The Deposition of Polyunsaturated Fatty Acids in the Rat Fed Partially Hydrogenated Vegetable Oil[1]

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Abstract

The composition of deposited polyenoic fatty acids in rats fed liquid or partially hydrogenated corn oil was determined by gas chromatography, which did not distinguish isomeric forms, and by lipoxidase which reacted with the *cis,cis*-methylene-interrupted acids. The two methods gave similar results for the liver fatty acids of rats fed either the unhydrogenated or partially hydrogenated oil. Of the fatty acids from the epididymal fat pads of rats fed the hydrogenated product, an appreciable quantity of linoleate isomers did not react with lipoxidase. The total amount of linoleic acid deposited was related to the total fatty acid pattern of the dietary oil. It appeared that the *trans*-acids were mostly metabolized and that the original *cis,cis*-linoleic acid remaining in the partially hydrogenated product was preferentially incorporated into tissues.

Introduction

Upon partial hydrogenation, a vegetable oil acquires a greater variety of fatty acids than found in the original oil. The new species of geometric and positional isomers of unsaturated fatty acids then become part of the food supply, and appear largely in the form of margarines and shortenings. Incorporation of these fatty acids, particularly the *trans* isomers, into the lipid components of tissues, has intrigued many investigators.

Elaidic acid, the most studied *trans* isomer, was found in rats and pigs in the primary positions of the depot triglycerides (1–3). Compared to this *trans*-monounsaturated fatty acid, the isomers of linoleic acid have posed more problems. The *trans, trans* form, believed to be produced only in negligible quantities during the commercial hydrogenation of vegetable oils, was esterified in the primary position of triglycerides and of lecithins (4,5). Privett et al. (4) demonstrated that *cis,trans*-linoleate tended to have a similar fate to the original *cis,cis* form and appeared in the 2 position of glyceryl lipids. Furthermore, Lands et al. (6) showed that the selectivity in the enzymic transfer of *cis,cis*-linoleate into the 2 position of lecithin was followed by that of the *cis, trans* isomer which was, however, less readily incorporated. In the feeding study from which Privett et al. (4) and Lands et al. (6) derived their material, rats were deprived of all fat for four weeks and were assumed to be virtually depleted of the *cis,cis* isomer. Then, when the various geometric isomers were fed, the octadecadienoic acid found was considered to be the isomer administered.

A convenient method of analyzing the original *cis,cis*-linoleate is by the use of lipoxidase as described by McGee (7). The enzyme reacts with the *cis*-methylene-interrupted polyunsaturated fatty acids having the terminal double bond at the (n-6)-position and the methylene group at the (n-8)-position (8,9,10).

To determine the extent to which the polyunsaturated fatty acids in tissues of rats fed either liquid or partially hydrogenated corn oil contain the usual all *cis* configuration, results obtained by lipoxidase were compared with those obtained by packed column, gas liquid chromatography (GLC).

Materials and Methods

Animals and Diet

Male Wistar rats of 42 to 56 g were obtained from Woodlyn Farms, Guelph, and fed ad lib. the diet shown in Table I. The oil, obtained from Canada Packers Ltd., and the other ingredients, obtained from Nutritional Biochemicals Corp., were stirred for 30 min in a 20 qt Hobart mixer. The corn oil had been hydrogenated at 350 F, 20 psi hydrogen pressure with 0.15% nickel catalyst, type Selectol B (Drew Chemical Co.). Information about the fatty acid composition of the dietary oils (Table II) was obtained by GLC (11) which provided no information about isomers, by lipoxidase (7) which reacted with the *cis,cis*-linoleate, by argentation thin layer chromatography (TLC) (12) with development in 15% hexane in benzene (Fig. 1) and by IR spectroscopy (13).

Tissue Analyses

At 0, 2 and 14 days, nonfasted rats were killed, the livers removed and extracted by the method of Bligh and Dyer (14) and suitable aliquots of the chloroform extract used for phosphorus (15), for preparation of methyl esters for GLC (11) with methyl arachidate as an internal standard, for reaction with lipoxidase (7), and for precipitation of phospholipids (11). The lipoxidase procedure was standardized with liquid peanut oil or corn oil after the linoleate content had been determined by gas chromatography. In other

TABLE I
Diet

Ingredient	% By wt.
Liquid or hydrogenated corn oil	10
Casein	20
Sucrose	60
Salt mixture, U.S.P. XIV	4
Vitamin mixture[a]	1
Alphacel	5
Total	100

[a] Described in Can. J. Biochem. *47*, 257 (1969).

TABLE II
Fatty Acid Composition of Liquid Corn Oil (LCO) and Partially Hydrogenated Corn Oil (HCO)

Method	Fatty acid	LCO, wt %	HCO, wt %
GLC			
	16:0	10.7	10.7
	16:1	0.9	1.0
	18:0	1.5	3.9
	18:1	26.5	62.0
	18:2	58.1	20.7
	20:0	0.5	0.7
	18:3	1.8	1.0
	20:1		
IR	Total *trans*, as methyl elaidate	0	24.9
UV	Conjugated dienes	0.3	0.5
Lipoxidase	Total *cis*-PUFA's	53.3	8.0

[1] One of 10 papers to be published from the Symposium "Hydrogenation" presented at the AOCS Meeting, New Orleans, April 1970.

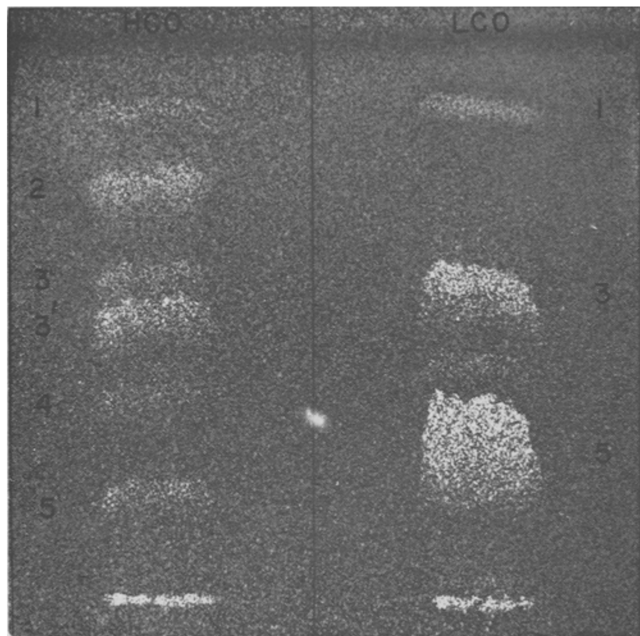


FIG. 1. Photograph of TLC of methyl esters of fatty acids sprayed with 0.2% 2′,7′-dichlorofluorescein in ethanol and viewed under UV. HCO, hydrogenated corn oil; LCO, liquid corn oil; 1, saturates; 2, *trans*-monoenes; 3 and 3′, *cis*-monoenes; 4, mixture of *cis,trans*- and *trans,cis*-dienes (tentative); 5, *cis,cis*-diene.

experiments, epididymal fat pads were also analyzed by both procedures.

Results

The hydrogenation of the corn oil appeared to alter about 85% of the original *cis,cis*-linoleic acid, for liquid corn oil had 58% but the partially hydrogenated product only 8% of this fatty acid and 12.7% of isomeric forms (the difference between that obtained by gas chromatography and by lipoxidase), as shown in Table II. The large increase in monoenoic acids after hydrogenation appeared to be partly accounted for in the *trans* fraction as shown in Figure 1.

At 2 and 14 days, the rats fed the liquid corn oil and those fed the partially hydrogenated corn oil had similar liver weights, total lipid phosphorus and fatty acids (Table III). The liquid oil permitted a greater deposition of polyunsaturated acids than did the hydrogenated product (Table IV), the difference being apparent in the neutral lipid fraction (Table V).

In a comparison of the results obtained by gas chromatography and by lipoxidase (Table V), it is seen that the two methods gave similar values for polyunsaturated fatty acids in the total lipids, in the phospholipids and in the neutral lipids of liver. It was surprising that the two methods still provided similar results after two days of feeding the rats either the liquid or partially hydrogenated corn oils.

TABLE III

Liver Weight, Lipid Phosphorus and Total Fatty Acids of Rats Fed Liquid Corn Oil (LCO) or Partially Hydrogenated Corn Oil (HCO)

Days	Dietary fat	Weight of liver, g	Liver lipid P, mg	Liver fatty acids, mg
0		1.85 ± 0.20[a]	1.61 ± 0.19	63.2 ± 9.8
2	LCO	3.14 ± 0.21	1.42 ± 0.14	91.9 ± 8.6
	HCO	3.22 ± 0.16	1.72 ± 0.08	108.3 ± 7.6
14	LCO	5.52 ± 0.39	2.65 ± 0.16	165.3 ± 9.4
	HCO	5.42 ± 0.96	2.67 ± 0.30	133.8 ± 19.4

[a] Mean ± S.E.M. of five rats.

TABLE IV

Polyunsaturated Fatty Acids (PUFA's) Deposited in the Liver of Rats Fed Liquid Corn Oil (LCO) or Partially Hydrogenated Corn Oil (HCO)

Day	Dietary fat	Liver PUFA's	
		mg/g tissue	mg/mg phosphorus
0		16.5 ± 1.8[a]	18.0 ± 0.9
2	LCO	12.2 ± 0.4	27.5 ± 1.8
	HCO	8.9 ± 0.3	16.8 ± 0.6
14	LCO	14.5 ± 0.6	30.2 ± 1.1
	HCO	9.8 ± 0.5	18.4 ± 1.5

[a] Mean ± S.E.M. of five rats.

By 14 days, the amounts of polyunsaturated acids determined by either gas chromatography or lipoxidase, had doubled, and gave no indication of the significant deposition of isomeric forms from the partially hydrogenated dietary oil.

These results were essentially replicated in a second experiment (Table VI) in which the polyunsaturated fatty acids found in the liver after 14 days also reacted with lipoxidase. When the epididymal fat tissue from these rats was analyzed, not all of the polyunsaturated acids (essentially all linoleic) reacted with lipoxidase.

The amount of *cis,cis*-linoleic acid deposited in adipose tissue appeared to be related to the pattern of dietary fatty acids (relative proportions) rather than to the total amount available in the diet under the conditions shown in Table VII. When the amount of dietary safflower oil was reduced to supply the same amount of linoleate as 20% corn oil in the diet, safflower oil still led to a significantly higher level of linoleic acid in the fatty acids of the epididymal fat pads than did corn oil.

Discussion

Although the partial hydrogenation of corn oil eliminated about 85% of the original *cis,cis*-linoleic acid and produced an appreciable quantity of isomeric forms of that acid, the polyenoic acids deposited in liver tissue did not seem to be significantly diluted with the isomeric forms. On the other hand, adipose tissue did contain significant amounts of polyenoic acids which did not react with lipoxidase. Without distinguishing between monoenes and dienes, earlier investigators found more dietary *trans* acids in adipose than in liver tissue (16). The liver appeared to be more selective in the incorporation of dienoic or other polyenoic acids into its fatty acids.

It has been demonstrated that the total fatty acid composition of a dietary oil is an important factor in

TABLE V

Liver Polyunsaturated Fatty Acids (PUFA's) Determined by Lipoxidase or GLC From Rats Fed Liquid or Partially Hydrogenated Corn Oil

Day	Fraction	Lipoxidase PUFA, mg/liver	GLC PUFA, mg/liver
0	Total lipid	29.9 ± 3.9[a]	33.9 ± 4.7
	Phospholipid	8.3 ± 2.0	10.6 ± 1.2
	Neutral lipid	20.4 ± 3.1	19.7 ± 2.9
Liquid corn oil			
2	Total lipid	38.3 ± 2.0	42.1 ± 3.4
	Phospholipid	6.6 ± 0.7	9.5 ± 0.5
	Neutral lipid	29.5 ± 2.0	32.3 ± 1.8
14	Total lipid	79.6 ± 4.2	82.1 ± 4.0
	Phospholipid	15.6 ± 2.2	17.4 ± 1.5
	Neutral lipid	56.5 ± 8.8	63.5 ± 4.8
Hydrogenated corn oil			
2	Total lipid	28.8 ± 1.6	34.6 ± 2.8
	Phospholipid	8.2 ± 1.8	10.1 ± 1.2
	Neutral lipid	22.6 ± 6.8	22.4 ± 2.4
14	Total lipid	52.7 ± 9.2	54.9 ± 8.5
	Phospholipid	13.8 ± 3.8	19.6 ± 5.9
	Neutral lipid	33.0 ± 2.2	38.0 ± 3.7

[a] Mean ± S.E.M. of 5 rats.

TABLE VI

Fatty Acids in Epididymal Fat Pads and in Liver From Rats Fed Liquid Corn Oil (LCO) or Partially Hydrogenated Corn Oil (HCO) for 14 Days

Tissue	Dietary oil	Total FAs by GLC, mg/g tissue	PUFA's by GLC, mg/g tissue	PUFA's by lipoxidase, mg/g tissue
Adipose				
	LCO	673 ± 16[a]	221 ± 7	248 ± 11
	HCO	627 ± 28	92 ± 3	72 ± 5[b]
Liver				
	LCO	30.3 ± 1.3	13.1 ± 0.4	13.0 ± 0.7
	HCO	34.0 ± 2.6	9.5 ± 0.5	8.4 ± 0.2

[a] Mean ± S.E.M. for seven rats.
[b] Different from results obtained by GLC at 1% level of significance.

influencing the extent of linoleate deposition in adipose tissue. Other fatty acids tend to decrease its incorporation into tissue lipids, but there is evidence that linoleic acid is preferentially retained by the animal (17,18).

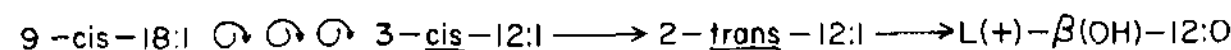
The utilization of *cis* and *trans* isomers of unsaturated acids for energy appeared to occur through the usual β-oxidation of fatty acids to acetyl CoA and the subsequent production of carbon dioxide (19–22). In a study of the oxidation of geometric isomers by rat liver mitochondria, Anderson (23,24) found that the rate of CO_2 production for the C_{18} monoenes was greater with oleic than with elaidic acid, but for the dienes, the *trans* isomers were more readily metabolized. The explanation for the differences in the metabolism of the CoA derivatives of these fatty acids is detailed in Figure 2. With a *trans* double bond in the original Δ^9 position, six carbons are readily removed before the β-oxidation sequence presumably slows down at the stage where there is a *trans*-Δ^3 substrate. According to Stoffel et al. (25) it must be isomerized to the *trans*-Δ^2 compound. The mitochondrial isomerase functions more readily with the *cis*-Δ^3 than with the *trans*-Δ^3 substrate. A different situation is encountered when a double bond occurs at Δ^{12}. After five cycles of β-oxidation, the substrate is Δ^2-octanoic acid. A *cis* double bond in this compound is hydrated during the β-oxidative sequence to D(−)-β-OH-octanoic acid which requires a critical epimerization to the L(+)-form, whereas the corresponding *trans*-Δ^2-octanoic acid directly forms the required intermediate. On this basis the most readily oxidized linoleate isomer should be *cis*-Δ^9, *trans*-Δ^{12}, followed by the *trans,trans*, then *trans,cis*, and then the *cis,cis* forms. Accordingly, Anderson (24) found that his mixture of *cis,trans* and *trans,cis* isomers gave similar results to the all *trans* isomer which would rank between them. In the experiments reported here, the linoleic acid containing a *trans* double bond may have been more readily oxidized, and the all *cis* form, which was in lower concentration in the diet, was more readily incorporated into tissue lipids.

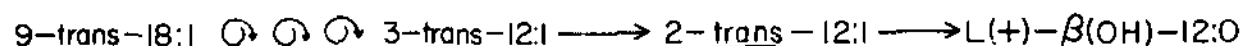
TABLE VII

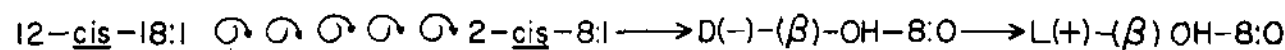
Influence of Pattern of Dietary Fatty Acids on Deposition of *Cis,Cis*-Linoleate in Epididymal Fat Pads

Diet	Per cent *cis,cis*-linoleate	
	in diet	in epididymal fatty acids
20% Safflower oil[a]	16	48.0 ± 0.9[b]
12.5% Safflower oil	10	46.4 ± 1.6
20% Corn oil	10	34.9 ± 0.8

[a] Oil added to basal diet at expense of carbohydrate.
[b] Mean ± S.E.M. for 10 rats.







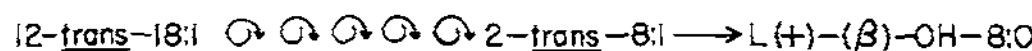


FIG. 2. Steps in β-oxidation of fatty acids containing *cis* and *trans*-double bonds as described by Anderson (23,24).

There is still a scarcity of analytical data on the fatty acid composition of partially hydrogenated vegetable oils. To separate the methyl esters on the basis of unsaturation, prior to argentation chromatography, Kuemmel and Chapman (26) employed a column of alumina and Scholfield et al. (27) a column of vulcanized rubber. Both of these workers reported the presences of dienes containing one *trans* double bond, but Holmer and Aaes-Jorgensen (12) who found such compounds in partially hydrogenated soybean oil, detected none in partially hydrogenated arachis oil after TLC on silver nitrate plates. The characterization of the specific fatty acids produced during hydrogenation of edible oils requires further attention.

In these experiments it has been demonstrated that liver polyenoic acids of rats fed partially hydrogenated corn oil were not significantly different when determined by gas chromatography and by lipoxidase but that those in adipose tissue did contain an appreciable quantity of linoleate isomers which did not react with the enzyme.

ACKNOWLEDGMENTS

Canada Packers Ltd, Toronto, supplied the liquid and partially hydrogenated corn oils. R. H. Hollywood provided technical assistance in chemical analyses and Claude Desloges in animal care.

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[Received April 29, 1970]