Effect of Carnitine on Uptake, Oxidation and Esterification of Palmitate by the Perfused Rat Heart[1] (34607)

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The role of (—)-carnitine (β-hydroxy-γ-trimethyl ammonium butyrate) as a requirement in fatty acid oxidation has been well established (1–3). Carnitine acyl transferase (acyl CoA: carnitine *o*-acyl transferase, EC 2.3.1.7), located in the inner mitochondrial membrane (4), catalyzes the formation of long-chain acyl carnitine from acyl CoA and this reaction is considered to be rate-limiting for fatty acid oxidation by mitochondria (5, 6). The isolation of mitochondria from tissue homogenates results in a loss of carnitine (7, 8), and decreased ability to oxidize added acyl CoA. Thus, subsequent addition of carnitine in increasing concentrations results in a proportional increase in fatty acid oxidation (9).

Data on the effect of carnitine on fatty acid metabolism in intact tissue or *in vivo* has been less consistent than those obtained with tissue homogenates or isolated mitochondria. Miller and Krake (10) have reported that in mice injected with carnitine, there is a 25–50% increase in oxidation of palmitic acid. Shipp (11) was unable to demonstrate any effect of carnitine on palmitate oxidation by isolated perfused rat heart *in vitro*. Spector (12) reported that intact Ehrlich ascites tumor cells, which readily metabolize fatty acids, were able to take up labeled carnitine from the incubation medium. Although the uptake of carnitine and formation of acyl carnitine were influenced by exogenous fatty acid, there was no increase in fatty acid oxidation by the intact cells. However, when isolated mitochondria from these cells were used, carnitine did stimulate fatty acid oxidation.

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During the course of studies on factors affecting fatty acid synthesis, oxidation and esterification in intact perfused rat heart, it became of interest to reinvestigate the influence of circulating carnitine on certain aspects of palmitate uptake and metabolism. A preliminary report of a portion of this study has been presented (13).

Materials and Methods. Palmitic acid-1-^{14}C was obtained from Nuclear-Chicago Corporation and was checked for purity by gas liquid radio-chromatography. (*d,l,*) - Carnitine-methyl-^{14}C-HCl was purchased from Tracerlab. Unlabeled palmitic acid (> 99% purity) was obtained from the Hormel Institute, and other unlabeled compounds were from California Corporation of Biochemical Research (Calbiochem) or from Pabst Biochemicals. Fraction V bovine serum albumin (Nutritional Biochemical Corp.) was used without purification.

Male albino rats of the Wistar strain (Microbiological Associates) were maintained on stock diet *ad libitum* prior to a 18- to 24-hr period fasting. The animals were anesthesized with intraperitoneal Nembutal before exposure of the thoracic cavity and removal of the heart.

The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (14), pH 7.4, containing half (1.27 m*M*) the suggested concentration of $CaCl_2$. The medium contained 0.5 m*M* palmitate complexed to 0.43 m*M* bovine serum albumin, as described previously (15). Where applicable, *d, l*-carnitine HCl was added to a final concentration of 0.5 m*M* or 5 m*M*. All perfusion media were equilibrated with O_2–CO_2 (95:5, v/v) before use.

The perfusion apparatus and procedure for

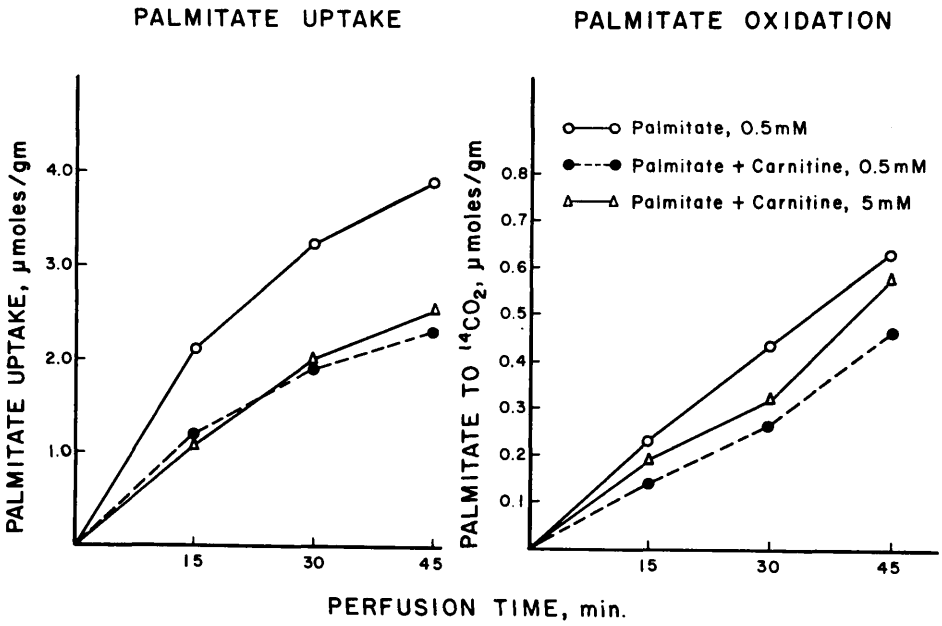

FIG. 1. Effect of *d,l*-carnitine on palmitate-1-^{14}C uptake and oxidation to $^{14}CO_2$ by isolated perfused rat heart. The initial concentration of carnitine was 0.5 or 5.0 m*M* and of palmitate was 0.5 m*M*. Each curve is derived from mean values of five perfusions. Other conditions are described in the text.

aortic perfusion in the closed recirculating system have been described elsewhere (16). Sampling of the perfusion medium, extraction and determination of palmitic acid in the perfusion medium, and determination of $^{14}CO_2$ were described in detail previously (15, 16).

At the end of perfusion, the heart and cannula were removed from the apparatus, 5 ml of Krebs buffer were forced through the coronaries to remove labeled substrate, and the heart was immediately frozen on dry ice. The hearts were then stored at —40° until extraction. The frozen heart was weighed and placed in 20 vol of chloroform–methanol (2:1, v/v). This was homogenized and then sonicated for 3 min, and the crude extract was carried through the separation and washing procedures of Folch *et al.* (17). The chloroform phase was evaporated to dryness under nitrogen, and the residue was redissolved in 20 ml of petroleum ether. Radioactivity in the total heart lipids was determined by evaporation of 1 ml of the petroleum ether extract in a scintillation vial, addition of 10 ml of scintillant, and counting as above. The heart lipids in petroleum ether were fractionated on thin-layer silicic acid chromatoplates using a solvent system containing hexane:diethyl ether:acetic acid (86:13:2, v/v). The areas corresponding to individual lipid classes were scraped into scintillation vials and counted after adding 1 ml of methanol and 10 ml of toulene scintillation mixture containing 2, 5-diphenyloxazole (4 g/liter) and *p*-bis-2′-(5′phenyloxazolyl) benzene (500 mg/liter). All isotope data were corrected to dpm by the channels ratio method (18).

The uptake of labeled carnitine was determined by chloroform–methanol extraction of hearts perfused for 45 min with Krebs buffer containing 5 m*M* glucose and 5 m*M* ^{14}C-methyl-carnitine HCl (1 μCi). After separation of the chloroform and methanol–water phases (17), aliquots of each phase were used for liquid scintillation counting.

Palmitate uptake is expressed as μmoles substrate taken up g wet weight heart/45 min. Oxidation to $^{14}CO_2$ is expressed as μmoles substrate equivalents converted to $^{14}CO_2$ per time period.

TABLE I. Effect of Carnitine on Palmitate Uptake, Oxidation, and Incorporation into Myocardial Lipids.

Group[a]	Substrate uptake	$^{14}CO_2$		Substrate to total lipids		Isotope recovery
		(μmoles/g)	(%)	(μmoles/g)	(%)	
Palmitate (0.5 m*M*)	3.90 ± 0.51[b]	0.94 ± 0.11	24	1.29 ± 0.06	33	57[c]
Palmitate + *d,l*-carnitine (0.5 m*M*)	2.26 ± 0.11[d]	0.68 ± 0.06	33	1.56 ± 0.15	69[d]	102
Palmitate + *d,l*-carnitine (5 m*M*)	2.66 ± 0.14[d]	1.02 ± 0.12	38	1.58 ± 0.34	59[d]	97

[a] Hearts from fasting animals were placed on the aortic cannula and preperfused with 5 ml of Krebs bicarbonate buffer. Perfusions were carried out for 45 min in an open-recirculating apparatus under continuous gassing with 95% O_2–5% CO_2 and exit gasses were trapped in 6 *N* KOH. At termination of experiments, hearts were rapidly flushed free of isotope, using buffer, and were frozen in dry ice. Subsequent homogenization and extraction of lipids are described in the text.

[b] Values represent means ± SEM.

[c] The remainder of the isotope was recovered in the aqueous methanol phase after Folch extraction (17).

[d] $p < .05$.

Results and Discussion. In preliminary experiments, addition of 1 m*M* *d, l*-carnitine to heart homogenates or washed mitochondria produced a 3- to 4-fold increase in the oxidation of labeled palmitate to $^{14}CO_2$. This effect is quantitatively similar to that reported by Fritz and Yue (19). The effect of carnitine on the uptake and oxidation of palmitate-1-^{14}C by the perfused rat heart is shown in Fig. 1. The presence of 0.5 m*M* or 5 m*M* carnitine in the perfusing medium produced a marked depression of palmitate uptake by the heart throughout the entire experimental period. There also appeared to be a slight depression of $^{14}CO_2$ production measured in successive 15-min periods; however, this was not statistically significant. The cumulative uptake, oxidation of palmitate-1-^{14}C, and its incorporation into the total heart lipid is summarized in Table I. These data show a significant reduction in palmitate uptake by perfused hearts in the presence of either level of carnitine. It is possible that carnitine complexed with palmitate in the perfusate, thereby preventing uptake, but attempts to show formation of acyl carnitine or salt formation in the perfusing medium were unsuccessful. Thus, the nature of this effect remains to be elucidated.

There was no significant effect of carnitine on $^{14}CO_2$ production from palmitate when calculated as μmoles palmitate equivalents/g of tissue or as percentage of the substrate taken up in the heart. The failure of these levels of circulating carnitine to stimulate palmitate oxidation by the intact perfused rat heart substantiates recent findings by Shipp (11) with perfused rat heart, and by Spector (12) with intact Ehrlich ascites tumor cells. However, as shown in Table II, only about 1% of available circulating *d, l*-^{14}C-methyl carnitine (5 m*M*) was taken up by the perfused heart during the experimental period, and if this were uniformly distributed, the total tissue *l*-carnitine concentration would be approximately 0.5 μmoles/g wet weight tissue. In studies on long-chain fatty acid oxidation by myocardial homogenates (1 ml = 50 mg tissue), the labeled carnitine taken up represents about 25 μmoles/reaction mixtures (approx. $10^{-5}M$), and this is well below the concentration needed to optimally stimulate fatty acid oxidation by mitochondria (8).

Carnitine, at either concentration, had no significant effect on the absolute amount of palmitate incorporated in heart total lipids; however, when incorporation of label was calculated as a percentage of substrate taken up, both concentrations of carnitine in perfusion medium caused a significant increase. Thus, in the absence of carnitine, only 57% of the palmitate-1-^{14}C taken up was accounted for as $^{14}CO_2$ and as extractable total lipids (the rest of the isotope was recovered

TABLE II. ^{14}C-Methyl-Carnitine Uptake by Perfused Rat Heart.

Additions to perfusion medium[a]	Distribution of carnitine in heart extract[b]		Total uptake (%)
	Aqueous extract (μmoles)	Lipid extract (μmoles)	
d,l-^{14}C-methyl carnitine (5 m*M*)	0.77 ± 0.01	0.04 ± 0	0.81 ± 0.01
d,l-^{14}C-methyl carnitine + palmitate (0.5 m*M*)	0.94 ± 0.01	0.04 ± 0	0.98 ± 0.01

[a] The basic perfusion medium consisted of 20 ml of Krebs bicarbonate buffer containing 150 mg/100 ml of glucose. Perfusions were carried out for 45 min in 95% O_2–5% CO_2. Extraction and counting procedures are described in the text.

[b] Each value is the mean of three perfusions ± SE.

in the aqueous-methanol phase), while in the presence of either level of carnitine, essentially all of the palmitate taken up was recovered in these two fractions. Determination of the distribution of radioactivity among the various lipid fractions (Table III) showed that 5 m*M* carnitine significantly decreased the percentage of label in unesterified fatty acids and diglyceride, and resulted in an increase in the proportion of radioactivity in phospholipids ($p < 0.05$) and triglycerides ($p < 0.05$).

To obtain additional data on the possible effect of carnitine on stimulation of fatty acid esterification, perfusion of 0.5 m*M* palmitate-1-^{14}C for 45 min with and without 5 m*M* carnitine was followed by separation of the individual classical subcellular fractions (20) of heart homogenates. Each tissue fraction was then extracted with chloroform–methanol, as described under Methods. The addition of carnitine to the perfusion medium had no effect on the distribution of palmitate among subcellular fractions. However, when the various major lipid classes of each tissue fraction were analyzed for radioactivity, intracellular effect of carnitine was evident. There was a significant increase in palmitate incorporation into mitochondrial triglyceride from 72 to 82% ($p < 0.01$) of the total radioactivity. A similar increase in triglyceride radioactivity was found in the microsomal fraction (61% to 74%, $p < 0.01$). The radioactivity in other subcellular fractions was not influenced by the presence of carnitine. These data are compatible with the results of Bressler and Friedberg (21), who reported that 5 m*M* carnitine not only stimulated fatty acid oxidation in guinea pig heart homogenates, but also resulted in a twofold stimulation of palmitate incorporation into phospholipids. When KCN was also included in incubations to prevent oxidation of palmitate, and ATP was included to enhance fatty acid activation, palmitate incorporation into phospholipids was stimulated 9-fold by added carnitine. These authors were not able to show a carnitine stimulation of fatty acid esterification in the isolated microsomal fraction and concluded that the stimulation ob-

TABLE III. Distribution of Radioisotope among Heart Lipid Fractions after Perfusion with Palmitic Acid-1-^{14}C.[a]

Group	% of ^{14}C recovered as:			
	Fatty acid	Phospholipid	Diglyceride	Triglyceride
Palmitate (0.5 m*M*)	7.2 ± 1.6[b]	3.8 ± 0.3	7.0 ± 0.7	79.7 ± 2.1
Palmitate + *d,l*-carnitine (5 m*M*)	2.2 ± 0.7[c]	6.0 ± 1.0[c]	2.9 ± 0.4[d]	86.4 ± 1.7[c]

[a] Condition as described in Table I and text.

[b] Values represent means ± SEM.

[c] $p < 0.05$.

[d] $p < 0.01$.

served in isolated mitochondria did not represent *de novo* synthesis of lecithin. However, we have recently observed 3-fold stimulation by carnitine of palmitate incorporation into esterfied lipids using the microsomal fraction of a heart homogenate. Current studies are directed toward determining the mechanism of this stimulation.

Summary. The effect of carnitine on the uptake, oxidation, and esterification of circulating palmitate has been studied with the isolated perfused rat heart. At concentrations of 0.5 m*M* and 5.0 m*M*, carnitine depressed the extraction of palmitate by perfused heart throughout the 45-min perfusion period. There was no significant effect of perfusing carnitine on the oxidation of labeled palmitate to $^{14}CO_2$, but this could be explained by the low level of carnitine uptake under the same experimental conditions. Despite this, both levels of carnitine resulted in an increased percentage of recovery of extracted palmitate in the esterified lipids of the heart. Among the various lipid fractions of cardiac tissue, 5 m*M* carnitine perfusion caused a decrease in the percentage of label in unesterified fatty acids and diglycerides, and a significant increase in label recovered as phospholipids and triglycerides. This apparent increase in esterification of labeled palmitate was confirmed by separation of the subcellular fractions of heart after perfusion with palmitate in the absence and presence of carnitine. In the presence of carnitine, there was significant increases in palmitate incorporation into mitochondrial and microsomal triglycerides, while the other cell fractions were not affected.

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