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Metabolism of methyl elaidate*

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SUMMARY

Methyl elaidate-1-C^{14} was fed to fat-deficient guinea pigs. Very little incorporation of the radioactivity was found in the glycerides or phospholipids under these conditions. Methyl ester seemed to have been absorbed without complete hydrolysis and deposited in body tissues as the methyl ester. Intestinal lipids from these animals showed the presence of unhydrolyzed free methyl esters even after a period of 4 hours. Percentage distribution of the radioactivity in the stearic acid isolated from these animals shows direct *in vivo* hydrogenation of elaidic acid to stearic acid.

During recent years, the possibility of increased human ingestion of fatty acids containing trans double bonds has led to considerable interest in their metabolism and possible effect on the animal body.

In a previous communication from this laboratory (1), it was shown that guinea pigs, when fed an elaidic acid-coconut oil diet, showed normal growth. In earlier studies, Sinclair and Smith (2) found that elaidic acid comprised 35% of the fatty acids in the intestinal lipids of cats; its incorporation into the body lipids could not be easily investigated at that time. The present experiments were undertaken to evaluate the incorporation of carboxy-labeled methyl elaidate into various lipid components and the transformation, if any, of elaidic acid to other fatty acids.

EXPERIMENTAL METHODS

Methyl elaidate-1-C^{14} (specific activity 0.56 mc/mM) prepared according to the method of Nevenzel and Howton (3) was found to be greater than 99% pure by gas-liquid chromatography.[1] The labeled ester was diluted with 1.5 ml of methyl oleate and administered by mouth to six fat-deficient[2] guinea pigs (total activity fed, 447 μc). After 4 hours, the animals were anesthetized and blood was withdrawn by direct heart puncture. The livers were flushed with normal saline and the pooled livers, kidneys, lungs, hearts, spleens, and adipose tissue of all six animals were excised and extracted by the Folch method (4). An aliquot of the total lipids was chromatographed on a silicic acid column (1). A portion of each fraction was then further purified by thin-layer silicic acid chromatography (5) using 16% ether in hexane. This procedure gave good separation of triglycerides and free fatty acids, cholesterol and phospholipids. Pure samples, 7–12 mg in weight, were collected by using several plates, and the activity of each was determined by using a scintillation counter[3] (Table 1).

Fraction I, which normally contains cholesterol esters, when fractionated further by thin-layer chromatography using 1.5% ether in hexane as solvent, proved to be a mixture of methyl and cholesterol esters of fatty

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[1] Gas-liquid chromatographic analysis was performed by using a Wheelco Model 10 (Barber Coleman Company, Rockford, Illinois) apparatus with either a 6-foot, 6-mm ID column of ethylene glycol-succinate polyester on 80 to 120 mesh siliconized chromosorb at 185° to 200°, or a 3-foot column of 0° to 29° silicone rubber gum SE 30 (General Electric Company) on 80 to 120 mesh glass beads at 185° to 200°.

[2] Fat deficiency was indicated by scaly paws and loss of hair on the abdomen.

[3] All counting was performed with a single-channel scintillation counter of the type described by Hodgson, Gordon, and Ackerman (13) operated at 1150 volts with 77 ± 0.2% efficiency. Samples were weighed into 11-ml vials and dissolved in 5 ml of toluene containing 5 g per liter of phenyl biphenyl oxidazole and 0.1 g of 1,4-di-(2-(5-phenyloxazolyl))-benzene per liter.

TABLE 1. INCORPORATION OF ELAIDIC ACID INTO LIPID COMPONENTS OF POOLED TISSUES AS SHOWN BY SILICIC ACID CHROMATOGRAPHY

Eluent	Component Identified by Thin-Layer Chromatography	Specific Activity
		dps/mg
2% Ether in pentane	Cholesterol esters	0.5
	Methyl esters	708
5% Ether in pentane	Triglycerides	4.1
20% Ether in pentane	Sterols	0.4
Methanol	Phospholipids	3

acids. Further, it was found that almost all the radioactivity was concentrated in the methyl ester portion.[4] This unusual finding was confirmed in a repeat feeding experiment (identical to the original one) with two fat-deficient guinea pigs fed a total of 2×10^5 dps of methyl elaidate-1-C^{14}. Total body lipids were isolated and analyzed as before, and again most of the activity was found in the methyl ester fraction.

The remaining total lipids, 6.6 g, were hydrolyzed with 1 N KOH, and the nonsaponifiable fraction, primarily cholesterol, was separated in the usual manner. The cholesterol was purified by repeated crystallization. The total fatty acids in the saponifiable fraction were purified by passage through a silicic acid column before determination of radioactivity (Table 2).

The total fatty acids were separated into three fractions by low temperature crystallization from acetone. Fraction I, insoluble at $-20°$, consisted mainly of saturated acids and gave 46 dps per mg. However, small amounts of elaidic acid, which is also insoluble at $-20°$, had to be removed from this mixture. For this purpose, the fatty acids were methylated by using 1% methanolic sulphuric acid, and the methyl esters were brominated at 0° in carbon tetrachloride. The dibromides, formed from either oleic or elaidic acid, were separated from the saturated fatty acid methyl esters by chromatography on alumina (6).

Examination of the purified saturated fatty acid methyl esters by gas-liquid chromatography on a non-polar silicone column revealed that the mixture did not contain any dibromides. The mixture, consisting largely of palmitic and stearic acids (specific radioactivity 11.1 dps per mg) was further separated by using liquid-liquid reversed phase chromatography on siliconized Celite with paraffin as the stationary phase and acetone-water as the mobile phase (7). Stearic acid (recrystallized to constant radioactivity) was further

[4] Further experiments concerned with this finding will be reported in a separate communication.

TABLE 2. SPECIFIC RADIOACTIVITIES OF TOTAL BODY LIPIDS, CHOLESTEROL, AND FATTY ACIDS

Component	From Animals Injected with Sodium Acetate-1-C^{14}	From Animals Fed Methyl Elaidate-1-C^{14}
	dps/mg	*dps/mg*
Total lipids	18.8	66.4
Cholesterol	28	0.93
Total fatty acids		73.5
Saturated fatty acids	28	11.1
Palmitic acid	43	3.6
Stearic acid	19	15.8
Oleic acid		7.7

degraded by the Dauben procedure, with minor modifications (8), to give margaric and benzoic acids. The benzoic acid, which represented the carboxyl group of the original stearic acid, was purified by sublimation and recrystallization to constant radioactivity. Margaric acid was recrystallized to constant radioactivity first with methanol and then with petroleum ether. From the molar radioactivities of margaric and benzoic acid, the distribution of the radioactivity in the original stearic acid was calculated (Table 3).

Fraction II, insoluble at $-55°$, gave 137 dps per mg. In order to remove contamination by small amounts of elaidic acid, equal amounts of inactive elaidic acid were added to this fraction and the mixture was subjected to crystallization at $-20°$. The precipitated elaidic acid was removed and counted. Repeated crystallizations were carried out until constant radioactivity of the original fraction was obtained, and the crystallized elaidic acid no longer contained any radioactivity. Examination of the purified fraction by gas-liquid chromatography showed that it contained oleic and palmitoleic acids only. The two acids were separated from each other by reversed-phase chromatography (7). Pure oleic acid was hydrogenated to stearic acid

TABLE 3. PERCENTAGE DISTRIBUTION OF RADIOACTIVITY IN STEARIC ACID

Stearic Acid Sample	Benzoic Acid (C_1 of Stearic Acid)	Heptadecanoic Acid (C_2 through C_{18} of Stearic Acid)
From animals injected with sodium acetate-1-C^{14}	60.7	39.3
From animals fed methyl elaidate-1-C^{14}	98.6	1.3
Obtained by hydrogenation of oleic acid from animals fed methyl elaidate-1-C^{14}	99.6	0.4

TABLE 4. FRACTIONATION OF THE LIPIDS OF INTESTINAL CONTENTS BY SILICIC ACID CHROMATOGRAPHY

Eluent	Component Identified by Thin-Layer Chromatography	Specific Activity	
		dps/mg	
Pentane	Hydrocarbon	...	
2% Ether in pentane	Methyl esters of fatty acids (cholesterol esters absent)	2262	
5% Ether in pentane	Triglycerides (less than 1%)	23.4	
15% Ether in pentane	Free fatty acids	536	Bromo esters* 915
25% Ether in pentane	Diglycerides	4.8	Saturated esters 50.6
	Sterols	2.0	
Ether	Monoglycerides	10.2	
Methanol	Phospholipids	7.2	

* All the fractions, except the methyl ester fraction, were pooled, transmethylated, brominated, and separated on alumina.

by using palladium on charcoal as catalyst. The stearic acid, crystallized to constant radioactivity, was degraded by the Dauben procedure and the percentage of radioactivity in the carboxyl group determined as before (Table 3).

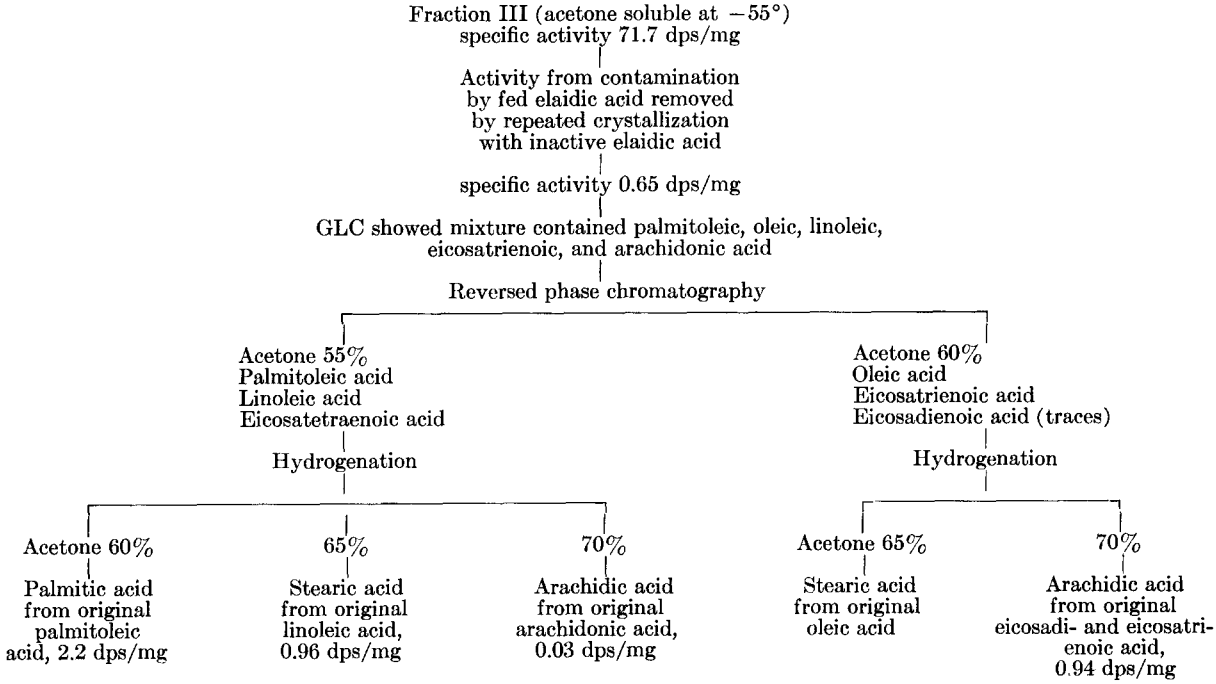
Fraction III, soluble at −55°, was separated into palmitoleic, linoleic, arachidonic, eicosadienoic, and eicosatrienoic acids by using reversed phase chromatography as outlined in Scheme 1.

The intestines of the guinea pigs fed methyl elaidate were removed and the contents washed with normal saline. Lipids were extracted from the contents in the usual manner, and a portion was chromatographed on a silicic acid column (Table 4). Aliquots of each fraction from the silicic acid column were further purified by thin-layer chromatography and counted in the scintillation counter. The methyl ester fraction, when analyzed by thin-layer chromatography, gave only one spot, indicating the absence of cholesterol esters. Gas-liquid chromatography revealed that the fraction contained elaidic acid and possibly a small amount of oleic acid. No traces of stearic acid were found. All the fractions except the methyl ester fraction were pooled and transmethylated as described previously (1). These methyl esters were then brominated, and the saturated methyl esters were separated from the bromo esters on alumina as before and their radioactivity measured (Table 4).

To determine if the radioactivity of the carboxyl group of stearic acid was derived from acetyl CoA obtained by breakdown of the fed elaidate, six fat-deficient guinea pigs were given injections of sodium acetate-1-C^{14} (total radioactivity, 1 mc). The animals were sacrificed, and the total lipids were extracted and hydrolyzed as before. Stearic acid was isolated and purified to constant radioactivity (Table 2). It was

SCHEME I

Fractionation of polyunsaturated fatty acid-rich fraction



then degraded as before to determine the percentage radioactivity in the carboxyl group (Table 3).

DISCUSSION

The incorporation of radioactivity of fed methyl elaidate into major lipid fractions seemed unusual in that almost all the radioactivity was located in the cholesterol ester fraction eluted with 2% ether from the silicic acid column. Earlier workers (9) had noticed that, when carboxy-labeled fatty acids were fed, the radioactivity was found mainly in the tissue phospholipids and triglycerides. The unusual concentration of radioactivity in the cholesterol ester fraction raised doubt as to the purity of this fraction; this doubt was confirmed when thin-layer chromatography revealed that the fraction contained two components identified as methyl and cholesterol esters of fatty acids, both of which would be eluted from the silicic acid column with 2% ether. After separation of the methyl and cholesterol esters, it was noticed that almost all the radioactivity of the original fraction was due to methyl esters.[4]

The presence of fed methyl ester in the intestine even after 4 hours clearly showed that the free methyl ester of elaidic acid resists complete hydrolysis and opens the possibility of its absorption as the methyl ester. Repeat experiments with methyl elaidate-1-C^{14} were carried out to confirm this observation and to ascertain that there had been no accidental contamination of the tissue lipids by the fed ester. It is evident from these results that, under circumstances in which fatty acid methyl esters are not readily hydrolyzed in the intestine, they may be absorbed and deposited in the tissues, a finding not in agreement with those of previous workers (10, 11).

Normal absorption of the fed elaidate resulted in the incorporation of considerable activity into the total fatty acids of the body lipids. When elaidate was removed from the fatty acid mixture, however, the activity of the saturated as well as unsaturated fatty acids fell considerably, showing rather poor transformation, either direct or through breakdown, to other fatty acids.

It was revealing to find that palmitic acid was less radioactive than stearic acid in animals fed elaidate since the results were the reverse when acetate was injected (Table 2). Thus, it was suspected that elaidate might undergo a direct transformation rather than β-oxidation to acetyl CoA with subsequent total biosynthesis of stearic acid. This transformation by direct enzymatic reduction to stearic acid, if it occurred, would result in a great preponderance of activity in the carboxyl carbon. Degradation studies indicated that this was indeed the case.

In an earlier study (1), it had been noticed that animals fed coconut oil plus elaidic acid showed about the same incorporation of acetate into body lipids as those fed coconut oil (mostly saturated acids) alone. It was also noticed that the amount of stearic acid in the total depot fat of the elaidate-fed animals was higher than that in coconut-oil-fed animals. The present study, indicating biohydrogenation of elaidate to stearate, suggests a possible explanation for that observation. Zabin (12) has reported that stearic acid isolated from fasted rats injected with acetate-1-C^{14} had a high percentage of the radioactivity in the carboxyl group. Thus, there could be two pathways leading to high radioactivity in the carboxyl group of stearic acid. One would be by direct biohydrogenation of elaidic acid and the other by addition of acetyl CoA (derived by breakdown of elaidic acid) to preformed palmitic acid. Stearic acid isolated from animals injected with active acetate, however, was less radioactive than palmitic acid and did not have all the radioactivity in the carboxyl group. Thus, direct biohydrogenation of elaidic acid seems to be the major pathway of transformation under these conditions.

The low radioactivity in the polyunsaturated fatty acids also supports the postulate that, at least within 4 hours, elaidic acid is not catabolized to acetate at a high rate.

The high radioactivity in the carboxyl group of oleic acid (7.7 dps per mg) may be the result of either direct isomerization of elaidic acid or dehydrogenation of the stearic acid. The relatively low radioactivity of oleic acid, however, bears approximately the usual relationship to that of stearic acid (15.8 dps per mg). In the absence of any evidence for *in vivo* isomerization of this type, there is reason to favor dehydrogenation rather than isomerization as the probable mechanism.

The question of whether the biohydrogenation occurred in the body or in the intestines by the microbial flora cannot be answered decisively. Although careful examination of the methyl ester fraction of the intestinal lipids failed to show even traces of stearic acid, the saturated methyl esters isolated from total methyl esters of these lipids did have some activity (Table 4). However, the specific radioactivity was too low in comparison with that of the fed elaidic acid to permit any conclusions.

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