Enhancement of Mitochondrial Carnitine and Carnitine Acylearnitine Translocase-mediated Transport of Fatty Acids into Liver Mitochondria under Ketogenic Conditions*

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The rates of hepatic fatty acid utilization are markedly influenced by the delivery rate of free fatty acids to liver of normal and ketotic rats alike. Livers from such rats take up similar amounts of fatty acids from perfusion medium but the subsequent pattern of intracellular fatty acid utilization differs between the two. In livers from normal fed rats most of the fatty acid taken up is directed toward triglyceride formation whereas in ketotic rats the fatty acid is oxidized. This is probably due to the changes in the activity of carnitine acyltransferase and carnitine acylcarnitine translocase. Fasting and alloxan diabetes influenced the rates of carnitine acylcarnitine translocase-catalyzed transport of carnitine in liver mitochondria. Measurements of Km for carnitine transport and intramitochondrial carnitine concentrations showed that carnitine acylcarnitine translocase normally remains subsaturated with respect to the level of matrix carnitine and that the enhancement of transport on fasting and alloxan diabetes results from an elevation of the intramitochondrial carnitine. These results indicate that the ability of liver to transport fatty acids into mitochondria is increased under ketogenic conditions. The intramitochondrial carnitine content was found positively related to liver carnitine in a variety of conditions.

Fasting for up to 48 h had little effect on serum total carnitine but decreased the urinary excretion of carnitine and deoxycarnitine and appeared to enhance carnitine retention in body. The ratio of esterified to free carnitine, in serum as well as urine, rose on fasting. It seems that under conditions of active hepatic fatty acid oxidation, like acetoacetate and β-hydroxybutyrate, short chain acylcarnitines are produced in and exported out of liver to serve as fuel for extrahepatic tissues.

The rates of hepatic fatty acid oxidation are increased in ketotic states and have been shown to be positively related to liver carnitine (11, 13). Recently, increases in hepatic carnitine concentrations were found in fasting (14) and diabetes (15). McGarry et al. (11) have shown further that the increases in hepatic carnitine in these conditions, as well as on glucagon infusion, correlate well with the ketogenic ability of liver and have proposed that increased carnitine levels promote ketogenesis by increasing the flux of fatty acids through the carnitine acyltransferase reaction, presumably by activating that carnitine acyltransferase which is situated on the inner side of the inner mitochondrial membrane. Their more recent observations suggest that the ketogenic adaptation causes activation of some carnitine-dependent step(s) related to fatty acid transport but involved subsequent to the formation of acylcarnitine.

The possibility that the ketogenic adaptation involves enhancement of carnitine palmitoyltransferase activity has been examined and it is now believed that marginal increases in the activity of this enzyme in ketogenic states do not correlate well with the differences in the rates of fatty acid oxidation observed between livers of normal and ketotic rats (11, 13). Recently, increases in hepatic carnitine concentrations were found in fasting (14) and diabetes (15). McGarry et al. (11) have shown further that the increases in hepatic carnitine in these conditions, as well as on glucagon infusion, correlate well with the ketogenic ability of liver and have proposed that increased carnitine levels promote ketogenesis by increasing the flux of fatty acids through the carnitine acyltransferase reaction, presumably by activating that carnitine acyltransferase which is situated on the inner side of the inner mitochondrial membrane. Their more recent observations suggest that the flux through carnitine palmitoyltransferase may be controlled by the levels of malonyl-CoA, an inhibitor of carnitine palmitoyltransferase (16).

Our findings (17, 18) and those of Ramsay and Tubbs (19), largely with heart mitochondria, have shown that besides carnitine acyltransferases, carnitine-dependent transport of fatty acids for oxidation requires participation of a mitochondrial carnitine acycarnitine translocase system. This translocase, by catalyzing an exchange diffusion of acylcarnitines for 

* This work was supported by grants from the Medical Research Council of Canada (MT-4264) and the Quebec Heart Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ Unless stated otherwise, carnitine refers to (−)-carnitine.
carnitine, permits a transport of fatty acyl groups into mitochondria. We present here data, first, to show that carnitine acylcarnitine translocase is demonstrable in liver mitochondria. A brief documentation of this appeared desirable because of the inferences (20, 21) that, unlike in heart, carnitine acylcarnitine translocase may not be present in liver mitochondria. Secondly, we show that one of the sites activated under ketogenic conditions is the carnitine acylcarnitine translocase reaction of liver mitochondria.

**EXPERIMENTAL PROCEDURES**

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**Materials**—[methyl-14C]Carnitine was synthesized according to the method of Stokke and Bremer (32) and purified by chromatography on a Dowex 50W-X8 utilizing the conditions of Bohmer and Bremer (33). Of the various sources of other chemicals were as previously stated (17, 18, 24).

**Animals and Their Treatment**—Sprague-Dawley male rats, weighing 190 to 200 g unless indicated otherwise, were housed in individual metabolic cages and fed a Purina rat chow diet that according to the supplier, contained 10.5% fat, 28% protein, and vitamins. All rats had free access to food and water at all times except that the fasted rats were deprived of food for 24 or 48 h. Alloxan diabetes was induced by the intraperitoneal injection of 100 mg of alloxan/kg of body weight 30 h prior to the experiment. Glucagon infusion was carried out as described by McGarry et al. (7). With the fasted rats, the latter was given at 5 h intervals. Each rat was provided a special diet containing at least 20% protein, 5% fat with adequate minerals and vitamins. The fasted rats were deprived of food for 24 or 48 h. Alloxan diabetes was induced by the intraperitoneal injection of 100 mg of alloxan/kg of body weight 30 h prior to the experiment. Glucagon infusion was carried out as described by McGarry et al. (7). With the fasted rats, the latter was given at 5 h intervals. Each rat was provided a special diet containing at least 20% protein, 5% fat with adequate minerals and vitamins.

**Preparation of Liver Mitochondria**—The livers of the rats were minced, washed with chilled 210 mM mannitol, 10 mM Tris/HCl (pH 7.4), and homogenized using fresh medium of the same composition and a Potter-Elvehjem homogenizer to obtain 10% (w/v) homogenate. The preparation of homogenate as described above, 3 ml was centrifuged at 500 g for 30 s. Any contaminating material was aspirated off and the pellet was gently rinsed twice with a small volume of homogenizing medium to free it from the lightly packed material. The pellet was washed by being suspended in fresh homogenizing medium followed by 5 min of centrifugation at 8,700 x g and then rinsed as described above. The rinsed pellet was suspended in fresh homogenizing medium to obtain a preparation of mitochondria. The above operations were carried out at 0–4°C. The yield of mitochondria varied between 7 to 11 mg of protein for each gram of liver. These mitochondria showed good respiratory control ratios (7.5 ± 0.3) and ADP-stimulated respiratory activity of carnitine palmitoyltransferase of the two preparations was determined. In three such experiments, carried out at different occasions, the two methods gave mitochondrial preparations that exhibited carnitine palmitoyltransferase specific activities differing by no more than ±25%. Examples such as carnitine palmitoyltransferase appear as an acceptable marker for mitochondria in liver (25), the purity of the mitochondria obtained by the silicone oil method appeared suitable for analyzing the pool size of intramitochondrial carnitine.

**Influx Measurements**—The reaction system in a final volume of 200 μl contained 100 mM KCl, 50 mM mannitol, 20 mM Tris/HCl, pH 7.4, 0.5 μg of rotenone, 1.25 μg of oligomycin, and 0.25 μg of antimycin A. A (–)[methyl-14C]carnitine at the desired concentration and freshly isolated mitochondria (2.5 to 3 mg of protein/tube) from rat liver. Unless otherwise stated, uptake was initiated by the addition of mitochondria to rapidly stirring incubation mixture and was arrested by the addition of 20 μl of 25 mM mersalyl in 200 mM mannitol, 50 mM Tris/HCl 10 s after the addition of mitochondria. In controls, addition of mersalyl preceded that of mitochondria. A portion of the reaction mixture, usually 150 μl, was transferred to 1.5-ml Eppendorf tube containing from bottom to top, 50 μl of a formic acid/water (1:1:1, v/v) mixture, about 150 μl of a silicone oil (specific gravity 1.048) and 800 μl of 120 mM KCl, 50 mM mannitol, 5 mM Tris/HCl, pH 7.4, containing 45 mg of dextran of Mr = 170,000. Mitochondria were separated by rapid centrifugation and then processed as described in Ref. 36.

**RESULTS AND DISCUSSION**

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**Some Properties of Carnitine Acylcarnitine Translocase in Liver Mitochondria and Kinetics of Carnitine Influx**—Previously one of us mentioned, without presenting data, that carnitine transport in liver mitochondria seemed to proceed in a manner analogous to that in heart mitochondria. Suggestion has since been made that, unlike in heart, carnitine acylcarnitine translocase may not be present in liver mitochondria (20, 21), presumably because liver mitochondria had once been reported (27) to have little carnitine and mitochondria of liver were used in those experiments which had earlier led to the consensus that carnitine was unable to cross the mitochondrial inner membrane (27–30). When experiments are set up to detect an exchange diffusion either by following an efflux of carnitine from mitochondria previously loaded with radioactive carnitine, under conditions previously described for heart mitochondria (17, 18, 31), or by following carnitine influx, as presently outlined, carnitine transport becomes measurable in liver as in heart mitochondria. However, because isolated mitochondria from livers of normal rats exhibit much smaller (≤ one-eighth) carnitine pool size as compared to those from heart, carnitine acylcarnitine translocase is technically less readily assayable in liver than in heart mitochondria. Using a sensitive assay (24) we find, as described below (a) that reproducibly measurable amounts of carnitine are found associated with isolated liver mitochondria, (b) that such carnitine association appears to result largely from the presence of carnitine in mitochondrial matrix, and (c) that the exact value of mitochondrial carnitine depends on the method employed for the isolation of mitochondria and as well as on the physiologic state of rats. Carnitine transport in liver mitochondria, measured as carnitine influx, is inhibited by low concentrations of decanoyl(+)carnitine, mersalyl, and N-ethylmaleimide, the same compounds previously recognized as effective inhibitors of the transport, measured as carnitine efflux, in heart mitochondria (17, 18). The
concentration of these three inhibitors required for 50% inhibition (2 mg of mitochondria were incubated with the inhibitors for 60 s and then [14C]carnitine was added to initiate influx) was estimated as 1.5, 4, and 30 μM, respectively. Data of Table I with liver mitochondria show, in agreement with that described for heart earlier (17, 18) (a) that naturally occurring carnitine and its esters were more effective in transport than were the corresponding (+) isomers, (b) that low, near-physiological, concentrations of carnitine and acylcarnitines served as substrates for carnitine acylcarnitine translocase, (c) that acylation of carnitine enhanced its ability to serve as a substrate, and (d) that within the limits examined an increase in the chain length of the acyl portion of acylcarnitine further increased its effectiveness as substrate (compare data with decanoylcarnitine and acetylcarnitine at 0.05 mM).

In experiments utilizing heart mitochondria and when the transport was followed as efflux of mitochondrial carnitine, carnitine acylcarnitine translocase catalyzed exchange was found to follow first order reaction kinetics (18). Fig. 1 shows that first order reaction kinetics apply for the initial influx of carnitine in liver mitochondria also. These observations together with the findings (see below, Fig. 1 and Table III) that the rapid uptake of medium [14C]carnitine apparently ceased with time when the amount of entered [14C]carnitine nearly matched that of endogenous mitochondrial carnitine show that as in heart, carnitine acylcarnitine translocase of liver mitochondria catalyzes a mole to mole exchange diffusion of carnitine. In the experiment of Fig. 1 a marked deviation from first order reaction kinetics was observed for time points beyond 30 s (not shown). The intramitochondrial carnitine pool in freshly isolated liver mitochondria consisted of free carnitine as well as of appreciable amounts of acylated carnitine (Table II), and it is possible that different rates of influx of medium carnitine result at different time points depending upon whether external carnitine is exchanging with internal free carnitine, acylated carnitine, or a mixture of the two, composition of which is likely to change progressively with increasing influx of free carnitine. Moreover, it is likely that as with other metabolites (cf. 32) not all the intramitochondrial carnitines equilibrate rapidly enough to constitute a homogeneous pool. On prolonging incubation for 20 min the uptake of carnitine, measured from radioactivity and taking into account the slight dilution of the specific radioactivity of added labeled carnitine by the carnitine associated with mitochondria, was usually >90% of that expected from the amount of endogenous total carnitine found initially present in isolated mitochondria (Fig. 1 and Table III). In a similar experiment with heart mitochondria, where 3 mM external carnitine was present, near equilibrium of exchange was attained in 20 min and at this point the uptake of carnitine, based on radioactivity, corresponded to 105% of the amount of endogenous carnitine initially found in mitochondria. These data show that almost the entire endogenous carnitine of both liver and heart mitochondria is exchangeable with external carnitine.

**Table I**

| Addition to incubation medium | Efflux of mitochondrial carni-
|-----------------------------|-----------------------------|
|                            | (-) Isomer | (+) Isomer |
| Carnitine                  | 0.05       | 52         | 11          |
|                            | 0.5        | 382        | 89          |
|                            | 2.5        | —          | 414         |
| Acetylcarnitine            | 0.05       | 94         | 7           |
|                            | 0.5        | 654        | 46          |
|                            | 2.5        | —          | 128         |
| Decanoylcarnitine          | 0.01       | 578        | 116         |
|                            | 0.05       | 1128       | 152         |

* Reliable values were not obtained because all of the mitochondrial (-)-[3H]carnitine had effluxed out.

**Fig. 1.** Time course of carnitine uptake by liver mitochondria of a fasted rat. Influx of carnitine was determined as described under “Experimental Procedures.” The concentration of carnitine was 0.5 mM and the temperature was 0°C. Total uptake of carnitine at equilibrium, carnitine total, was estimated by extrapolation of data in A, to be 0.42 nmol/mg of mitochondrial protein. Total endogenous carnitine, associated with mitochondria at the time of assay, was determined by carrying out incubations identical with that for following influx except that carnitine and mersalyl were not added and that formic acid was not present below silicone oil. Total carnitine of the mitochondrial pellet in silicone oil was analyzed as described in Ref. 24 and was found to be 0.40 nmol/mg of mitochondrial protein. C, represents the uptake of carnitine at time 0. All incubations were in duplicate and the individual values are shown. From B, the first order rate constant was calculated to be 1.25 min⁻¹ and hence the initial rate of carnitine influx was determined from the first order rate equation \( v = K \times \text{carnitine} \) (total) to be 0.5 nmol/min/mg of mitochondrial protein.
level of the translocase itself. Estimations showed (Table II) that fasting and diabetes do not only increase the total carnitine in whole liver, as is known (11, 14, 15), but also in the mitochondrial fraction. These increases in mitochondrial carnitine did not result from possible increased mitochondrial adsorption of long chain acylcarnitines, concentrations of which are elevated in ketogenic states (33). This is indicated, first, by the finding (Table II) that increases in mitochondrial total carnitine in fasting and alloxan diabetes accompanied parallel increase in the free carnitine content; mitochondrial free carnitine, expressed as per cent of total mitochondrial carnitine, constituted 71% for fed, 67% for fasted, and 68% for alloxan diabetic rats. Secondly, if fasting increases the endogenous intramitochondrial content of carnitine, then in influx experiments despite an apparent leveling off of the net uptake of radioactivity on prolonged incubation (e.g. Fig. 1A), the amount of carnitine taken up at such time points, calculated from the specific activity of radioactive carnitine being used, should be greater for mitochondria from livers of fasted as compared to those from fed rats. Table III shows not only that this was observed but that the net uptake off [14C]carnitine approached closely the endogenous total carnitine content of mitochondria. This indicates that most of the carnitine associated with isolated mitochondria was present in the matrix.

The observed increase in the rate of carnitine transport described above could result from elevation of intramitochondrial carnitine if the intramitochondrial carnitine in livers of fed rats normally remains below saturating for the operation of carnitine acylcarnitine translocase. Results of kinetic experiments showed (Table IV) that the apparent \( K_a \) for carnitine for the carnitine-carnitine exchange of mitochondria from livers of fed rats was 1.8 mM. Fasting had little effect on \( K_a \) but increased the \( V_{\text{max}} \) of carnitine transport (Table IV) in agreement with the other observations described above. Matrix volume of isolated liver mitochondria from fed and fasted rats, determined as described in Ref. 26, was found to be 1.11 ± 0.08 and 1.23 ± 0.07 (S.E., \( n = 5 \)) \( \mu l/\text{mg of protein, respectively. Accordingly, based on the known mitochondrial carnitine content (Table II), the concentration of carnitine in matrix, assuming uniform distribution and accessibility of all carnitine to entire matrix water volume, was calculated to be about 0.19 and 0.29 mM for fed and fasted groups, respectively. These data indicate that carnitine acylcarnitine translocase of liver mitochondria remains subsaturated with respect to the level of matrix carnitine assuming that the translocase would exhibit about the same affinity for the matrix carnitine as observed for the medium carnitine.

If increases in intramitochondrial carnitine are related to the ketogenic ability of liver then these should be seen in other conditions of enhanced ketogenesis as well. McGarry et al. (11) have reported that glucagon infusion readily converts a normal liver to a ketogenic liver. We found (Table V) that glucagon infusion not only increased total carnitine in liver, as previously described by McGarry et al. (11), but also the amount of carnitine associated with mitochondria. The relationship between total liver carnitine and mitochondrial carnitine was analyzed further and showed (Fig. 2) that the latter was positively and linearly related to the former under a variety of conditions. A silicone oil technique was worked out for rapid isolation of mitochondria for these analyses and showed higher mitochondrial carnitine content compared to the conventionally isolated mitochondria presumably because losses of carnitine were lower in the former method that required less preparative handling of mitochondria than the conventional isolation procedure. Experiments to determine the mechanism by which intramitochondrial carnitine adjusts to total liver carnitine are currently underway.

**Effect of Fasting on Carnitine and Deoxycarnitine in Serum and Urine**—To elucidate the mechanism(s) responsible for the elevation of liver carnitine, effect of fasting on serum and urinary carnitine was determined. Table VI shows that 1-

### Table II

| Experimental group | Rate of mitochondrial carnitine-carnitine exchange* nmol/mg protein | Total (-)-carnitine in liver nmol/g wet wt | Mitochondrial (-)-carnitine nmol/mg protein |
|--------------------|----------------------------------------------------------|----------------------------------------|----------------------------------------|
| Normal (6)         | 0.71 ± 0.076                                             | 268 ± 14.0                             | 0.21 ± 0.016                           |
| Fasted (7)         | 1.38 ± 0.101                                             | 463 ± 30.016                           | 0.36 ± 0.025                           |
| Alloxan diabetic (5)| 1.23 ± 0.084                                             | 439 ± 30.16                            | 0.34 ± 0.026                           |

* \( p < 0.025 \) compared to normal.

**Table III**

Correspondence between maximal [14C]carnitine uptake at steady state and endogenous carnitine in liver mitochondria of fed and fasted rats

| Pooled mitochondria from livers of four rats in each group were used. Conditions for influx were as described under “Experimental Procedures,” except that 0.2 mM [14C]carnitine with 2 mg of mitochondrial protein was present and incubations were for 20 or 30 min. For the determination of endogenous carnitine, additional incubations were set up simultaneously and differed from influx incubations in that [14C]carnitine and mersalyl were not added. Following 20 or 30 min of incubation at 0°C, mitochondria were washed with a wash layer and a silicone oil layer, as described for influx under “Experimental Procedures,” except that formic acid was not present below the silicone oil. The washed mitochondria obtained below the silicone oil were suspended in water and portions analyzed for total carnitine (24). Values shown below are average of duplicate incubations.

| Liver mitochondria from | Incubation period | Total carnitine associated with mitochondria based on [14C]-carnitine nmol/mg mitochondrial protein |
|-------------------------|------------------|-----------------------------------------------------------------------------------------------|
| Fed rats                | min              | nmol/mg mitochondrial protein                                                                |
| 20                      | 0.22             | 0.25                                                                                         |
| 30                      | 0.23             | 0.24                                                                                         |
| Fasted rats             | 20               | 0.43                                                                                         |
| 30                      | 0.42             | 0.42                                                                                         |
were also observed for the urinary deoxycarnitine (Table VI) on Day 1 and 2 of fasting, respectively, shows that carnitine excretion to 356 and 230 nmol/100 g body weight/day/100 g body weight. The observed decrease of urinary and per day food intake of 5.3 g/100 g of body weight. Thus, fed rats at steady state synthesized about 483 nmol of carnitine/day for 5.3 g/100 g of body weight, were, respectively, as nanomoles per g wet tissue, 1091 ± 24 and 1190 ± 54 (mean ± S.E, n = 5 in each group) for skeletal muscle (p > 0.1), 1328 ± 79 and 1415 ± 44 (n = 8 in both) for heart (p > 0.2), and 667 ± 38 and 555 ± 49 (n = 5 in both) for kidney (p > 0.1). The fasting-induced increase of liver carnitine did not result from any increase in the level of the enzyme γ-butyrobetaine hydroxylase as the activity of this enzyme, assayed using post-mitochondrial supernatant fraction of liver homogenates, expressed as nanomoles of carnitine formed per min per mg of protein, was 0.25 ± 0.011 for fed and 0.24 ± 0.008 for fasted (n = 5 in both) group.

Table VII shows that although up to 48-h fasting did not affect total serum carnitine, the concentration of free carnitine markedly declined on 24- and 48-h starvation and consequently the ratio of esterified to free carnitine rose at these time points. This increase in esterified to free carnitine ratio was also seen in urine (Table VI) and is in agreement with the reports (35, 37) that appeared while this work was in progress.

We thank Dr. Mitchell L. Halperin of the University of Toronto for suggesting this interpretation.

**Table IV**

| Condition | $K_a$ (nm) | $V_{max}$ (nmol/min/mg) |
|-----------|-----------|-------------------------|
| Fed (5)* | 1.8 ± 0.24 | 1.2 ± 0.16 |
| Fasted (5) | 1.6 ± 0.19 | 2.3 ± 0.17* |

* Number of rats (in parentheses).

**Table V**

| Experimental group | Total carnitine in Liver (nmol/g wet wt) | Mitochondria (nmol/mg protein) |
|--------------------|----------------------------------------|--------------------------------|
| Saline-infused (6)* | 256 ± 10.9* | 0.45 ± 0.025 |
| Glucagon-infused (6) | 428 ± 16.7* | 1.07 ± 0.030* |

* Number of rats in each group (in parentheses).

Values shown are mean ± S.E.

*p value < 0.001.

Fig. 2. Relationship between total carnitine of liver and that of mitochondrial fraction under a variety of conditions. Details of fed, fasted, diabetic, saline, and glucagon infusion were as described under "Experimental Procedures." The points shown for baby rats are for pooled samples from four or six, 1- to 2-day-old, rats. Mother rats are those that had given birth 1 to 2 days earlier.

day starvation decreased urinary total carnitine excretion from 1157 to 365, i.e. by 801 nmol/100 g of body weight for the 24-h period. Of this decrease only 318 nmol can be ascribed to the lack of intake of preformed carnitine from the diet based on the average determined carnitine content of diet as 60 nmol/g diet (range 55 to 73 for four batches analyzed) and per day food intake of 5.3 g/100 g of body weight. Thus, fed rats at steady state synthesized about 483 nmol of carnitine/day/100 g body weight. The observed decrease of urinary carnitine excretion to 356 and 230 nmol/100 g body weight (Table VI) on Day 1 and 2 of fasting, respectively, shows that fasting decreased net carnitine synthesis in body by about one-fourth and one-half by 24 and 48 h of fasting. Decreases were also observed for the urinary deoxycarnitine (Table VI) suggesting that fasting lowered deoxycarnitine synthesis as well. The contribution of dietary deoxycarnitine to this decrease was minimal because the rat diet contained little deoxycarnitine (<3% of that of carnitine).

Since liver is considered to be the chief site of carnitine biosynthesis in rats (34), it follows from the above that fasting caused a lowering of hepatic carnitine synthesis. However, despite this, an increase in carnitine content of liver, expressed on per g liver weight (Table II), occurred. This suggests, therefore, that fasting caused a slightly increased retention of carnitine in liver. However, Brass and Hoppel (35) have reported that increase in liver carnitine on fasting, expressed on per g of liver wet weight, could be accounted for solely by a conservation of carnitine resulting from a fasting-induced loss of liver weight, relative to body weight, occurring without concurrent losses of hepatic carnitine. Whereas in accord with Brass and Hoppel (35), we find that the data themselves lend to such an interpretation, we believe that it does not represent the actual mechanism involved. A strong suggestive evidence supporting this belief comes from our observation that glucagon infusion markedly (67%) increased liver carnitine (Table V) without causing any noticeable change in liver weights of rats; the liver weights, expressed on grams per 100 g body weight, were 4.1 ± 0.08 for saline (0.9% NaCl solution) infused and 3.9 ± 0.09 (n = 6 for both) for glucagon-infused rats. Moreover, we found that as in fasting, glucagon infusion-mediated increase in liver carnitine occurred without any significant alteration of serum total carnitine (data not elaborated), indicating that increased extraction of carnitine from serum was probably not the mechanism involved.

The observation that urinary carnitine excretion declined without concurrent decrease in serum carnitine (Table VII) indicates that fasting increased carnitine retention. This is clearly illustrated by the fractional excretion rate of carnitine and acylcarnitines, calculated according to glomerular filtration rate of 0.77 ml/min/100 g body weight (36); fractional excretion of total carnitine thus came to 1.8% for both fed days but decreased to 0.7% on Day 1 of fasting and to 0.4% on Day 2 of fasting. This decrease in the fractional excretion of total carnitine was shared by similar decreases in the fractional excretion of both free as well as of esterified carnitine (data not elaborated).

The possibility that increased mobilization of carnitine from peripheral tissues to liver might explain the increase in liver carnitine on fasting is not supported. Fasting for 48 h did not affect total carnitine in skeletal muscle, heart, and kidney; the total carnitine values of these three tissues of fed and fasted rats were, respectively, as nanomoles per g wet tissue, 1091 ± 24 and 1190 ± 54 (mean ± S.E, n = 5 in each group) for skeletal muscle (p > 0.1), 1328 ± 79 and 1415 ± 44 (n = 8 in both) for heart (p > 0.2), and 667 ± 38 and 555 ± 49 (n = 5 in both) for kidney (p > 0.1). The fasting-induced increase of liver carnitine did not result from any increase in the level of the enzyme γ-butyrobetaine hydroxylase as the activity of this enzyme, assayed using post-mitochondrial supernatant fraction of liver homogenates, expressed as nanomoles of carnitine formed per min per mg of protein, was 0.25 ± 0.011 for fed and 0.24 ± 0.008 for fasted (n = 5 in both) group.

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**Mitochondrial Carnitine in Fatty Acid Transport**

**TABLE VI**

| Fasting period | Total deoxycarnitine | Carnitine | Esterified/free carnitine ratio |
|----------------|----------------------|-----------|--------------------------------|
|                | nmol/100 g body weight in 24 h | Total | Free | Esterified/fre carnitine |
| **Fed, Day 1** | 107 ± 14             | 1093 ± 67 | 902 ± 53  | 191 ± 17 | 0.21 ± 0.03 |
| **Fed, Day 2** | 103 ± 16             | 1157 ± 63 | 928 ± 40  | 220 ± 21 | 0.25 ± 0.03 |
| **Fasted, Day 1** | 54 ± 11^a | 356 ± 30^a | 198 ± 11^a | 158 ± 20^a | 0.80 ± 0.11^a |
| **Fasted, Day 2** | 33 ± 6              | 230 ± 15^a | 131 ± 9^a  | 101 ± 9^a  | 0.77 ± 0.09^a |

^a p < or <= 0.05 versus fed.
^b p < or <= 0.025 versus fasted Day 1.

**TABLE VII**

| Fasting period | Carnitine |
|----------------|-----------|
|                | Total | Free | Esterified/fre carnitine |
| h | nmol/ml |          |                      |
| 0 | 50 ± 2  | 40 ± 2  | 0.3 ± 0.04 |
| 6 | 50 ± 3  | 45 ± 3  | 0.2 ± 0.03 |
| 24 | 48 ± 3 | 21 ± 2^a | 1.2 ± 0.08^a |
| 48 | 52 ± 2  | 18 ± 1^a  | 1.9 ± 0.08^a |

^a p < 0.001 compared to the prefasting value.

We suggest that increased hepatic oxidation of fatty acids such as in fasting leads to the export of acetyl group not only as ketone bodies but also as short chain acylcarnitines to serve as a fuel for extrahepatic tissues just like ketone bodies are known to do. Experiments to ascertain this aspect are in progress. This suggestion is in line with the observations that when hepatic ketogenesis is stimulated by the inclusion of octanoylcarnitine, acetyl carnitine appears in the perfusate (38), and that acetyl carnitine is a physiological substrate for the carnitine-transporting system of heart cells (39). We believe that the results described in this manuscript are consistent with the idea that an enhancement of carnitine-dependent transport of fatty acids into mitochondria occurs under known ketogenic conditions and that this could contribute to the control of ketogenesis. Amatruda et al. (40) have recently reported that the uptake of palmitate by liver mitochondria specially in the presence of carnitine is markedly enhanced by fasting and diabetes. Whereas this effect was considered important for the control of ketone body production, the mechanism of enhanced fatty acid uptake was concluded as being unknown. Our findings strongly indicate that the enhancement of palmitate uptake in the experiments of Amatruda et al. (40) resulted largely from the enhancement of mitochondrial carnitine acylcarnitine translocase reaction in fasting and diabetes.

**Acknowledgments**—We thank Mr. Antoine Brault and Miss Var-timer Doghramadjian for excellent technical help.

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Enhancement of mitochondrial carnitine and carnitine acylcarnitine translocase-mediated transport of fatty acids into liver mitochondria under ketogenic conditions.
R Parvin and S V Pande

*J. Biol. Chem.* 1979, 254:5423-5429.

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