly distilled ether. Because there was overlapping of some compounds, particularly the two tetraene fractions, D1 and D, from the phospholipids of the *cis*-9,*trans*-12-linoleate group, each concentrate was then rechromatographed. The final concentrates were chromatographed with 10% ether in petroleum ether on silicic acid-coated plates to remove the small amount of spray reagent that accumulated in them. With this solvent system, the spray reagent remained at the base of the plate. Recoveries of the esters from the plates were approximately 90%. The process was repeated until sufficient amounts (about 10 mg) of those components in small concentrations were obtained for infrared, structure, and GLC analyses.

For GLC analyses, an F & M Scientific Corp. Model 609 flame ionization instrument equipped with a 6-ft x $^1/_8$-in. column, packed with Chromosorb W containing 15% ethylene glycol succinate polyester, was

TABLE 1. GROWTH OF ANIMALS

Diet or Supplement	Wt. of Animals		Wt. Gain
	Start of Expt.	End of Expt.	
	g	*g*	*g*
Fat-free	263 ± 21*	272 ± 28.8	7.2 ± 9.5
Methyl *cis*-9,*trans*-12-linoleate	272 ± 20	274 ± 25	2.0 ± 4.3
Methyl *cis*-9,*cis*-12-linoleate	260 ± 11	312 ± 18.6	52.2 ± 14.0†
Methyl "*cis-trans*"-linolenate	264 ± 4.7	307 ± 20	42.8 ± 19.3†

* Standard deviation.
† Significant at 99% probability.

used. The conditions of each analysis are described with the results. The variation from linearity was less than 2% as judged by the analysis of The Hormel Foundation GLC reference mixtures No. 1 (consisting of equal weights of methyl palmitate, stearate, oleate, linoleate, and linolenate) and No. 5 (consisting of equal weights of 16, 18, 20, and 22 normal straight-chain saturated methyl esters).

The structures of unsaturated fatty acids and positional isomer composition of mixtures of polyunsaturated fatty acids were determined by reductive ozonolysis in conjunction with GLC and TLC as previously described (10, 16).

Infrared analysis of the fatty acids isolated in the form of methyl esters from the rat livers was carried out on liquid films with a Perkin-Elmer Model 21 spectrophotometer. The *trans* unsaturation was calculated on the basis of the intensity of the peak at 10.33 μ, using the ester carbonyl band to normalize the values. The absorption of *trans* double bonds was assumed to be constant, independent of position, and equal to that of the *trans* double bonds in a pure sample of methyl elaidate (11).

Nutritional Experiments. Male rats, 23 days old, of the Sprague-Dawley strain, were fed *ad libitum* a fat-free diet of the following composition: 16% vitamin-test casein, 4% α-cellulose, 74% sucrose, 4% Wesson salt mixture, 1% of a mixture of vitamins in casein, and 1% of a mixture of choline chloride in casein (1). Vitamins A and E were mixed into the diet in a diethyl ether solution; the ether was removed by evaporation under reduced pressure.

After 6 months on this diet, the animals exhibited gross symptoms of EFA deficiency. Twenty of the animals were selected for this investigation and divided into four groups of five animals each. One group was continued on the fat-free diet while the other three groups received supplements of 200 mg/animal/day

of methyl linoleate, methyl *cis,trans*-linoleate, and methyl "*cis-trans*"-linolenate.

After 17 days on the supplemented diets, the animals were killed by exsanguination after light ether anesthesia. The livers in each group were pooled and extracted three times with 100 ml chloroform–methanol 2:1 (v/v) in a Servall Omni-Mixer. The solvent was evaporated under reduced pressure nearly to dryness, leaving a crude fatty-aqueous residue that was dissolved in petroleum ether (bp 35–60°) and dried over anhydrous sodium sulfate. The lipids were recovered from the petroleum ether solution by evaporation of the solvent.

RESULTS

Weights of each group of animals at the beginning and the end of the nutritional experiment are presented in Table 1. The animals receiving the *cis,trans*-linoleate and the "*cis,trans*"-linolenate, as well as those on the fat-free diet, exhibited gross signs of EFA deficiency. The dermal symptoms of the animals receiving the methyl *cis,cis*-linoleate were greatly improved, but not completely cured, at the end of the experiment. Even over the short period of 17 days, the growth of the animals receiving the *cis,cis*-linoleate and "*cis,trans*"-linolenate supplements was significantly greater than those on the fat-free diet and those receiving the *cis,trans*-linoleate. These observations are in accord with our previous studies on the nutritional effect of methyl *cis,trans*-linoleate (3) in which this ester exhibited no growth-stimulating properties on EFA-deficient rats.

The lipid and fatty acid analyses of the liver lipids are presented in Table 2, which shows that the polyenoic acids were concentrated in the phospholipids. Methyl esters of the phospholipid fatty acids, fractionated by preparative TLC as described above, were subjected to GLC, the results of which are presented in Fig. 2 and 3.

The dienes and trienes of the *cis,cis*-linoleate and *cis,trans*-linoleate groups, which proved to be 18- and 20-carbon chain esters, were obtained free of other classes, as illustrated in Fig. 2. The all-*cis*-tetraene obtained from the *cis,cis*-linoleate group and from the *cis,trans*-linoleate group (D, Fig. 2) were also uncontaminated by other classes of unsaturated esters. The all-*cis*-tetraene from the fat-free group previously reported (10) was contaminated with triene (methyl 5,8,11-eicosatrienoate). This contamination, which resulted because of the large concentration of methyl 5,8,11-eicosatrienoate in the sample, made the purification of the tetraene extremely difficult. Purification of *trans*-tetraene (D1, Fig. 1) in the esters of the *cis,trans*-linoleate group was also difficult because its R_F value was only slightly lower than that of methyl 5,8,11-eicosatrienoate. In fact, it was recovered from the trailing edge of the methyl 5,8,11-eicosatrienoate

TABLE 2. ANALYSIS OF RAT LIVER LIPIDS

Diet or Supplement →	Fat-Free			Methyl *cis*-9,*trans*-12-Octadecadienoate			Methyl *cis*-9,*cis*-12-Octadecadienoate			Methyl "*cis,trans*-" Linolenate*		
	SE†	TG	PL	SE	TG	PL	SE	TG	PL	SE	TG	PL‡
% in Total Lipid →	7.5	20.7	62.3	3.6	33.0	54.5	3.0	30.5	54.3	1.9	28.1	58.4
						% of fatty acids						
14:0	1.1	1.6	0.3	0.6	1.6	tr.	2.5	1.5	tr.	2.7	1.8	tr.
16:0	26.0	35.1	21.6	23.9	39.0	22.2	35.8	33.4	23.8	33.8	36.8	29.2
16:1	21.1	13.4	8.5	15.2	10.2	4.6	7.9	15.2	5.4	9.9	12.5	7.5
18:0	3.3	2.7	22.6	5.6	2.3	25.0	5.8	2.1	24.0	7.3	2.5	24.4
18:1	46.9	47.2	24.2	52.7	46.9	18.8	48.0	47.7	18.6	46.3	46.4	21.6
18:2	1.6		1.5	2.0		2.8	tr.	tr.	8.3	tr.		1.0
18:3											tr.	2.0
20:3			18.1			19.0			4.7			5.2
20:4			3.1			7.6			15.2			0.8
20:5												8.3
% in Total Lipid	FFA§ = 2.5 Sterol = 7.0			FFA = 2.2 Sterol = 6.8			FFA = 0.8 Sterol = 11.4			FFA = 0.8 Sterol = 10.8		

* Contained 30% of the double bonds in the *trans* configuration and consisted primarily of all-*cis* and *cis.trans* isomers.
† SE = sterol esters, TG = triglycerides, PL = phospholipids.
‡ Contains traces of 22:5 and 22:6 also.
§ FFA = free fatty acids.

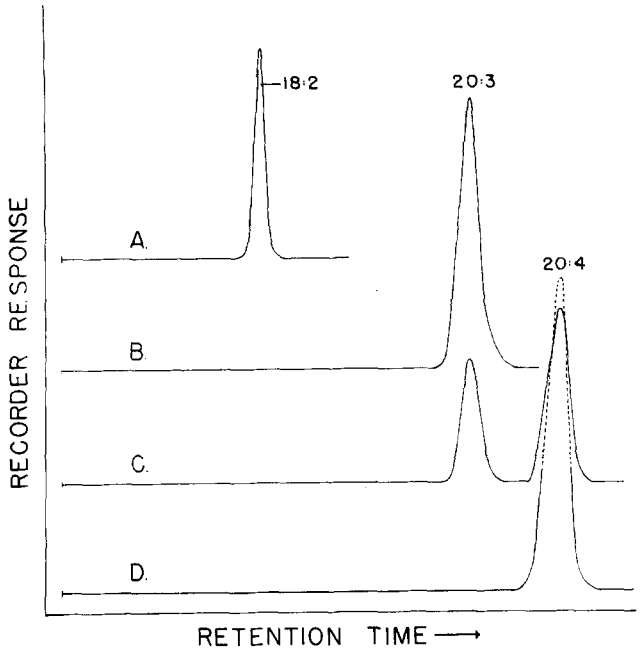

FIG. 2. GLC analyses of fractions isolated by $AgNO_3$–TLC from methyl *cis*-9, *trans*-12-linoleate-fed animals: A, diene; B, triene, C, polyene (D1, Fig. 1); D, polyene. Conditions: column 6′ x 1/8″, 15% ethylene glycol succinate polyester on Chromosorb W at 180°.

(Fig. 1). No attempt was made to separate the individual components of the higher polyenoic esters isolated from the *"cis,trans"*-methyl linolenate group; GLC analysis (Fig. 3) showed that the mixture contained tetra-, penta-, and hexaenoic esters, the major component being methyl eicosapentaenoate.

Figure 4 shows the infrared spectra, in the region of *trans* absorption (10.33 μ), of the esters isolated by $AgNO_3$–TLC. These results demonstrated that the methyl *cis,trans*-linoleate had been converted to a *trans*-eicosatetraenoic acid (D1, Fig. 1; C, Fig. 2; and C, Fig. 4) and that the *"cis,trans"*-methyl linolenate

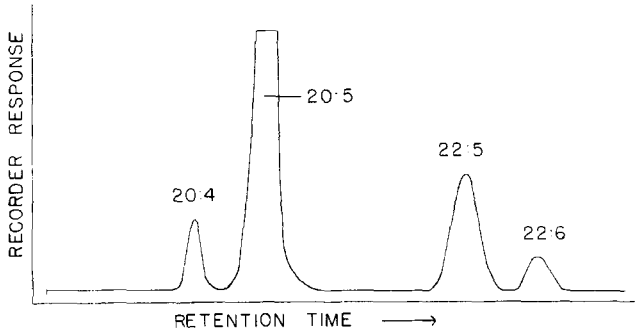

FIG. 3. GLC analysis of the polyene fraction isolated by $AgNO_3$–TLC from the animals fed methyl *"cis,trans"*-linolenate. Conditions: column 6′ x 1/8″, 15% ethylene glycol succinate polyester on Chromosorb W at 200°.

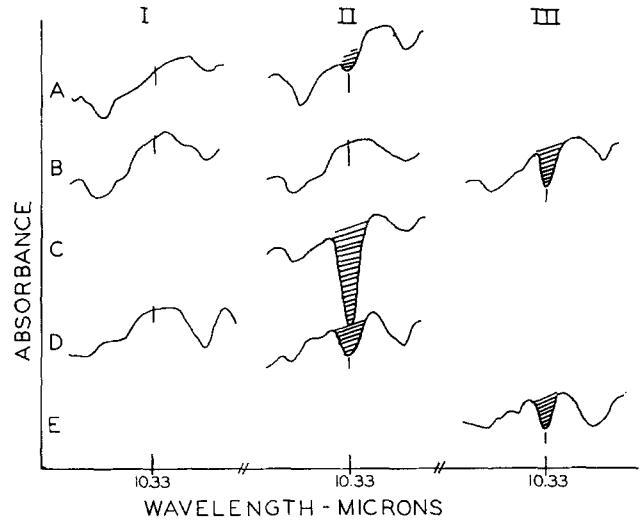

FIG. 4. Infrared spectra in the *trans* region of the spectrum: I, group fed methyl linoleate; II, group fed methyl *cis*-9,*trans*-12-linoleate; III, group fed methyl *"cis,trans"*-linolenate. A, dienes; B, trienes; C, *trans*-tetraene (D1, Fig. 1); D, tetraene; E, polyenes of the methyl *cis,trans* linolenate group.

was converted to polyenoic acids consisting mainly of eicosapentaenoic acid (D, Fig. 1; Fig. 3). The esters isolated from the animals fed *cis,cis*-linoleate were also analyzed (Fig. 4) to demonstrate that the *trans* unsaturation in the polyenoic acids was not due to artifacts. The validity of the analysis was also demonstrated by the absence of *trans* unsaturation in methyl eicosatrienoate isolated from the animals receiving methyl *cis,trans*-linoleate (BII, Fig. 4).

The infrared absorption of methyl eicosatetraenoate (D1, Fig. 1), isolated from the *cis,trans*-linoleate group of animals, indicated a content of one *trans* double bond per molecule. It was assumed that the eicosatrienoate impurity present in this fraction did not contain any double bonds of the *trans* configuration. This assumption appears to be valid since there was no *trans* unsaturation in the major part of the methyl 5,8,11-eicosatrienoate (BII, Fig. 4).

Figure 5 shows the GLC analyses of the fragments obtained by reductive ozonolysis (10, 16) of the tetraene fraction of the group fed *cis,cis*-linoleate and the *trans*-tetraene fraction of the group fed *cis,trans*-linoleate. These results showed that the double bonds of both of the major eicosatetraenoic acids were in the 5,8,11,14 positions. In addition, both of these fractions contained a small amount of 4,7,10,13-eicosatetraenoate, as evidenced by the presence of heptanoic aldehyde and methyl butanoate-4-al (peaks 3 and 4, respectively, Fig. 5). Corresponding results for the fat-free group of animals, which have been previously reported (10), are presented in the lower half of Fig.

6. The 4,7,10,13-eicosatetraenoic acid makes up a larger proportion of the tetraene in the liver phospholipids of the fat-free group of animals, as shown by the larger size of the peaks corresponding to heptanoic aldehyde and methyl butanoate-4-al relative to those of hexanoic aldehyde and methyl pentanoate-5-al, respectively.

The results in the upper part of Fig. 6 show that the 18:2 acids from liver phospholipids of the animals in the fat-free group contained appreciable amounts of the 8,11 isomer of linoleic acid, presumably a precursor of 4,7,10,13-eicosatetraenoic acid.

DISCUSSION

The detection of *trans* double bonds in eicosatetraenoic and other polyenoic acids suggests that methyl *cis,trans*-linoleate and methyl *"cis,trans"*-linolenate undergo the same transformations as their all-*cis* isomers. Since the *trans*-eicosatetraenoic acid is presumably synthesized by elongation of methyl *cis*-9,-*trans*-12-linoleate, its *trans* double bond is probably in the 14 position. The *trans* double bonds in the polyenoic acid fraction of the group of animals on the *"cis,trans"*-methyl linolenate diet are probably, for the most part, located in the eicosapentaenoic acid because this acid is the major product of the metabolism of linolenic acid (Fig. 3) (17). The positions of the *trans* double bonds in methyl *"cis,trans"*-linolenate were unknown, so that it is obviously impossible to predict the positions of the *trans* double bonds in the eicosapentaenoic acid.

Positional isomer compositions of monoenoic, dienoic, and trienoic acids, synthesized in rats on a fat-free diet and on diets supplemented with linoleic acid, have been well established (18, 19, 20). Klenk and Oette (21) have also presented evidence for the occurrence of 4,7,10,13-eicosatetraenoic acid in the livers of animals receiving a fat-free diet. This isomer of arachidonic acid is apparently synthesized from palmitoleic acid. The small amount of 4,7,10,13-eicosatetraenoic acid in the linoleate-fed animals is probably a carry-over from the fat-deficient state and would be reduced to a smaller concentration in the course of a longer period of supplementation.

The inability of methyl *cis,trans*-linoleate to cure an EFA deficiency apparently does not arise from the inability of chain-lengthening enzymes to use this compound as a substrate in the same manner as *cis,cis-*

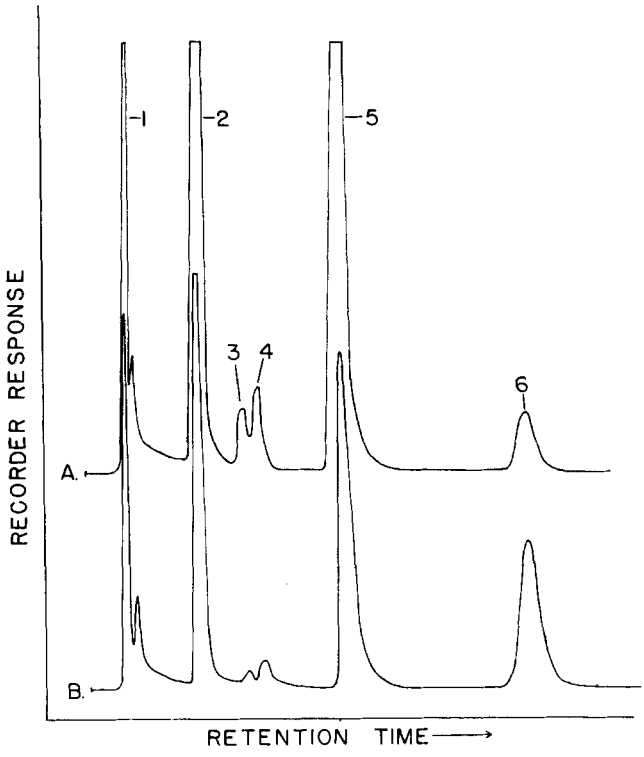

FIG. 5. GLC of fragments in the structural analysis of: A, methyl eicosatetraenoates from the animals fed methyl linoleate; B, methyl *trans*-eicosatetraenoate (D1, Fig. 1) from the group fed methyl *cis,trans*-linoleate. 1, malonaldehyde and artifact; 2, C_6 aldehyde; 3, C_7 aldehyde; 4, methyl butanoate-4-al; 5, methyl pentanoate-5-al; 6, C_9 aldehyde (from methyl 5,8,11-eicosatrienoate impurity). Conditions: column 12′ x 1/8″, 30% silicone on Chromosorb W at 150°. Solvent, methyl decanoate.

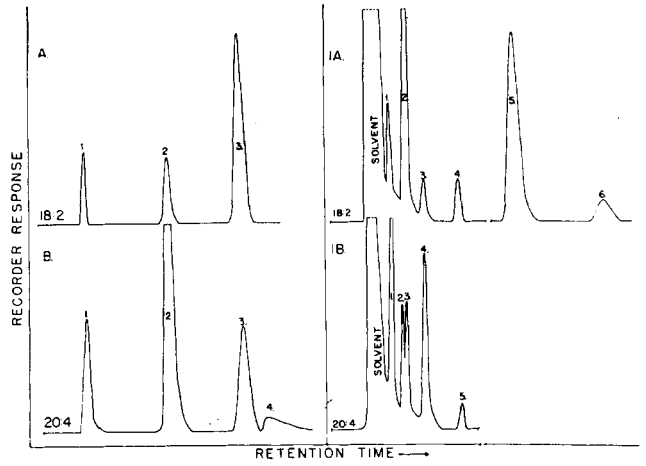

FIG. 6. Structural analysis of the 18:2 and 20:4 fatty acids isolated from fat-free animals. Left: 12′ x 1/4″ column of 20% silicone on Chromosorb W at 125°; solvent, methyl decanoate. Right: same column at 200°; solvent, methylene chloride. *18:2, A:* 1, malonaldehyde; 2, C_6 aldehyde; 3, C_7 aldehyde. *1A:* 1, C_6 aldehyde; 2, C_7 aldehyde; 3, methyl pentanoate-5-al; 4, C_9 aldehyde; 5, methyl octanoate-8-al; 6, methyl nonanoate-9-al. *20:4, B:* 1, malonaldehyde; 2, C_6 aldehyde; 3, C_7 aldehyde; 4, methyl butanoate-4-al. *1B:* 1, C_6 aldehyde; 2, C_7 aldehyde; 3, methyl butanoate-4-al; 4, methyl pentanoate-5-al; 5, C_9 aldehyde.

linoleic acid. It seems that *trans* isomers of arachidonic acid fail to perform or be utilized in essential functions. These studies show that part of the all-*cis* or EFA-active arachidonic acid might be replaced by isomeric *trans* forms of arachidonic acid on the consumption of *cis,trans*-linoleic acid. The extent of replacement of active arachidonic acid by the inactive *trans*-eicosatetraenoic acid will obviously depend on a number of factors. Should *cis,trans*-linoleic acid compete effectively with *cis,cis*-linoleic acid as a substrate for the enzymes involved in the synthesis of arachidonic, it could conceivably be harmful in the diet.

Mattson (5) found that *trans* isomers of ethyl linoleate, fed to EFA-deficient rats together with ethyl linoleate, did not interfere with the utilization and growth-stimulating properties of the latter. Since the present studies show, however, that the geometrical isomers can be elongated to *trans* polyunsaturated acids, it is possible that *prolonged* administration of such isomers, even in the presence of adequate supplies of linoleate, might have an adverse effect.

In contrast to methyl elaidate, which is deposited unchanged in the tissues of guinea pigs (7), there was no evidence of the presence of methyl esters in the liver lipids of the rats in our experiments. Furthermore, no methyl octadecadienoate could be detected in the feces of animals receiving the methyl *cis,trans*-linoleate supplement. Thus, the metabolism of methyl *cis,trans*-linoleate appeared to be similar to that of methyl *cis,cis*-linoleate, except perhaps for quantitative aspects. In the latter regard, it was noted that methyl *cis,trans*-linoleate was not nearly as effective in lowering the triene to tetraene ratio (22, 23) as methyl *cis,cis*-linoleate. Furthermore, the percentage accumulation of dienoic and tetraenoic acids in the liver phospholipids of the animals receiving the methyl *cis,trans*-linoleate supplement was considerably lower than in those receiving the methyl *cis,cis*-linoleate. This difference cannot be explained at present. It is possible, in view of the studies of Dhopeshwarkar and Mead (7), that a portion of the methyl *cis,trans*-linoleate is reduced in the animal body. Another possibility is that the methyl *cis,trans*-linoleate is catabolized more readily than methyl *cis,cis*-linoleate. These aspects of the metabolism of *trans* fatty acids are currently under investigation in our laboratory.

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