Effect of Carnitine on Fatty Acid Synthesis in Perfused Rat Heart[1] (34627)

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In a previous communication (1), the effect of *d,l*-carnitine on palmitic acid-1-^{14}C metabolism was studied in the intact perfused rat heart. The main effects of carnitine in this model system were: (1) To decrease the uptake of palmitic acid; and (2) to increase the percentage recovery of palmitate in esterified lipids. Carnitine itself was taken up by the perfused heart, and there was an increase in the esterification of palmitic acid in various subcellular fractions during carnitine perfusion. However, at the low levels of carnitine taken up by the perfused heart, there was no effect of carnitine on palmitic acid oxidation to $^{14}CO_2$.

In addition to the effect of carnitine on fatty acid oxidation and esterification, this compound has been found to influence fatty acid synthesis. Bressler and Katz (2) have reported that carnitine stimulates hepatic conversion of glucose or pyruvate to long-chain fatty acids *in vivo* and in guinea pig liver homogenates. It was suggested that the increase in fatty acid synthesis due to carnitine was the result of stimulation of acetyl group transport from intramitochondrial sites of acetate production to extramitochondrial sites of fatty acid synthesis. Fritz and Hsu (3) presented evidence suggesting that palmityl carnitine stimulates fatty acid synthesis from acetate in rat liver soluble fraction; this effect appeared due to stimulation of the enzyme acetyl CoA carboxylase which catalyzes the formation of malonyl CoA. Although this mechanism would explain stimulation of cytoplasmic fatty acid synthesis from malonyl CoA in liver, it has been reported by others that carnitine stimulates intramitochondrial fatty acid synthesis in incubated intestinal slices (4), a process which appears independent of malonyl CoA formation (5).

In the present study, the effect of carnitine on myocardial fatty acid synthesis from acetate and pyruvate has been studied using the isolated perfused rat heart and cell-free preparations.

Materials and Methods. Materials. Sodium acetate-1-^{14}C and sodium pyruvate-2-^{14}C were obtained from Nuclear Chicago Corp. and diluted with unlabeled acetate and pyruvate purchased from California Corporation of Biochemical Research (Calbiochem).

Perfusion studies. Male albino rats (Microbiological Associates, Wistar strain) were fasted for 18–24 hr prior to use. The procedures for cannulation of the aorta and perfusion in an open, recirculating system have been described earlier (1, 6). The basic perfusion media consisted of Krebs–Henseleit bicarbonate buffer (7) containing 1.27 m*M* Ca^{2+}. The medium, pH 7.4, contained 5 m*M* acetate or pyruvate, and *d,l*-carnitine HCl was added to a final concentration of 5 m*M* in certain groups. All perfusion media were equilibrated with 95% O_2–5% CO_2 before use. Perfusions were carried out for 45 min at 37° with an initial perfusate volume of 25 ml and pressure of 50 mm Hg. The system was continuously gassed with O_2–CO_2 (95:5 v/v), and the effluent gasses were passed through 6 *N* KOH.

The perfusion medium was sampled (1 ml) initially and at 15-min intervals for determinations of substrate uptake and $^{14}CO_2$ production. Perfusate pyruvate was determined spectrophotometrically by measurement of NADH oxidation after addition of lactic de-

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hydrogenase (8). Perfusate acetate was determined by isotope analysis after acidification of the sample to eliminate $^{14}CO_2$. The method was verified by thin-layer silicic acid chromatography of the perfusate in a solvent system of ethanol:ammonium hydroxide (100:1 v/v) and quantitative recovery of isotope in the silicic acid area corresponding to authentic acetate-1-^{14}C.

The methods for removal and freezing of the heart, extraction of lipids, protein determination, $^{14}CO_2$ analysis, separation of lipid classes, and lipid analysis have been described earlier (1). Chloroform-methanol extracts of heart homogenates were checked for possible contamination by labeled acetate and pyruvate by addition of these compounds to homogenates of nonperfused hearts, and complete recovery of the isotopes in the methanol–water phase of the Folch separation (9).

In addition to determining isotope distribution in the individual lipid fractions, the percentage of isotope incorporation into water-insoluble (fatty acids) and water-soluble (mainly glycerol) fractions of total lipids was determined. A sample of the lipid extract was saponified with 1 ml of 5% ethanolic KOH at 60° for 30 min. The mixture was acidified (1 ml 7 *N* H_2SO_4) and repeatedly extracted with ether. The combined ether extracts and remaining aqueous hydrolyzate were separately assayed for isotope, and the ratio of ether-soluble:water-soluble radioactivity was calculated.

Acetate or pyruvate uptake is expressed as micromoles uptake per gram wet weight heart per 45 min. Oxidation to $^{14}CO_2$ is expressed as micromoles substrate equivalents per time period or as micromoles per gram wet weight heart per 45 min. Specific activities are expressed as disintegrations per minute per micromole. Values represent means ± SEM.

Studies with high-speed supernatant fractions. Fatty acid synthesis was determined by measuring incorporation of acetate-1-^{14}C into long-chain fatty acids using the high-speed supernatant fraction (HSS), prepared by centrifugation of the tissue homogenate at 105,000*g* for 1 hr in a Spinco ultracentrifuge (Model L-2). The basic incubation medium was similar to that described by Abraham *et al.* (10) and contained the following components in 2-ml volume: 240 μmoles glycylglycine, pH 7.4; 70 μmoles $MgCl_2$; 1 μmole $MnCl_2$; 10 μmoles $KHCO_3$; 70 μmoles potassium citrate; 50 μmoles ATP (K^+); 0.2 μmole CoA; 1 μmole NADPH; 60 μmoles glutathione; 150 μmoles potassium phosphate, pH 7.4; and 6.2 μmoles potassium acetate-1-^{14}C (2×10^{-6} dpm).

The incubation medium (2 ml) was placed in 25-ml Ehrlenmeyer flasks, and either 0.1 ml of distilled water or 0.1 ml of *d,l*-carnitine HCl was added to a final concentration of 1 m*M* (3.6 μmoles). To this was added 1.5 ml of the HSS fraction (7.5 mg protein) to give a final volume of 3.6 ml, and the flask was sealed with a commercial rubber septum containing a polyethylene center well (Kontes). Incubations were at 37° for 2 hr with shaking. One minute before termination of the reaction, 0.3 ml of hyamine hydroxide was carefully injected through the septum into the center well. The reaction was terminated by injecting 0.5 ml of 6 *N* H_2SO_4 into the medium, and incubation at 37° was continued for 30 min to trap evolved $^{14}CO_2$ in the hyamine hydroxide (11). The methods for extraction of lipids, separation of lipid fractions by thin-layer chromatography, protein determination, and radioactive counting were described previously (1).

Results and Discussion. The effect of *d, l*-carnitine on the uptake and oxidation of acetate-1-^{14}C by isolated perfused rat heart is shown in Fig. 1 and Table I. At 0.5 m*M* concentration, carnitine did not affect either the uptake or oxidation of acetate. However, at 5 m*M* concentration, carnitine significantly increased both acetate uptake and its oxidation to $^{14}CO_2$. In this latter case, the oxidation of acetate was increased to 75% of the acetate taken up, compared to approximately 60% oxidation of the acetate taken up by control hearts.

In the case of perfusion with 5 m*M* pyruvate-2-^{14}C, shown in Fig. 2, ciruclation of 5 m*M* carnitine significantly depressed pyruvate uptake by heart but had no effect on

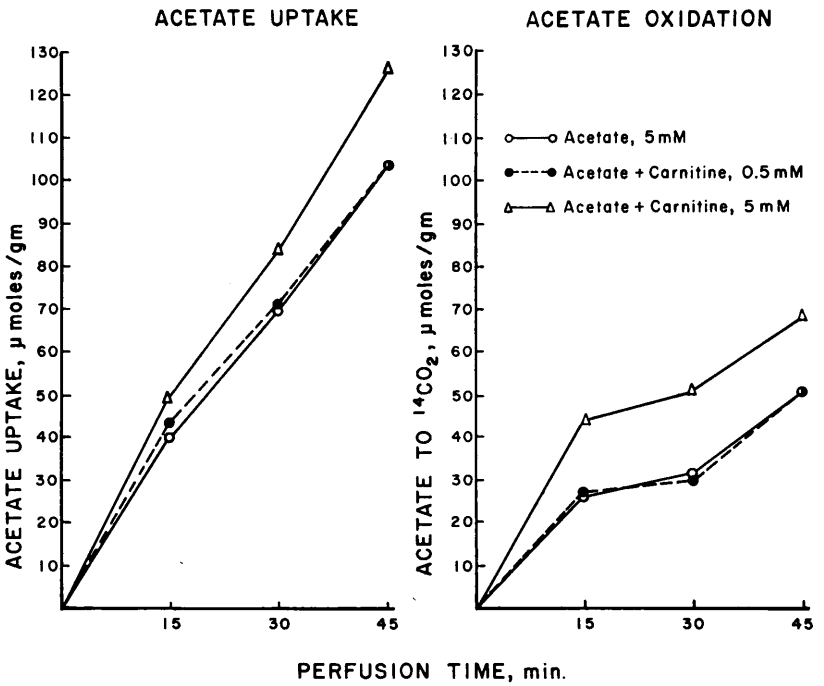

FIG. 1. Effect of *d,l*-carnitine on acetate-1-^{14}C uptake and oxidation to $^{14}CO_2$ by isolated perfused rat heart. The initial concentration of carnitine was 0.5 m*M* or 5 mM and of acetate was 5 m*M*. Each curve is derived from mean values of 4–5 perfusions. Other conditions are described in the text.

the absolute level of $^{14}CO_2$ produced during the perfusion period. These data are qualitatively similar to the results obtained after perfusion with 0.5 m*M* palmitate (1).

The incorporation of acetate-1-^{14}C into lipids, shown in Table I, was markedly depressed in the presence of either level of perfusing carnitine (from 0.72 μmoles/g in the control to 0.38 and 0.35 μmoles/g with 0.5 m*M* and 5 m*M* carnitine, respectively). In contrast, there was no effect of circulating 5 m*M* carnitine on the absolute amount of pyruvate incorporated into heart lipids.

In order to determine the relative incorpo-

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

Group[a]	Substrate uptake (μmoles/g)	Substrate to $^{14}CO_2$ (μmoles/g)	Substrate to total lipids (μmoles/g)
Acetate-1-^{14}C, 5 m*M*	104.2 ± 9.9[b]	57.4 ± 2.4	0.72 ± 0.08
Acetate + carnitine (5×10^{-4} *M*)	104.2 ± 6.1	60.8 ± 6.0	0.38 ± 0.05[d]
Acetate + carnitine (5×10^{-3} *M*)	126.7 ± 2.3[c]	93.7 ± 3.8[d]	0.35 ± 0.02[d]
Pyruvate-2-^{14}C, 5 m*M*	84.8 ± 2.8	14.9 ± 4.9	0.11 ± 0.01
Pyruvate + carnitine (5×10^{-3} *M*)	66.5 ± 5.1[c]	21.7 ± 4.7	0.19 ± 0.04

[a] Hearts from fasting rats 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 with continuous gassing with 95% O_2–5% CO_2; exit gasses were trapped in 6 *N* KOH. At the termination of experiments, hearts were rapidly flushed free of isotope and were frozen in Dry Ice. Subsequent homogenization and extraction of lipids were described in the text.

[b] Values represent means ± SEM.

[c] $p < .05$ from noncarnitine controls.

[d] $p < .01$ from noncarnitine controls.

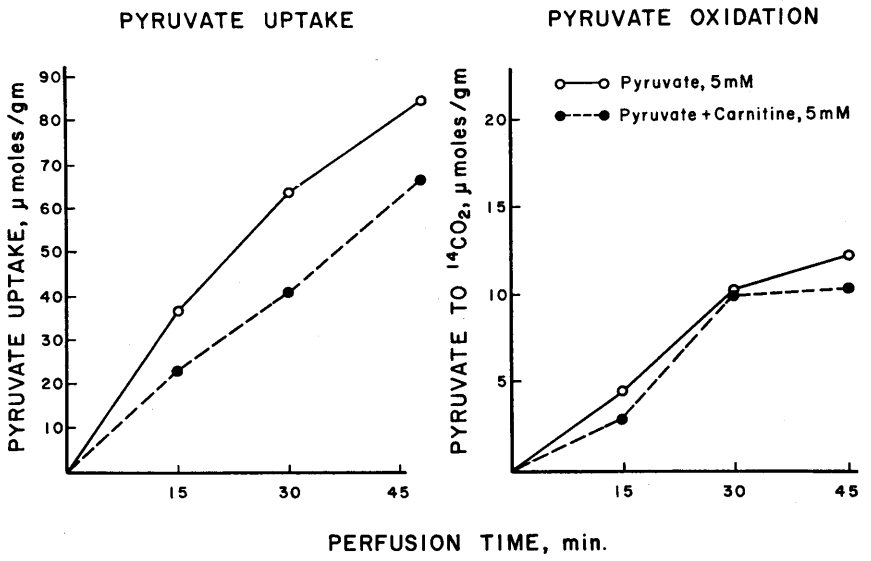

FIG. 2. Effect of *d,l*-carnitine on pyruvate-2-^{14}C uptake and oxidation to $^{14}CO_2$ by isolated perfused rat heart. The initial concentrations of carnitine and pyruvate were each 5 m*M*. Each curve is derived from mean values of four perfusions. Other conditions are described in the text.

ration of acetate and pyruvate into the fatty acid and glycerol moieties of esterified lipids, a portion of the lipid extract of perfused hearts was treated with 5% ethanolic KOH for 30 min at 60°. The mixture was repeatedly extracted with ether after acidification, and portions of the ether extract and the remaining aqueous hydrolyzate were counted separately. Radioactivity in the ether extract was taken to represent primarily fatty acids, while that in the aqeuous portion was taken to represent glycerol. After perfusion with acetate, the ratio of ^{14}C in ether to that in water was 3.0, indicating that 75% of the radioactivity of acetate-1-^{14}C was incorporated into fatty acids and only 25% into the glycerol (and other water-soluble materials) backbone. When carnitine and acetate were perfused, this ratio dropped to 1.1, which was due primarily to a reduction in ether-soluble radioactivity. These results support the earlier findings which indicate that carnitine inhibition of acetate incorporation into lipids is due primarily to an effect on fatty acid synthesis.

After pyruvate perfusion, the ratio of lipid-soluble-^{14}C/water-soluble-^{14}C was 0.5, showing that two-thirds of the radioactivity was associated with aqueous extracts after hydrolysis of esterified lipids. The perfusion of carnitine with pyruvate had no effect on either this ratio or on the absolute level of pyruvate incorporated into extractable lipids.

The specific activities of individual lipid fractions after acetate-1-^{14}C perfusion is summarized in Table II. As in the first study, carnitine at concentrations of 0.5 m*M* and 5 m*M* caused a significant depression of acetate incorporation into myocardial lipids. This was due primarily to a marked decrease in the specific activities of the free fatty acid fraction and the triglyceride fraction. There was no significant change in the specific activity of phospholipid fraction which makes up the majority (60–70%) of heart lipids, or in any of the minor lipid fractions, *i.e.*, diglyceride, monoglyceride, cholesterol, or cholesterol esters.

The distribution of isotope between lipid fractions after pyruvate-2-^{14}C perfusion is shown in Table III. Of the radioactivity recovered in the total lipid extract, about 6% was found in free fatty acids, while 86% was present in the three major esterified lipid fractions. These data are consistent with results above, indicating that the majority of radioactivity in lipids after pyruvate perfusion is probably in the glycerol backbone of

TABLE II. Radiospecific Activities of Heart Lipid Fractions After Acetate-1-^{14}C and Carnitine Perfusion *in Vitro*.

Group[a]	% Substrate uptake to total lipids	Specific activity of lipid fractions, (dpm/μmole)		
		Fatty acids	Phospholipids	Triglycerides
Acetate-1-^{14}C, 5 m*M*	0.70 ± 0.09[b]	560 ± 65	322 ± 60	1.98 ± 0.15
Acetate + carnitine (5×10^{-4} *M*)	0.37 ± 0.05[c]	121 ± 55[d]	183 ± 9	0.82 ± 0.30[c]
Acetate + carnitine (5×10^{-3} *M*)	0.29 ± 0.01[c]	178 ± 24[d]	206 ± 30	0.58 ± 0.11[d]

[a] Perfusion conditions are described in Table I and the text.
[b] Values represent means ± SEM.
[c] $p < .05$ compared to noncarnitine control.
[d] $p < .01$ compared to noncarnitine control.

esterified lipids. The perfusion of carnitine with pyruvate-2-^{14}C resulted in a redistribution of radioactivity among esterified lipids, with no significant change in the percentage incorporation into fatty acids. In this case, 72% of the radioactivity in total lipids was recovered in the triglyceride fraction, with decreases in the recovery in diglycerides and phospholipids. These results agree well with previous data in which carnitine perfusion resulted in an increase in palmitate-1-^{14}C esterification in the isolated perfused heart (1).

The effect of *d,l*-carnitine on acetate incorporation into fatty acids was also studied in a cell-free system. The high-speed supernatant fractions of rat liver and heart were prepared and tested for acetate incorporation into fatty acids. With liver, there was a consistent increase in fatty acid synthesis due to 1 m*M* carnitine (average 42%) which is comparable to the levels reported previously (3). However, carnitine depressed fatty acid synthesis in heart to the extent of 15–60% (average 36%). The changes in individual lipid fractions in liver and heart due to carnitine are shown in Table IV. With liver high-speed supernatant fraction, carnitine stimulation of fatty acid synthesis was reflected in a significant increase in the isotope incorporation into free fatty acid (+44%), while changes in the diglyceride and phospholipid fractions were insignificant. With heart tissue, carnitine depression of acetate incorporation into lipids was due to significant decreases in isotope incorporation into the fatty acid (−28%) and diglyceride fractions (−29%).

TABLE III. Distribution of Label in Heart Lipids After Perfusion with Pyruvate-2-^{14}C.

Lipid fraction	% Distribution of label in total lipids after perfusion with:[a]	
	Pyruvate	Pyruvate + carnitine
Fatty acids	5.7 ± 3.8[b]	4.4 ± 1.4
Phospholipids	19.6 ± 4.0	11.6 ± 2.1
Diglycerides	27.0 ± 2.8	8.6 ± 0.2[c]
Triglycerides	39.7 ± 1.2	72.0 ± 3.2[c]

[a] Perfusion conditions are described in Table I and the text.
[b] Values represent means ± SEM.
[c] $p < .01$ compared to noncarnitine control.

Fritz and Hsu (3) have reported that hepatic fatty acid synthesis from acetate is enhanced 5- to 10-fold by (+) palmityl carnitine or *d,l*-palmityl carnitine. Pearson and Tubbs (12) have also shown that acetate perfusion of rat heart results in a decreased concentration of free carnitine and increased levels of acetyl carnitine. Based on these results and data derived from several other tissues, it has been suggested that acetyl carnitine formation in certain tissues may represent a mechanism for buffering rapid changes in acetyl CoA concentrations (12). Studies are currently under way to determine the significance of acetyl carnitine formation in heart under the conditions used in the present report.

Summary. The effect of carnitine on fatty

TABLE IV. Distribution of Labeled Carbon from Acetate-1-^{14}C in Lipid Fractions of Heart and Liver Soluble Fraction.

Lipid fraction	Change due to carnitine 1×10^{-3} *M* (mμmoles/mg protein)	(%)	Significance
Liver (4)			
Fatty acid	+0.532	+44	$p < .01$
Diglyceride	+0.048	+24	NS
Phospholipid + monoglycerides	+0.037	+52	NS
Heart (4)			
Fatty acid	−0.873	−28	$p < .05$
Diglyceride	−0.113	−29	$p < .01$
Phospholipid + monoglycerides	−0.060	−12	NS

The incubation medium contained 6.2 μmoles potassium acetate-1-^{14}C (2×10^{-6} dpm), 240 μmoles glycylglycine, pH 7.4; 50 μmoles ATP(K^+); 0.2 μmoles CoA; 1 μmole TPN; 70 μmoles $MgCl_2$; 1 μmole $MnCl_2$; 10 μmoles $KHCO_3$; 75 μmoles potassium citrate; 60 μmoles glutathione; 150 μmoles potassium phosphate, pH 7.4. The final volume was 2 ml. The incubation medium was placed in 25-ml Ehrlenmeyer flasks and either 0.1 ml of distilled water or 0.1 ml of *d,l*-carnitine was added to give a final concentration of 1 m*M*. To this was added 1.5 ml of the high-speed supernatant fraction of heart or liver (7–10 mg protein) to give a final volume of 3.6 ml. Incubations were at 37° for 2 hr with shaking. Extraction of lipid and isotope analysis are described in the text.

acid synthesis in myocardium has been studied with isolated perfused rat hearts. Circulating carnitine (0.5 m*M* or 5 m*M*) depressed acetate (5 m*M*) incorporation into heart lipids in perfused heart preparations, and this effect was due primarily to reduced incorporation into tissue fatty acids and triglycerides. There was also stimulation of acetate oxidation to $^{14}CO_2$ by the higher level of perfusing carnitine. Carnitine significantly depressed pyruvate (5 m*M*) uptake by perfused rat heart but had no effect on oxidation of pyruvate to $^{14}CO_2$, nor on incorporation of pyruvate into heart lipids.

With 105,000*g* supernatant fraction of heart, fatty acid synthesis from acetate was markedly depressed in the presence of carnitine, while with liver supernatant fraction there was a 40% stimulation of fatty acid synthesis by carnitine. These changes were again reflected primarily in the unesterified fatty acid fraction in each tissue.

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