Studies on the Metabolism of Linoelaidic Acid in the Essential Fatty Acid-Deficient Rat[1]

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Abstract

Male weanling rats of the Sprague-Dawley strain were made essential fatty acid (EFA)-deficient by feeding them a fat-free diet for five months. The animals were then fed a supplement of methyl *trans*-9,*trans*-12-octadecadienoate (methyl linoelaidate), as 5% of the dietary calories (approximately 400 mg/animal/day) for 19 days, and killed by exsanguination. The composition of the liver, kidney, epididymal and plasma lipids was determined and compared with that obtained from EFA-deficient rats given oral supplements of methyl *cis*-9,*cis*-12-octadecadienoate (linoleate) and methyl *cis*-9,*trans*-12-octadecadienoate. Linoelaidic acid was deposited in the phospholipids, sterol esters and triglycerides in all of the tissues examined. Isolation analysis of the fatty acids showed no evidence that linoelaidic acid was converted to higher polyunsaturated fatty acids in the EFA-deficient rat.

Introduction

THE INABILITY of *trans* isomers of linoleic acid to cure an EFA deficiency in the rat has been well documented (1,2,3,4,5,6). Not so clear is the metabolism of these compounds. Studies by Holman and Aaes-Jørgensen (3) indicated that unnatural *trans* acids were deposited in the tissues of rat, and appeared to worsen the condition of animals on a fat-free diet. These observations suggested that *trans* acids may increase the requirements for essential fatty acids (7). Dhopeshwarker and Mead found that methyl elaidate was deposited unchanged in the tissues of guinea pigs (8), and that it had no adverse effect on the animals (9). Mattson (6) found no adverse effect of *trans* isomers of ethyl linoleate fed in mixtures with all-*cis*-linoleate to rats. We demonstrated that whereas methyl *cis*-9,*trans*-12-octadecadienoate neither cured an EFA deficiency (5), nor served as a substrate for lipoxidase (10), it was converted to higher polyunsaturated fatty acids (11).

The work reported here shows that in contrast to *cis*-9,*trans*-12-octadecadienoic acid, *trans*-9-*trans*-12-octadecadienoic (linoelaidic) acid is not converted to higher polyunsaturated fatty acids in the animal body.

Experimental

Materials. Highly purified methyl *cis*-9,*cis*-12-octadecadienoate (hereafter designated as *cis,cis*-linoleate) was obtained from The Hormel Institute. This compound was prepared from safflower seed oil by physical methods and was >99% pure. Methyl *trans*-9,*trans*-12-octadecadienoate (linoelaidate) was prepared by elaidinization of methyl *cis,cis*-linoleate according to the procedure described by McCutcheon et al. (12). The crude preparation of methyl linoelaidate was dissolved in petroleum ether and purified by passing it through a 2-in. thick pad of silicic acid in a Buchner funnel, followed by about five recrystallizations of the product from acetone at ca. −20C. The product was then distilled through a Podbielnaik Hyper-Cal column at 3 mm pressure. IR analysis of the final product by the AOCS Official and Tentative Method (13) gave 1.94 times the absorptivity of methyl elaidate at 10.33 μ. No isomeric fatty acid impurities could be detected in this preparation by thin-layer chromatography (TLC) on silver nitrate coated plates (15,16). Also, no fatty acids differing in degree of unsaturation or chain length could be detected in this preparation by gas-liquid chromatography (GLC). Structural analysis of the final preparation (15) showed that the double bonds resided, exclusively, in the 9 and 12 positions.

The silicic acid used in this work was prepared in our laboratory as previously described (14); commercial preparations of this absorbent were contaminated with organic matter which created a problem when TLC was used for preparative purposes. The pure reference lipids used in this study were obtained from The Hormel Institute.

Methods. Fatty acid composition was determined with an F & M Scientific Corp. Model 609 flame ionization instrument equipped with a 6 ft x ¼ in. column, packed with Chromosorb W containing 15% ethylene glycol succinate polyester at 185C. The variation from linearity in the analysis of standard reference mixtures of long-chain methyl esters obtained from The Hormel Institue was less than ± 2 percentage unit. Thus, percent composition was calculated on the basis of the proportion of the peak areas.

Quantitative analysis of the major lipid classes was carried out by TLC as previously described (11, 14). The fatty acid composition of the lipid classes was determined on material isolated by TLC as follows. Approximately 100 mg lipid was applied in a row of spots on a 20 x 20 cm silicic acid chromatoplate. The components were separated with 15% diethyl ether in petroleum ether (bp 35–60C) containing 1% acetic acid. The phospholipids and related compounds remained at the origin on the plate; 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 phopholipids, triglycerides and sterol esters were scraped from the chromatoplate in a band of adsorbent and converted to methyl esters. The methanolysis of these compounds was carried out by heating them(with adsorbent) in refluxing dry 1N HCl-methanol for about two hr. The crude esters were extracted into petroleum ether, washed with distilled water, dried over anhydrous sodium sulfate, filtered and recovered by evaporation of the solvent. GLC analysis was carried out as described above.

A Perkin-Elmer Model 21 IR spectrophotometer was used for the IR analyses. *Trans* unsaturation was calculated from the intensity of the peak at 10.33 μ with reference to pure methyl elaidate (10% solution in CS_2).

Nutritional Experiment. Male weanling rats of the Sprague-Dawley strain were fed *ad libitum* for five months on a fat-free diet of the following com-

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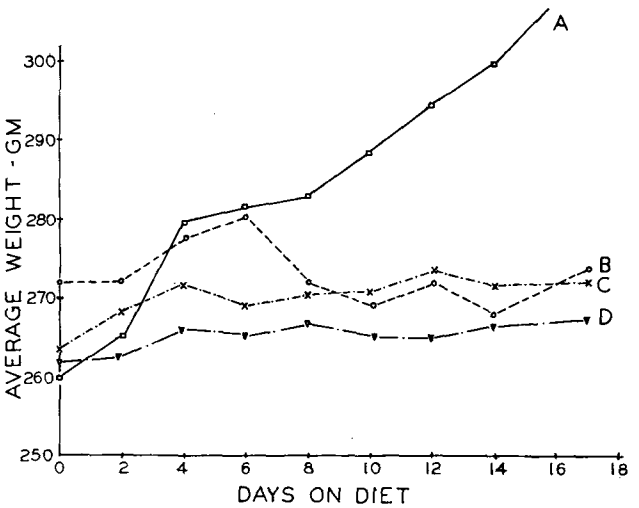

FIG. 1. Growth rate of groups of animals during the supplementation period with: A. *cis,cis*-linoleate; B. *cis,trans*-linoleate; C. fat-free group; and D. linoelaidate.

position: vitamin test casein—16%, sucrose—74%, α-cellulose—4%, Wesson salt mixture—4%, 1% of a mixture of vxitamins 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. A group of five animals was given a supplement of methyl linoelaidate consisting of 5% of the total calories (approximately 400 mg/animal/day) in place of sucrose for 19 days. In an earlier experiment, which has been reported in part (11), two groups of 5 EFA-deficient animals of ca. the same wt and ages, reared on the same diet as in the present experiment, were given oral administrations of 200 mg/day *cis,cis*-linoleate and methyl *cis*-9,*trans*-12-octadecadienoate (hereafter designated as *cis,trans*-linoleate), respectively, for 17 days, and another group was maintained on a fat-free diet to serve as a control. The analyses of the tissue lipids of these animals are reported here for comparison with those of the linoelaidate supplemented group.

All animals were killed by exsanguination and the livers, kidneys and epididymal pads, as well as samples of plasma, were excised for analyses of the lipids. The lipid of each of the pooled tissues was extracted with chloroform-methanol (2:1, v/v) in a Servall Omni-Mixer and recovered as follows. Most of the methanol and chloroform was evaporated leaving an aqueous-fatty residue. This residue was extracted with low boiling petroleum ether (35–60C); the solution was then washed with distilled water, dried over anhydrous sodium sulfate and filtered. The fat was recovered by evaporation of the solvent. The plasma lipids were obtained from the clear supernatant of the centrifuged heparin-treated blood by extraction with alcohol-ether (2:1, v/v) and recovered in a similar manner.

TABLE I

Analysis of Tissue Lipid Classes*

	Liver			
	A	B	C	D
Steryl esters	3.0	3.6	5.2	7.5
Triglycerides	30.5	33.0	34.3	20.7
Free fatty acids	0.8	2.2	.....	2.5
Phospholipids	54.3	54.4	48.4	62.3
Sterols	11.4	6.8	12.0	7.0
	Kidney			
	A	B	C	D
Steryl esters	1.7	1.5	6.4	6.2
Triglycerides	20.9	19.6	20.4	28.0
Free fatty acids	1.4	1.3	.....	6.2
Phospholipids	66.6	65.5	60.0	43.7
Sterols	9.4	12.1	13.2	15.9
	Plasma			
	A	B	C	D
Steryl esters	14.6	16.4	15.3	20.0
Triglycerides	11.7	15.6	13.2	18.7
Free fatty acids	2.9	2.2	3.0	3.3
Phospholipids	58.0	57.0	62.4	51.0
Sterols	12.8	8.8	6.1	7.0

A - *cis,cis*-linoleate group.
B - *cis,trans*-linoleate group.
C - *trans,trans*-linoleate group.
D - Fat-free diet group.
* Average of triplicate analyses on pool samples of lipid.

Results

Figure 1 shows a comparison of the growth rates of the groups of animals during the supplmentation period. The growth of the *cis,cis* group was significantly greater than the fat-free and the *cis,trans*-linoleate supplemented group. Methyl linoelaidate

TABLE II

Fatty Acid Composition of Lipids*
(% by wt)

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:3	20:4
Liver phospholipids									
A	tr.	23.8	5.4	24.0	18.6	8.3	.....	4.7	15.2
B	tr.	22.2	4.6	25.0	18.8	2.8	.....	19.0	7.6
C	tr.	17.3	10.1	18.9	33.0	7.3	1.5	10.1	1.8
D	0.3	21.6	8.5	22.6	24.2	1.5	.....	18.1	3.1
Liver triglycerides									
A	1.5	33.4	15.2	2.1	47.7	tr.	.....	.....	.....
B	1.6	39.0	10.2	2.3	46.9	.....	.....	.....	.....
C	1.5	29.9	13.9	2.3	48.6	2.9	.....	.....	.....
D	1.6	35.1	13.4	2.7	47.2	.....	.....	.....	.....
Epididymal fat									
A	2.7	29.6	20.5	1.7	44.3	1.2	.....	.....	.....
B	tr.	29.6	17.7	2.5	50.2	.....	.....	.....	.....
C	1.8	23.0	14.1	1.1	56.3	3.7	.....	.....	.....
D	2.2	32.0	16.7	3.2	46.0	.....	.....	.....	.....
Liver sterol esters									
A	2.5	35.8	7.9	5.8	48.0	tr.	.....	.....	.....
B	0.6	23.9	15.2	5.6	52.7	2.0	.....	.....	.....
C	0.8	20.4	23.0	3.0	39.8	11.3	0.7	.....	.....
D	1.1	26.0	21.1	3.3	46.9	1.6	.....	.....	.....
Kidney lipid									
A	1.2	24.7	6.4	13.6	32.3	7.8	tr.	1.2	12.8
B	1.1	25.4	7.5	14.4	29.5	5.0	.....	8.3	9.0
C	0.7	21.8	7.5	11.0	40.0	7.0	.....	9.1	2.9
D	0.5	20.8	6.1	15.0	33.6	1.6	.....	15.1	7.3

A - *cis,cis*-linoleate group.
B - *cis,trans*-linoleate group.
C - *trans,trans*-linoleate group.
D - Fat-free diet group.
* Average of triplicate analyses on pooled methyl esters.

TABLE III

Fatty Acid Composition of Plasma Lipids*
(% wt)

	A			B			C			D		
	SE	TG	PL	SE	TG	PL	SE	TG	PL	SE	TG	PL
14:0	0.4	1.7	0.5	0.6	1.8	0.7	tr.	2.5	0.2	0.3	1.2	0.5
16:0	7.8	30.6	29.6	10.0	33.6	21.6	11.9	23.4	19.6	7.9	30.0	27.1
16:1	17.0	10.7	3.3	19.4	10.8	5.4	29.0	7.9	5.3	24.3	12.8	4.1
18:0	0.6	3.0	18.5	1.3	3.2	21.0	0.6	9.4	19.7	0.4	2.0	17.2
18:1	18.7	50.5	21.0	30.0	47.7	23.0	39.2	37.8	32.0	27.4	54.0	25.4
18:2	11.7	3.5	6.3	7.3	2.9	6.4	4.9	19.0	12.0	6.1	tr.	.....
18:3	1.4	.....	.....	1.6	.....	.....	1.6	.....	.....	2.1	.....	.....
20:3	10.3	.....	8.9	17.7	.....	13.9	12.8	.....	11.2	28.2	.....	23.9
20:4	32.1	.....	11.9	12.5	.....	8.0	tr.	.....	.....	3.3	.....	1.8

SE = Steryl esters; TG = Triglycerides; PL = Phospholipids.
A - *cis,cis*-linoleate group.
B - *cis,trans*-linoleate group.
O - *trans,trans*-linoleate group.
D - Fat-free diet group.
* Average of triplicate analyses on pool samples of lipid.

gave no improvement in the condition of the animals, nor did it promote growth.

Supplementation of EFA-deficient rats with the isomers of linoleate for even a short period of 17–19 days produced some effects on the relative amounts, as well as the fatty acid composition of the major lipid classes of the tissues, as shown in Tables I,II and III. The results of the analysis of the liver lipids of the *cis, cis*-linoleate, *cis,trans*-linoleate and fat-free groups have been previously reported and are presented here for comparison with those on the other tissues, as well as with the linoelaidate supplemented group of animals.

The main influence of the supplements on the lipid classes was an increase in the triglycerides, and a decrease in the phospholipids. The sterol level of the *cis,cis*-linoleate and linoelaidate groups was higher than that of the fat-free and *cis,trans*-linoleate groups. The linoleate isomers gave a reduction of the sterol esters and triglycerides, and an increase in the phospholipids in the kidney tissues. There was a slight decrease in the level of the triglyceride and sterol esters, and an increase in the phospholipids in the plasma lipids of the animals receiving the supplements. In general, the effect of the *cis,trans*- and *trans,trans*-octadecadienoate isomers on the lipid class composition of the tissues was the same as that of the natural *cis,cis* isomer.

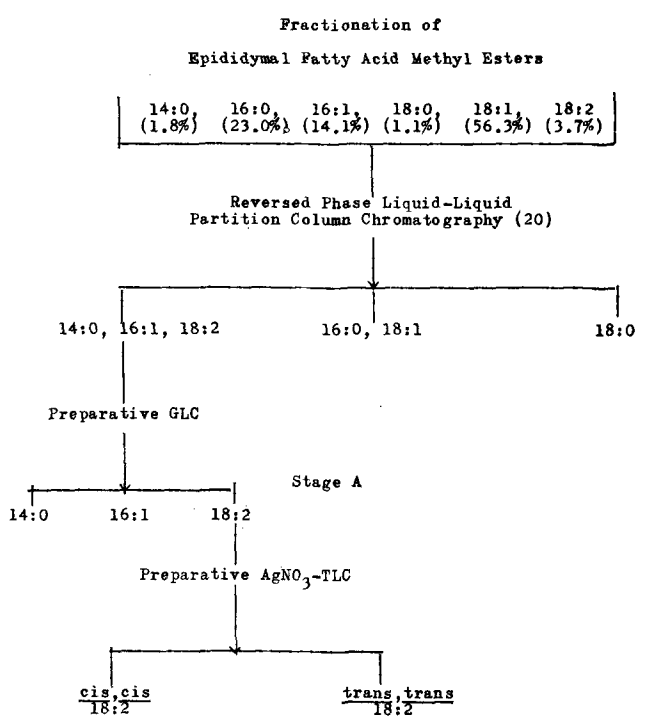

FIG. 2. Fractionation procedure for the isolation of 18:2 acids (as methyl esters).

The fatty acid composition analyses of the major lipid classes of the tissue lipids of the four groups of animals are shown in Tables II and III.

Although there were some variations in the proportions of the saturated and monounsaturated fatty acids, the main influence of the linoleate supplements was on the polyunsaturated fatty acid composition. *Cis,cis*-linoleate decreased the 20:3 and increased the 20:4 acids as expected from the observations of Holman and co-workers (17,18,19). The 20:4 acid content was increased in the animals receiving the *cis, trans*-linoleate, mainly as a result of the conversion of this acid to tetraene (11). The methyl linoelaidate supplement lowered the 20.3 content; the 20.4 content was not raised, however, and no conversion of linoelaidic acid to higher polyunsaturated fatty acids appeared to take place. The 18:2 acids were higher in all tissues of the animals receiving the linoleate isomers as expected.

Charactrization of the 18:2 and 20:4 acids in the tissue lipids of the linoelaidate group. Using the information on the fatty acid composition as a guide, the 18:2 and 20:4 acids were isolated from the linoelaidate group of animals for further characterization. The isolation of the 18:2 acid was carried out from the epididymal fat because of the small amount of fat in the other tissues. The general procedure employed for the isolation of the 18:2 acids is shown in Figure 2.

Liquid-liquid partition (20) (Fig. 2) was used mainly to isolate the 18:2 quantitatively from the 18:1 fraction. Since the 18:1 was present in roughly 19 times the concentration of the 18:2, other methods, including GLC, could not effect this separation as efficiently as liquid-liquid partition. The critical pairs 14:0 and 16:1, which contaminated the 18:2 fraction, were readily separated by preparative GLC. Preparative GLC was carried out on 30 mg amounts of sample with a 10 ft x 3/8 in. column of 15% ethylene glycol succinate polyester at 185C, in the instrument described above, equipped with an F & M Scientific Corp. stream splitter inserted between the column and detector. As the fractions emerged from the splitter, they were collected in small tubes of cold (−10C) chloroform. Yields of ester of the order of 90% were obtained by this technique. The esters were recovered from the chloroform and purified from column bleed, in particular, by TLC on 20 x 20 cm silicic acid coated plates with 7% diethyl ether in petroleum ether. TLC analysis with $AgNO_3$ coated plates (15) of the 18:2 acid fraction isolated

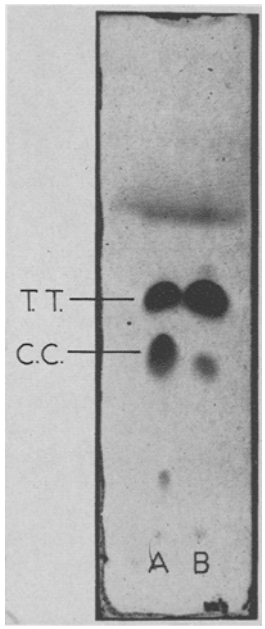

FIG. 3. Chromatoplate of the TLC analysis of the total 18:2 acids (in the form of methyl esters) isolated from the epididymal fat (Stage A, Figure 2) of animals on the linoelaidate supplemented diets by $AgNO_3$-TLC. A = standard of methyl *trans*-9,*trans*-12-octadecadienoate (TT), and methyl *cis*-9,*cis*-12-octadecadienoate (CC). B = sample of 18:2 fraction of epididymal fat.

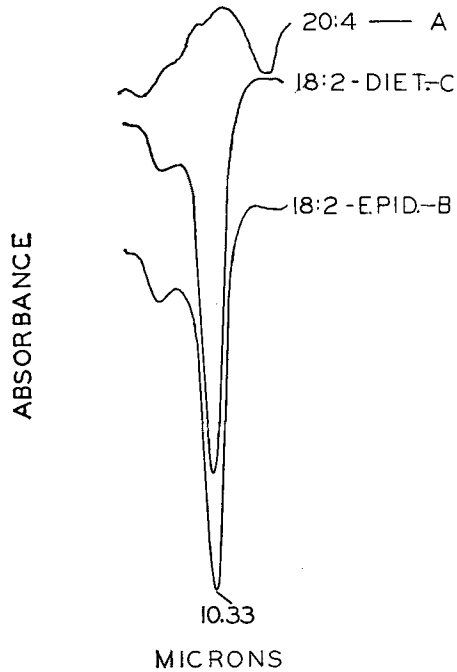

FIG. 4. IR spectra (10% solution in CS_2). A, 20:4 acids (as methyl esters) isolated from the pooled esters of kidney and liver lipids of the linoelaidate group. B, linoelaidic acid (as methyl esters) isolated from the epididymal fat of the linoelaidate group of animals. C, dietary methyl linoelaidate.

at this point is shown in Figure 3. Quantitative analysis of this preparation by densitometry of the charred spots (14) indicated that it consisted of 79.5% *trans,trans*-18:2.

The small amount of *cis,cis*-18:2 in this preparation was separated by preparative TLC with silver nitrate coated plates as previously described (11,15). The intensity of the band at 10.33 μ in the IR spectrum of the final preparation of 18:2 was 1.92 times that given by methyl elaidate (Fig. 4) indicating that it was essentially pure *trans,trans* diene.

Structural analysis of this ester by reductive ozonolysis (15) showed that the double bonds resided in the 9 and 12 positions. These results showed that 79.5% of the 18:2 acids of the epididymal fat of the animals in the *trans,trans*-linoleate group consisted of linoelaidic acid. No effort was made to determine the structures of the *cis,cis*-18:2 acids in the tissue lipids. However, they should consist of a mixture of *cis*-9,*cis*-12- and *cis*-8,*cis*-11-octadecadienoic acids, the latter acid being synthesized from palmitoleic acid.

Evidence that the 18:2 acids in the other tissue lipids of this group of animals also contained linoelaidic acid was obtained by a combination of GLC and IR analyses of the methyl esters. The total esters were fractionated by preparative TLC with $AgNO_3$ coated plates, as illustrated in Figure 5, which shows a chromatoplate with the appropriate standards of the methyl esters of the epididymal fat. The band corresponding to the standard methyl oleate (2, Fig. 5) contained not only methyl oleate, but also *trans,trans*-linoleate, because these compounds cannot be separated by this technique as illustrated in Figure 6. Although it is not so evident from Figure 6, the fraction containing the *trans, trans*-octadecadienoate (2, Fig. 5) can also be readily separated from methyl elaidate, as well as from methyl *cis,cis*-linoleate by this technique. The amount of *trans,trans*-linoleate in fraction 2, Figure 5 (and hence the original lipid) can be readily determined by GLC. If all the 18:2 in this fraction consists of *trans,trans*-18:2, the GLC analysis for 18:2 should agree with the IR spectral analysis of this fraction for *trans,trans*-18:2. The results of the analysis of

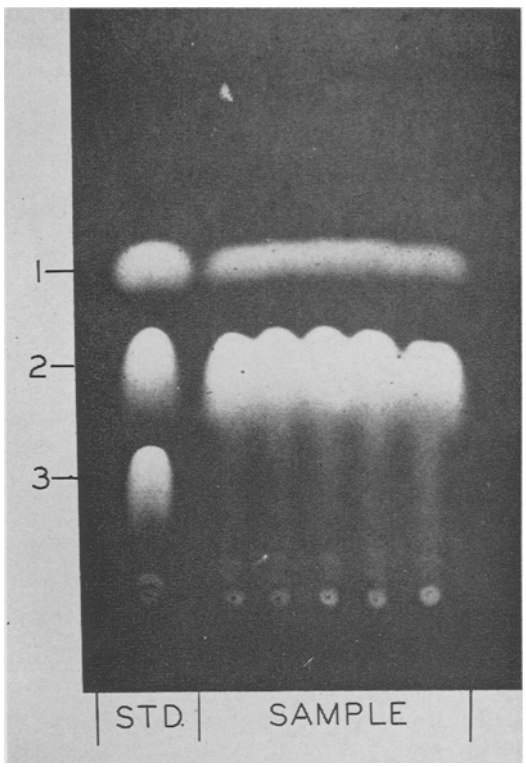

FIG. 5. Chromatoplate of the fractionation of the methyl esters of the epididymal fat by $AgNO_3$-TLC. Standard 1, methyl palmitate; 2, methyl oleate; 3, methyl *cis*-9,*cis*-12-linoleate. Sample: 1, saturates; 2, *cis*-monoenes and *trans, trans*-dienes. Spots are made visible by spraying them with 2,7-dichlorofluorescein and photography under UV light. Solvent: 20% ethyl ether in petroleum ether.

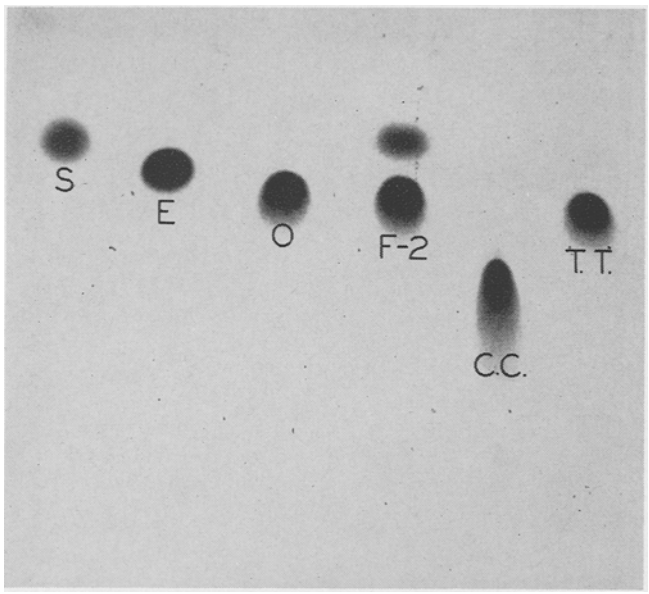

FIG. 6. Chromatoplate of the analysis of Fraction No. 2 esters of the epididymal fat isolated by $AgNO_3$-TLC in Figure 5 with standards. S = saturate; E = elaidate; O = oleate; F-2 = Fraction No. 2, Figure 5; CC = *cis,cis*-linoleate; TT = linoelaidate. Solvent: 20% ethyl ether in petroleum ether.

a number of tissues reported in Table IV show, in fact, that the GLC analyses do agree well with the IR analysis and, thus, provide further proof for the deposition of linoelaidic acid in the tissues.

Since the amount of 20:4 acids in the *trans,trans*-linoleate group of animals was less than that in the fat-free group, there appeared to be little, if any, conversion of this acid to higher polyunsaturated fatty acids. In order to substantiate this hypothesis, the 20:4 fraction was isolated for IR analysis. Because there was little 20:4 acid in the tissues, the isolation was carried out from the pooled esters of the phospholipids and kidney fat. The pooled esters were fractionated directly by preparative $AgNO_3$-TLC using 30% diethyl ether in petroleum ether. One fraction was recovered from the area of the 20:3 esters, and another fraction from the area adjoining it containing the 20:4 acid. The reason for selecting the two fractions as such was to be certain of isolating any *trans*-20:4 which ordinarily would reside in the *cis*-20:3, as well as between the *cis*-20:3 and *cis*-20:4 esters. GLC analysis of these fractions showed that the first fraction was devoid of 20:4; it also contained no *trans* unsaturation. The other fraction consisted of 22% 20:3 and 78% of 20:4. IR analysis of this fraction (Fig. 4) showed that it was also devoid of *trans* unsaturation. Thus, none of the linoelaidic acid was converted to 20:4 acids.

Discussion

The present study showed that linoelaidic acid was not converted to higher polyunsaturated fatty acids in contrast to *cis*-9,*trans*-12- and *cis,cis*-octadecadienoic acids. However, linoelaidic acid was deposited in the major lipid classes in all the tissues examined. Moreover, it appeared to exert an influence on the composition of the lipid classes and their constituent fatty acids. The influence of linoelaidic and *cis*-9,*trans*-12-octadecadienoic acids on the lipid classes and fatty acid composition resembled that of *cis*-9,*cis*-12-octadecadienoic acid (natural linoleic acid), although neither of these acids cured the EFA deficiency, nor stimulated growth of the animals.

TABLE IV

GLC and IR Analysis of Esters in Fractions Isolated by $AgNO_3$-TLC (Fr. No. 2, Fig. 5) (% wt)

Source	GLC (18:2) %	Infrared (trans,trans-18:2) %
Liver phospholipids	12.3	12.4
Liver triglycerides	4.0	4.7
Liver steryl esters	13.9	16.5
Epididymal fat	3.9	3.5
Kidney fat	8.2	7.5

Quackenbush and co-workers (21) observed that the *trans* isomers of octadecadienoic acid exerted a similar, but less pronounced effect as natural linoleic acid on the cholesterol levels of hypercholesteremic rats.

The relatively high amount of 18:2 of the tissue lipids, particularly the triglycerides of the animals receiving methyl linoelaidate as compared to the other supplemented groups, may be attributed, in part, to the higher level of this ester added to the diet of these animals. However, the higher level in the tissue lipids, particularly the sterol esters, may also result from its "piling up" in the tissues, because it is not converted to higher polyunsaturated fatty acids.

It has been observed by Holman and co-workers (17,18,19) that natural linoleic acid given to EFA-deficient animals effects a marked lowering of the 20:3 acids simultaneously with the production of 20:4 (arachidonic) acid. The low ratio of the 20:3 to 20:4 was, therefore, regarded as a good index of the nutritional requirement of the animal for EFA. The present study shows that linoelaidic acid also effected a lowering of the 20:3 acids, but since it was not converted to higher polyunsaturated fatty acids, the triene-to-tetraene ratio of fatty acid was not greatly altered. However, the *cis*-9,*trans*-12-octadecadienoic acid was converted to tetraene and gave an appreciable lower triene-to-tetraene ratio, in spite of the fact that it was fed for only a short period at a low level. It was not as effective in this regard as natural linoleic acid, however, as shown by the results in Tables II and III. Whether or not the synthesis of 20:3 acids, particularly in the phospholipids, would have been suppressed more, and as a consequence reversed completely the triene-tetraene ratio from that of the animals in the fat-free group by feeding a larger amount of methyl *cis*-9,*trans*-12-octadecadienoate, must await the results of further experiments. One can only speculate on the reason why *cis,trans*-octadecadienoate is less effective than the *cis,cis*-isomer in the suppression of the 20:3 acids. This acid may be catabolized more readily than the *cis,cis*-isomer, or it may undergo biohydrogenation somewhat like methyl elaidate (8). In both cases, the apparent effect would be the same as providing less of this ester in the diet, in accordance with the low values for 18:2 in the phospholipids.

Since the *trans* isomers of linoleic acid resemble natural linoleic acid in their effects on the component lipids and fatty acid composition, and *cis*-9,*trans*-12-octadecadienoic acid is converted to "*trans*" arachidonic acid (11), enzymatic interconversions of fatty acids do not appear to be involved *per se* in the physiological role of essential fatty acids.

The results of this study also indicate that the *trans* isomers of linoleic acid compete with the corresponding *cis* isomers as substrates in the course of being metabolized. Thus, one might expect that *trans* acids will substitute for a portion of the *cis*

acids on prolonged feeding and, conceivably, could produce a *de facto* essential fatty acid deficiency. What other nutritional effects *trans* acids may have is not known. However, it is pertinent that when they are consumed as a dietary ingredient, they are found in all the common lipid classes in the tissues of the animal.

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Analysis of Detergent Mixtures Containing Amine Oxides[1]

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Abstract

Long chain alkyldimethylamine oxides in detergent mixtures have not only been difficult to determine but also interfere with anionic active analysis by the usual quaternary titration with methylene blue indicator. Titration methods have been devised for the quantitative analysis of amine oxides and anionic actives in the presence of each other and low molecular wt sulfonates. A gas chromatographic method has also been developed for determining molecular distribution in alkyldimethylamine oxide mixtures. Analytical data are presented for a series of alkyldimethylamine oxides of different mol wt and for several experimental detergent formulations. The titration method is rapid and amenable to both solid and liquid detergent formulations.

Introduction

CONSIDERABLE INTEREST has developed recently in long chain alkyldimethylamine oxides, such as dodecyldimethylamine oxide, as detergents and detergent additives. These compounds are usually made by oxidation of the corresponding amines (3), and consequently contain amines as impurities.

Several analytical methods (1,2,3,5,6,7) have been reported for amine oxides in general. However, these methods which are based either on acidimetry or reductometry are mainly for determining product purity and are not amenable to analysis of detergent mixtures. Furthermore, the presence of amine oxides in detergents interferes with the usual anionic active determination by quaternary titration with methylene blue indicator (4). Because of growing interest in these materials for detergent use, methods for determining anionic actives and amine oxides in detergents are highly desirable.

In the procedure presented, total anionic active is determined by quaternary titration of an aliquot of sample solution with bromocresol green indicator. Total amine oxide is then determined by adding a stoichiometric amount of quaternary to a second aliquot (to complex with anionic actives), extracting the mixture with chloroform, and titrating the extracted amine oxides with a standard alkylbenzenesulfonate solution and methylene blue indicator. If the mol wt of amine oxides is unknown, it can be determined by the gas chromatographic method given.

Accuracy of these methods is shown by analytical data on known compounds and both liquid and solid detergent formulations.

Experimental

Bromocresol Green (BCG) Method for Anionic Actives

Reagents

Quaternary Hyamine 1622 solution (ca. 0.00450 N). About 2.1 g Hyamine 1622 (Rohm and Haas, mol and equivalent wt, 466.1) is dissolved in water, diluted to 1 liter, and standardized against dodecylbenzenesulfonic acid as described by House and Darragh (4).

Bromocresol Green indicator solution (buffered at pH 9.5). Prepared from 0.040 g bromocresol green (3′,3″,5′,5″-tetrabromo-m-cresolsulfonephthalein, Eastman 1782), 70 g sodium sulfate (CP), 3.09 g boric acid ("Baker Analyzed" Reagent), 34.4 meq of sodium hydroxide solution (0.5 N), sufficient Hyamine solution (ca. 13 ml 0.00450 N) to give zero titer for 10 ml distilled water, and water to make up to 1 liter.

Procedure

A weighed detergent sample (ca. 0.0025 equivalent of active) is dissolved in water and diluted to 250 ml. A 5-ml aliquot is placed in a 100-ml stoppered graduate cylinder with 5 ml water, 15 ml chloroform (CP), and 25 ml BCG solution and then titrated with Hyamine solution. The mixture is shaken vigorously after each increment of titrant. Initially the blue color of BCG concentrates in the aqueous (top) layer, and at the end point is equally distributed between the aqueous and chloroform layers. A correction curve for titers of less than 10 ml is given in Figure 1.

Calculation

$$\text{Milliequivalent anionic active/gram sample} = H \times F \times N_H \times 50/W$$

where H = ml Hyamine 1622 solution
F = correction factor from Figure 1
N_H = normality of Hyamine 1622 solution
W = sample wt in g

Amine Oxide Method

Reagents

Inorganic Salt solution (buffered). Same as BCG

[1] Presented at the AOCS Meeting in Minneapolis, 1963.