ASBMB
JOURNAL OF LIPID RESEARCH

A comparison of the metabolism of *cis*, *cis*-linoleic, *trans*,*trans*-linoleic, and a mixture of *cis*,*trans*- and *trans*,*cis*-linoleic acids in the rat

ROBERT H. COOTS

The Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio

Downloaded from www.jlr.org by guest, on June 19, 2012

SUMMARY A comparison has been made of the metabolism of *cis*,*cis*-linoleic, *trans*,*trans*-linoleic, and a mixture of *cis*,*trans*- and *trans*,*cis*-linoleic acids in the rat. The data show that linoleic acid and its geometric isomers were well absorbed. These acids were readily oxidized to CO_2 with no apparent difference in rate or extent of catabolism between the *trans*-isomers. However, the *trans*-isomers of linoleic acid were catabolized to CO_2 to a somewhat greater extent than was *cis*,*cis*-linoleic acid. Although these differences were small, they are consistent with the fact that the geometric isomers have no essential fatty acid activity and, therefore, might conceivably be more available as an energy source than the *cis*,*cis*-isomer. The *trans*-linoleic acids, like the *cis*,*cis*-linoleic acid, were transported in the lymph mainly as glycerides. There were no major differences in the distribution of the various acids among the lymph lipid classes, indicating that the rat does not distinguish among the linoleic acid isomers as far as digestion and absorption are concerned. Linoleic acid and its *trans*-isomers were metabolized in an efficient and apparently normal manner.

THE EFFECT ON THE metabolic fate of an unsaturated fatty acid which might result from a change in the geometric configuration, from *cis* to *trans*, has long been of interest. It has been shown conclusively that changing the geometry of one or both of the double bonds of linoleic acid from the *cis*-configuration to the *trans*-configuration results in the loss of essential fatty acid activity (1–4). However, although the geometric isomers of linoleic acid no longer possess essential fatty acid activity, they do not interfere with that of the *cis*,*cis*-isomer (4). Whether or not the geometric isomers of linoleic acid differ metabolically in other respects is not known. Therefore, it was the purpose of this study to compare the absorption and catabolism of these isomers in the rat. The compounds under investigation were the following 1-C^{14}-labeled acids: *cis*,*cis*-linoleic acid, *trans*,*trans*-linoleic acid, and a mixture of *cis*,*trans*- and *trans*,*cis*-linoleic acids.

MATERIALS AND METHODS

The *cis*,*cis*-linoleic acid-1-C^{14} used in these experiments was purchased from Isotopes Specialties Company, Burbank, Calif. After suitable dilution with unlabeled carrier, the acid was purified by liquid–liquid countercurrent distribution, 400 transfers, using the two-phase solvent system resulting from a mixture of heptane, dimethyl formamide, and glacial acetic acid, 3:2:1 (v/v).

The geometric isomers of linoleic acid were prepared by SO_2 isomerization. *cis*,*cis*-Linoleic acid was dissolved in liquid SO_2 at a concentration of 0.1 g/ml and maintained at 38° for 9 hr. The geometric isomers were purified by repeated crystallization from 10 volumes of acetone, *trans*,*trans*-linoleic acid at −32° and the mixture of *cis*,*trans*- and *trans*,*cis*-isomers at −50°. This procedure produces no detectable positional isomers.

Chemical purity of the fatty acids was established by gas–liquid chromatography using an F & M 500 gas chromatograph. The conditions were: column length, 305 cm (0.4 cm i.d.); packing, 15% (w/w) stabilized succinic acid diethylene glycolate on 60–70 mesh Anakrom ABS; temperature, 170°; and helium flow rate, 60–70 ml/min, standard temperature and pressure. Peak identification was based upon the chromatographic behavior of reference fatty acids. All of the linoleic acids were shown to be at least 96% octadecadienoic acid.

Radiochemical purity of the fatty acids was established by reversed-phase paper chromatography on Whatman

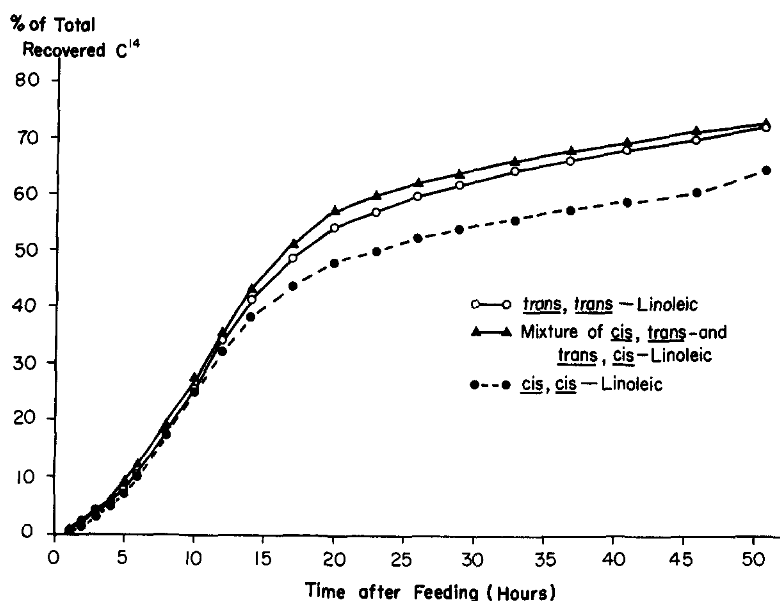

FIG. 1. Appearance of C^{14} in the respiratory CO_2 following the feeding of labeled fatty acids.

No. 1 paper. The solvent system was 75% aqueous acetic acid and the chromatograms were developed for 7 and 16 hr in a descending direction at 38°. After development each paper was scanned for radioactivity using an end-window G-M tube with a $^1/_4$ inch slit-opening to locate the C^{14} peaks. Quantification was achieved by cutting the chromatograms into $^1/_4$ inch strips and counting, after immersion in scintillation solution. The radiochemical purity of the acids was 99%.

The isomeric purity of the methyl esters of the linoleic isomers was determined using infrared spectroscopy. The instrument used was a Perkin-Elmer 112, single beam infrared spectrophotometer. The average number of *trans* double bonds per molecule was calculated using pure methyl elaidate as a standard. The *cis,trans-* and *trans,cis*-linoleic acid mixture contained 1.0 *trans* double bonds per molecule and the *trans,trans*-linoleic acid contained 1.9. Theoretical values would be 1.0 and 2.0, respectively.

The experimental fats were prepared by randomly incorporating the labeled acids into the triglycerides of soybean oil as previously described (5). These dietary fats had the following specific activities: *cis,cis*-linoleic, 7.7 μc/g; *trans,trans*-linoleic, 6.0 μc/g; and the *cis,trans-* and *trans,cis*-mixture, 5.7 μc/g. The labeled soybean oils were fed as part of a liquid diet (5). Each rat was given about 5.5 g of the diet, the actual amount fed being determined for each animal.

The experimental animals were young, adult, male albino rats of the Holtzman strain. In the catabolism experiments groups of six animals were used; in the absorption studies groups of five animals were used except for the group receiving *cis,cis*-linoleic acid which was made up of four animals. The feeding regimen and the experimental procedures for both the catabolism and the absorption studies have been described in detail previously (5).

Radioactivity measurements were made with a "Tri-Carb" liquid scintillation counter (6). When CO_2 was to be counted, it was converted to barium carbonate which was suspended in "Thixin" scintillation gel (7).

RESULTS AND DISCUSSION

The appearance of C^{14} in the respiratory CO_2, as a function of time, after feeding the various experimental fats is shown in Fig. 1. The excretion curves for the *trans,trans*-linoleic acid and the mixture of *cis,trans-* and *trans,cis*-linoleic acids are quite similar and represent an essentially identical rate of catabolism for these acids.

During the first 10 hours after feeding, the rate of excretion of $C^{14}O_2$ by the group fed the *cis,cis*-linoleic acid-1-C^{14} was identical with the rate of excretion by the groups fed the geometric isomers of linoleic acid. After 10 hr, however, the excretion of $C^{14}O_2$ by the *cis,cis*-linoleic acid group fell behind that by the groups fed the linoleic acid isomers. As a consequence, at the end of the 51 hr experimental period 64% of the recovered C^{14} had been eliminated in the CO_2 of the *cis,cis*-linoleic acid group while 72% of the recovered C^{14} had been eliminated in the CO_2 of the groups fed the isomeric linoleic acids. This difference was statistically significant at the 90% confidence level ($P = 0.1$).

While the differences observed among the *cis,cis*-linoleic acid and the *trans*-isomers were not great, they are compatible with a known difference in the metabolic

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH JLR

TABLE 1 DISPOSITION OF C^{14} BY THE RAT AFTER FEEDING THE GEOMETRIC ISOMERS OF LINOLEIC ACID*

C^{14} Isomer	% of Recovered Activity				
	CO_2	Urine	Feces	Gastrointestinal Contents	Carcass
cis,cis†	64.2 ± 1.9‡	—	1.3 ± 0.2	0.8 ± 0.2	33.8 ± 1.9
trans,trans	72.1 ± 3.0	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	25.8 ± 2.7
cis,trans and *trans,cis*	72.1 ± 3.2	0.8 ± 0.1	0.9 ± 0.2	0.6 ± 0.1	25.6 ± 3.2

* Duration of experiment was 51 hr.
† Urine and feces collected together.
‡ Standard error of the mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

function of these acids. It has been conclusively established that the geometric isomers of linoleic acid possess no essential fatty acid activity (2–4); one might, therefore, expect that that portion of the ingested isomeric linoleic acids which was not capable of substituting for linoleic acid in this particular metabolic function would be readily available as an energy source. Thus, it is reasonable that the geometric isomers of linoleic acid should contribute somewhat more heavily to the CO_2 output of the animal than would *cis,cis*-linoleic acid.

The rate of catabolism of *cis,cis*-linoleic acid in normal mice observed by Mead et al. (8) (31% of the fed C^{14} appeared in the respiratory CO_2 in 10 hr) was somewhat greater than was observed in these studies. Bernhard et al. (9) reported that in essential fatty acid-deficient rats 38% of the fed *cis,cis*-linoleic acid was catabolized to CO_2 in 24 hr, somewhat less than the 50% reported here for normal rats.

The final distribution of the recovered activity for all groups at the end of the 51 hr experimental period is shown in Table 1. These data are the averages of the values obtained from six animals. The actual C^{14} recoveries ranged from 95 to 98%. The low amount of C^{14} recovered in the gastrointestinal tract contents and the feces of the animals indicates that absorption of linoleic acid and its geometric isomers was essentially complete and that no differences in absorption occurred among the various acids.

A comparison of the C^{14} distribution obtained for *cis,cis*-linoleic acid and its isomers (Table 1) shows that no difference existed in the observed catabolism of the *trans*-isomers of linoleic acid. However, the *cis,cis*-linoleic acid was converted to CO_2 to a lesser extent ($P = 0.1$) and was retained in the carcass to a greater extent ($P = 0.05$) than were the *trans*-isomers. These data tend to strengthen the hypothesis that the *trans*-isomers of linoleic acid are less able to serve in essential structural capacities than is the *cis,cis*-isomer, but they show that the *trans*-isomers serve readily as an energy source. The *trans*-linoleic acid isomers were oxidized to CO_2 to an extent similar to that of oleic, elaidic, and palmitic acids (5).

The appearance of C^{14} in the lymph of animals fed the various C^{14}-fatty acids was also followed and the data obtained are shown graphically in Fig. 2. Each point on the curves for the *trans*-linoleic acids represents the average of the values from five animals and on the curve for *cis,cis*-linoleic acid the average of the values from four animals. The C^{14} recovered in the lymph lipids ranged

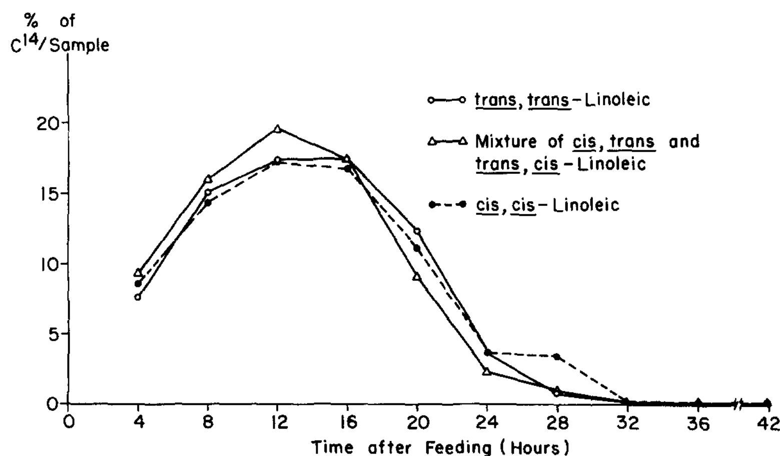

FIG. 2. Appearance of C^{14} in the lymph lipids following the feeding of labeled fatty acids.

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH JLR

TABLE 2 Incorporation of the Geometric Isomers of Linoleic Acid into the Various Lymph Lipid Classes of the Rat

C^{14} Isomer	% of Recovered C^{14}		
	Glycerides	Phospholipids	Sterol Esters
cis, cis,	97.0	3.0	0.1
trans,trans	97.4	2.2	0.3
cis,trans and *trans,cis*	96.9	3.0	0.2

from 82 to 91%. No significant differences were noted in absorption behavior among the various acids. Over-all absorption, as indicated by residual C^{14} in the gastrointestinal tract and feces, exceeded 98% for all groups. The difference in absorption values obtained by using the amount of C^{14} appearing in the lymph lipids versus the amount of C^{14} disappearing from the gut is the result of a combination of factors, among which are a less than 100% efficient extraction of C^{14} from the collected lymph and absorption of a portion of the fed C^{14} fatty acids by a non-thoracic duct pathway, the latter amounting to 5–10% of the fed C^{14}.

The distribution of the C^{14} fatty acids among the various lymph lipid classes (glyceride, phospholipid and sterol ester), obtained from the composite lipid samples by silicic acid column chromatography, is shown in Table 2. In general, it would appear that the rat did not discriminate between isomers as far as the processes of digestion and absorption are concerned. It is obvious that linoleic acid and its geometric isomers were transported in the lymph mainly as glycerides. This behavior of the *cis,cis*-linoleic acid is in agreement with the human studies of Blomstrand et al. (10) and studies with rat lymph chylomicrons by Whyte et al. (11). A small difference was observed in the amount of *trans,trans*-linoleic acid esterified in the phospholipid fraction as compared with *cis,cis*-linoleic acid and the mixture of *cis,trans*- and *trans,cis*-linoleic acids. The physiologic significance, if any, of this difference is obscure. Although the absolute values for the various sterol ester fractions differ slightly, the amount of these fractions isolated was too small to allow any conclusions to be drawn at this time.

In general, the rat seems to metabolize the geometric isomers of linoleic acid in an efficient and normal manner. If one assumes that the unsaturated fatty acids are oxidized via β-oxidation, it should not be surprising that the *trans*-fatty acid isomers are oxidized as readily by the animal as are the *cis*-fatty acids because the double bond resulting from the action of an acyl coenzyme A dehydrogenase (green enzyme) is reported to be of the *trans*-configuration (12). Thus, in the β-oxidation sequence, *trans*-double bonds seem to be the normal substrate for the subsequent hydrating action of crotonase.

The author wishes to express his appreciation to Mr. E. J. Hollenbach for capable technical assistance and to Mr. R. G. Folzenlogen for preparing the *trans*-isomers of linoleic acid.

Manuscript received February 17, 1964; accepted April 1, 1964.

References

1. Holman, R. T. *Proc. Soc. Exptl. Biol. Med.* **76:** 100, 1951.
2. Privett, O. S., F. J. Pusch, and R. T. Holman. *Arch. Biochem. Biophys.* **57:** 156, 1955.
3. Holman, R. T., and E. Aaes-Jørgensen. *Proc. Soc. Exptl. Biol. Med.* **93:** 175, 1956.
4. Mattson, F. H. *J. Nutr.* **71:** 366, 1960.
5. Coots, R. H. *J. Lipid Res.*, **5:** 468, 1964.
6. Davidson, J. D., and G. Feigelson. *Intern. J. Appl. Radiation Isotopes* **2:** 1, 1957.
7. White, C. G., and S. Helf. *Nucleonics* **14** (10): 46, 1956.
8. Mead, J. F., W. H. Slaton, Jr., and A. B. Decker. *J. Biol. Chem.* **218:** 401, 1956.
9. Bernhard, K., M. Rothlin, J.-P. Vuilleumier, and R. Wyss. *Helv. Chim. Acta* **41:** 1017, 1958.
10. Blomstrand, R., O. Dahlback, and E. Linder. *Proc. Soc. Exptl. Biol. Med.* **100:** 768, 1959.
11. Whyte, M., A. Karmen, and DeW. S. Goodman. *J. Lipid Res.* **4:** 322, 1963.
12. Green, D. E., *Biol. Rev. Cambridge Phil. Soc.* **29:** 330. 1954

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 19, 2012