A comparison of the metabolism of elaidic, oleic, palmitic, and stearic acids in the rat

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SUMMARY A comparison has been made of the metabolism of 1-C^{14}-labeled oleic, elaidic, palmitic, and stearic acids in the rat. Each acid was fed as a component of randomly rearranged soybean oil. All the acids were more than 96% absorbed. The rate of catabolism and the extent to which elaidic acid was excreted in the respiratory CO_2 were essentially the same as those of oleic acid and both of these acids were similar to palmitic acid. Oleic, elaidic, and palmitic acids were catabolized to CO_2 to a greater extent than was stearic acid. Elaidic acid seemed to be incorporated into the lymph phospholipids to a slightly greater extent than was oleic acid during the peak of absorption; otherwise, elaidic acid was similar to oleic acid in its absorption and distribution among the lymph lipids. Stearic acid was incorporated into the lymph phospholipids to a considerably greater degree (~8%) than were the other acids (~2–3%). The major transport of each fatty acid was via the lymph glycerides, being ~90% for stearic acid and ~97% for the other acids.

THE METABOLISM of the saturated fatty acids has been thoroughly studied and the catabolic fate of the carbon chain of these acids is known. However, the metabolism of the unsaturated fatty acids has not been studied as completely. For example, the effect of the geometry of the double bond in the unsaturated acids upon the catabolic fate of that acid in vivo has not been studied fully.

The evidence is clear that the animal is capable of metabolizing *trans* fatty acids such as elaidic acid. Early work by Sinclair (1, 2) and Barbour (3) has shown that dietary elaidic acid can be absorbed and incorporated into the body tissues. A later report by Johnston et al. (4) confirmed these findings. It was also shown by these investigators (1, 3, 4) that, once incorporated, the isomeric acids disappear from the tissues on cessation of their incorporation into the diet. Also, Dhopeshwarkar and Mead (5) have reported the direct reduction of elaidic acid to stearic acid in vivo. That animal tissues possess the metabolic machinery for catabolizing a *trans* fatty acid, elaidic acid, was shown by Kennedy and Lehninger (6), who demonstrated that elaidic acid could be oxidized by rat liver mitochondria. However, the specific behavior of the *trans* fatty acids in vivo is unknown. Therefore, an investigation was initiated to compare the metabolic behavior in vivo of the geometric isomers of some of the unsaturated fatty acids to one another and to well-known reference compounds. The present paper describes such a comparison between oleic and elaidic acids with reference to palmitic and stearic acids studied under the same experimental conditions.

MATERIALS AND METHODS

1-C^{14}-Labeled palmitic, stearic, and oleic acids were prepared chemically by decarboxylation of the unlabeled acid using a procedure similar to that of Anker (7) and recarboxylation via the Grignard reaction. The palmitic and stearic acids were purified by crystallization and the oleic acid by silicic acid column chromatography. Elaidic acid was prepared by SO_2 isomerization of the purified oleic acid-1-C^{14} (8). This procedure produces no detectable positional isomers.

The four experimental fats used in this study consisted of soybean oil labeled with radioactive oleic, elaidic, palmitic, or stearic acid. These fats were prepared as follows. The methyl ester of each labeled fatty acid was transesterified with a 10-molar excess of dry glycerol using an alkaline catalyst (9). The mono- and diglycerides were then isolated by silica gel column chromatography (10). The mono- and diglyceride mixture of the C^{14}-fatty acid was transesterified with soybean oil, up to 350 mg of partial glycerides per 10 g of soybean oil, using an alkaline catalyst. The triglycerides isolated from the

TABLE 1 COMPOSITION OF LIQUID DIETS

Constituent	Fat-Free Diet	Experimental Diet
	%	%
Sucrose	27	16
Non-fat dry milk solids	15	18
Vitamin mix (in sucrose)*	3	4
Salt Mix U.S.P. 14	2	2
Water	53	33
Fat	—	27

* The vitamin mix contained the following in mg/g: 0.06 menadione, 0.08 thiamine, 0.20 riboflavin, 0.40 niacin, 0.40 calcium pantothenate, 0.005 folic acid, 0.08 pyridoxine, 0.003 B_{12}, 60 choline chloride, 40 inositol, 2 ascorbic acid, 2 *p*-aminobenzoic acid, 0.005 biotin.

reaction mixtures (10) will be referred to as the experimental fats. This procedure produces a random incorporation of the labeled fatty acid into the soybean oil glycerides.

The experimental fats were incorporated into a liquid diet (Table 1) which was fed to the rats by stomach tube. Each animal was fed about 5.5 g of the experimental diet; the actual amount fed was determined for each animal. The dietary fats had the following specific activities: oleic acid, 11.2 μc/g; elaidic acid, 11.9 μc/g; palmitic acid, 12.3 μc/g; stearic acid, 11.5 μc/g.

The experimental animals were young, adult, male albino rats of the Holtzman strain. Groups of four animals were used except in the catabolism experiments with oleic acid and palmitic acid, in which respectively three and seven animals were used. The animals were trained to consume their daily ration of food in a 30 min period each morning. This allowed precise knowledge as to when each animal had eaten last and, therefore, helped to provide a more uniform set of experimental conditions. In all cases, the daily feeding was omitted on the day the experiment was begun, thus providing a 24 hr fast. Animals weighed 220–265 g at the start of an experiment.

When the catabolism of the labeled fatty acid to CO_2 was to be followed, the animals were fed the experimental diets and then were placed immediately in metabolism chambers which were designed to allow separate collection of feces, urine, and respiratory CO_2. The latter was trapped in 5% KOH. During the collection period the animals were allowed free access to water but were given no food. After the 51 hr experimental period the animals were killed, the gastrointestinal contents were recovered, and the carcasses were frozen and ground. The gastrointestinal contents, feces, and carcass were burned to CO_2 prior to radioassay. Urine was radioassayed directly by scintillation counting.

When the absorption of the labeled fatty acid and its distribution among the various lipid classes of the lymph were studied, each rat was provided with a thoracic duct cannula by a procedure similar to that of Bollman et al. (11). The animals were fed a fat-free diet (Table 1) following their recovery from the anesthesia. This facilitated the removal of residual dietary fat from the gastrointestinal tract. The experimental diets were fed on the following day. All animals had access to 0.9% saline from 24 hr prior to surgery until the end of the experiment, 42 hr after feeding the experimental diets. Food was withheld during the lymph collection period. Lymph was collected in oxalated tubes using an automatic fraction collector. The lipids were extracted from the lymph by the method of Bixby et al. (12), and radioassayed. Composite lipid samples were prepared which represented different portions of the absorption curve: 0–4 hr, 4–16 hr (the peak of absorption), 16–24 hr, and 24–42 hr (the return to a basal lymph) for the palmitic and stearic acid groups and 0–5, 5–17, 17–25, and 25–43 hr for the oleic and elaidic acid groups. Failure of the fraction collector to make the first change caused the slight difference in collection times between these acid pairs. The composite samples were separated into three lipid classes: glyceride, phospholipid, and sterol ester by silicic acid column chromatography (13). Each composite sample represents the pooled lipids from four rats.

Radioactivity measurements were made with a Tri-Carb liquid scintillation counter (14). When CO_2 was to be counted, it was converted to barium carbonate which was suspended in Thixcin scintillation gel for counting (15).

RESULTS AND DISCUSSION

The appearance of radioactivity in the respiratory CO_2 following the feeding of the various experimental fats is shown in Fig. 1. The excretion curves for the oleic and

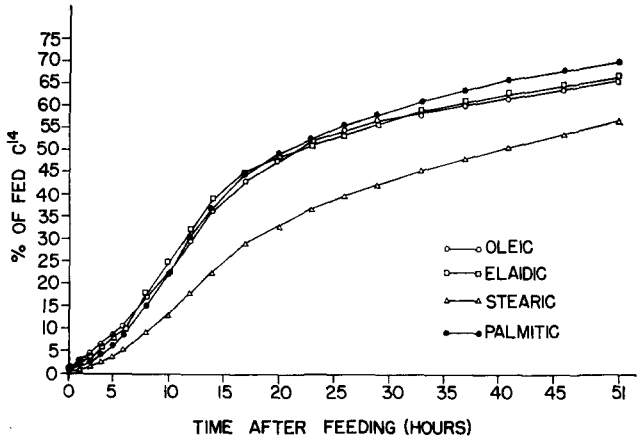

FIG. 1. Appearance of C^{14} in the respiratory CO_2 following the feeding of labeled fatty acids.

TABLE 2 DISPOSITION OF C^{14} BY THE RAT AFTER FEEDING VARIOUS 1-C^{14}-FATTY ACIDS*

C^{14}-Fatty Acid	% of Administered Activity				
	CO_2	Urine	Feces	Gastro-intestinal Contents	Carcass
Oleic	66.0 ± 4.5†	0.9 ± 0.0	1.4 ± 0.4	2.5 ± 0.7	24.3 ± 2.8
Elaidic	66.3 ± 2.0	0.7 ± 0.1	2.1 ± 0.6	2.4 ± 0.7	32.0 ± 1.6
Palmitic	70.2 ± 2.3	0.6 ± 0.1	2.2 ± 0.2	1.4 ± 0.3	31.1 ± 1.2
Stearic	57.1 ± 0.8	0.5 ± 0.0	2.4 ± 0.4	1.3 ± 0.3	38.5 ± 2.1

* Duration of experiment was 51 hr.

† Standard error of the mean $= \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

elaidic acid groups are superimposable over the entire 51 hr experimental period; thus, the geometry of the double bond did not affect the excretion of the C^{14} (from the carboxyl carbon) into the respiratory CO_2.

The rate of excretion of $C^{14}O_2$ in the palmitic acid group was quite similar to that in the oleic and elaidic acid groups. At the end of 51 hr, 70% of the administered C^{14} had been eliminated in the CO_2 in the palmitic acid group while 66% had been eliminated in the oleic and elaidic acid groups. Similar values for oleic acid were obtained by Bernhard et al. (16); they found that when free oleic acid, dissolved in corn oil, was fed to essential fatty acid-deficient mice, 46.7% of the administered C^{14} appeared in the respiratory CO_2 in 24 hr. However, Mead et al. (17) reported a much more rapid catabolism rate for oleic acid in mice. In their study approximately 49% of the administered oleic acid-C^{14} was excreted as CO_2 in 10 hr, when it was fed as the methyl ester.

When the stearic acid-C^{14} fat was fed, a different excretion pattern was observed. The rate of appearance of C^{14} in the respiratory CO_2 during the first 15 hr of the experiment was perceptibly lower for this group than for the palmitic, oleic, and elaidic acid groups. In the final hours of the experiment, however, the rate was equal to or greater than that for the other groups. Even so, at the end of the 51 hr experimental period only 57% of the fed C^{14} had been excreted as $C^{14}O_2$ by those animals fed the stearic acid-labeled soybean oil. Mead et al. (17) have reported that, at the end of 10 hr, mice fed methyl oleate-C^{14} had excreted approximately 2.5 times as much of the fed C^{14} into the respiratory CO_2 as had mice fed methyl stearate-C^{14}, a somewhat greater difference than seen here.

The final distribution of the administered activity at the end of 51 hr for all groups is shown in Table 2. These data are the averages of the values from three animals for the oleic group, four for the elaidic and stearic groups, and seven for the palmitic group. The C^{14} recoveries ranged from 95 to 105%. The low amount of C^{14} recovered in the gastrointestinal contents and feces of the animals indicates that the labeled fatty acids were well absorbed in all cases.

The C^{14} distribution was very similar for the oleic, elaidic, and palmitic acid groups, the only apparent difference being the unexplainably low value for carcass C^{14} in the oleic acid group. On the other hand, the stearic acid group showed a much lower elimination of C^{14} into the respiratory CO_2 and a concomitant increase in the amount of C^{14} remaining in the carcass. Thus, it would appear that stearic acid had the lowest catabolic rate among the acids studied.

The appearance of C^{14} in the lymph of animals fed the various labeled fatty acids was followed. These data are shown graphically in Fig. 2. Each point on these curves represents an average of the values from four animals. The C^{14} recovered in the lymph lipids ranged from 83 to 90% for the various groups. The weight of lipid recovered ranged from 1.3 to 1.6 g per animal. The absorption behavior of all the experimental fats was similar; variation among groups at any point during the absorption period was no greater than the variation among animals within the groups. Absorption, as calculated from residual C^{14} in the gastrointestinal tract and feces, exceeded 96% in all groups. The difference in absorption values obtained by using the amount of C^{14} appearing in the lymph lipids vs. the amount of C^{14} disappearing from

TABLE 3 SPECIFIC ACTIVITIES OF THE VARIOUS LIPID CLASSES OBTAINED FROM THE LYMPH OF ANIMALS FED LABELED OLEIC, ELAIDIC, PALMITIC, OR STEARIC ACID

Time after Feeding	Specific Activity		
	Glyceride	Phospholipid	Sterol Ester
hr	*μc/g*	*μc/g*	*μc/g*
	*Oleic Acid Group**		
0–5	8.6	1.7	1.5
5–17	9.5	2.9	2.2
17–25	9.1	2.3	2.2
29–43	0.5	0.2	0.4
	*Elaidic Acid Group**		
0–5	9.0	1.7	1.2
5–17	10.4	5.2	1.6
17–25	9.2	3.0	1.0
29–43	0.5	0.2	0.3
	*Palmitic Acid Group**		
0–4	10.9	2.3	0.6
4–16	12.2	4.8	2.1
16–24	10.8	3.9	0.6
28–43	1.8	1.4	0.3
	*Stearic Acid Group**		
0–4	7.1	6.2	0.4
4–16	8.6	14.3	5.6
16–24	9.3	10.7	0.9
28–43	2.7	3.5	1.1

* Specific activities of the dietary fats were: oleic acid, 11.2; elaidic acid, 11.9; palmitic acid, 12.3; stearic acid, 11.5 μc/g. The periods 4–16 and 5–17 hr represent the peak of absorption.

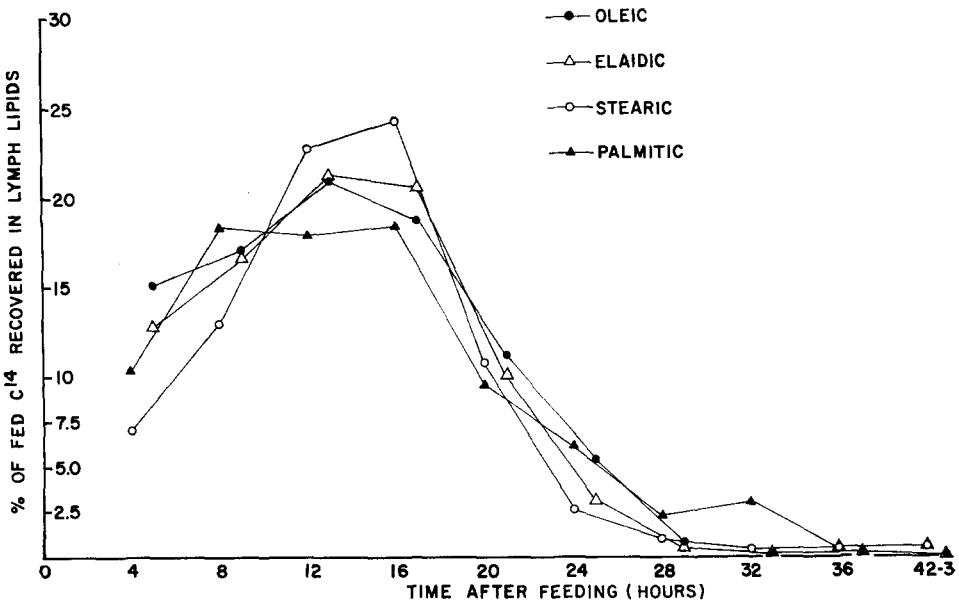

FIG. 2. Appearance of C^{14} in the lymph lipids following the feeding of labeled fatty acids.

the gut is probably a composite of an incompleteness of the extraction of lipid from the lymph and of the absorption of a portion of the C^{14}-fatty acids via a pathway other than the thoracic duct.

The specific activities of the glycerides, phospholipids, and sterol esters, obtained from the composite lymph lipid samples by silicic acid column chromatography, are shown in Table 3. The distribution of the C^{14} fatty acids among these classes, on a per cent of recovered activity basis, is shown in Table 4. A comparison of these data for oleic and elaidic acids shows that these compounds were very similar in their behavior except that, during the peak of absorption, elaidic acid showed a slightly greater incorporation into the lymph phospholipid fraction than did oleic acid (Tables 3 and 4). A similar tendency for elaidic acid to be incorporated into the phospholipid fraction of blood and other tissues was seen by Sinclair (1, 2), who reported that elaidic acid was incorporated into these phospholipids at the expense of the saturated fatty acid rather than at the expense of oleic acid.

The data in Tables 3 and 4 show stearic acid to have a strong tendency to enter the phospholipid fraction of lymph. This is in agreement with reports by Blomstrand et al. (18) and Bergström et al. (19). Thus, elaidic acid tends to be like stearic acid in this respect. This resemblance between stearic and elaidic acids might be explained on the basis of carbon number and conformation. The *trans* configuration of the elaidic acid double bond causes this acid to assume an 18-carbon, straight-chain conformation similar to stearic acid. Oleic acid, in which the double bond is *cis*, has a folded conformation, quite different from that of stearic acid.

The significant incorporation of stearic acid into phospholipids may provide an explanation for the slower catabolism of this acid to CO_2 (Fig. 1). Olivecrona (20) has shown, in vitro, that phospholipids turn over more slowly than do triglycerides. In addition, lecithin heterogeneity has been demonstrated by Collins (21) and by Harris et al. (22). This heterogeneity is evidenced by both a difference in turnover rate and in fatty acid composition of two different lecithin fractions separated by silicic acid column chromatography. The fraction higher in stearic acid had the slower turnover rate (24). If this preferential incorporation of stearic acid into phospholipids carried over to the tissues, as the evidence suggests that it does (23, 24), it is conceivable that the slower turnover rate of such a phospholipid fraction might explain the slower catabolism observed for the stearic acid-C^{14}.

Palmitic acid was found to be incorporated primarily into the triglyceride fraction of the lymph lipids (Table 3). Its general distribution was very similar to that found for oleic acid (Table 4).

The incorporation of the fatty acids into the sterol fraction of the lymph was minor, on a per cent incorpora-

TABLE 4 INCORPORATION OF C^{14}-FATTY ACIDS DURING THE PEAK OF ABSORPTION INTO THE VARIOUS LYMPH LIPID CLASSES AFTER FEEDING LABELED SOYBEAN OILS TO RATS

	Per Cent of Recovered C^{14}		
C^{14}-Fatty Acid	Glycerides	Phospho-lipids	Sterol Esters
Oleic	97.8	2.0	0.3
Elaidic	97.0	2.9	0.2
Palmitic	97.4	2.1	0.5
Stearic	89.2	7.8	3.1

tion basis (Table 4), except for stearic acid. The sterol ester fraction contained 3% of the recovered activity in the stearic acid group. Recently, Karmen et al. reported a slight preferential incorporation of oleic acid into the cholesterol esters of lymph chylomicrons (25). The reason for the apparent disagreement with these authors is not clear, but may be partly because of the very small proportion of the fed C^{14} which enters this group of lipids (<1%). With the exception of the sterol esters, the distribution of oleic, palmitic, and stearic acids among the lymph lipids was similar to that reported by Whyte et al. (26).

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