Metabolism of arachidonic acid-1-^{14}C in the rat

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SUMMARY The metabolism of arachidonic acid-1-^{14}C has been studied in the rat. The acid was fed as a component of randomly rearranged soybean oil; its absorption was greater than 96%. The rate of catabolism of arachidonic acid was significantly lower than that previously seen for linoleic acid and other long-chain fatty acids. A marked tendency for arachidonic acid to be incorporated into phospholipids was observed. Arachidonic acid-containing phospholipids have been shown to have a slower turnover rate than linoleic and palmitic acid-containing phospholipids. It is proposed that this slower turnover rate is a reflection of the "essential" character of arachidonic acid and is a rational explanation for the slower catabolic rate of this acid.

KEY WORDS metabolism · arachidonic acid · rat · absorption · catabolism

THE BIOSYNTHESIS OF ARACHIDONIC acid has been investigated by Mead and his co-workers, and these studies have been reviewed by Mead (1). Recently Van Dorp, Beerthuis, Nugteren, and Vonkeman (2) and Bergström, Danielsson, and Samuelsson (3) have shown arachidonic acid to be a precursor for the biosynthesis of prostaglandin E_2, a compound possessing a hormone-like activity in vivo. In 1959, Brown and Tappel (4) reported that the mitochondria of both rat and carp liver were capable of oxidizing arachidonic acid in vitro. The rate of oxidation of arachidonic acid by carp liver mitochondria was roughly the same as that for other long-chain fatty acids tested with the exception of linolenic acid, which was oxidized more rapidly. Rat liver mitochondria, on the other hand, oxidized arachidonic acid and linolenic acid at the same rate. Recently Coniglio, Davis, and Aylward (5) have reported the results of a short-term study on the metabolism of arachidonic acid in the rat. They found that 10% or less of the absorbed acid was excreted as CO_2 in 6 hr, depending on the nutritional state of the animal. It was the purpose of the study reported here to investigate the absorption and catabolism of arachidonic acid in vivo and compare its metabolic fate with that of other long-chain fatty acids.

A preliminary report of this work has been presented orally: *Federation Proc.* **22**: 303, 1963 (abstract).

MATERIALS AND METHODS

The arachidonic acid-1-^{14}C used in these experiments was the generous gift of Hoffmann-La Roche, Inc., Nutley, N. J. It had a specific activity of 3.85 μc/mg and a purity of ~89% by gas–liquid chromatography. The analytical data were supplied by Hoffmann-La Roche, Inc.

The experimental fat was prepared by randomly incorporating the labeled arachidonic acid into the triglycerides of soybean oil. Details of this procedure are described in an earlier publication (6). Specific activity of the resulting fat was 7.5 μc/g. The labeled soybean oil was fed as part of a liquid diet (6). Each animal was given about 5.3 g of the diet, the actual amount fed being determined for each rat. The amount of arachidonic acid received by each animal under these conditions was ~3 mg in a total fat intake of ~1.5 g.

Young, adult, male albino rats of the Holtzman strain were used in these studies. Training and feeding procedures have been described elsewhere (6).

In the catabolism study a group of 4 animals was used. Respiratory CO_2, urine, and feces were collected for 51 hr. At the end of this experimental period the animals were killed and the disposition of the ^{14}C was determined as previously described (6). In addition to the fractions listed above, the ^{14}C content of the gastrointestinal contents and the remaining carcass was also determined.

In the absorption study a group of 5 animals was used. Lymph was collected from each animal for a total of 42 hr following administration of the experimental diet.

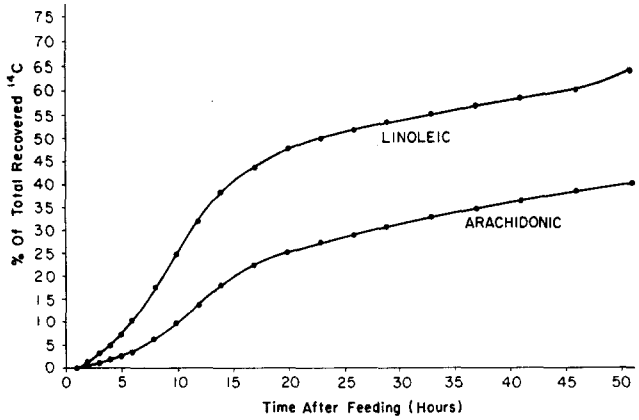

FIG. 1. Appearance of ^{14}C in the respiratory CO_2 following the feeding of arachidonic acid-1-^{14}C or linoleic acid-1-^{14}C.

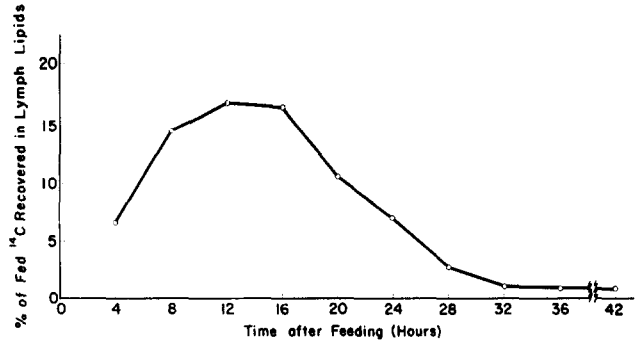

FIG. 2. Appearance of ^{14}C in the lymph lipids following the feeding of arachidonic acid-1-^{14}C.

The lymph lipids were isolated and separated into glyceride, phospholipid, and sterol ester classes. The experimental details have been reported in an earlier publication (6). Free fatty acids were removed from the glyceride fraction with Dowex-2 ion-exchange resin.

Radioactivity measurements were made using a "Tri-Carb" liquid scintillation counter (7). When CO_2 was to be counted, it was converted to barium carbonate which was suspended in "Thixin" scintillation gel (8).

RESULTS

Figure 1 shows the cumulative rate of appearance of ^{14}C in the respiratory CO_2, as a function of time, following the feeding of the arachidonic acid-labeled soybean oil. For comparison purposes similar data for *cis,cis*-linoleic acid from a previous paper (9) are also presented. The catabolism experiment data are normalized to 100% recovery, that is, they are expressed as a percentage of the total activity recovered in all fractions. Actual total recoveries in these studies averaged 96% for the arachidonic acid group and 97% for the linoleic acid group. During the 51-hr experimental period only 40% of the recovered ^{14}C was excreted as respiratory CO_2 by the group fed arachidonic acid. This amount was considerably less ($P = 0.001$) than the value of 64% obtained under similar conditions with *cis,cis*-linoleic acid-1-^{14}C.

The disposition of radioactivity 51 hr after the feeding of the labeled acid is shown in Table 1. Similar data for *cis,cis*-linoleic acid, previously reported (9), are given for comparison. Absorption of the arachidonic acid was excellent (>96%), as indicated by the small amount of ^{14}C recovered from the gastrointestinal tract contents and the feces. The amount of ^{14}C recovered in the carcasses of the rats fed arachidonic acid was considerably greater ($P = 0.001$) than that seen with the rats fed linoleic acid. Since the rate of catabolism of arachidonic acid was low, the greater incorporation into body lipids was reasonable.

The appearance of ^{14}C in lymph of animals fed arachidonic acid-labeled soybean oil is shown in Fig. 2; each point on the curve represents the average of the values from 5 animals. Peak of absorption occurred 12–16 hr after feeding.

In Table 2 is shown the distribution of the arachidonic acid-^{14}C among the various lipid classes of the lymph, glyceride, phospholipid, and sterol ester, which were isolated from the composite lymph lipids by silicic acid column chromatography. The data are reported as a percentage of the total activity recovered from the chromatographic column. Recovery of ^{14}C applied to the column was 96.3%. Similar data for linoleic acid (9)

TABLE 1 DISPOSITION OF ^{14}C BY THE RAT AFTER INGESTING ARACHIDONIC ACID-1-^{14}C OR LINOLEIC ACID-1-^{14}C*

	% of Recovered Activity				
Fatty Acid Fed	CO_2	Urine	Feces	Gastro-intestinal Contents	Carcass
Arachidonic†	40.2 ± 0.5‡	0.8 ± 0.3	1.2 ± 0.4	1.9 ± 0.1	56.0 ± 0.9
Linoleic§	64.2 ± 1.9	—	1.3 ± 0.2 ‖	0.8 ± 0.2	33.8 ± 1.9

* Duration of experiment was 51 hr.
† Data are the averages of the values from 4 animals.
‡ Standard error of the mean = $\pm\sqrt{\Sigma d^2/n(n-1)}$.
§ Linoleic acid data taken from reference 9.
‖ Urine and feces collected together.

TABLE 2 DISTRIBUTION OF FATTY ACIDS AMONG THE VARIOUS LIPID CLASSES OF THE LYMPH

Acid Fed	% of Recovered ^{14}C		
	Glycerides	Phospholipids	Sterol Esters
Arachidonic	90.0	9.9	0.1
Linoleic*	97.0	3.0	0.1
Stearic†	89.2	7.8	3.1

* Linoleic acid data taken from reference 9.
† Stearic acid data taken from reference 6.

and stearic acid (6) are given for comparison. The major portion of the arachidonic acid, 90%, was transported in the lymph as glyceride even though the specific activity of this fraction was only about one-half that of the phospholipid fraction. During the peak of absorption the specific activities of the glyceride, phospholipid, and sterol ester fractions were 6.0, 12.4, and 1.1 μc/g, respectively.

In one experiment the amount of ^{14}C in the respiratory CO_2 of a thoracic duct-cannulated animal was measured. It was found that 5.4% of the recovered ^{14}C was excreted as $^{14}CO_2$. This is in agreement with a report by Blomstrand (10) indicating a similar level of $^{14}CO_2$ excretion when methyl linoleate-^{14}C and methyl palmitate-^{14}C were fed to thoracic duct-cannulated rats. Thus, long-chain fatty acids can presumably be absorbed by a pathway other than the main thoracic duct. This absorption could be via the portal circulation or via a collateral lymphatic circulation.

DISCUSSION

The extent to which arachidonic acid was catabolized to CO_2 was markedly lower than that we have reported for other long-chain fatty acids (6, 9). It is probable that this represents a conservation of arachidonic acid for essential structural requirements within the cell since this acid was incorporated to a large extent into the carcasses of the experimental animals. It would appear from a comparison of the extents to which arachidonic and linoleic acids are catabolized to $^{14}CO_2$ that arachidonic acid may be the more important acid from the standpoint of the economy of the cell. One might suspect that the difference in catabolism of arachidonic and linoleic acids was a reflection of the absolute quantities of the two acids in the diet. This does not seem to be an adequate explanation, however, because varying the linoleic acid content of the diet from 42 to 0.8% did not drastically affect the percentage of the administered linoleic acid-^{14}C which was oxidized to $^{14}CO_2$ (unpublished data). The low level of linoleic acid represented an intake of ~12 mg as compared with ~3mg for arachidonic acid in these studies. In addition, Coniglio et al. (5) saw no difference in $^{14}CO_2$ production between rats deficient in essential fatty acid and receiving only 0.5 mg of arachidonic acid-^{14}C and those which received a 300 mg per day supplement of arachidonic acid for 2 or 5 days prior to the study. This preferential retention of arachidonic acid is in agreement with the work of Thomasson (11) who showed that the essential fatty acid activity of arachidonic acid was greater than that of linoleic acid. These data are also in agreement with the findings of Coniglio et al. (5) who report a low catabolic rate for arachidonic acid, ~10% of the absorbed ^{14}C being excreted as CO_2 in 6 hr.

The absorption of arachidonic acid, as measured by the appearance of ^{14}C in the thoracic duct lymph of the experimental animals, was similar to the absorption of linoleic acid and other long-chain fatty acids (6, 9). However, the distribution of ^{14}C among the various lymph lipid classes when arachidonic acid-^{14}C was fed was different from that of linoleic acid and the other long-chain acids which we have studied (6, 9), except for stearic acid. Arachidonic acid showed a pronounced specificity for the phospholipid fraction of the lymph lipids, being incorporated into this lipid class to a more than 3-fold greater extent than was linoleic acid (~10% of the recovered ^{14}C vs. 3%, see Table 2). The specific activities of the phospholipids were 4.6 and 12.4 μc/g for the linoleic and arachidonic groups, respectively, during the peak of absorption. The incorporation of arachidonic and stearic acids into the phospholipids of lymph was similar on a percentage basis, being ~10% for arachidonic acid and ~8% for stearic acid (Table 2).

A marked tendency for arachidonic acid to enter the body phospholipid pool would offer a rational explanation for the rather low catabolic rate for this acid. Phospholipids are important to the structural integrity of the cell and are known to turn over more slowly than do triglycerides, for example (12). In addition, lecithins containing arachidonic and stearic acids have been shown to have a slower turnover rate than lecithins containing other fatty acids such as palmitic acid (13, 14). Thus, a high percentage of the total body arachidonic acid being present as tissue phospholipid together with a low turnover rate for that fraction could account for the lower rate of catabolism for this acid. Evidence is available which shows that arachidonic acid does occur primarily in the phospholipid fraction of tissues. Veerkamp, Mulder, and Van Deenen (15) have shown that the arachidonic acid content of tissue neutral lipid is low (0–2%) while that in phospholipids is relatively high (9–21%). Coniglio et al. (5) reported that 65–75% of the arachidonic acid-^{14}C in liver was present in the phospholipids. In our own experience 95% of the ^{14}C found in the liver of rats 8 hr after feeding arachidonic acid-^{14}C is in the phospholipids, and qualitatively similar (un-

published) data were obtained for kidney. Therefore, the low catabolic rate of arachidonic acid seems to be intimately connected with its high incorporation into the tissue phospholipids. This concept reinforces the belief that arachidonic acid plays a major structural role in the body, since phospholipids are known to be important to the integrity of the cell.

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