The Metabolism of (-)-Octanoylcarnitine in Perfused Livers from Fed and Fasted Rats

EVIDENCE FOR A POSSIBLE REGULATORY ROLE OF CARNITINE ACYLTRANSFERASE IN THE CONTROL OF KETOGENESIS*

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SUMMARY

In confirmation of previous findings it was shown that perfused livers from fasted rats converted oleic acid into ketone bodies far more efficiently than did livers from fed animals, whereas differences in rates of ketogenesis from octanoate were much less pronounced. However, relative rates of ketone body production from (-)-octanoylcarnitine resembled those seen with oleic acid rather than those obtained with free octanoic acid as substrate. In addition, (+)-octanoylcarnitine, an inhibitor of carnitine acyltransferase, was without effect on the oxidation of octanoic acid, but caused a profound and quantitatively similar depression in the oxidation of both oleic acid and (-)-octanoylcarnitine. The data support the concept that the carnitine acyltransferase system of the liver is under strict dietary, or hormonal control, or both, and that it may constitute a primary site for the regulation of hepatic fatty acid oxidation and ketogenesis.

There is now good reason to believe that of the intrahepatic factors responsible for the regulation of ketone body production, those that govern the partitioning of incoming fatty acids between the pathways of esterification and oxidation are of primary importance (1-10). Available evidence would support the view that the principal control on the ultimate disposition of fatty acids in liver tissue is exerted on the oxidative side of this metabolic branchpoint (3-8). We have suggested that a key regulatory site might reside at the first step specific for long chain fatty acids in liver tissue, namely, the carnitine acyltransferase reaction (4, 8). This conclusion was based on the fact that octanoic acid, which does not require esterification to carnitine for transfer across the mitochondrial membrane (11), was oxidized at equivalent rates in livers from fed, fasted, and diabetic rats (2), while long chain fatty acids were converted into ketone bodies much more rapidly in livers from ketotic animals (3, 8). Further, when the carnitine acyltransferase reaction was inhibited with (+)-decanoylcarnitine, the pattern of fatty acid metabolism in ketotic livers was rapidly reversed to that seen in normal hepatic tissue (9), indicating that no fundamental defect exists in the esterification system of the ketogenic liver.

Consistent with this interpretation were the observations of Norum (9) and Aas and Daae (10) that hepatic carnitine acyltransferase activity was elevated in ketotic states. However, for reasons yet unexplained we have been unable to confirm these findings. Moreover, in our hands, homogenates prepared from fed and fasted rats, when suitably fortified with cofactors, synthesized ketone bodies equally well and at rates that actually exceeded those in perfused livers from severely ketotic alloxan diabetic animals (12). For this reason we have attempted to assess the role of this enzyme in the regulation of ketone body production in the isolated perfused rat liver. The studies reported below are based on the fact that free octanoic acid enters the β oxidation sequence directly while octanoic acid esterified to carnitine requires an initial carnitine acyltransferase step prior to entry into the oxidative pathway. The experiments clearly show that in contrast to free octanoic acid, (-)-octanoylcarnitine behaves functionally as a "long chain fatty acid" in terms of its strikingly different capacity to support ketogenesis in livers from normal and ketotic animals. The results provide additional support for the postulated regulatory function of carnitine acyltransferase in hepatic fatty acid metabolism and ketogenesis.

EXPERIMENTAL PROCEDURE

Animals Male Sprague-Dawley rats weighing approximately 100 g were used in all experiments. Animals were fed a diet containing 58.5% sucrose, 21% casein, and less than 1% fat, and all necessary vitamins and minerals (General Biochemicals, Chagrin Falls, O.). The term "normal" as used in the text refers to rats that had free access to food up to the time the experiments were initiated, which was generally between 9:30 and 10:00 a.m. Fasted rats, sometimes referred to as "ketotic," were deprived of food for 24 hours before use.

Liver Perfusion—Livers were perfused with recirculating medium using the apparatus and techniques described previously (9, 3). The perfusion medium was composed of aged, human erythrocytes suspended to an hematocrit of 20% in Krebs bicarbonate buffer, pH 7.4, containing 3% bovine albumin (Fraction V, Armour Pharmaceutical Co.). In all experiments the red cells...
were dialyzed for 24 hours against 0.9% sodium chloride at 4° prior to use in order to remove lactate (8). Additions of substrates and inhibitors will be described in the text.

Analyses on Perfusion Fluid and Liver—As in previous reports (2, 3, 8) all analyses were carried out either on the cell-free perfusion fluid or on the neutralized perchloric acid extracts of livers that had been rapidly frozen in liquid N2 at the termination of experiments. The method employed for determination of free (—)carnitine was based on the spectrophotometric assay for coenzyme A described by Srere (13). The basic reaction mixture contained in a volume of 1.0 ml: Tris-Cl, pH 7.5, 100 µmol; sodium citrate, 10 µmol; MgCl2, 15 µmol; acetyl-CoA, 1 µmol; NADH, 0.15 µmol, and an appropriate aliquot of the sample. Any oxalacetate or coenzyme A that might have been present was first allowed to react by the addition of malate dehydrogenase and citrate cleavage enzyme. The presence of (—)carnitine was then detected by the fall in extinction at 340 nm due to the subsequent addition of carnitine acetyltransferase, which under these conditions results in the stoichiometric release of coenzyme A described by Srere (13). The basic reaction mixture contained in a volume of 1.0 ml: Tris-Cl, pH 7.5, 100 µmol; sodium citrate, 10 µmol; MgCl2, 15 µmol; acetyl-CoA, 1 µmol; NADH, 0.15 µmol, and an appropriate aliquot of the sample. Any oxalacetate or coenzyme A that might have been present was first allowed to react by the addition of malate dehydrogenase and citrate cleavage enzyme. The presence of (—)carnitine was then detected by the fall in extinction at 340 nm due to the subsequent addition of carnitine acetyltransferase, which under these conditions results in the stoichiometric release of coenzyme A described by Srere (13).

Materials—(—)- and (—)-Octanoylcarnitine were either synthesized from octanoyl chloride (Eastman Kodak) and the appropriate isomer of carnitine by the method of Bremer (16), or were generously provided by Dr. Yuzo Kawashima of the Otsuka Pharmaceutical Factory, Naruto, Tokushima, Japan, from whom we also received (—)-carnitine. (—)-Carnitine was from General Biochemicals. [1-14C]Octanoyl chloride, which was used in the synthesis of (—)[1-14C]octanoylcarnitine, was synthesized from [1,14C]octanoic acid and unlabeled octanoyl chloride by a slight modification of the method described by Borgström and Krabich (17). With the exception of citrate cleavage enzyme, which was the kind gift of Dr. Paul A. Srere, of this institution, all other enzymes were purchased from Boehringer Mannheim. The sources of other materials have been given in earlier reports.

RESULTS

Ketogenesis from Oleate and Octanoylate in Perfused Livers from Fed and Fasted Rats and Influence of (+)-Octanoylcarnitine—Consistent with earlier reports (3, 8) it is seen from the data of Fig. 1 that the rate of ketone body production from oleic acid was greatly enhanced in perfused livers from fasted rats compared with those from fed animals. It is now well established that this difference in response of the two types of liver to long chain fatty acids results primarily from a switch in the partitioning of substrate between the pathways of esterification and oxidation, a phenomenon that occurs abruptly in favor of the latter sequence during the early stage of fasting (8). It is also apparent from the figure and other studies (3, 8, 18) that the increased capacity for long chain fatty acid oxidation characteristic of livers from fasted rats does not require exogenous fatty acid to be manifest, implying that hepatic triglyceride content and lipase activity are sufficient to supply the necessary substrate for the accelerated ketogenesis of fasting.

1 In none of the experiments reported here was CO2 production measured. However, previous studies (2, 3) have shown that under these experimental conditions the major products of hepatic fatty acid oxidation are acetoacetate and β-hydroxybutyrate. Thus, throughout the text rates of ketone production will be taken as a reflection of the activity of the β oxidation process.

We have demonstrated recently that (+)-octanoylcarnitine is a potent inhibitor of carnitine acyltransferase and offers the distinct advantage over (+)-decanooylcarnitine, the more commonly used homologue (8, 19–22), that it exhibits markedly less surface activity (23). The potent antiketogenic action of this compound in livers from fasted rats perfused either in the presence or absence of added oleic acid is illustrated in Fig. 1. In experiments not shown it was clearly established that the effect of this (+)-carnitine ester on the metabolism of [14C]-labeled oleate was identical with that of (+)-decanooylcarnitine seen in earlier studies (8) in that the presence of the inhibitor caused virtually total diversion of the fatty acid from the β oxidation pathway into esterification reactions. The end result was a pattern of fatty acid metabolism in livers from ketotic animals which resembled that of normal hepatic tissue.

When octanoyl acid is supplied as substrate for ketogenesis in place of oleic acid (Fig. 2) two differences are noted. First, the initial ketogenic response in livers from normal rats is more brisk. Second, the medium chain fatty acid overcomes the block in fatty acid oxidation imposed by (+)-octanoylcarnitine. We have previously shown (2) that β oxidation of octanoic acid proceeds at similar rates in livers from normal, fasted, and alloxan diabetic rats. The difference in ketogenesis in fed and fasted livers subsequent to the addition of octanoate (Fig. 2A versus Fig. 2B) reflects primarily the greater contribution of endogenous fatty acids in the latter group, a point that will be emphasized below.

Ketogenesis from (—)-Octanoylcarnitine in Perfused Livers from Fed and Fasted Rats and Influence of (+)-Octanoylcarnitine—The data discussed so far would be entirely consistent with the thesis...
under "Experimental Procedure." When used (+)-octanoylcarnitine and absence of (+)-octanoylcarnitine on ketogenesis in perfused livers were perfused as described under "Experimental Procedure." When used (+)-octanoylcarnitine (+)-OC was present in the perfusion fluid at zero time in a concentration of 0.5 mM. Sufficient (-)-octanoylcarnitine (-)-OC was added at 15 min to bring its initial concentration to 1.25 mM. Values represent means ±S.E. for four perfusions in each group.

It has been shown previously that (-)-octanoylcarnitine ((-)-OC) was present in the perfusion fluid at zero time. Sufficient (-)-octanoylcarnitine ((-)-OC) was added at 15 min to bring its initial concentration to 1.25 mM. Values represent means ±S.E. for four perfusions in each group.

To answer these questions, livers from fed and fasted rats were perfused for 15 min in the presence or absence of 0.5 mM (+)-octanoylcarnitine, at which time, (-)-octanoylcarnitine was introduced into the perfusion fluid to provide an initial concentration of 1.25 mM. Values represent the means of two or three perfusions in each group.

Repeated in the presence of (+)-octanoylcarnitine (Fig. 3B) the normal livers, not surprisingly, again showed little or no response to (-)-octanoylcarnitine. However, despite the fact that endogenous fatty acid oxidation was now profoundly inhibited in the fasted livers (Δ 15 to 60 min, 27 μmol/100 g body weight), ketone production in this group rose sharply after an initial lag period subsequent to the addition of (-)-octanoylcarnitine (Δ 15 to 60 min, 124 μmol/100 g body weight).

The immediate implications of these observations were that (-)-octanoylcarnitine can support ketone production in the intact rat liver, and that its rate of oxidation is greatly enhanced in livers from fasted animals, thus resembling the situation with long chain fatty acids and in marked contrast to that observed when free octanoic acid was used as substrate. However, it also appeared that under circumstances where the long chain carnitine acyltransferase of the ketotic liver was severely inhibited by (+)-octanoylcarnitine, the enzyme responsible for the conversion of (-)-octanoylcarnitine into octanoyl-CoA was largely unaffected. This latter observation was puzzling since we had expected on a priori grounds that the oxidation of (-)-octanoylcarnitine would be inhibited by the (+)-octanoylcarnitine to a degree at least equivalent to that seen for long chain fatty acids. To determine whether the ketone bodies produced under these conditions arose from the added (-)-octanoylcarnitine, perfusions similar to those depicted in Fig. 3B were carried out with livers from fasted animals with the exception that a tracer quantity of [1-14C]oleate was included in the perfusion medium at zero time as a marker for endogenous fatty acid oxidation. Both the total quantity of ketone bodies synthesized and the radioactivity associated with them was then determined. The results of these experiments are shown in Fig. 4 from which it may be seen that in the presence of (+)-octanoylcarnitine both the rate of endogenous ketogenesis (Fig. 4B) and the incorporation of

- Fig. 3. The effect of (-)-octanoylcarnitine in the presence and absence of (+)-octanoylcarnitine on ketogenesis in perfused livers from fed and fasted rats. Livers were perfused as described under "Experimental Procedure." In all cases (+)-octanoylcarnitine (+)-OC was present in the perfusion fluid at zero time in a concentration of 0.5 mM. Sufficient (-)-octanoylcarnitine (-)-OC was added at 15 min to bring its initial concentration to 1.25 mM. Values represent means ±S.E. for four perfusions in each group.

- Fig. 4. Effect of various additions on ketogenesis in livers from fasted rats perfused in the presence of (+)-octanoylcarnitine. Livers were perfused as described under "Experimental Procedure." In all cases (+)-octanoylcarnitine (0.5 mM) and sodium [1-14C]oleate (20 μCi; 0.3 μmol) were added to the perfusion fluid at zero time. All additions made at 15 min were in a concentration of 1.25 mM. The abbreviation "(-)-OC" refers to (-)-octanoylcarnitine. Values represent the means of two or three perfusions in each group.

(8, 22) that the enhanced capacity for long chain fatty oxidation seen in livers from ketotic animals reflects primarily an increased activity of the carnitine acyltransferase reaction in the mitochondrial membrane. However, such a conclusion rests on the assumption that the only difference in the oxidation of oleic and octanoic acids resides in the requirement of the former for carnitine acyltransferase for entry into the mitochondrion. To test this assumption, (-)-octanoylcarnitine was used as substrate in place of octanoate. It has been shown previously that (-)-octanoylcarnitine is oxidized readily by mitochondria from various tissues of the rat (24, 25); furthermore, it is believed to be transported across the inner mitochondrial membrane under the influence of a carnitine acyltransferase (25, 26). By utilizing this particular (-)-carnitine ester we wished to address the following questions.

1. Can this compound support ketogenesis in the intact perfused liver?

2. If so, would the relative rates of ketone production from (-)-octanoylcarnitine in livers from fed and fasted rats resemble the pattern seen with oleate, or that observed when octanoate served as substrate?

3. Would the oxidation of (-)-octanoylcarnitine be inhibited by (+)-octanoylcarnitine?

To answer these questions, livers from fed and fasted rats were perfused for 15 min in the presence or absence of 0.5 mM (+)-octanoylcarnitine, at which time, (-)-octanoylcarnitine was introduced into the perfusion fluid to provide an initial concentration of 1.25 mM. Perfusion was continued for a further 45 min and the output of ketone bodies was determined. The initial results are shown in Fig. 3A where it can be seen that the addition of (-)-octanoylcarnitine had little effect on the very low rate of ketogenesis in livers from fed rats, values for this parameter over the 15- to 60-min period being 12 and 25 μmol/100 g body weight in the absence and presence of the substrate, respectively. In contrast, over the same time interval the (-)-carnitine ester caused a marked acceleration of the already high rate of ketone production in livers from fasted animals (from 109 to 170 μmol/100 g body weight). When these experiments were
[1-14C]oleate into ketones (Fig. 4A) were increasingly inhibited during the course of the perfusion (cf. fasted controls, Fig. 3A). As expected, addition of octanoate resulted in the usual increased rate of ketone production but had no effect on the disposition of the tracer oleate. A plausible mechanism for this effect stems from the observation by Deslisle and Fritz (20) that in pigeon liver homogenate. A plausible mechanism for this effect stems from the observation by Deslisle and Fritz (20) that in pigeon liver homogenate. However, the isotopic experiment revealed an almost 7-fold increase in the capacity of the ketotic liver to oxidize (-)-octanoylcarnitine to ketone bodies (Fig. 5B). Also illustrated are the effects of two different concentrations of (-)-octanoylcarnitine on the metabolism of the fasted liver. When present at 0.5 mM the (+)-carnitine ester resulted in a 50% inhibition of the conversion of the labeled (-)-octanoylcarnitine into ketones (Fig. 5B). Once again, however, endogenous ketogenesis, which would normally have been almost completely inhibited by this concentration of (-)-octanoylcarnitine (see Fig. 3B), was stimulated markedly after the addition of (-)-octanoylcarnitine (Fig. 5C), thus confirming the data of Fig. 4. When the inhibitor was added in a concentration of 2 mM it reduced the oxidation of isotopic (-)-octanoylcarnitine to ketone bodies by 83% resulting in a rate essentially equal to that observed in livers from fed animals (Fig. 5B). A similar effect was noted on endogenous ketogenesis over the 15- to 30-min interval, although this rate again began to accelerate as the perfusion was continued (Fig. 5C).

When experiments equivalent to those described in Fig. 5 were repeated using [1-14C]octanoate as the ketogenic substrate a very different pattern emerged (Fig. 6). First it is seen that total ketone production responded briskly to the addition of this fatty acid in both fed and fasted livers (Fig. 6A), and that endogenous ketogenesis was again markedly greater in the latter group (Fig. 6C). The major point of interest here, however, is that over the 15- to 30-min interval the conversion of labeled octanoate into ketones was almost as rapid in the fed livers as in the fasted group. Although in both cases the rate subsided over the latter half of the experimental period (due to substrate depletion (2)), the final quantity of octanoate incorporated by the fed livers was still 73% of that found in the fasted group. Although the

![Graph](http://www.jbc.org/jbc/content/263/12/7987/DC1/Fig-5/Fig-5.jpg)

**Fig. 5.** The metabolism of (-)-[1-14C]octanoylcarnitine in perfused livers from fed and fasted rats and the effect of (+) octanoylcarnitine. Livers were perfused as described under "Experimental Procedure." When used, (+)-octanoylcarnitine ((+)-OC) was present in the perfusion fluid at zero time at the concentration indicated. At 15 min, 100 μmol of (-)-[1-14C]octanoylcarnitine (specific activity, 0.5 μCi per μmol) was added to bring its initial concentration to 1.25 mM. The term "labeled C2 units → ketones" represents the micromoles of labeled substrate converted into ketones, multiplied by 4. The term "unlabeled C2 units → ketones" equals the total quantity of ketones formed, multiplied by 2, minus the contribution of labeled C2 units. Values represent means ± S.E. for four or five perfusions in each group.
The data refer to the perfusion experiments described in Figs. 5 and 6. The term "relative ketone specific activity" represents the fractional contribution of the labeled substrate to the total quantity of ketone bodies formed between the 15-min time point and that indicated (2). Values are given as means or means ± S.E. for the number of livers shown in parentheses.

<table>
<thead>
<tr>
<th>State of animal</th>
<th>Concentration of (+)-octanoylcarnitine at zero time (mM)</th>
<th>Added at 15 min</th>
<th>Total ketone production at 15-60 min (μmol/100 g body wt)</th>
<th>Relative ketone specific activity at 30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed (5)</td>
<td>0</td>
<td>(–)-[1-14C]Octanoylcarnitine</td>
<td>26 ± 3.5</td>
<td>0.21 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Fasted (5)</td>
<td>0</td>
<td>(–)-[1-14C]Octanoylcarnitine</td>
<td>145 ± 2.7</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Fasted (4)</td>
<td>0.5</td>
<td>(–)-[1-14C]Octanoylcarnitine</td>
<td>89 ± 4.3</td>
<td>0.20 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Fasted (4)</td>
<td>2.0</td>
<td>(–)-[1-14C]Octanoylcarnitine</td>
<td>42 ± 2.5</td>
<td>0.24 ± 0.04</td>
<td>0.16 ± 0.01</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Fed (2)</td>
<td>0</td>
<td>[1-14C]Octanoate</td>
<td>97</td>
<td>0.88</td>
<td>0.81</td>
<td>0.74</td>
</tr>
<tr>
<td>Fasted (2)</td>
<td>0</td>
<td>[1-14C]Octanoate</td>
<td>194</td>
<td>0.63</td>
<td>0.57</td>
<td>0.51</td>
</tr>
</tbody>
</table>

The final set of experiments was concerned with the fate of the (–)-carnitine presumed to be formed in livers oxidizing (–)-octanoylcarnitine. Since this tissue is known to contain carnitine acetyltransferase (27) and since a general parallelism exists between rates of ketogenesis and hepatic acetyl-CoA levels (28) a correlation might be expected between the flux of labeled C2 units (derived from the (–)-[1-14C]octanoylcarnitine) into ketones and the formation of (–)-acetyl carnitine. To test for such a relationship the livers used in the experiments of Figs. 5 and 6 were freeze-clamped at the 60-min time point and analyzed for their content of free (–)-carnitine and (–)-acetyl carnitine as described under "Experimental Procedure." In addition, the perfusion media were also assayed for these compounds. In Fig. 7 the individual values found for the concentration of (–)-acetyl carnitine in each liver and the quantity released into the perfusion medium have been plotted against the respective rates of conversion of (–)-[1-14C]octanoylcarnitine into ketone bodies. A positive correlation was seen between the capacity of the liver membrane to the two compounds or, alternatively, it might reflect the rate-limiting nature of the transferase involved in the conversion of (–)-octanoylcarnitine into octanoyl-CoA. We tend to favor the former explanation in view of the finding by Lee and Fritz (25) that in isolated rat liver mitochondria (–)-octanoylcarnitine actually supported higher rates of ketogenesis than did the free fatty acid.
described in Fig. 5, livers and perfusion fluid were analyzed for from fed and fasted rats. At the termination of the experiments pmol per g for the fed and fasted livers, respectively, and no liver (-)-acetylcarnitine levels were much lower, 0.08 and 0.12 accumulation of (-)-acetylcarnitine in the tissue and perfusion to oxidize the 8-carbon (-)-carnitine ester, and the subsequent period. (+)-OC, (+)-octanoylcarnitine. units converted into ketones during the 15- to 60-min perfusion in each liver (A) and the amount released into the perfusion their content of (-)-acetylcarnitine as described under "Ex-

Fig. 7. The relationship between (-)-octanoylcarnitine oxidation and the formation of (-)-acetylcarnitine in perfused livers from fed and fasted rats. At the termination of the experiments described in Fig. 5, livers and perfusion fluid were analyzed for their content of (-)-acetylcarnitine as described under "Experimental Procedure." The concentration of this metabolite in each liver (A) and the amount released into the perfusion medium (B) have been plotted against the quantity of labeled C2 units converted into ketones during the 15- to 60-min perfusion period. (+) OC, (+)-octanoylcarnitine.

to oxidize the 8-carbon (-)-carnitine ester, and the subsequent accumulation of (-)-acetylcarnitine in the tissue and perfusion fluid. In the experiments with [1-14C]octanoate (data not shown) liver (-)-acylcarnitine levels were much lower, 0.08 and 0.12 μmol per g for the fed and fasted livers, respectively, and no (-)-acetylcarnitine was released into the medium. Interestingly, all of the livers perfused with (-)-[1-14C]octanoylcarnitine contained similar concentrations of free (-)-carnitine, the values for fed controls, fasted controls, fasted plus 0.5 mM (+)-octanoylcarnitine, and fasted plus 2 mM (+)-octanoylcarnitine being 0.80 ± 0.04, 0.98 ± 0.09, 0.78 ± 0.06, and 0.77 ± 0.05 μmol per g, respectively. Using the same assay sensitivity, however, virtually no free (-)-carnitine could be detected in the livers perfused with [1-14C]octanoate.

DISCUSSION

The initial experiments reported here (Figs. 1 and 2) emphasize the conclusion drawn from earlier studies (2, 3, 8) that a major regulatory feature applies to the oxidation of long chain fatty acids in livers from normal and ketogenic animals that is not operative when the medium chain fatty acid, octanoic acid, serves as the ketogenic substrate. While both types of fatty acid support high rates of ketogenesis in the fasted state, only the shorter chain species is oxidized efficiently in the fed state. The experiments also establish the fact that the previously documented inhibitory effect of (+)-decanoylcarnitine on long chain fatty acid oxidation and the insensitivity of octanate oxidation to this agent (8, 21) are paralleled by (+)-octanoylcarnitine, a compound that offers distinct advantages over the 10-carbon (+)-carnitine ester in studies of this nature (23).

These observations, coupled with the growing body of evidence that the enhanced hepatic oxidation of long chain fatty acids in the ketotic state cannot be ascribed to a defect in the esterification capacity of the liver (3–8), would be consistent with the postulate that primary physiological control of hepatic ketone production is exerted at the site of carnitine acyltransferase (4, 8). However, as indicated above, direct proof for this concept has proved elusive since the total assayable quantity of the transferase enzyme has been found to be either unchanged (12) or only marginally increased (9, 10) in liver homogenates from ketogenic animals. The present experiments were designed to test the possibility that in the intact hepatocyte larger differences in the activity might be revealed. Two important points emerged. First, it was shown that (-)-octanoylcarnitine, which is known to be readily oxidized by isolated mitochondria from rat tissues (24, 25), is also metabolized in the intact rat liver. Second, and critical to the main theme of this investigation, the relative rates of ketone production from (-)-octanoylcarnitine in the two experimental groups of livers paralleled those seen with long chain fatty acids. In other words, in the fed state ketogenesis from both types of substrate was greatly depressed (about 80%) when compared with the rates seen in livers from fasted rats, whereas octanoic acid supported high rates of keto-
gensis irrespective of the nutritional state of the donor animal. These findings indicated that the ability of both long chain fatty acids and the medium chain (-)-carnitine ester to gain entry into the mitochondrion of the liver cell was severely restricted in the fed state. The results are compatible with a limitation in the oxidation of both types of substrate at the level of carni-
tine octanoyltransferase. Assuming this interpretation to be correct, it cannot be deduced from these studies whether the same or a different species of transferase is involved in the metabolism of long chain fatty acids and (-)-octanoylcarnitine. However, because of the recent discovery of a transferase in liver and other tissues of the rat that shows highest activity toward medium chain (-)-carnitine esters, it is possible that this enzyme, termed "carnitine octanoyltransferase" (25, 26), was responsible for the conversion of (-)-octanoylcarnitine into octanoyl-CoA in our experiments. Of interest in this respect was the finding that the oxidation of endogenous fatty acids and of the added (-)-octanoylcarnitine showed similar sensitivities to (+)-octanoylcarnitine, at least in the early stages of perfusion (Fig. 5). However, the subsequent release of this inhibition, as (-)-carnitine accum-
ulated in the tissue, appeared to be more pronounced in the case of long chain fatty acids (Fig. 5; Table I). Whether this phenomenon reflects a difference in the competitive interactions of the (+)-acylcarnitine and (-)-carnitine with the long and medium chain transferases remains to be established.

Additional evidence that the greatly reduced rate of ketone formation from (-)-octanoylcarnitine in livers from fed animals resulted directly from a diminished flow of carbon through the β oxidation pathway is furnished by the close correlation between the extent of conversion of the (-)-carnitine ester into aceto-
cetate and β-hydroxybutyrate and the quantity of (-)-acetyl-
carnitine that accumulated in the tissue and perfusion fluid (Fig. 7). Apparently, under these experimental conditions the enhanced rate of (-)-acylcarnitine synthesis in livers from fasted animals reflected the removal of acetyl groups from the mitochondria (catalyzed by carnitine acetyltransferase) under circumstances of increased "acetyl pressure," which in turn was governed by the flux of carbon through the β oxidation sequence (29). It might be mentioned, parenthetically, that in these livers the acetyl-CoA:CoA-SH ratio, which was not measured experimentally, would be expected to be proportional to the observed (-)-acetylcarnitine to (-)-carnitine ratio, provided that the carnitine acetyltransferase reaction was operating under...
Fig. 8 The relationship between the tissue (−)-acetyl carnitine to (−)-carnitine ratio and ketone production from (−)-octanoylcarnitine ((−)-OC) in the perfused rat liver. The data refer to the experiments of Fig. 5. ( ) Acetyl carnitine and (−)-carnitine levels were measured in perchloric acid extracts of livers after 60 min of perfusion and the ratio is plotted against the quantity of labeled (−)-octanoylcarnitine converted into ketone bodies during the 15- to 60-min interval. Values represent means ± S.E. (+)-OC, (+)-octanoylcarnitine.

near equilibrium conditions. That this was so can be surmised from the striking similarity between the curve relating the rate of ketogenesis to the (−)-acetylcarnitine to (−)-carnitine ratio and ketone production from the experiments of Fig. 8, and that showing the relationship between acetocacetae formation and the acetyl-CoA : CoA-SH ratio in Bremer's studies with disrupted rat liver mitochondria (30).

It is noteworthy that the carnitine acyltransferase reaction had previously been considered to act as the rate-limiting step in the β oxidation of fatty acids in isolated mitochondria from various tissues (27, 31). This viewpoint has since been challenged on the basis of the finding that the measured activity of the enzyme in rat liver mitochondria exceeded the capacity of these particles for β oxidation (32, 33). It is important to recognize, however, that conditions found to yield maximal rates of enzyme reactions in broken cell preparations are undoubtedly very different from those prevailing in the intact cell. For this reason the studies cited (32, 33) are not necessarily incompatible with the thesis that the carnitine acyltransferase step constitutes a major site for the physiological control of hepatic fatty acid metabolism, a concept that we believe to be supported strongly by the data presented here. Implicit in this formulation is that under conditions of carbohydrate or insulin deficiency the enzyme is "turned on," with the result that a major fraction of the concomitantly increased load of fatty acids reaching the liver is efficiently converted into acetocacetae and β-hydroxybutyrate. It is also apparent that should separate enzymic steps be involved in the transfer of long and medium chain (−)-carnitine esters across the mitochondrial membrane, their activities are subject to coordinate control.

One final point deserves comment. It has been previously shown that the activity referred to in this paper as "long chain carnitine acyltransferase" likely encompasses two distinct enzyme activities (transferases I and II (34-38)). While our experiments with (−)-octanoylcarnitine strongly implicate transferase II, which catalyzes the conversion of acylcarnitine into acyl-CoA, as an important locus of metabolic control, the possibility cannot be excluded that transferase I, which is responsible for the prior formation of acylcarnitine from acyl-CoA, also plays a regulatory role in the oxidation of physiological fatty acids.

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