The effect of glucagon treatment and starvation of virgin and lactating rats on the rates of oxidation of octanoyl-L-carnitine and octanoate by isolated liver mitochondria

Victor A. ZAMMIT

Department of Biochemistry, The Hannah Research Institute, University of Glasgow, Ayr KA6 5HL, Scotland, U.K.

(Received 22 February 1980)

1. Oxygen-consumption rates owing to oxidation of octanoate or octanoylcarnitine by isolated mitochondria from livers of fed, starved and glucagon-treated virgin or 12-day-lactating animals were measured under State-3 and State-4 conditions, in the presence or absence of L-malate and inhibitors of tricarboxylic acid-cycle activity (malonate and fluorocitrate). 2. Mitochondria from fed lactating animals had a slightly lower rate of octanoylcarnitine oxidation than did those of fed virgin animals, whereas the rates of octanoate oxidation were unaffected. 3. Starvation of virgin animals for 24 h or 48 h resulted in a large (70–100%) increase in mitochondrial octanoylcarnitine oxidation; rates of octanoate oxidation were either unaffected (24 and 48 h starvation in the absence of malonate and fluorocitrate) or diminished by 30% (48 h starvation in the presence of inhibitors). In lactating animals, 24 h starvation resulted in a smaller increase in the rate of octanoylcarnitine oxidation than that obtained for mitochondria from virgin rats. 4. Glucagon treatment (by intra-abdominal injection) of fed virgin and lactating rats increased the rate of mitochondrial oxidation of both octanoylcarnitine and octanoate. Injection of glucagon into 48 h-starved virgin rats did not increase further the already elevated rate of octanoylcarnitine oxidation, but reversed the inhibition of octanoate β -oxidation observed for these mitochondria in the presence of malonate and fluorocitrate. 5. It is suggested that glucagon activates octanoylcarnitine oxidation by increasing the activity of the carnitine/acylcarnitine transport system [Parvin & Pande (1979) *J. Biol. Chem.* **254**, 5423–5429] and that the increase in octanoate oxidation by mitochondria from glucagon-treated animals is caused by the increased rate of ATP synthesis in these mitochondria. 6. The results are discussed in relation to the increased capacity of the liver to oxidize long-chain fatty acids and carnitine esters of medium-chain fatty acids under conditions characterized by increased ketogenesis.

The liver utilizes fatty acids for triacylglycerol synthesis and for oxidation (to ketone bodies and CO_2). Although the rate of fatty acid utilization is determined by their rate of delivery to the liver (see, e.g., Owen *et al.*, 1969), the partition of fatty acids between esterification and oxidation depends on the physiological state of the animal. Under ketogenic conditions (e.g. starvation, diabetes) the liver has an increased inherent ability to oxidize long-chain fatty acids (McGarry & Foster, 1972; McGarry *et al.*, 1973) and carnitine esters of medium-chain fatty acids (e.g. octanoylcarnitine) (McGarry & Foster, 1974). This increase can be reproduced relatively rapidly (within 30–60 min) by injection of glucagon or anti-insulin serum into fed animals (Balasse *et al.*,

1972; McGarry *et al.*, 1975), but not by injection of carnitine (Brass & Hoppel, 1978). Although the rate of ketogenesis from octanoate (the oxidation of which by mitochondria is not carnitine-dependent) is increased by about 60% in starved and diabetic animals, the overall rate of octanoate oxidation is unaffected by the physiological state of the animals (McGarry & Foster, 1971, 1974).

Most aspects of these findings have been confirmed in experiments using isolated hepatocytes (Woodside, 1978; Witters & Trasko, 1979). In addition, it was found that hepatocytes from 12-day-lactating rats had a lower rate of ketogenesis from long-chain fatty acids than did those from virgin rats (Whitelaw & Williamson, 1977,

1978). However, the rates from substrates the oxidation of which is carnitine-independent (e.g. butyrate) was virtually unaltered. Thus lactation is a physiological state in which the rate of ketogenesis from long-chain fatty acids is decreased further than in the fed virgin animal.

The results from these studies suggest that, in states characterized by increased rates of hepatic ketogenesis, there is a stimulation of a carnitine-dependent reaction that occurs after the formation of acylcarnitine, but before β -oxidation in the ketogenic pathway (see, e.g., McGarry & Foster, 1974). This step could be either the carnitine/acylcarnitine exchange carrier system, which transports acylcarnitines across the inner mitochondrial membrane (Ramsay & Tubbs, 1975; Pande, 1975; Pande & Parvin, 1976), or carnitine acyltransferase II, which is situated on the inner side of the inner membrane (see Kopeck & Fritz, 1973; Brosnan *et al.*, 1973). However, other studies have indicated that any such effects may occur in addition to changes in the rate of acylcarnitine synthesis itself caused either by changes in the concentration of malonyl-CoA (which is a potent inhibitor of carnitine palmitoyltransferase I) (McGarry *et al.*, 1977, 1978a,b,c; McGarry & Foster, 1979) or by changes in the total activities of carnitine palmitoyltransferase I (EC 2.3.1.21) and mitochondrial acyl-CoA-*sn*-glycerol 3-phosphate *O*-acyltransferase (EC 2.3.1.15) (such as are induced during starvation and re-feeding of animals: Aas & Daae, 1971; Van Tol, 1974; Borrebaek, 1975).

Previous experiments with isolated mitochondria have produced conflicting results on the effects of starvation and diabetes (ketogenic conditions) on the rates of fatty acid oxidation *in vitro*. Thus Harper & Saggerson (1975) and Di Marco & Hoppel (1975) found no effect of starvation or diabetes on the rates of oxidation of palmitoylcarnitine or palmitoyl-CoA plus carnitine. However, Harano *et al.* (1972) found that mitochondria from diabetic rats oxidized palmitate (in the presence of carnitine) about 60% faster than did those from control rats. Similarly, Armatruda *et al.* (1978) have reported an increased 'association' of [^{14}C]palmitate with mitochondria from livers of starved and diabetic animals.

In the present study, the rates of oxidation of octanoate and octanoyl-L-carnitine by mitochondria isolated from livers of fed, starved and glucagon-treated virgin and lactating rats were investigated. The metabolism of these two substrates in mitochondria differs only up to the point of formation of octanoyl-CoA. Therefore the experiments were aimed at determining whether different physiological conditions and glucagon treatment have direct effects on the mitochondrial oxidation of a medium-chain fatty acid, as distinct from those on the oxidation of the carnitine ester of the same fatty

acid. The results are presented in this paper and discussed in relation to changes in hepatic fatty acid oxidation that occur *in vivo*. While this work was in progress, a report has appeared (Parvin & Pande, 1979) which suggests that under conditions of increased ketogenesis the mitochondrial carnitine/acylcarnitine carrier is activated. The results of the present work lend support to this suggestion.

Materials and methods

Chemicals

Octanoic acid, L-malic acid, ADP, DL-fluorocitrate (barium salt), malonic acid, fatty acid-free bovine serum albumin, Tris and Mops (4-morpholinepropanesulphonic acid) were obtained from Sigma (London) Chemical Co. (Poole, Dorset BH17 7NH, U.K.). Octanoyl-L-carnitine was a gift from Otsuka Chemical Co., Naruto, Japan. Crystalline glucagon was either bought from Calbiochem (Los Angeles, CA, U.S.A.) or a gift from Lilly Pharmaceutical Co. (Indiana, IN, U.S.A.). All other chemicals were of the highest purity available from BDH Chemicals (Poole, Dorset BH12 4NN, U.K.). Octanoic acid, malic acid and malonic acid were converted into their respective potassium salts before use. DL-Fluorocitrate (barium salt) was converted into the potassium salt as described by Harper & Saggerson (1975).

Animals

These were female Wistar rats (200–300g) obtained from A. Tuck and Son, Battlesbridge, Essex, U.K. Fed virgin and lactating rats were maintained on Oxoid rat breeding diet (Oxoid, Basingstoke, Hants. RG24 0PW, U.K.). Lactating animals (300–350g) whose litter had been restricted to eight pups were used on day 12 of lactation. Starved virgin and lactating rats were deprived of food for 24 or 48 h, as indicated, before death. Lactating rats were maintained with their litters throughout the period of starvation. Animals were supplied with water at all times. Rats were killed at 10:00h, 1 h after injection with either 0.9% NaCl (control) or a solution of glucagon (see below).

Glucagon treatment

Animals treated with glucagon were given an intra-abdominal injection of 50 μg of glucagon/100g body wt. as a solution in 0.2 ml of 1 mM-NaOH in 0.9% NaCl as described by Adam & Haynes (1969). Control animals received an injection of 0.2 ml of the alkaline saline only. Glucagon-treated and control animals were left for 60 min after injection. At the time of death, animals were anaesthetized by an intra-abdominal injection of 0.2 ml of pentobarbitone (60 mg/ml) (May and Baker, Dagenham, Essex, U.K.) and the livers were

quickly excised and placed into cold extraction buffer.

Preparation of mitochondria

These were prepared by differential centrifugation as described by Chappell & Hansford (1972) in an extraction buffer containing 0.25 M-sucrose, 5 mM-Tris and 1 mM-EGTA at pH 7.4. The final mitochondrial pellet was resuspended to give a final concentration of 30–35 mg of mitochondrial protein/ml and was stored on ice. Mitochondria were used immediately on preparation, usually within 80 min of the animals being killed.

Measurement of mitochondrial respiration

Oxygen uptake was measured polarographically by using a Clark-type oxygen electrode (Rank Bros., Bottisham, Cambridge, U.K.) in conjunction with a multi-range potentiometric pen recorder. The polarizing voltage was 0.6 mV. The oxygen electrode was standardized by determination of the change in the output from the electrode on addition of portions of spectrophotometrically standardized NADH to 2.2 ml of 'basal medium' (see below) containing lysed mitochondria (5 mg of mitochondrial protein) and 1 mg of cytochrome c.

For oxygen-consumption measurements, 0.1 ml samples of mitochondrial preparations were incubated at 30°C in a final volume of 2.2 ml in the water-jacketed electrode chamber in a medium containing 125 mM-KCl, 0.2 mM-MgCl₂, 5 mM-KH₂PO₄, 20 mM-Mops, 1 mM-EGTA and 10 mg of albumin (fatty acid-free). The final pH was 7.3. This is referred to subsequently as 'basal medium' in legends to Figures and Tables. Further additions to this basal medium were made (care being taken not to alter the pH) as indicated in the legends.

Two protocols were used for measurement of State-3 and State-4 respiration of mitochondria (Chance & Williams, 1956). When only the octanoate- or octanoylcarnitine-stimulated rate of State-3 respiration was to be measured, ADP (final concn. 0.35 mM) was added to the basal medium (plus other additions as indicated) containing the mitochondria in suspension, and the oxygen uptake was allowed to reach a steady state. Portions (10 µl) of solutions containing octanoate (100 µM) or octanoylcarnitine (100 µM) were then added and the State-3 respiration rate was recorded. When the ADP/O ratio was to be determined, addition of small portions of ADP (see below) was followed by addition of octanoate or octanoylcarnitine. When O₂ consumption had stabilized, 75 nmol of ADP was added.

Mitochondrial respiration was measured under two experimental conditions: (i) in the absence of any metabolic inhibitors and (ii) in the presence of malonate (5 mM) and DL-fluorocitrate (10 µM). In both instances respiration was measured in the

presence or absence of added L-malate (2.5 mM). When measurements were performed in the absence of inhibitors, mitochondria were first depleted of endogenous oxidizable substrates by sequential additions of small amounts (65 nmol) of ADP as described by Di Marco & Hoppel (1975), after which octanoylcarnitine- or octanoate-stimulated respiration was measured. Oxidation rates obtained under these conditions gave a measure of the rate of oxygen consumption owing to β -oxidation and oxidation of acetyl-CoA through the tricarboxylic acid cycle. In the presence of malonate and fluorocitrate the rate of cycle activity was minimized. Although it was appreciated that oxygen consumption measured in the absence of inhibitors (particularly malonate) resulted in higher endogenous respiratory rates (10–15% of substrate-stimulated respiration), it was necessary to perform these measurements because of the different sensitivity of octanoate oxidation to tricarboxylic acid-cycle inhibition of mitochondria from fed and starved animals (see the Results section). The ADP/O and respiratory-control ratios were determined as described previously (Estabrook, 1967).

Determination of mitochondrial protein

This was performed by the method of Lowry *et al.* (1951), with fatty acid-free albumin as a standard.

Expression of results

For any one given set of conditions, each determination of oxygen consumption (expressed as nmol of O₂/min per mg of mitochondrial protein) reported in the Results section was made on a separate preparation of mitochondria. Statistical significance was determined by Student's *t* test.

Results

The respiratory-control and ADP/O ratios of the mitochondria oxidizing octanoate or octanoylcarnitine were measured routinely. Experimental treatments of animals which resulted in an increase in State-3 respiration (e.g. glucagon treatment; see below) also resulted in higher respiratory-control ratios for octanoate and octanoylcarnitine, primarily owing to an increase in State-3 respiration. The increase was significant ($P < 0.01$) after glucagon treatment (results not shown). Similar observations were made by Yamazaki (1975) on the oxidation of succinate, pyruvate and α -oxoglutarate by mitochondria isolated from livers of glucagon-treated animals. The ADP/O ratio was constant irrespective of the treatments received by the animals (results not shown).

Table 1. *Effect of glucagon treatment and starvation of virgin and lactating rats on the octanoate- or octanoylcarnitine-stimulated rate of oxygen consumption (nmol/min per mg of mitochondrial protein at 30°C) of isolated liver mitochondria, measured in the absence or presence of metabolic inhibitors*

Values are means $\pm$ S.E.M. for the numbers of separate determinations given in parentheses. 'Inhibitors' were 5 mM-malonate + 10 μ M-DL-fluorocitrate.

Substrates	Inhibitors	Virgin			Lactating		
		Fed	Fed + glucagon	Starved (24h)	Starved (48h)	Starved (48h) + glucagon	Starved (24h)
Octanoyl-L-carnitine (100 μ M)	—	34.7 $\pm$ 1.6 (12)	47.2* $\pm$ 3.5 (10)	52.1* $\pm$ 3.4 (6)	60.8* $\pm$ 6.2 (7)	61.2* $\pm$ 5.7 (6)	35.2 $\pm$ 0.9 (5)
Octanoate (100 μ M)	—	28.5 $\pm$ 0.9 (12)	47.0* $\pm$ 4.2 (9)	28.4 $\pm$ 2.7 (6)	30.6 $\pm$ 1.9 (6)	35.2* $\pm$ 0.9 (6)	28.7 $\pm$ 2.5 (6)
Octanoyl-L-carnitine (100 μ M) plus L-malate (2.5 mM)	—	47.9 $\pm$ 2.2 (12)	79.5* $\pm$ 10.2 (10)	63.5 $\pm$ 5.4 (6)	80.9* $\pm$ 9.9 (6)	83.3* $\pm$ 5.4 (6)	52.1 $\pm$ 2.1 (6)
Octanoate (100 μ M) plus L-malate (2.5 mM)	—	41.7 $\pm$ 3.5 (12)	64.1* $\pm$ 3.8 (10)	42.3 $\pm$ 5.2 (6)	40.1 $\pm$ 2.6 (7)	66.3* $\pm$ 5.9 (6)	40.1 $\pm$ 2.0 (6)
Octanoyl-L-carnitine (100 μ M)	+	23.3 $\pm$ 2.5 (11)	41.9* $\pm$ 4.0 (12)	37.9* $\pm$ 3.0 (10)	42.9* $\pm$ 4.2 (6)	42.6* $\pm$ 2.8 (6)	26.8 $\pm$ 1.8 (5)
Octanoate (100 μ M)	+	16.7 $\pm$ 1.3 (10)	21.9* $\pm$ 1.8 (12)	17.0 $\pm$ 2.0 (6)	12.8* $\pm$ 0.8 (6)	16.8 $\pm$ 1.4 (6)	17.0 $\pm$ 1.1 (5)
Octanoyl-L-carnitine (100 μ M) plus L-malate (2.5 mM)	+	36.3 $\pm$ 2.0 (11)	61.0* $\pm$ 5.4 (12)	48.1* $\pm$ 4.3 (10)	57.9* $\pm$ 3.1 (6)	57.7 $\pm$ 3.3 (6)	35.7 $\pm$ 2.0 (5)
Octanoate (100 μ M) plus L-malate (2.5 mM)	+	27.9 $\pm$ 1.5 (10)	37.2* $\pm$ 3.0 (12)	28.1 $\pm$ 2.6 (6)	17.2* $\pm$ 0.8 (6)	26.3 $\pm$ 2.4 (6)	24.9 $\pm$ 2.4 (5)

* O₂ consumption significantly different ($P < 0.01$) from that for liver mitochondria from fed virgin rats.

Table 2. Effect of starvation and glucagon treatment on the ATP concentration and the rate of oxygen consumption owing to oxidation of succinate in the presence of ADP or ascorbate in the presence of *NNN'*-tetramethyl-*p*-phenylenediamine (TMPD, 0.3 μ M) and uncoupler [carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP, 0.2 μ M)]

ATP content of mitochondria was measured as described by Halestrap (1978a). Mitochondria were incubated for 15 min with octanoate (100 μ M) or octanoyl-L-carnitine (100 μ M) in the 'basal' medium (see the Materials and methods section). At the end of the incubation the mixture was acidified and the ATP concentration was measured in the neutralized protein-free supernatant. Values are means $\pm$ s.e.m. for the numbers of separate determinations given in parentheses.

Parameter	Substrate	Virgin				Lactating		
		Fed	Fed + glucagon	Starved (24 h)	Starved (48 h)	Starved (48 h) + glucagon	Fed	Fed + glucagon
Mitochondrial ATP concentration (nmol/mg of protein)	Octanoate (100 μ M)	1.10 $\pm$ 0.32 (5)	6.51* $\pm$ 0.62 (3)	1.20 $\pm$ 0.12 (4)	0.95 $\pm$ 0.22 (4)	2.82* $\pm$ 0.28 (4)	1.27 $\pm$ 0.13 (3)	5.13* $\pm$ 0.60 (4)
	Octanoyl-L-carnitine (100 μ M)	1.75 $\pm$ 0.35 (5)	3.37* $\pm$ 0.51 (3)	1.82 $\pm$ 0.38 (4)	2.12 $\pm$ 0.35 (4)	4.30* $\pm$ 0.58 (4)	2.50 $\pm$ 0.30 (3)	3.82* $\pm$ 0.41 (4)
O ₂ consumption (nmol/min per mg of protein) at 30°C	Succinate (10 mM) + ADP (1 mM)	55.7 $\pm$ 5.2 (4)	74.80* $\pm$ 4.8 (5)	63.5 $\pm$ 4.7 (4)	60.1 $\pm$ 2.8 (4)	68.5 $\pm$ 3.7 (3)	53.9 $\pm$ 4.1 (4)	79.3* $\pm$ 6.1 (4)
	Ascorbate (10 mM) + TMPD (0.3 mM) + FCCP (0.2 μ M)	77.1 $\pm$ 3.4 (4)	75.0 $\pm$ 2.8 (5)	78.0 $\pm$ 4.3 (4)	74.3 $\pm$ 3.8 (4)	77.6 $\pm$ 6.1 (3)	76.3 $\pm$ 3.6 (4)	75.4 $\pm$ 4.1 (4)
								57.3 $\pm$ 4.7 (4)
								78.1 $\pm$ 3.2 (4)

* Value significantly different ($P < 0.01$) from that for mitochondria from fed virgin rats.

Oxidation of octanoate and octanoylcarnitine by mitochondria from livers of fed virgin and fed lactating rats

The State-3 mitochondrial respiratory rates owing to octanoyl-L-carnitine oxidation were 10% lower for mitochondria from livers of fed lactating rats than for those from fed virgin animals. Although the difference was small, it was statistically significant ($P < 0.01$) and was observed in both the presence and absence of tricarboxylic acid-cycle inhibitors. There was no difference in the rate of oxidation of octanoate by mitochondria from fed lactating and virgin rats under any conditions studied. As expected, the oxygen consumption owing to either substrate was 30–40% higher in the presence of added L-malate.

Effect of starvation

Mitochondria from livers of 24 h- and 48 h-starved virgin rats oxidized octanoylcarnitine at a markedly higher rate than did those from livers of fed virgin animals (Table 1). This stimulation of octanoylcarnitine oxidation was more pronounced in the presence of added malate, but was not affected by the addition of tricarboxylic acid-cycle inhibitors. Starvation for 48 h resulted in a 20% greater increase than did 24 h starvation of virgin rats. For lactating rats, 24 h starvation also resulted in an increase in mitochondrial oxidation of octanoylcarnitine, but to a much smaller extent than observed for virgin animals. Thus the oxygen consumptions owing to β -oxidation of octanoylcarnitine in the presence of malonate and fluorocitrate were 23.3 and 38.0 nmol of O_2 /min per mg of protein by mitochondria from fed and starved virgin rats, and 18.8 and 26.8 nmol of O_2 /min per mg of protein by mitochondria from fed and starved lactating animals, respectively.

There was no change in the rate of octanoate-stimulated oxygen consumption by mitochondria from virgin and lactating animals after 24 h (or 48 h for virgin) starvation, when respiratory rates were measured in the absence of inhibitors. However, rates of octanoate oxidation by mitochondria from fed and 48 h-starved virgin rats differed substantially when measured in the presence of inhibitors. Thus the rate of octanoate oxidation by mitochondria from 48 h-starved virgin rats was 30% lower than that for mitochondria from fed virgin animals. Therefore oxidation of octanoate was more sensitive to inhibition of the tricarboxylic acid cycle in mitochondria from 48 h-starved rats. Addition of malate did not relieve this inhibition. Mitochondria from 24 h-starved virgin and lactating rats did not show any significant inhibition of oxidation of octanoate in the presence of malonate and fluorocitrate.

Effects of glucagon treatment

Mitochondria isolated from livers of glucagon-treated fed virgin and lactating animals oxidized octanoylcarnitine at a significantly higher rate than did those isolated from control animals (Table 1). This increase was observed when respiratory rates were measured either in the absence or in the presence of malonate and fluorocitrate. The rates of octanoylcarnitine oxidation obtained after glucagon treatment of fed virgin rats were similar to those obtained after 24 h starvation of the animals. Glucagon treatment of 48 h-starved virgin rats did not increase mitochondrial octanoylcarnitine oxidation further. Glucagon treatment of fed virgin and lactating rats also resulted in an increase in the rate of mitochondrial oxidation of octanoate. In addition, glucagon treatment resulted in partial reversal of the inhibition of oxidation of octanoate (in the presence of inhibitors) by mitochondria from 48 h-starved virgin rats (see above). Thus the rates of oxygen consumption owing to octanoate oxidation by mitochondria from fed virgin rats, 48 h-starved and 48 h-starved animals injected with glucagon were 16.7, 12.8 and 16.8 nmol of O_2 /min per mg of mitochondrial protein respectively.

Effects of different experimental treatments of animals on other mitochondrial parameters

The mitochondrial content of ATP was increased in preparations from livers of animals injected with glucagon. This increase was observed when mitochondria were incubated with either octanoate or octanoylcarnitine as substrate. However, it was particularly pronounced in mitochondria from fed animals when incubated with octanoate. Glucagon treatment of 48 h-starved animals had a smaller effect on mitochondrial ATP concentrations than treatment of fed animals, when octanoate was used as substrate (Table 2).

Glucagon treatment (but not starvation or lactation) resulted in a stimulation of succinate oxidation by mitochondria, but it had no effect on the oxygen consumption of uncoupled mitochondria in the presence of ascorbate and *NNN'*-tetramethyl-*p*-phenylenediamine (TMPD) (Table 2; cf. Yamazaki, 1975; Halestrap, 1978b).

Discussion

The results of the present study show that the rate of oxidation of the carnitine ester of a medium-chain fatty acid (octanoylcarnitine) by isolated rat liver mitochondria can be modulated independently from that of the oxidation of the unesterified fatty acid itself (octanoate) by subjecting the whole animals to different experimental conditions. Thus, whereas the octanoylcarnitine-stimulated respiratory rate was higher for mitochondria from livers of 24 h-starved virgin and lactating rats than for mitochondria from

fed animals, the rate of octanoate oxidation remained unaltered. The difference in the response of the oxidation of octanoylcarnitine and of octanoate to starvation was even more striking in 48 h-starved virgin rats; starvation resulted in a doubling of the rate of mitochondrial octanoylcarnitine oxidation, but in a 30% decrease in the rate of octanoate oxidation when tricarboxylic acid-cycle activity was inhibited by addition of malonate and fluorocitrate. Moreover, whereas mitochondria from livers of lactating rats oxidized octanoate at the same rate as did those from virgin rats irrespective of the experimental treatment given to the animals, the rate of octanoylcarnitine oxidation by mitochondria from lactating rats was always lower for any given condition studied. The increases in the rate of oxidation of octanoylcarnitine after starvation and glucagon treatment did not result from uncoupling of the mitochondria, as indicated by the high respiratory-control ratios obtained throughout (5.24 and 6.18, with octanoylcarnitine as substrate, for mitochondria from fed and glucagon-treated virgin rats, respectively). Nor was the increase due to changes in the uncoupled rate of oxygen consumption (Table 2). Also the increased rates of oxidation of octanoylcarnitine could be attributed neither to an increased rate of β -oxidation (since oxidation of acetyl-carnitine was similarly affected; V. A. Zammit, unpublished work) nor to increased activity of the tricarboxylic acid cycle (since the increases were observed even in the presence of fluorocitrate and malonate; Table 1).

It is noteworthy that the directions of the changes in octanoylcarnitine oxidation induced by starvation, lactation or glucagon treatment are the same as those expected to occur from the changes in hepatic fatty acid oxidation observed in intact animals, perfused livers and isolated hepatocyte preparations. Thus, under ketogenic conditions (e.g. starvation, diabetes, glucagon excess), the hepatic capacity for oxidation of long-chain fatty acids and carnitine esters of medium-chain fatty acids to ketones is higher than in the normal fed animals (Williamson, 1967; Liljenquist *et al.*, 1974; McGarry & Foster, 1974). Conversely, hepatocytes from lactating rats have a lower rate of ketogenesis from oleate than do those from virgin animals, and this difference persists even in starved animals (Whitelaw & Williamson, 1977, 1978).

The different effects of physiological conditions such as starvation and lactation on the oxidation of octanoylcarnitine and octanoate by isolated mitochondria either in the presence or in the absence of inhibitors indicate that these effects are probably independent from possible actions that these treatments may have on tricarboxylic acid-cycle activity (oxygen consumption in the absence of inhibitors) or on β -oxidation. Therefore these results confirm the

conclusions reached from studies *in vivo* and on intact perfused livers that the rate of oxidation of a medium-chain fatty acid by the liver can be altered by the physiological state of the animal if the acid is esterified to carnitine (McGarry & Foster, 1974). The results obtained *in vivo* also indicated that the activity of a carnitine-associated reaction that occurs after acylcarnitine formation but before β -oxidation is regulated under different physiological conditions to produce changes in the rate of hepatic oxidation of long-chain fatty acids and carnitine esters of medium-chain acids (McGarry & Foster, 1974; McGarry *et al.*, 1975). The results of the present study indicate that these regulatory changes are retained by isolated mitochondria and support the suggestion (Parvin & Pande, 1979) that the activity of the carnitine/acylcarnitine carrier is increased under ketogenic conditions.

The magnitude of the increases in the rate of mitochondrial octanoylcarnitine oxidation were of the same order as the increases in the ketogenic rate from long-chain fatty acids and octanoylcarnitine observed for intact liver when animals were subjected to the same experimental conditions (McGarry & Foster, 1974). Therefore, if the effects observed *in vitro* also occur *in vivo*, they could be of major importance in mediating the observed alterations in the rate of oxidation of fatty acids in the liver under conditions of increased ketogenesis. Inherent differences in the rates of oxidation of acylcarnitines by liver mitochondria may explain why hepatocytes from starved rats still have a higher rate of ketogenesis than those from fed animals even after inhibition of lipogenesis and depletion of glycolytic intermediates (see Williamson, 1979).

However, it has to be appreciated that changes in acylcarnitine utilization by mitochondria may occur in addition to changes in the rate of acylcarnitine formation itself [i.e. by changes in the total activities of carnitine acyltransferase I and acyl-CoA:sn-glycerol 3-phosphate 1-acyltransferase (Aas & Daae, 1971) and/or modulation of activity of carnitine palmitoyltransferase I by malonyl-CoA (McGarry *et al.*, 1977, 1978a,b,c)]. Thus the rate of octanoylcarnitine oxidation by isolated mitochondria was only slightly decreased during lactation, whereas it has been shown that hepatocytes from lactating animals have a 70% lower capacity for ketogenesis from long-chain fatty acids (Whitelaw & Williamson, 1977, 1978). Therefore changes in the rate of acylcarnitine utilization by liver mitochondria may play only a small part in decreasing the rate of long-chain fatty acid oxidation by the liver below that of the fed virgin animal. It is suggested that adaptations in ketogenic rate in livers of lactating rat are due primarily to changes in the rate of acylcarnitine synthesis. However, the observation that octanoylcarnitine oxidation by mitochondria

from lactating animals can still be increased (albeit to a smaller degree than in virgin animals) by starvation or glucagon treatment indicates that the regulatory mechanism for acylcarnitine oxidation by the mitochondria is unimpaired in liver of lactating animals and may be used to mediate the increased rate of fatty acid oxidation in liver during starvation in these animals.

One important difference between the results obtained with isolated mitochondria and those obtained previously with perfused livers or hepatocyte preparations is that glucagon treatment resulted in an increase in the rates of oxidation of both octanoylcarnitine and octanoate. This is in contrast with the results obtained by McGarry & Foster (1974), who found that, although ketogenesis from octanoate was increased by 60% under ketogenic conditions, the overall rate of hepatic octanoate oxidation was unaffected by the increased ketogenic capacity of the liver (primarily owing to a decrease in fatty acid synthesis under ketogenic conditions). It is possible that, *in vitro*, the oxidation of octanoate is limited by the rate of octanoyl-CoA synthesis and that glucagon treatment enhances its oxidation by increasing the rate of ATP synthesis in liver mitochondria (Table 2 and Halestrap, 1978b). Similarly, it is not known why glucagon and starvation have different effects on octanoate oxidation by isolated mitochondria. The greater inhibition of octanoate oxidation by tricarboxylic acid-cycle inhibition in mitochondria from 48 h-starved rats suggests that GTP generated by substrate-level phosphorylation plays a more important role in octanoyl-CoA synthesis in mitochondria from 48 h-starved than in those from fed animals.

I thank Professor J. A. F. Rook and Dr. W. W. Christie for their interest and encouragement throughout this work, and Mrs. S. C. Eyley for able technical assistance.

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