Effect of Hydrogenated Triolein on Utilization of Essential Fatty Acids in the Rat.* (23274)

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It has been reported(1) that the isomerization which occurs during the catalytic hydrogenation of linoleic acid and methyl linoleate produces cis and trans 9, 10, 11 and 12 monounsaturated fatty acids. It is therefore not surprising that trans fatty acid isomers have been found to occur in margarines and shortenings(2,3), since in their production, oils containing large amounts of unsaturated fatty acids—and a large proportion of these unsaturated fatty acids are the essential fatty acids, linoleic and linolenic—are either selectively or non-selectively hydrogenated. The nutritional fate of the trans fatty acids in hydrogenated fat has been the subject of several reports. Elaidic acid, trans 9, 10 octadecenoic acid, which is the geometric isomer of oleic acid, is not changed to oleic acid in the animal body. However, Barbour(4) reported that "isooleic acid" (a mixture of elaidic acid, Δ-12, 13 octadecenoic acid, and other isomers of oleic acid formed during the hydrogenation process), found in hydrogenated cottonseed oil, is deposited in the adipose tissues of the rat and is eventually utilized by the animal body as efficiently and effectively as other dietary fatty acids. Sinclair(5) used elaidic acid and trielaidin in studies of intermediary fat metabolism and reported that these compounds behaved exactly as did the normal fat components. Kohl(6) also reported on efficient utilization of elaidic acid laid down in adipose tissues and phospholipids. In a study of the positional and stereoisomers of the unsaturated fatty acids produced on hydrogenation of vegetable oils, Melnick and Deuel(7) found that these isomers are not antimetabolites for natural oleic acid, but are used as nutrients. Microbiological methods were used in the studies concerned with the oleic acid isomers, and biological studies with the polyunsaturated fatty acid materials. Recently, Holman and Aaes-Jorgensen(8) reported that trans fatty acid isomers of linoleic acid fed to rats on an essential fatty acid deficient diet, containing cholesterol and hydrogenated coconut oil, did not induce recovery of the severe testicular degeneration which occurs in essential fatty acid deficiency. These trans isomers under the test conditions selected also worsened the skin condition and were found to be deposited in the animal fat. In experiments conducted in this laboratory directed toward the study of possible essential fatty acid antagonists, the presence of hydrogenated coconut oil has been shown to shorten considerably the time of depletion of essential fatty acids in the rat(9). In other long-term experiments(10) in which margarine oil, containing 35.3% trans fatty acid isomers, was fed in varying levels to rats for long periods of time, no interference attributable to this large concentration of trans fatty acids was observed when the criteria of growth, reproduction, lactation, survival, plasma and liver cholesterol levels and tissue pathology were studied. There was the possibility that the concomitant presence of adequate amounts of linoleic acid in this fat masks or counteracts any antimetabolite action which may reside in the trans fatty acids contained in the margarine oil; the average ratio of trans to biologically active linoleic acid content in the test oils was 7:1. With this in mind, a partially hydrogenated triolein, containing no linoleic

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acid whatsoever but with a trans fatty acid content of 32.8% was prepared for use in feeding studies with rats. During this hydrogenation of oleic acid, migration of the double bond takes place so that positional cis and trans isomers also occur(11). The physical constants of this triglyceride are listed in Table I.

Methods. Male rats of the University of Southern California strain were placed at weaning on a diet adequate in all respects but deficient in fat. The diet contained 20% casein, 72% sucrose, 4% cellulose, 4% salt mixture and all required vitamins in adequate amounts.‡ The weight of the animals was recorded weekly. Animals were judged to be deficient in essential fatty acids when a plateau in weight and the appearance of typical skin symptoms (*i.e.* scaliness of tail and paws, rough coat and general unthrifty appearance) were observed, which usually occurred in from 12 to 16 weeks. After 16 weeks on the fat-free diet, the animals were divided into the following groups and placed in individual cages. Each animal received daily the supplement listed:

Group I	Control — continued on fat-free diet, no supplement
II	Fat-free diet + 250 mg hydrogenated triolein
III	Fat-free diet + 500 mg hydrogenated triolein
IV	Fat-free diet + 20 mg linoleate
V	Fat-free diet + 250 mg hydrogenated triolein + 20 mg linoleate
VI	Fat-free diet + 500 mg hydrogenated triolein + 20 mg linoleate
VII	Fat-free diet + 50 mg linoleate
VIII	Fat-free diet + 250 mg hydrogenated triolein + 50 mg linoleate
IX	Fat-free diet + 500 mg hydrogenated triolein + 50 mg linoleate

The animals were dosed for a period of 8 weeks during which time they were weighed weekly. The results are shown in Table II.

‡ A vitamin mixture containing 38.57% p-amino benzoic acid, 31.88% inositol, 12.75% ascorbic acid, 4.59% thiamine hydrochloride, 3.82% niacin, 3.82% Ca pantothenate, 1.72% riboflavin, 1.72% pyridoxine, 0.64% folic acid, 0.32% menadione, 0.16% biotin and 0.00004% B_{12} was added to the diet at a level of 0.19%. A Nopsol solution containing 100,000 I.U. of vit. A/g and 20,000 I.U. of D/g was included at a level of 0.012%. 0.012% of α-tocopherol was added as well.

TABLE I. Chemical and Physical Properties of Hydrogenated Triolein.

Wiley melting point	97.7°F
Iodine value (Wijs)	67.2
Saponification value	195.8
Free fatty acids (%)	<0.05 as oleic acid
Fatty acid composition (%)	
Saturated (stearic)	20.9
Trans fatty acids (infra-red analysis)	32.8
Linoleic acid	.0
Linolenic acid	.0
Oleic acid (by diff.)	46.3

Results. It is apparent from a consideration of the weight increments of the rats at various time intervals during the assay period, that there is no interference with growth as a result of the administration of 250 mg or 500 mg of a hydrogenated triolein to the animals in the various groups. In fact, with animals receiving no other fat in the diet, supplementation with this hydrogenated triolein product results in a slight gain in weight over the 8-week period (Groups II and III, Table II) as compared with the animals continued on the fat-free ration (Group I). The gains in weight of the groups in which both linoleate and hydrogenated triolein are fed to the animals are remarkably similar; at the lower level of linoleate at the end of the 8-week assay period the weight increment was 65.6 g when the linoleate was fed alone, and 63.6 g and 63.8 g when either 250 mg or 500 mg of hydrogenated triolein were administered concomitantly (Groups IV, V, VI). The weight gain at the higher level of linoleate was 73.4 g, and 70.8 g and 81.1 g when the 2 levels of hydrogenated triolein were given concomitantly (Groups VII, VIII, IX). These studies demonstrate that the trans isomers of oleic acid in the hydrogenated triolein have no antimetabolite activity for the essential fatty acids either in the presence or absence of linoleic acid.

When animals are placed on either fat-free diets alone or on fat-free diets containing additives which are known to deplete more rapidly the essential fatty acid stores of an animal, it is conceivable that the further addition of any substance which does not possess essential fatty acid activity, but which might

TABLE II. Effect of Various Supplements over an 8 Week Period on the Growth of Male Rats Previously Depleted of Essential Fatty Acids.

Group	Supplement* T	Supplement* L	No. of animals	Avg wt at start (g)	Wt increment (g) after 3 wk	6 wk	8 wk
I			15	232	− 15.2	− 7.5	− .3
II	250		11	238	− 5.4	+ 8.9	+ 14.8
III	500		10	237	− 11.3	− 5.9	+ 2.0
IV		20	11	242	+ 16.6	+ 42.1	+ 65.6
V	250	20	11	235	+ 19.4	+ 45.4	+ 63.6
VI	500	20	13	250	+ 19.9	+ 46.8	+ 63.8
VII		50	9	249	+ 22.6	+ 51.1	+ 73.4
VIII	250	50	8	238	+ 26.4	+ 49.6	+ 70.8
IX	500	50	9	232	+ 26.9	+ 60.4	+ 81.1

* T = Hydrogenated triolein; L = Methyl linoleate.

ordinarily be used efficiently as a nutrient, might lead to an accentuation of the essential fatty acid deficiency symptoms. In fact, addition of certain growth factors might induce a more complete deficiency of essential fatty acids due to production of certain stresses. Experiments performed in our laboratories have shown that animals deficient in essential fatty acid accumulate cholesterol esters and total lipid in the liver leading to a typical "fatty" liver condition(12). The subsequent administration of the methyl ester of essential fatty acid, linoleic, alleviates this condition within 4 weeks. Supplementation with methyl oleate for the same length of time, however, not only does not reduce the cholesterol levels in the liver but tends to accentuate the accumulation of both the cholesterol esters and the total lipid in this organ.§ In view of the fact that oleic acid does not possess essential fatty acid activity, this result is not surprising. To label as antimetabolites or as antagonists substances which do not alleviate the deficiency symptoms in an animal caused by a lack of essential fatty acids is certainly to be avoided. It must be demonstrated that these substances increase the requirement for essential fatty acids or in the absence of the essential fatty acids aggravate the deficiency syndrome. The trans isomers, the oleic acid and the stearic acid in the hydrogenated triolein are not anti-metabolites for the essential fatty acids according to these criteria.

Summary. Rats which were depleted of essential fatty acids after 16 weeks on a fat-free diet were maintained for 8 weeks thereafter on either a continued fat-free diet, fat-free diets containing 2 levels of hydrogenated triolein (containing 33% of trans isomers and no essential fatty acids), fat-free diets containing 2 levels of methyl linoleate, or fat-free diets supplemented with both linoleate and the hydrogenated triolein at the 2 levels. The animals receiving the linoleate supplement alone or the linoleate plus the hydrogenated triolein showed equivalent weight gains over an 8-week period. In the absence of the linoleate there was no aggravation of the deficiency syndrome. No observable antimetabolic activity of the hydrogenated triolein was evident.

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1. Allen, R. R., and Kiess, A. A., *J. Am. Oil Chemists' Soc.*, 1956, v33, 355.
2. Mabrouk, A. F., and Brown, J. B., *ibid.*, 1956, v33, 92.
3. Sreenivasan, B., and Brown, J. B., *ibid.*, 1956, v33, 341.
4. Barbour, A. D., *J. Biol. Chem.*, 1933, v101, 63.
5. Sinclair, R. G., *ibid.*, 1935, v111, 515.
6. Kohl, M. F. F., *ibid.*, 1938, v126, 709.
7. Melnick, D., and Deuel, H. J., Jr., *J. Am. Oil Chemists' Soc.*, 1954, v31, 63.
8. Holman, R. T., and Aaes-Jorgensen, E., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 175.
9. Deuel, H. J., Jr., Alfin-Slater, R. B., Wells, A. F., Kryder, G. D., and Aftergood, L., *J. Nutrition*, 1955, v55, 337.
10. Alfin-Slater, R. B., Wells, A. F., Aftergood, L., and Deuel, H. J., Jr., *ibid.*, in press.
11. Allen, R. R., and Kiess, A. A., *J. Am. Oil Chemists' Soc.*, 1955, v32, 400.
12. Alfin-Slater, R. B., Aftergood, L., Wells, A. F., and Deuel, H. J., Jr., *Arch. Biochem. Biophys.*, 1954, v52, 180.

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