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Labeling of Serum, Liver, and Testicular Lipids Following the Injection Of Arachidonic-1-C^{14} Acid.* (31840)

LEON SWELL AND M. D. LAW
Veterans Administration Hospital and Department Biochemistry, Medical College of Virginia, Richmond, Virginia

It has been demonstrated that arachidonic acid is synthesized from linoleic acid and that arachidonic acid is the precursor of prostaglandin E_2, highly polar metabolites, and certain long chain polyunsaturated fatty acids (1-4). Recent studies(5,6) have shown that arachidonic acid is well absorbed in the rat and appears in the lymph principally as triglyceride. Examination of the liver lipids from several animals fed arachidonic-1-C^{14} acid showed that 65-95% of the C^{14}-arachidonic acid was present in the phospholipid fraction. Also, conversion of arachidonic acid to CO_2 was shown to be slower than that of linoleic acid, suggesting a slower rate of metabolism of arachidonic acid. In view of the limited information available on the metabolism of arachidonic acid, it was of interest to study the handling of injected arachidonic-1-C^{14} acid. Data are presented on the turnover of arachidonic acid and the appearance of arachidonic-1-C^{14} acid in the different lipid fractions of serum, liver, and testes.

Methods and materials. Methyl arachidonate-l-C^{14} (4 μc/mg) was the generous gift of Hoffmann-LaRoche, Inc., Nutley, N.J. It was checked for purity by gas-liquid chromatography and gas-liquid radiochromatography (7). Purity by mass was 97% and radioactive purity was 96%. Fasting, male rats, (Wistar strain) maintained on Purina pellet chow, and weighing 150-185 g were injected into the saphenous vein with 1 μc of methyl arachidonate-1-C^{14} in 0.5 ml rat serum. The solution for injection was prepared by dispersing (by ultrasonic disintegration for 2 minutes at 5°C) 0.25 mg (1 μc) methyl arachidonate-1-C^{14} in 0.5 ml rat serum. The arachidonate was found to be uniformly dispersed in the serum and no chemical changes in the arachidonate-1-C^{14} was noted when the serum was subsequently extracted and analyzed by thin-layer and gas-liquid radiochromatography. The animals remained fasting following the injection until time of sacrifice. Groups of 5 animals were sacrificed at 1, 4, and 24 hours after the injection. At time of sacrifice, the liver, testes, and blood were obtained. The liver and testes were quick-frozen with dry ice, pulverized, and extracted with 2:1 chloroform-methanol. The serum was separated and extracted with 20 volumes 2:1 chloroform-methanol. The tissue lipids were separated by silicic acid column chromatography(8). Cholesterol esters were eluted with 1% diethyl ether-petroleum ether. Glycerides and fatty acids with 25% diethyl ether-petroleum ether, and phospholipids with methanol. The C^{14}-activity of the lipid fractions was determined in a liquid scintillation

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TABLE I. Recovery of Injected C^{14}-Activity in Tissues.

Time hours	% injected C^{14}-arachidonate recovered*		
	Serum†	Liver	Testes
1	2.2 ± 1.0	35.2 ± 4.5	.2 ± .1
4	1.4 ± .4	24.1 ± 6.0	.2 ± .1
24	.5 ± .2	9.1 ± 1.8	.2 ± .1

* Values represent average ± S.D. of 5 animals.
† Estimated from serum volume based on body weight.

counter. Further separation of the lipid fractions was carried out by thin-layer chromatography on silica gel G with a solvent mixture of 90:10:1 hexane diethyl-ether acetic acid. Identification of the C^{14}-fatty acids in the tissue lipids following the injection of arachidonic acid-1-C^{14} was carried out with the aid of gas-liquid radiochromatography as previously described(9).

Results. The recovery of injected C^{14}-activity in the tissues is shown in Table I. Of the tissues examined, the liver contained the greatest percentage of the C^{14}-activity injected. The total recovery of C^{14}-activity in the tissues (serum, liver, and testes) at the end of 1 hour was only 37%. Therefore, the remainder of the arachidonic-1-C^{14} acid must have been distributed in the other body tissues. Oxidation of arachidonic-1-C^{14} acid to $C^{14}O_2$ could only account for a very small portion of the C^{14}-activity in the 1-hour period (5,6). With time there was a substantial decrease in the percentage of recovered arachidonic-1-C^{14} acid in the tissues. At the end of 24 hours, only 9% of the injected dose was present in the liver and less than 1% in the serum and testes. The distribution of the C^{14}-activity in the tissue lipids is shown in Table II. In the early time period (1 hour) a substantial proportion of the C^{14}-activity was associated with phospholipid fraction of the liver and testes. In the serum at 1 hour, 84% of the C^{14}-activity was found in the glyceride-fatty acid fraction. When the glyceride plus fatty acid fraction of the serum and liver was separated by thin-layer chromatography into glycerides and fatty acids, it was observed that 40-60% of the C^{14}-activity was in the free fatty acids 1 hour after the injection; only 5-10% of the C^{14}-activity was in the free fatty acid fraction at 4 and 24 hours. The presence of appreciable amounts of C^{14}-activity in the free fatty acids was a result of the splitting of injected methyl arachidonate-l-C^{14}. At the end of 24 hours, 95% of the C^{14}-activity in the liver lipids, and 83% of the C^{14}-activity in the testes lipids was present in the phospholipid fraction. The distribution of C^{14}-activity in the serum lipids at 4 and 24 hours indicated that a major proportion of the total C^{14}-activity was present in the cholesterol ester fraction; after 24 hours, 55% of the total C^{14}-activity was present in this serum fraction.

The identification of the C^{14}-fatty acids in the 24-hour tissue lipid samples was checked by gas-liquid radiochromatography. In the serum lipids, 96% of the C^{14}-activity was found to be in the form of C^{14}-arachidonic acid. In the liver, the phospholipid C^{14}-fatty acids consisted of 93% C^{14}-arachidonic acid and the remainder of the C^{14}-activity was present as C^{14}-long chain polyunsaturated fatty acids. The testes phospholipid C^{14}-fatty acids consisted of 75% C^{14}-arachidonic acid and 25% C^{14}-long chain polyunsaturated fatty acids. This latter observation is in agreement with earlier findings(4).

Discussion. The data of the present study indicate that there is a preferential incorporation with time of arachidonic acid into the phospholipid fraction of the liver and testes. The preferential utilization of arachidonic acid for tissue phospholipid synthesis

TABLE II. Distribution of C^{14}-Activity in Tissue Lipids.

Time hr	% of total C^{14}-activity recovered*		
	Cholesterol ester	Glyceride + fatty acids	Phospholipids
		Liver	
1	.3	32.7	67.0
4	.7	9.5	89.8
24	.7	4.3	95.0
		Testes	
1	2.5	18.8	78.7
4	2.8	12.8	84.4
24	3.9	13.0	83.1
		Serum	
1	4.8	83.9	11.3
4	39.7	22.8	37.5
24	55.0	16.8	28.2

* Values represent the analysis (in duplicate) of a pool of tissues from 5 animals.

may be related to the requirement of certain phospholipids as structural elements of the cell. This has been suggested(6) as a possible explanation for the slow catabolism of arachidonic acid as compared to other long chain fatty acids. The additional possibility exists that the tissue phospholipids could serve as a reservoir and furnish the arachidonic acid necessary for the formation of the prostaglandins, long chain polyunsaturated fatty acids, cholesterol arachidonate, and other metabolites.

In contrast to the liver and testes, the serum C^{14}-lipid distribution pattern indicates a preferential incorporation, with time, of arachidonic acid into the cholesterol ester fraction. The cholesterol ester fraction of rat serum(10) has a high proportion of arachidonic acid (50-60%) and this serum lipid fraction may be an important form for the transport of arachidonic acid to the tissues. The turnover time of liver and serum arachidonic acid, as estimated from the disappearance of C^{14}-activity, was 18-24 hours. Rapid turnover of liver arachidonic acid would be consistent with the view that this tissue may furnish a major part of the tissues with arachidonic acid.

Examination of the C^{14}-fatty acids present in the tissues following the injection of arachidonic-1-C^{14} acid indicated that in the serum and liver, very little transformation of arachidonic acid to fatty acids of 20 carbons and longer had occurred. However, in testes, the phospholipid fraction contained appreciable amounts of C^{14}-polyunsaturated fatty acid (20 carbons and longer). A major acid formed from arachidonic acid in testes has been shown to be a docosopentaenoic acid (4). The adrenal gland has also been shown to contain appreciable amounts of long chain polyunsaturated fatty acids(11); the structure of these acids indicates that arachidonic acid may be the principal precursor. The function of the long chain polyunsaturated fatty acids in tissues such as testes and adrenal is not known, but it may be related to the role of these tissues in steroid hormone synthesis.

Summary. Rats were injected with arachidonic-1-C^{14} acid and the serum, liver, and testicular lipids examined at different time periods. The liver, at 1 hour, was found to contain 35% of the injected C^{14}-activity. Arachidonic acid was preferentially incorporated into the liver and testicular phospholipids and the serum cholesterol esters. A rapid turnover of liver and serum arachidonic acid was observed (turnover time 18-24 hours). Long chain fatty acids derived from arachidonic acid were present in appreciable amounts only in the testes. The significance of these findings in relation to the metabolism of arachidonic acid is discussed.

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