Incorporation of Infused Palmitic-1-C^{14} and Linoleic-1-C^{14} into Plasma Lipid Fractions.* (26330)

RODNEY D. ORTH,† MELWYN B. FINE‡ AND ROBERT H. WILLIAMS
Department of Medicine, University of Washington School of Medicine, Seattle

In recent years, there has been increasing recognition of the major contribution of free fatty acids (FFA) to the energy needs of nearly all body tissues. Several studies have clearly shown that infused FFA disappear rapidly from the general circulation, and are oxidized, in part, directly by tissues. The metabolic fate of that portion which does not undergo direct oxidation has been the subject of many studies. It has been shown that a considerable portion of administered palmitic acid-1-C^{14} is incorporated by the liver into triglycerides and, to a lesser degree, into phospholipids(1,2). These studies have involved primarily an analysis of liver lipids rather than plasma lipids. Laurell(3), on the other hand, reported the disappearance and recycling, as esterified fatty acid, of injected palmitic acid-1-C^{14} and found that peak incorporation into plasma triglycerides occurred at about 40 minutes. Fredrickson and Gordon(4) infused palmitic-1-C^{14}, linoleic-1-C^{14}, and oleic-1-C^{14} acids into humans and found initial disappearance rates to be similar. Although their flux data indicate that basic differences do exist between the metabolism of linoleic and palmitic acids, they did not find significant differences in incorporation of these acids into the non-phospholipid-containing neutral lipid fraction. In a study in which acetate-1-C^{14} was administered orally to humans, Lipsky(5) was able to follow the metabolic fate of the fatty acids synthesized. He found that palmitic-C^{14} acid incorporation into triglycerides, phospholipids, and sterol esters occurred maximally at 2, 24, and 48 hours respectively. Although peak incorporation of linoleic-C^{14} acid into triglycerides occurred at 2 hours also, the activity of sterol esters and phospholipids derived from this fatty acid was too low to permit conclusions regarding its ultimate fate in plasma lipid fractions. The present study was undertaken to investigate possible differences in incorporation of a typical saturated (palmitic) and a typical unsaturated (linoleic) fatty acid into various plasma lipid fractions.

Materials and methods. Mongrel dogs, weighing 10-20 kg, and fasted overnight were used for this study. Forty microcuries (0.7 mg) of palmitic-1-C^{14} or linoleic-1-C^{14} bound to dog plasma were infused intravenously into three or more dogs over a 2-3 minute period. Blood samples were taken at varying intervals up to 72 hours after infusion. The animals were fed after the 6-hour sample, and all subsequent samples were taken at least 3 hours after a meal. The blood was placed in chilled, citrated tubes in an ice bath, and promptly centrifuged at 4°C in the cold room. A 1-cc portion of plasma was extracted by the method of Freeman(6). Extracts were evaporated, taken up in hexane, and placed on silicic acid columns. The various lipid fractions were eluted using the solvent systems described by Dittmar(2). The fraction containing triglycerides and FFA was extracted 3 times with water-isopropyl alcohol (1:2) made basic (pH 9.0) with dilute NaOH. Subsequent acidification of the aqueous extract with 0.1 N HCl and extraction with 3 volumes of hexane removed the FFA. All eluted fractions were then evaporated to dryness, and counted in toluene-PPO in a liquid scintillation counter.

Results. Both the linoleic-C^{14} and the palmitic-C^{14} disappeared from the plasma very rapidly—more than 90% in one hour. The palmitic-C^{14} had largely disappeared after 5 hours and the linoleic-C^{14} slightly more slowly. Each were incorporated into plasma triglyceride at a comparable rate and continued at similar concentrations over 72

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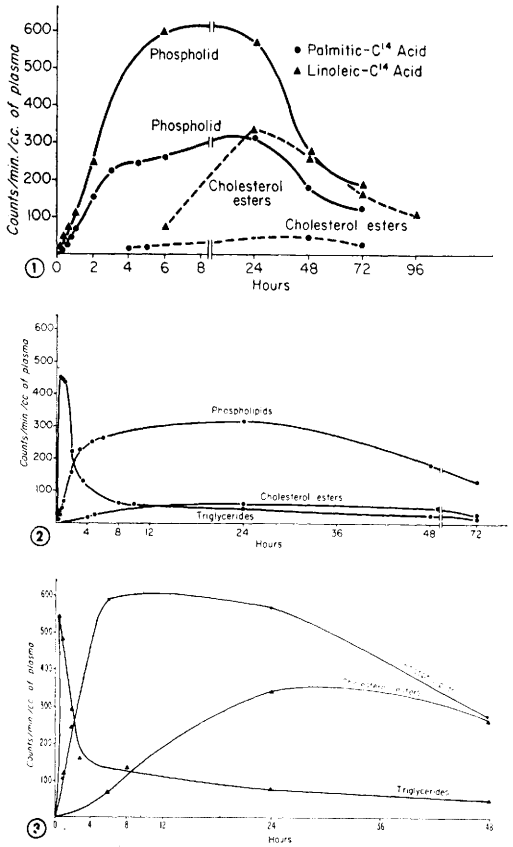

FIG. 1. Each labeled fatty acid is incorporated much more rapidly into phospholipid than into cholesterol esters. More linoleic-C^{14} than palmitic-C^{14} is incorporated into both lipids.

FIG. 2. Palmitic-C^{14} is incorporated very rapidly into triglycerides, more slowly into phospholipids, and very slowly into cholesterol esters.

FIG. 3. Linoleic-C^{14} is incorporated rapidly into triglyceride, more slowly into phospholipids, and very slowly into cholesterol esters. Comparison with Fig. 2 demonstrates that linoleic-C^{14} is incorporated into phospholipid and cholesterol esters faster than palmitic-C^{14}.

hours. The highest labeled triglyceride concentrations were attained between one-half and one hour.

Much greater concentrations of labeled plasma phospholipid and cholesterol esters were obtained after administration of linoleic-C^{14} than after palmitic-C^{14} (Fig. 1). In each instance the incorporation of the labeled material occurred most rapidly in triglyceride, then phospholipid and then cholesterol esters (Fig. 1, 2, 3). Incorporation into cholesterol esters is very slow, no significant activity being found until after 2 or 3 hours; in each instance maximum activity was reached in about 24 hours. On the other hand, significant activity was found in the phospholipid fraction within 10 minutes of the infusion of the FFA, and peak incorporation occurred at about 6 hours.

Discussion. The major difference found in this study of incorporation of palmitic-C^{14} and linoleic-C^{14} acids into various plasma lipid fractions consisted of a quantitatively greater incorporation of linoleic-C^{14} acid into sterol esters and phospholipids. Lipsky(5) has shown that linoleic-C^{14} acid synthesized after oral administration of acetate-1-C^{14} to humans is preferentially incorporated into sterol esters and the phospholipid fraction of serum lipids, while palmitic-C^{14} acid appears to be primarily esterified with glycerol. It was also noted in his study that peak incorporation of palmitate-C^{14} into triglycerides, phospholipids, and sterol esters occurred at 2, 24, and 48 hours respectively. The time course of events found in our experiments suggests that earlier peak activities (45 minutes, 6 hours, 24 hours) are found when the FFA (rather than acetate-1-C^{14}) are used as the starting material and are given intravenously.

Amount of fatty acid incorporation in various lipids may differ with route of administration, species, and other factors. For example, Hanahan and Blomstrand(7), administering FFA orally to rats, found that 10 times as much radioactivity was incorporated in lecithins after administration of palmitic-1-C^{14} acid as after administration of oleic-1-C^{14} acid.

Our observation that linoleic-C^{14} acid remains longer as a FFA following intravenous administration than palmitic-C^{14} acid has been described by Fredrickson and Gordon (4), whose data indicate that linoleic-C^{14} acid is oxidized less rapidly and recycles through the plasma compartment much more than either palmitic-C^{14} or oleic-C^{14} acids.

It is evident that more rapid oxidation of palmitic-C^{14} acid would result in diminished amounts available for synthesis into other lipid fractions, and hence could account for some quantitative differences observed in

subsequent synthetic processes. Such a mechanism cannot account for differences in slopes of the curves, however, and therefore certain preferential esterifying mechanisms for each of the fatty acids must be operative.

Summary. Palmitic-1-C^{14} and linoleic-1-C^{14} acids were infused into fasted dogs, and fractionation of the serum lipids carried out at time intervals up to 72 hours. Although many similarities were found in the metabolism of these 2 fatty acids, it was observed that linoleic-C^{14} acid appeared to 1) persist longer in the plasma as the FFA and 2) to be incorporated preferentially into cholesterol esters and phospholipids.

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Hypoxic Alteration of Righting Ability and Pain Threshold in Two Mammalian Species.* (26331)

ROBERT W. BULLARD AND JAMES L. SNYDER (Introduced by Ewald E. Selkurt)
Department of Physiology, Indiana University School of Medicine, Indianapolis

Although much experimental work has been done in man and animals on pain(1), there are only limited studies on the effects of hypoxia on thermal pain perception(2). Besides studying hypoxic effects on pain thresholds, we were also interested in using the response to pain along with tests of righting ability as indices of body function during hypoxic episodes.

Mammalian species that hibernate survive more severe hypoxia than do non-hibernating species(3,4). Two possible means by which hypoxic tolerance could be greater in one species than another are: a) the more tolerant mammal possesses greater capacity to maintain high functional levels by maintaining tissue oxygenation through greater utilization of physiological regulatory mechanisms or biochemical energy reserves. b) The more tolerant mammal by decreasing metabolism and function, reduces the oxygen requirement to parallel the reduced oxygen supply.

In the present study, an attempt was made to evaluate the above alternatives as to their role in species differences of hypoxic tolerance. Degree of hypoxic alteration of function as shown in pain threshold and righting ability changes was compared for one hibernating species and one non-hibernating species.

Methods. Experimentally naive, Harlan strain white rats (*Rattus norvegicus*) of 200 to 300 g body weight were the non-hibernators used. Thirteen lined ground squirrels (*Citellus tridecimlineatus*) of 150 to 250 g body weight were the hibernators used.

Pain threshold was determined by using a modification of the method described by D'Amour and Smith(5). The tails of the ground squirrels were shaved prior to the experiment. Animals of both species were restrained either in cylinders or taped to boards. A small hood was placed over the head of the animal through which 1 to 2 liters per minute of air, or an air mixture deficient in oxygen were pumped. The tail was then placed in a trough containing a resistance wire heater. The heater was turned on and the temperature increased at approxi-

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