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A Comparison of the Disposition of Intravenously-Injected, Albumin-Bound Stearic Acid-1-C^{14} and Palmitic Acid-1-C^{14} in the Rat.* (29975)

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A number of studies have demonstrated that a difference exists between the metabolism of stearic acid and other 16- and 18-carbon containing fatty acids. The following examples illustrate this point: (a) The rate of oxidation of the C^{14} of stearic acid-1-C^{14} to CO_2 in the intact rat is lower than that of palmitic acid-1-C^{14} when they are injected intravenously either as free fatty acids bound to albumin or as emulsified triglycerides(1); (b) after an intravenous injection of acetate-1-C^{14} into rats, Tove *et al*(2) found that the specific activity of stearic acid in the neutral fat fraction of the liver rose more slowly than did that of palmitic acid or those of unsaturated fatty acids; (c) Olivicrona *et al* (3) injected palmitic acid-labeled and stearic acid-labeled chylomicrons into rats and observed less recirculation of labeled glycerides and free fatty acids with the stearic acid than with the palmitic acid-labeled chylomicrons.

It has been reported that a greater proportion of lipid C^{14} is recovered in the liver as phospholipids and a smaller proportion as triglycerides when C^{14}-stearic acid is fed to rats than when either C^{14}-labeled palmitic, oleic or linoleic acid is fed(4). But since it has also been shown(5) that approximately 20% of the C^{14} of labeled stearic acid fed to rats appears in the thoracic duct lymph as phospholipids—as compared to 4% in experiments with labeled palmitic, oleic and linoleic acids—the distribution of the C^{14} among lipid fractions in the liver, after the feeding of these various fatty acids, does not necessarily establish that a preferential synthesis of phospholipids from stearic acid occurs in the liver.

To determine whether or not the preferential incorporation of stearic acid into phospholipids is restricted to incorporation during absorption from the intestines, we have in the present study injected intravenously into rats albumin-bound C^{14}-labeled stearic or palmitic acid and determined the distribution of the C^{14} in lipid fractions of liver, plasma, adipose tissue, skeletal muscle and heart at various time intervals up to 24 hours after the injections.

Experimental. Administration of labeled fatty acid. Palmitic acid-1-C^{14} (6.23 mc/mmole) and stearic acid-1-C^{14} (9.13 mc/mmole) obtained from New England Nuclear Corp., Boston, were purified on silicic acid columns(6). One hundred mg of crystalline bovine albumin (Armour Pharmaceutical Co., Kankakee, Ill.), dissolved in 0.9% NaCl, was added to 1.20 mg of the sodium salt of the labeled palmitic acid and to 1.33 mg of the sodium salt of the labeled stearic acid dissolved in isotonic saline. The final volume of each fatty acid solution was 10 ml. Sixteen male Long-Evans rats (240 to 290 g) were lightly anesthetized with ether, and 1.0 ml of the labeled palmitic or stearic acid solution was injected into a leg or tail vein of each rat. Thereafter, at intervals of 1, 4, 12 and 24 hours, a pair of rats from each group was again anesthetized and blood samples (7-10 ml) were drawn from the hearts into heparin-rinsed syringes. The following organs and tissues were then rapidly excised: liver,

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perirenal adipose, heart and gastrocnemius muscle.

Analytical procedures. The hearts were cut open and thoroughly rinsed with 0.9% NaCl. The livers were homogenized in a Waring Blendor with an ethanol-ethyl ether solution, 3:1 (v/v). Each of the other tissues was minced with a razor blade, and the mince was placed in the ethanol-ethyl ether solution, as was the plasma. Total lipids were extracted from tissues as previously described (7). In all cases, the extractions with the ethanol-ethyl ether solution were followed by an additional extraction for 2 hours with 30 volumes of a chloroform-methanol solution, 2:1 (v/v).

The lipid extracts prepared from adipose tissue were fractionated on silicic acid columns by the method of Barron and Hanahan (6). The extracts of all the other tissues, which do not contain such large amounts of triglycerides, were fractionated on Unisil columns by a more rapid micro procedure based on the method of Creech and Sewell(8). With both procedures the following fractions were separated: (a) cholesterol esters; (b) glycerides, free fatty acids and free cholesterol; and (c) phospholipids. When the Unisil columns were used, cholesterol esters were eluted with 15 ml of a benzene-hexane solution, 1:1 (v/v); free fatty acids, triglycerides, free cholesterol and most of the mono- and diglycerides were eluted with 20 ml of chloroform. This was followed by 10 ml of ethyl ether to ensure complete removal of the mono- and diglycerides. Phospholipids were eluted with 15 ml of methanol. Free fatty acids were separated from glycerides on Florisil columns(9). The lipid samples eluted from the columns were dried and dissolved in 15 ml of toluene containing 45 mg of 2,5-diphenyloxazole and 1.5 mg 1,4-bis-2-(5 phenyloxazolyl) benzene and assayed for C^{14} in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., La Grange, Ill.). An internal C^{14} standard was used to correct the activity in the phospholipid samples for color quenching. It was determined by digitonide precipitation that free cholesterol was not labeled in the course of the experiments.

Results. To estimate the total lipid C^{14} recovered in the 5 tissues studied, we have assumed that muscle constitutes 40%(10), adipose tissue 10%(11) and plasma 4.0% (12) of body weight. Incorporation of the injected C^{14} into total lipids of all 5 tissues was calculated to be 75%, 60%, 55% and 46% at 1, 4, 12 and 24 hours, respectively, after injection of palmitic acid, and 72%, 65%, 56% and 60% after injection of stearic acid.

Each bar in Fig. 1 and 2 shows percentages of the injected C^{14} recovered as total lipids (per g of tissue or per 10 ml of plasma) as well as the distribution of the lipid C^{14} among the 4 fatty acid fractions isolated. Each value is the average for 2 rats (individual values closely approximated the average). In both groups of rats, the percentages of C^{14} recovered in total lipids of liver and heart declined with time, but a greater proportion was lost in the palmitic than in the stearic acid-injected rats. Consistent differences between the stearic and palmitic acid-injected rats in total lipid C^{14} recovered were not observed in any of the other tissues examined.

In both groups of rats most of the C^{14} was recovered in the phospholipid and glyceride fractions. In adipose tissue practically all of the C^{14} was found as glycerides. In all tissues and at all intervals a greater proportion of the lipid C^{14} was recovered as phospholipids and less as glycerides in the stearic acid-injected rats than in those injected with palmitic acid.

Discussion. Our observation that a higher proportion of the C^{14} of the intravenously-injected stearic acid-1-C^{14} than of the palmitic acid-1-C^{14} was recovered as phospholipids in plasma and in all tissues studied indicates that the preferential incorporation of stearic acid into phospholipids is not limited to incorporation during absorption from the intestines into lymph.

Phospholipids are synthesized in many tissues(13-15). In dogs(16) and rats(17), after intravenous injection of a labeled fatty acid, plasma phospholipids are derived almost exclusively from the liver. In our study, therefore, the labeled phospholipids recovered in a given extrahepatic tissue could have been derived either from synthesis in that tissue or from recirculated liver phospholipids. Since

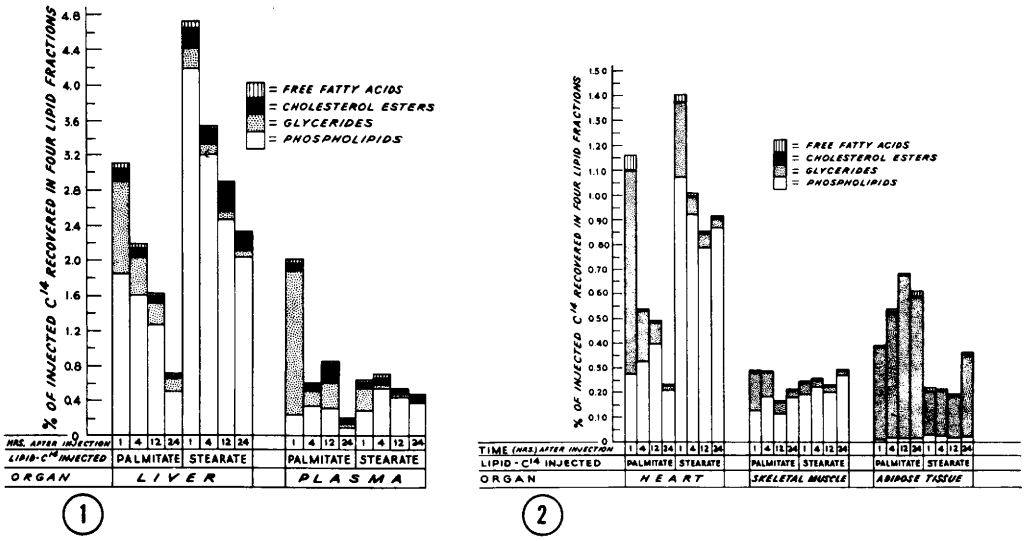

FIG. 1. Per cent of intravenously-injected, albumin-bound palmitic acid-1-C^{14} or stearic acid-1-C^{14} recovered as glycerides, phospholipids, cholesterol esters and free fatty acids in liver (per g) and plasma (per 10 ml) at 1, 4, 12 and 24 hr after injection. Each column shows total C^{14} in all 4 fractions and distribution of C^{14} among them.

FIG. 2. Per cent of intravenously-injected, albumin-bound palmitic acid-1-C^{14} or stearic acid-1-C^{14} recovered as glycerides, phospholipids, cholesterol esters and free fatty acids in heart, gastrocnemius muscle and perirenal adipose tissue (per g in each case) at 1, 4, 12 and 24 hr after injection. Each column shows total C^{14} in all 4 fractions and distribution of C^{14} among them.

we cannot determine the contribution of labeled liver phospholipids to the total labeled phospholipid content of a specific extrahepatic tissue, the present study does not necessarily establish that preferential incorporation of stearic acid into phospholipids occurs in a given extrahepatic tissue.

In the stearic acid-injected rats, the proportion of lipid C^{14} recovered as phospholipids did not change appreciably with time. In the palmitic acid-injected rats, on the other hand, distinct increases in the proportion of labeled phospholipid were observed. Conversion of palmitic acid to oleic and stearic acids has been shown to occur in the rat liver(4). Thus, the increase with time in the proportion of lipid C^{14} recovered as phospholipids in the palmitic acid-injected rats may have been due not only to a movement of labeled palmitic acid from triglycerides to phospholipids but also to conversion of the labeled palmitic to labeled stearic acid.

The observation that, between 1 and 24 hours, a greater percentage of the total lipid C^{14} appeared to be lost from all tissues combined in the palmitic than in the stearic acid-injected rats is consistent with the observation in our previous study(1) that intravenously-injected labeled palmitic acid is oxidized to CO_2 in intact rats more rapidly than is intravenously-injected stearic acid. The more rapid oxidation of palmitic as compared to stearic acid cannot be accounted for by a lesser dilution of palmitic acid since in rat tissues the concentration of palmitic acid in both unesterified and esterified forms is higher than that of stearic acid(18). It is possible that the lower rate of oxidation of stearic acid is related to its lower incorporation into triglycerides and higher incorporation into phospholipids. Although phospholipid fatty acids, glyceride fatty acids and free fatty acids do interchange, it has been observed in muscle that phospholipids are relatively more stable than glycerides(19). As compared to palmitic acid, therefore, stearic acid might be less readily converted to free fatty acid and oxidized.

Summary. Stearic acid-1-C^{14} or palmitic acid-1-C^{14} was injected intravenously into rats in the form of albumin-bound free fatty acids. At various intervals thereafter livers,

hearts and samples of adipose tissue, skeletal muscle and plasma were taken for determination of total C^{14} and its distribution among glycerides, phospholipids, cholesterol esters and free fatty acids. At all intervals the ratio (C^{14} in phospholipid/C^{14} in glycerides) was greater after injection of C^{14}-labeled stearic acid than after that of labeled palmitic acid. This indicates that preferential incorporation of stearic acid into phospholipids is not limited to incorporation during absorption from the intestines into lymph.

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Heterogeneity of Antinuclear Factors in Lupus Erythematosus and Rheumatoid Arthritis.* (29976)

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Antinuclear factors (ANF) of γ_1, γ_1A, and γ_1M immunoglobulin classes (IgG, IgA, and IgM)‖ have been found in the sera of children and adults with lupus erythematosus (LE) and rheumatoid arthritis (RA)(1). These 3 immunoglobulin classes were distinguished on the basis of their antigenically differing heavy (H) chains. All 3 immunoglobulin classes have similar light (L) chains in common. Antigenically, however, L chains and the individual immunoglobulin molecules which carry them may be divided into 2 distinct types, L I and L II(2) (κ and λ)‖. The present study demonstrates that immunoglobulins with an ANF activity include molecules of both L chain types, as detected by a 3-layer indirect immunofluorescence test.

Materials and Methods. The 3-layer im-

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