Role of Oleic Acid in the Metabolism of Essential Fatty Acids[1]

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Groups of young male guinea pigs were fed diets containing corn oil, coconut oil, coconut oil plus elaidic acid, and coconut oil plus oleic acid. The oleic acid-fed group showed signs of essential fatty acid deficiency after four weeks and severe signs after eight weeks. The elaidic acid-fed group did not show these symptoms. It is proposed that oleic acid competes competitively with linoleic acid as a substrate for the enzymes involved in linoleate transformations when only a very limited supply of linoleic acid is available to the animals and oleic acid is made available in relatively large amounts.

A detailed analysis of the serum, liver, and adipose tissue lipid and a study of the incorporation of acetate-1-C^{14} into different lipids is presented.

THE NUTRITIONAL properties of dietary fats have been studied extensively, particularly since the appearance of reports indicating that polyunsaturated fatty acids influence serum cholesterol levels (1,2). Although the geometric isomerism of the unsaturated fatty acids has been recognized for some time, the effect of such isomerism on the metabolism of the acids was not extensively investigated. Before the advent of radioisotopes, *trans*-isomers were used as tracers since they could be easily distinguished from their *cis*-isomers by differences in physical properties. Fat absorption was thus studied by R.G. Sinclair (3,4) with the aid of elaidic acid. However the consideration by H.M. Sinclair of the possible role of these unusual *trans*-isomers in the cholesterol regulatory metabolism aroused considerable interest in the metabolism of these compounds (5). More recently Holman and Aaes-Jorgensen studied the effects of feeding *trans* and conjugated isomers of the polyunsaturated fatty acids (6). These authors concluded, on the basis of a few animals, that the *trans*-acids might have an action antagonistic to that of the essential fatty acids, a finding which was not confirmed in the experiments of Mattson (7).

Isomerization of unsaturated fatty acids to *trans* isomers is known to occur during processing of edible fats and, because oleic acid is most abundantly found in natural oils and fats, this acid and its *trans*-isomer, elaidic acid, were chosen for this study. In earlier experiments (in press) it was found that elaidic acid, when fed in combination with coconut oils gave excellent growth and lower serum cholesterol levels whereas, the natural isomer, oleic acid, showed adverse effects. An attempt is made in the present study to find an explanation for this unexpected effect.

Experimental

Diets and Treatment of Animals. One-week-old, healthy, male guinea pigs were fed the semi-synthetic diet recommended by Reid and Briggs (8). Choice of these animals was on the basis of the possibility of separating the young from the mothers at only 2–3 days, leading to a much more rapid development of dietary deficiencies. A daily supplement of 10 mg. of ascorbic acid was given to all animals. Different groups, of 12 animals each, received the following types of lipids in the diet:

Group I, 7.3% corn oil
Group II, 7.3% coconut oil
Group III, 3.65% coconut oil plus 3.65% oleate (free acid or ethyl ester)
Group IV, 3.65% coconut oil plus 3.65% elaidic acid
Group V, 3.65% oleate only
Group VI, 3.65% elaidic acid only
Group VII, fat-free diet

Oleic acid obtained from Nutritional Biochemicals Company (Ohio) was purified and esterified with ethanol to give a mixture composed of 83% ethyl oleate and 13% ethyl palmitoleate. Elaidinization was carried out by heating in the presence of selenium. Several crystallizations at −20°C. gave chromatographically-pure elaidic acid.[2]

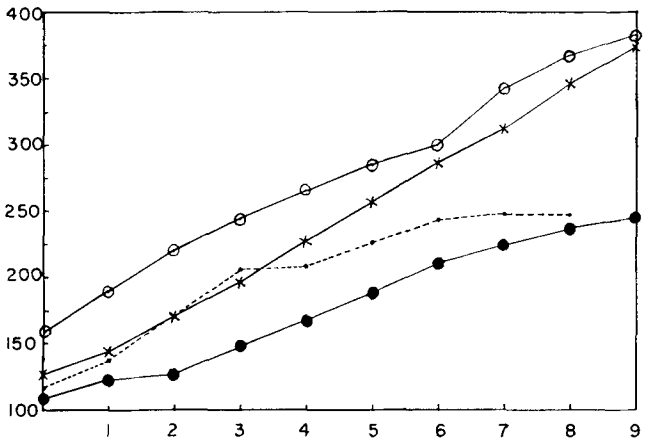

FIG. 1. Growth of animals on diets containing different fats.

In all cases the lipid component was doubled after four weeks, and the animals were maintained for a total period of 8–9 weeks. At the end of this experimental period the animals were given intraperitoneal injections of 15 μc of acetate-1-C^{14} and sacrificed after 1-,2-, and 4-hr. periods.

Animals were anaesthetized with Nembutal, and blood samples were withdrawn by direct heart puncture and heparinized; the separated plasma was stored at −20° until analyzed. The liver was first perfused with saline solution to remove most of the blood, then removed for extraction of lipids. Adipose tissue was removed, and the lipids were extracted.

Lipids were extracted from tissues by homogeniza-

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[2] Gas-liquid chromatographic analysis was performed, using a Wheelco Model 10 (Barber-Coleman Company) apparatus with a 6-ft., 6-mm. I.D. column of ethylene glycol succinate polyester on 80–100 mesh siliconized chromosorb at 185–192°C.

TABLE I

Composition of Eluents Used in Silicic Acid Column Chromatography

Eluent	Fraction eluted
100 ml. of n-pentane	Hydrocarbons
200 ml. of 2% ethyl ether in pentane	Sterol esters
200 ml. of 5% ethyl ether in pentane	Triglycerides
200 ml. of 20% ethyl ether in pentane	Free sterol
200 ml. of ethyl ether	Mono- and diglycerides
200 ml. of methanol	Phospholipids

tion in chloroform-methanol (2:1), followed by washing with saline, drying the chloroform layer with anhydrous sodium sulphate, and evaporating the solvent under nitrogen. The serum lipids were extracted according to the method of Folch *et al.* (9).

Separation and Analysis. The total lipids were first separated into different classes by silicic acid column chromatography. Silicic acid [3] was mixed with methanol in the ratio of 1:5 (weight:volume) in a large beaker and allowed to settle for 10 min. The methanol was then decanted, thereby removing the fine particles of silicic acid. This process was repeated four times, and the silicic acid was then spread over an aluminum tray and air-dried. The removal of the fine particles not only gives a desirable flow-rate but also eliminates the washing of the column by methanol to remove pigmented material from crude silicic acid. To 15 g. of this pretreated silicic acid were added 40 ml. of acetone; the resulting slurry was packed on a column (¾ x 14 in.) under gentle suction. Acetone, ether, and n-pentane (100 ml. of each) were passed through the column before the lipid sample, 100–150 mg., dissolved in minimum volume of pentane, was washed onto the column. The concentration of the eluents passed through the column, and the fractions collected are shown in Table 1. All the solvents were evaporated under nitrogen, and the samples were transferred to 3-dram vials for counting the radioactivity.[4]

Transmethylation of sterol esters, triglycerides, and phospholipids was carried out with 1% of methanolic sulphuric acid. Samples not more than 10 mg. in weight were dissolved in 9 ml. of methanolic sulfuric acid:benzene (2:1) in 15-ml. centrifuge tubes. The tubes were heated in refluxing methanol-benzene for 8–10 hrs. The methyl esters from the phospholipids and triglycerides were extracted with ether in the usual manner. In the case of sterol esters, the methyl esters were freed from free sterols by thin-layer silicic acid chromatography (11) and were analyzed by gas-liquid chromatography.[2]

Finally the sterol ester, triglyceride, free sterol, and phospholipid fractions were counted for radioactivity.[4]

Results

The growth of the various groups is shown in Figures 1 and 2. Growth was excellent with corn oil (Figure 2); coconut oil gave good growth (Figure 1), but the animals developed a rough coat. When half the proportion of coconut oil was replaced by oleic acid or ethyl oleate, the animals showed extremely poor growth and a higher percentage of mortality. After about four weeks all the animals lost the fur on the abdominal area, and the skin on their legs became rather rough and scaly. Although after about five weeks some of the animals had regained their fur, they ceased to gain weight and after 8–10 weeks 60–75% of the total number of the animals on this diet had died. When coconut oil was omitted and only oleate was fed, a similar response was observed, but the onset of the symptoms was apparent even earlier and the symptoms themselves were more severe. Doubling the fat content of the diet after four weeks definitely increased the severity of the symptoms. However, when half of the coconut oil was replaced by pure elaidic acid, no such symptoms were observed. The animals continued to gain weight, and there was no hair-loss or mortality until after nine weeks. Figure 2 illustrates the effect of feeding a supplement of methyl linoleate (100 mg. per day) to the oleic acid-fed group. No loss of hair or development of scaly skin was observed, and the animals apparently did not suffer from deficiency symptoms.

Finally animals on the fat-free diet also lost hair and developed dermal symptoms, but this was evident about one-and-a-half weeks later and the early mortality was much less than with the oleic acid-fed group.

Table II gives the percentage composition of the lipids of serum, liver, and the adipose tissue of the corn oil-fed group, coconut oil plus elaidic acid-fed group, and the coconut oil plus oleic acid-fed group.

The percentage composition of the serum lipids in the three groups does not differ very greatly although the total lipids (mg./100 ml.) of the oleic acid-fed group are significantly higher than those of the other groups.

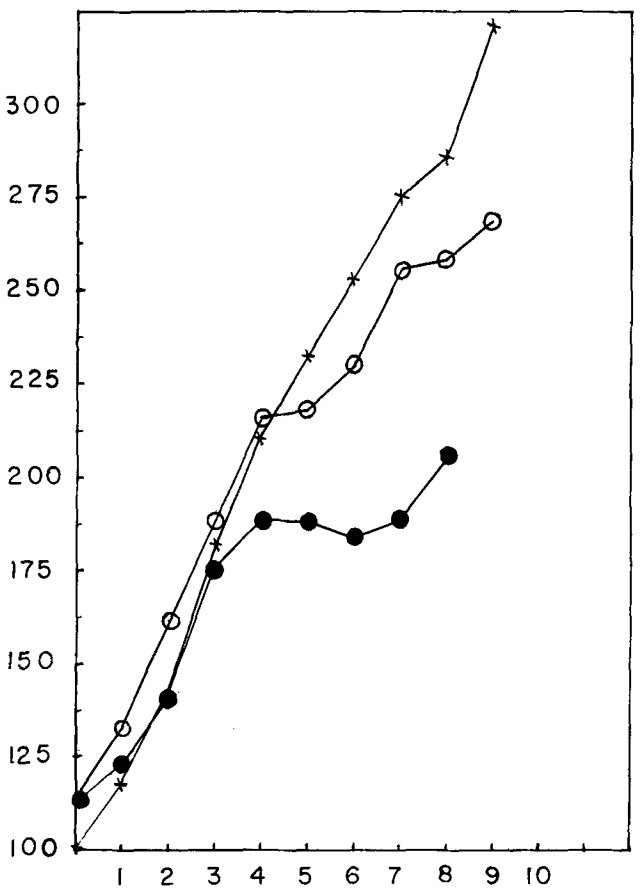

FIG. 2. Effect of linoleate on growth of animals on diet containing oleate.

●——● Oleic acid
○——○ Oleic acid + linoleic acid
X——X Corn oil

[3] Silicic acid, Baker analyzed reagent, from J.T. Baker Chemical Company, Phillipsburg, N.J., was used.

[4] All counting was performed with a single channel scintillation counter of the type described by Hodgson, Gordon, and Ackerman (10) at 1150 volts and 77 ± 0.2% sensitivity. Samples were weighed into 3-dram vials and dissolved in 5 ml. of toluene, containing 5 g. of phenylbiphenyl oxadiazole and 0.1 g. of "POPOP" per liter.

TABLE II

Composition of Serum, Liver, and Adipose Lipids

Dietary group		Total lipids mg. %	Hydrocarbons mg. %	Sterol esters mg. %	Triglycerides mg. %	Free sterol mg. %	Monoglycerides mg. %	Phospholipids mg. %
C.O.	Serum	222 ± 43	2.7 ± 2.4	26.9 ± 4.4	7.3 ± 3.2	14.9 ± 2.3	15.2 ± 4.1	33.3 ± 6.2
	Liver	53 ± 15	0.3 ± 0.05	1.4 ± 0.23	12.1 ± 6.7	7.3 ± 1.2	5.4 ± 1.4	73.0 ± 8.9
	Adipose tissue	639 ± 57	0.3 ± 0.2	0.9 ± 0.8	84.0 ± 3.6	4.2 ± 1.2	5.4 ± 3.3	4.7 ± 3.6
E.A.	Serum	228 ± 71	3.0 ± 2.4	35.5 ± 5.9	16.2 ± 4.9	15.5 ± 2.2	3.2 ± 0.8	27.6 ± 7.8
	Liver	57 ± 13	2.7 ± 1.1	1.5 ± 0.6	33.0 ± 14	5.0 ± 1.2	1.9 ± 0.3	57.0 ± 13
	Adipose tissue	226 ± 48	1.3 ± 0.9	1.3 ± 0.6	88.0 ± 3.4	1.8 ± 0.9	1.1 ± 0.3	7.0 ± 2.2
O.A.	Serum	346 ± 85	4.0 ± 0.9	32.8 ± 2.1	16.1 ± 2.3	12.3 ± 1.7	5.15 ± 1.5	29.6 ± 2.7
	Liver	40 ± 8.7	0.6 ± 0.4	1.9 ± 0.8	10.2 ± 5.2	6.8 ± 1.8	1.7 ± 0.9	79.0 ± 5.4
	Adipose tissue	243 ± 120	1.0 ± 0.07	2.6 ± 1.6	77.0 ± 13	5.1 ± 3.5	1.7 ± 1.0	12.1 ± 8.0

C.O., corn oil-fed group; E.A., elaidic acid-fed group; O.A., oleic acid-fed group.

The liver total lipids do not show significant changes despite the fact that the oleic acid-fed group showed symptoms of essential fatty acid deficiency. In the elaidic acid-fed group the phospholipid fraction seems to be reduced whereas the triglyceride fraction has increased.

The total lipid per g. of adipose tissue in both elaidic- and oleic acid-fed groups was reduced below the control values, a finding which was also reflected in the growth curves of these groups.

Table III shows the percentage composition of the fatty acids of sterol esters, triglycerides, and phospholipids. The most striking difference in the serum sterol ester, triglyceride, and phospholipid fatty acids is the lowering of linoleic acid in both the elaidic- and oleic acid-fed groups. There is a concomitant increase in the lauric and myristic acids. Moreover the ratio of phospholipid stearic acid to oleic acid is much lower in the elaidic acid-fed group than in the oleic acid-fed group while in the corn oil-fed group it appears to be reversed. Liver sterol esters formed only a very small percentage of the total lipids, hence were not studied with respect to fatty acid composition. To get the composition of the total fatty acids, the liver total lipids were saponified with alcoholic potassium hydroxide and the fatty acids were extracted in the usual manner. Both lauric acid and myristic acid from the triglycerides are much lower in the corn oil-fed group. Once again linoleic acid is much higher in the corn oil-fed group. The same holds true for arachidonic acid. The presence of eicosatrienoic acid in the oleic acid-fed group was confirmed by plotting the retention times of standard eicosamono-, tetra-, and pentaenoic acids.

The adipose triglycerides and total fatty acids seem to follow the same trend as the liver lipids.

The incorporation of acetate 1-C^{14} into the serum, liver, and adipose tissue (Table IV) cholesterol ester, triglyceride, and free cholesterol fractions of the oleic acid-fed group was greater than for the elaidic acid-fed group during the first hour. It remained more or less constant during the next three hours in the oleic acid-fed group whereas in the elaidic acid-fed group there was a rapid rise after four hours.

The activity of the phospholipid fraction was higher in the corn oil-fed group during the first-hour period compared with the other groups but decreased in the subsequent intervals. In the oleic- as well as elaidic acid-fed group however the activity seems to have been increasing during the two- and four-hour periods.

Discussion

From the results of these experiments it is evident that the animals receiving corn oil or coconut oil as a source of dietary fat showed reasonable growth and no obvious symptoms of dietary insufficiency. However replacement of half of the coconut oil by oleic acid or ethyl oleate led to poor growth, loss of hair, and high early-mortality rate. That these symptoms were due to a deficiency in essential fatty acids was shown by comparison with animals on a fat-free diet, also by the fact that they did not occur when a daily supplement of 100 mg. of methyl linoleate was given.

TABLE III

Composition of Serum, Liver, and Adipose Fatty Acids

	Dietary group		Lauric %	Myristic %	Palmitic %	Palmit-oleic %	Stearic %	Oleic %	Linoleic %	Linolenic %	Eicosatri-enoic %	Arachi-donic %
Sterol esters	C.O.	Serum	.....	.....	9.1 ± 1.7	0.9 ± 0.5	0.4 ± 0.2	10.6 ± 5.8	74.4 ± 9.8	Trace	.....	4.8 ± 2.5
	E.A.	Serum	.....	17.4 ± 1.8	12.3 ± 3.0	15.9 ± 2.7	Trace	33.5 ± 3.0	20.7 ± 1.8	.....	.....	.....
	O.A.	Serum	1.0 ± 0.3	12.8 ± 1.7	11.7 ± 1.9	18.3 ± 3.3	.....	35.4 ± 1.6	20.6 ± 4.0	.....	.....	.....
Phospho-lipids	C.O.	Serum	.....	Trace	17.9 ± 1.3	0.6 ± 0.3	36.0 ± 3.9	15.3 ± 4.3	24.2 ± 5.3	Trace	.....	6.8 ± 3.4
		Liver	.....	.....	13.2 ± 6.3	.....	35.7 ± 4.6	6.8 ± 2.4	36.3 ± 5.6	.....	.....	8.1 ± 2.9
	E.A.	Serum	.....	2.8 ± 0.6	17.9 ± 2.9	2.8 ± 0.9	18.7 ± 2.7	41.0 ± 5.2	15.0 ± 1.8	.....	.....	.....
		Liver	.....	1.3 ± 0.5	13.5 ± 2.1	3.3 ± 0.3	22.4 ± 2.0	39.0 ± 8.1	18.7 ± 5.3	.....	.....	2.1 ± 1.0
	O.A.	Serum	.....	2.0 ± 0.6	14.9 ± 1.6	3.0 ± 0.2	28.0 ± 2.5	34.8 ± 1.1	17.1 ± 4.2	.....	.....	2.1 ± 0.7
		Liver	.....	1.3 ± 0.4	9.6 ± 1.1	4.7 ± 0.8	32.1 ± 2.3	24.6 ± 1.0	23.7 ± 2.0	0.6 ± 0.1	0.5 ± 0.3	3.3 ± 0.8
Triglyc-erides	C.O.	Serum	.....	1.2 ± 0.5	24.8 ± 8.1	2.2 ± 1.1	4.5 ± 0.9	32.2 ± 3.3	36.2 ± 6.4	Trace	.....	Trace
		Liver	.....	1.0 ± 0.5	28.9 ± 9.6	1.4 ± 0.3	3.0 ± 1.5	22.4 ± 4.7	42.0 ± 15.0	1.8 ± 1.4	.....	Trace
		Adipose tissue	.....	0.9 ± 0.4	24.1 ± 11.8	1.1 ± 0.6	4.1 ± 0.8	28.4 ± 3.3	40.1 ± 17.0	1.3 ± 0.5	.....	.....
	E.A.	Serum	.....	14.1 ± 3.0	29.8 ± 0.8	7.3 ± 0.6	4.2 ± 0.9	41.9 ± 2.0	2.4 ± 0.3	.....	.....	.....
		Liver	3.8 ± 1.2	28.8 ± 5.1	27.7 ± 2.7	6.1 ± 0.4	.....	31.3 ± 6.5	2.3 ± 0.7	.....	.....	.....
		Adipose tissue	17.1 ± 6.2	28.2 ± 9.1	22.6 ± 5.8	2.5 ± 1.1	2.0 ± 0.7	25.9 ± 7.1	1.6 ± 0.8	.....	.....	.....
	O.A.	Serum	1.8 ± 0.7	14.6 ± 7.4	23.3 ± 7.7	10.3 ± 1.3	3.9 ± 0.9	44.0 ± 5.7	4.6 ± 0.8	.....	.....	.....
		Liver	2.6 ± 0.6	15.7 ± 3.4	19.8 ± 3.3	9.6 ± 0.4	2.3 ± 0.6	42.1 ± 6.7	6.1 ± 1.1	.....	.....	.....
		Adipose tissue	17.6 ± 2.8	21.6 ± 3.6	12.5 ± 2.5	6.4 ± 0.8	2.3 ± 1.4	36.0 ± 8.2	1.7 ± 0.7	.....	.....	.....
Total fatty acids	C.O.	Liver	0.3	1.0	19.2	0.9	21.8	12.2	39.4	2.9	.....	1.9
		Adipose tissue	.....	0.8	27.2	1.8	3.8	29.3	34.4	2.7	.....	.....
	E.A.	Liver	2.6	16.6	21.6	4.2	9.5	30.9	13.8	Trace	.....	0.8
		Adipose tissue	9.7	19.4	21.7	3.9	4.4	37.3	2.5	.....	.....	1.0
	O.A.	Liver	0.5	3.2	13.6	6.1	21.8	32.8	18.1	0.3	0.3	2.9
		Adipose tissue	17.4	18.1	11.5	5.1	1.2	43.9	1.6	.....	.....	0.4

C.O.,corn oil-fed group; E.A., elaidic acid-fed group; O.A., oleic acid-fed group.

TABLE IV

Incorporation of Acetate-1-C^{14} into Serum, Liver, and Adipose Lipids

Interval		Sterol esters Sp. Act. cpm./mg.			Triglycerides Sp. Act. cpm./mg.			Free sterol Sp. Act. cpm./mg.			Phospholipids Sp. Act. cpm./mg.		
		C.O.	E.A.	O.A.	C.O.	E.A.	O.A.	C.O.	E.A.	O.A.	C.O.	E.A.	O.A.
1 hour	Serum	1	2	4	191	202	228	88	32	37	28	49	14
	Liver	70	8	12	305	45	169	49	29	21	39	14	13
	Adipose tissue	43	14	34	35	4	14	34	19	37	64	23	44
2 hours	Serum	10	16	3	130	449	194	83	113	36	38	53	12
	Liver	19	51	12	89	235	110	43	48	21	20	27	17
	Adipose tissue	18	25	20	2	33	3	8	58	28	24	47	19
4 hours	Serum	3	12	3	33	74	146	18	46	28	17	85	31
	Liver	15	30	11	57	57	66	34	53	24	18	14	17
	Adipose tissue	6	27	24	4	7	11	5	50	42	24	22	31

C.O., corn oil fed-group; E.A., elaidic acid-fed group; O.A., oleic acid-fed group.

The observation that even this supplementation did not produce as good growth as replacement of the coconut oil by corn oil may be explained by the estimation that animals consuming corn oil were receiving 600–700 mg. of linoleate per day, an amount which may be more nearly optimal for these animals.

This adverse effect of feeding oleate was surprising, considering the fact that olive oil, which supports normal growth, has about 85% of oleic acid. It was more puzzling because none of the above symptoms were observed, and the animals appeared healthy and normal when oleate was replaced by elaidic acid. It was difficult to conceive that elaidic acid has any growth-promoting properties; however this was quickly cleared by observing that a group maintained on elaidic acid only did not show normal growth. In fact, the loss of hair and other symptoms were seen at about the same time as in the oleate-fed group although the gain in weight of the former group was slightly better.

Attention was thus focused on the possibility that oleic acid interfered with the normal utilization of some other component in coconut oil. As we were dealing with essential fatty acid deficiency symptoms, this component might be linoleic acid. Gas-liquid chromatographic analysis of coconut oil showed that it contained 1.5% of linoleic acid, furnishing about 20 mg. of linoleic acid per day per guinea pig. This amount, although small, can support almost normal growth without any deficiency symptoms as shown by feeding coconut oil alone. However, when comparatively large amounts of oleic acid are included in the diet, the animals no longer can utilize the small but significant amount of linoleic acid and deficiency symptoms are observed. When an additional supplement of linoleic acid was given, the equilibrium shifted towards normal utilization of linoleic acid. Thus it seems that when only small amounts of linoleic acid are available and oleic acid is fed in considerably larger amounts, oleic acid interferes with the utilization of linoleic acid. It may be possible that under these circumstances oleic acid competes with linoleic acid and acts as an inhibitor with some of the enzymes involved in arachidonic acid formation. If such is the case, metabolites of oleic acid would accumulate in the oleic acid-fed group. Eicosatrienoic acid, which is formed from oleic acid under conditions of fat deficiency, was found in the oleic acid-fed group and was absent in the other groups (Table III).

Using the same argument, it seems that the enzyme involved must have negligible affinity for the *trans*-isomer, elaidic acid, which therefore does not interfere with the utilization of the small amount of linoleic acid present in coconut oil; hence it remains inert so far as the enzyme is concerned. This explains the absence of trienoic acid in the liver fat, also a somewhat similar growth curve of the elaidic acid-fed group and the fat-deficient group.

The adverse effect of feeding oleate agrees in part with similar observations reported by Bosshardt and co-workers (13) for mice, and by Thomasson *et al.* (14) for rats.

Analysis of the fatty acids of sterol esters and phospholipids revealed a very low level of arachidonic acid in guinea pigs, agreeing with reports by Swell and co-workers (15). Tove and Smith (16) observed a decrease in palmitoleic acid and an increase in stearic acid when a high percentage of linoleic acid was fed. This agrees well with the corn oil-fed group in the present study. The higher percentage of lauric and myristic acids in the oleic- and elaidic-fed groups must be from the coconut oil in the diet. The higher percentage of palmitoleic acid may be due to either the deficiency state of the oleate-fed group (17) or to the fact that the oleic acid used in the diet contained about 12–13% of palmitoleic acid.

Reiser *et al.* (18) have reported the specific activities of various lipid components isolated from animals injected with acetate-1-C^{14} and sacrificed after 24 hrs. The plasma triglyceride activity in the cottonseed oil-fed group was minimum whereas those of the low-fat-fed animals had highest activity. In the present study the corn oil-fed group (comparable to the cottonseed oil-fed group) has minimum activity in the serum triglyceride fraction after four hours, and the oleic acid-fed group showing signs of fat deficiency (comparable to the low-fat-fed group) has the maximum activity. The same could be said about the phospholipid fractions. The sterol esters however do not show such similarities.

The liver and adipose triglycerides had the lowest activity in the corn oil-fed group after four hours in agreement with the observations on the cottonseed oil-fed group by Reiser *et al.* It may also be noted that the fall of activity of the triglycerides in the corn oil-fed group from one hour to four hours is considerable, indicating the possibility of further loss of activity at later time-intervals.

The elaidic acid plus coconut oil-fed group would be comparable to the saturated fat-fed group because the fatty acids of coconut oil are mostly saturated, and elaidic acid, with a higher melting-point than oleic acid, would be comparable to a saturated fatty acid. Moreover elaidic acid did not interfere with the utilization of essential fatty acids. Comparison of the specific activities of these two groups shows identical patterns of intermediate incorporation of activity in the serum and adipose triglycerides.

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Influence of Lauroyl and Myristoyl Peroxides and Oxidized Cottonseed Oil on Depot Fat and Liver Lipid Composition[1,2]

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In view of the interest in the biological properties of products of fat oxidation, lauroyl and myristoyl peroxides were fed and their nutritional effects compared with those of autoxidized cottonseed oil, which had been analyzed for its composition. Purified diets containing no fat + 2% of linoleic acid, 5% lauroyl or myristol peroxide, or 10% oxidized cottonseed oil were fed to weanling male albino rats for 73 to 98 days, after which they were killed and their organs weighed. Their sera, livers, and testicular fat bodies were used for lipid analysis.

With peroxides, growth was significantly depressed but not as much as when oxidized cottonseed oil was fed. Analysis of organ weight data showed that peroxides and oxidized cottonseed oil differed in their effects. Animals fed the latter had significantly heavier livers, kidneys, and hearts. The rats fed peroxides were also different from those fed the fat-free diet and those kept on restricted food intake. Gas chromatographic analysis of the testicular fat bodies revealed a greater deposition of oleate in the animals fed oxidized cottonseed oil, which suggested that these animals were unable to use the oxidized oil for depot fat formation. In the animals fed lauroyl and myristoyl peroxides, appreciable amounts of laurate and myristate, respectively, were found. The composition of the liver neutral fat of the animals fed peroxides was similar to that of the animals fed the low-fat diet + 2% linoleic acid. Serum cholesterol levels of the rats fed peroxides were about 70 mg. %, and of those fed oxidized cottonseed oil, 53 mg. %. The groups fed peroxides also had significantly higher liver cholesterol levels, which suggests that peroxides and oxidized cottonseed oil differed in their effects on cholesterol formation and transport.

THE NUTRITIONAL EFFECTS of substances developed in fats under the influence of heat and oxygen have been studied during the last decade. It has been pointed out that many of these materials are toxic (1,10,12). Later studies showed that oxidized fats or some of their fractions have other pharmacological properties, some of which seemed to invite further work (8,9,4).

Hardly any pharmacological studies of the materials formed in heated and aerated fats have been carried out because none of the polymers have been purified, and pure peroxides are not easily obtainable in sufficient quantities for nutritional work. In one such study, oleic peroxide was found not to influence growth (11). For the experiments reported, lauroyl and myristoyl peroxides were selected although they do not occur during autoxidation of fats. They are oxidation products of fatty acids and are readily available and relatively stable. Furthermore it seemed to us that biological data obtained with any products of fatty acid oxidation would be valuable. Their nutritional effects on rats were compared with those of a low-fat diet and one containing highly-autoxidized cottonseed oil in order to have some comparison of short-chain oxidation products and fat polymers. The animals were observed for body weight, organ weights, serum cholesterol levels, and composition of the neutral fat of the liver and testicular fat bodies.

TABLE I

Composition of Oxidized Cottonseed Oil

	%
Unsaponifiables	1.4
Fatty acids	51.5
Polymerized material	26.4
Carbonyls	9.4
Unknown	3.4
H_2O-soluble fragments	7.9

Fatty Acid Composition Based on Total Oil

	%
14 : 0 myristic	0.7
16 : 0 palmitic	20.4
16 : 1 palmitoleic	0.5
18 : 0 stearic	2.3
18 : 1 oleic	12.7
18 : 2 linoleic	14.5
20 : 0 arachidic	0.4
	51.5

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