Development of fatty acid oxidation in neonatal guinea-pig liver

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The capacity of foetal and neonatal liver to oxidize short-, medium- and long-chain fatty acids was studied in the guinea pig. Liver mitochondria from foetal and newborn animals were unable to synthesize ketone bodies from octanoate, but octanoylcarnitine and palmitoylcarnitine were readily ketogenic. The ketogenic capacity at 24 h after birth was as high as in adult animals. Hepatocytes isolated from term animals were unable to oxidize fatty acids, but at 6 h after birth production of $^{14}\text{CO}_2$, acid-soluble products and acetoacetate from 1- ^{14}C -labelled fatty acids was 40–50% of the rates at 24 h. At 12 h of age these rates had already reached the 24 h values and did not change during suckling in the first week of life. The activities of hepatic fatty acyl-CoA synthetases, which were minimal in the foetus or at term, increased to maximal values in 12–24 h. The data show that the capacity for β -oxidation and ketogenesis develops maximally in this species during the first 6–12 h after birth, and appears to be partly dependent on the development of fatty acid-activating enzyme.

Together with glycogenolysis and gluconeogenesis, fatty acid oxidation contributes a major portion of the energy needs of neonatal and suckling animals (Bailey & Lockwood, 1973; Warshaw, 1979a). Several previous published papers demonstrated the general lack of ability of tissues, especially liver and heart, of foetal and newborn animals to oxidize fatty acids (Warshaw, 1970; Warshaw & Terry, 1970; Bieber *et al.*, 1973; Foster & Bailey, 1976; Aprille, 1976; Yeh & Zee, 1979). Most of these studies have usually been on the oxidation of only long-chain fatty acids, specifically palmitate. In general, the results show that the ability of neonatal tissues to oxidize appreciable amounts of long-chain fatty acids is not coincident with birth, but becomes noticeable only during the first few days after birth (for reviews see Warshaw, 1979a,b). Much less attention has been given to the oxidation of short- and medium-chain fatty acids (Foster & Bailey, 1976), presumably because these fatty acids are not generally thought to occur in substantial amounts in plasma.

Livers of several species, notably the human and the guinea pig, have a high fat content at birth (Widdowson, 1950; Dobiasova *et al.*, 1964; Dymza *et al.*, 1964; Keen *et al.*, 1981). Foetal guinea-pig liver has been reported to take up both long- and short-chain fatty acid from the umbilical-vein blood (Jones, 1976), suggesting that both types of fatty

acids may be of metabolic importance to the fuel economy of newborn animals. Another source of fat is the milk of the mother, which is rich in fat in almost all species (Glass *et al.*, 1967). It has also been reported that fatty acids of medium chain length (C_8 – C_{12}) are preferentially hydrolysed from triacylglycerols in the stomach and the intestine of 9–10-day-old suckling rats (Fernando-Warnakulasuriya *et al.*, 1981) and that there is a preferential utilization of fatty acids of medium chain length in the liver (Staggers *et al.*, 1981). Appreciable amounts of short- and medium-chain fatty acids occur in the milk of some species of animals (Dermarne *et al.*, 1978). For example, about 35% by weight of the fatty acids in rat milk triacylglycerols are of medium chain length (Grigor & Warren, 1980; Staggers *et al.*, 1981), and both octanoate and palmitate are present in rabbit milk triacylglycerols (Dermarne *et al.*, 1978). These observations point to the possible importance of short- and medium-chain fatty acids as potential sources of oxidizable fuel during neonatal development.

In our previous study on phosphoenolpyruvate synthesis by mitochondria isolated from foetal and newborn guinea pigs, we noted that octanoylcarnitine inhibited the synthesis of phosphoenolpyruvate from malate, whereas octanoate was not inhibitory (Parameswaran & Arinze, 1981), suggesting that liver mitochondria of the foetal and

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newborn guinea pig may be deficient in the fatty acyl-CoA synthetase which activates short- and medium-chain fatty acids. In the present studies the acquisition of the capacity by hepatocytes and liver mitochondria to utilize short-, medium- and long-chain fatty acids has been assessed during neonatal development in the guinea pig. The results show that the capacity for β -oxidation and ketogenesis develops maximally in this species during the first 6–12 h after birth.

Materials and methods

Chemicals

Aquasol, 1- 14 C-labelled fatty acids and [methyl- 3 H]carnitine were purchased from New England Nuclear Corp., Boston, MA, U.S.A., and Amersham Corp., Arlington Heights, IL, U.S.A. Octanoic acid was purchased from Eastman Kodak Co., Rochester, NY, U.S.A. Hyamine hydroxide, sodium salts of palmitic acid and oleic acid, palmitoyl-CoA, octanoyl-CoA and fatty acid-free bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. L-Carnitine and carnitine esters of fatty acids were gifts from Dr. Yuzo Kawashima, Otsuka Pharmaceuticals Co., Tokushima, Japan. Analytical enzymes and co-factors were from Boehringer Mannheim Corp., Indianapolis, IN, U.S.A. Collagenase (type II) was purchased from Worthington Biochemicals Corp., Freehold, NJ, U.S.A. All other chemicals were of reagent grade and were obtained from readily available sources.

Animals

Hartley-strain pregnant guinea pigs were obtained from the Williams Kentucky Cavies, Fern Creek, KY, U.S.A., and from Dutchland Laboratory Animals, Denver, PA, U.S.A., at 30–40 days of gestation and fed on Purina Laboratory chow *ad libitum*. Fresh lettuce was supplied twice a week. Newborn animals used in these experiments weighed 90–120 g at birth. Adult animals weighed 400–500 g. Unless specified all animals used were allowed access to food and water at all times.

Isolation and incubation of mitochondria and hepatocytes

Mitochondria were isolated in sucrose/Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer, pH 7.4, and incubated for phosphoenolpyruvate synthesis as described by Parameswaran & Arinze (1981). For studies on ketogenesis, mitochondria were diluted to 20–40 mg of protein/ml and incubated at 37°C with octanoate, octanoyl-CoA or octanoylcarnitine. The incubation conditions and treatment of samples for

assay of metabolites were essentially as described previously (Parameswaran & Arinze, 1981). Phosphoenolpyruvate and 2-oxoglutarate were measured by standard enzymic methods (Czok & Lamprecht, 1974; Bergmeyer & Bernt, 1974). Acetoacetate and 3-hydroxybutyrate were measured with a metabolite fluorimeter (Instech Laboratories, Philadelphia, PA, U.S.A.) by the method of Williamson & Corkey (1969). Protein was determined by the biuret method (Layne, 1957) with bovine serum albumin as standard.

Hepatocytes, isolated as described previously (Kawai & Arinze, 1981), were incubated with 0.4 mM-1- 14 C-labelled fatty acids (1 Ci/mol) in the presence of fatty acid-free bovine serum albumin (molar ratio of fatty acid to albumin = 1.55:1) in a final volume of 2 ml of Krebs–Ringer bicarbonate buffer (Umbreit *et al.*, 1964), pH 7.4, at 37°C in 25 ml Erlenmeyer flasks. Flasks were gassed with O₂/CO₂ (19:1) and capped with rubber stoppers from which plastic centre wells were suspended. The flasks were shaken at 50–60 oscillations/min, and at the end of 10 min 0.4 ml of Hyamine hydroxide (1 M in methanol) and 0.5 ml of 70% (v/v) HClO₄ were injected into the centre well and flask respectively. The flasks were shaken for an additional 45 min to trap the 14 CO₂ produced. The radioactivity in CO₂ and in acid-soluble products was measured as described by McGarry *et al.* (1978b). Under our experimental conditions the rate of 14 CO₂ production was linear with time for up to 20 min with 20–80 mg wet wt. of cells.

Enzyme assays

A 10% (w/v) liver homogenate was prepared in 0.25 M-sucrose containing 1 mM-reduced glutathione. The homogenate was centrifuged at 100 000 *g*_{av} for 1 h and the pellet (particulate fraction) was resuspended in 0.25 M-sucrose containing 1 mM-reduced glutathione and 0.15 mM-Tris/HCl, pH 7.4, and kept frozen at –80°C until assay. The thawed sample was diluted 1:2 or 1:3 and assayed for fatty acyl-CoA synthetase activity by the hydroxamate trapping method of Kornberg & Pricer (1953) as modified by Pande & Mead (1968). In the experiments described in Table 3, mitochondrial fraction was prepared by differential centrifugation, washed twice, and frozen (–80°C) and thawed twice before assay of synthetase activities by a radiochemical method (Hosaka *et al.*, 1981). For the assay of carnitine acyltransferase liver mitochondria were prepared in sucrose/mannitol/Hepes buffer, pH 7.4, containing 0.5% fatty acid-free bovine serum albumin as described by Greenawalt (1974). The mitochondria were frozen at –80°C, thawed and assayed for total carnitine acyltransferase activity towards palmitoyl-CoA and octanoyl-CoA by the method of Bremer & Norum (1967), except that

10 mM-reduced glutathione was added to the assay. Octanoylcarnitine was extracted with isobutanol as described by Solberg (1972).

Results

Fatty acid oxidation has been shown to inhibit phosphoenolpyruvate synthesis from malate by isolated adult liver mitochondria (Somberg & Mehlmán, 1969; Garber & Ballard, 1970; Jomain-Baum & Hanson, 1975). This inhibition is thought to occur by redox-mediated decrease in intramitochondrial oxaloacetate concentrations (Garber & Hanson, 1971a,b; Jomain-Baum & Hanson, 1973). However, our previous study indicated a lack of effect of octanoate in foetal and newborn guinea-pig liver mitochondria (Parameswaran & Arinze, 1981). Similar experiments have been repeated in the present study with 2-oxoglutarate as substrate and are summarized in Table 1. Whereas octanoyl- and palmitoyl-carnitine inhibited phosphoenolpyruvate synthesis in both foetal and adult liver mitochondria, free octanoate was inhibitory only in adult liver mitochondria. Table 1 also shows that, although almost identical rates of ketogenesis occurred from octanoylcarnitine in foetal and adult liver mitochondrial preparations, ketogenesis from octanoate occurred only in adult liver mitochondria. These results essentially confirm earlier findings (Parameswaran & Arinze, 1981). Since the entry of octanoate into mitochondria is carnitine-independent, the data suggest that the observed poor ability of foetal and newborn guinea-pig liver mitochondria to oxidize octanoic acid may be localized at the activation step for short- or medium-chain fatty acids.

The addition of ATP usually results in higher rates of ketogenesis when isolated mitochondria are incubated with fatty acids (Jomain-Baum & Hanson, 1975). To ensure that ATP was not limiting the activation of fatty acid in our experiments, subsequent incubations were performed in the presence of 3.3 mM-ATP. Under this condition a baseline rate of 13.1 ± 1.1 nmol of ketone bodies/10 min per mg of protein was obtained with octanoate in mitochondria from term animals, as opposed to 50.1 ± 8.7 nmol/10 min per mg of protein with octanoylcarnitine as substrate (Table 2). The lower basal rate with octanoate was not substantially altered by the further addition of CoA and carnitine. Table 2 also shows the developmental profile for ketogenic capacity in the first week after birth. Clearly ketogenic capacity from octanoylcarnitine was the same at all neonatal ages studied, and, by comparison with the adult, indicates that the pathways of β -oxidation and ketogenesis from this substrate are already developed at birth. In contrast, the capacity to produce ketone bodies from octanoate or octanoyl-CoA was very low in the newborn and increased 2–3-fold within 1 day of birth. Ketogenesis from octanoate was highest in mitochondria isolated from 7-day-old animals. Carnitine addition did not appear to stimulate ketogenesis from octanoate, indicating that, as in adult animals, octanoate oxidation is independent of carnitine.

Since the data in Tables 1 and 2 support the general inference that fatty acid oxidation in the foetus and newborn may be limited at the fatty acid-activation step, we measured the activities of hepatic fatty acyl-CoA synthetase for both medium- and long-chain fatty acids, with octanoate and palmitate respectively as substrates. Initially these

Table 1. *Effect of octanoate, octanoylcarnitine and palmitoylcarnitine on phosphoenolpyruvate synthesis from 2-oxoglutarate by mitochondria isolated from livers of foetal and adult guinea pigs*

Mitochondria (10–15 mg of protein) were incubated in 2 ml of medium containing 160 mM-sucrose, 20 mM-potassium phosphate, 4 mM-Hepes, 6.6 mM-MgCl₂, 3.3 mM-ADP and 2 mM-2-oxoglutarate at pH 7.5 for 6 min at 30°C. Where indicated, 0.2 mM-octanoate, 0.2 mM-octanoylcarnitine or 0.1 mM-palmitoylcarnitine was added. Values (nmol/6 min per mg of protein) are the means $\pm$ S.E.M. for six experiments. Where S.E.M. values are not indicated, the number of measurements was less than three. Abbreviation: n.d. not detectable.

	Substrate	Phosphoenolpyruvate produced	Ketone bodies produced		2-Oxoglutarate used
			Acetoacetate	3-Hydroxybutyrate	
Foetus	None	8.9 ± 0.9	n.d.	n.d.	—
	2-Oxoglutarate	36.7 ± 3.9	n.d.	n.d.	375 ± 43
	+ Octanoate	30.7 ± 2.4	<1.0	n.d.	320 ± 7
	+ Octanoylcarnitine	13.8 ± 3.0	28.4 ± 4.4	8.8 ± 1.8	359 ± 42
	+ Palmitoylcarnitine	11.1 ± 1.0	15.1 ± 4.4	5.6 ± 0.9	300 ± 66
Adult	None	5.5 ± 0.8	n.d.	n.d.	—
	2-Oxoglutarate	41.3 ± 1.1	0.9 ± 0.1	n.d.	212
	+ Octanoate	18.4 ± 2.3	19.1 ± 0.1	3.6 ± 1.1	240
	+ Octanoylcarnitine	11.8 ± 1.3	22.0 ± 4.7	5.3 ± 1.6	—
	+ Palmitoylcarnitine	13.3 ± 1.9	18.8 ± 5.3	1.6 ± 0.7	123

Table 2. Ketone-body production from octanoic acid, octanoyl-CoA and octanoylcarnitine in mitochondria isolated from neonatal and adult guinea-pig liver

Mitochondria (4–8 mg of protein) were incubated in 3 ml of medium containing 160 mM-sucrose, 20 mM-potassium phosphate, 4 mM-Hepes, 1 mM-dithiothreitol, 6.6 mM-MgCl₂, 3.3 mM-ADP or -ATP and 0.5 mM-octanoate, 0.2 mM-octanoyl-CoA or 0.2 mM-octanoylcarnitine at pH 7.4, for 10 min at 37°C. Where indicated, 0.4 mM-carnitine and 20 μ M-CoA were added. Ketone bodies measured were acetoacetate and 3-hydroxybutyrate. Values are means $\pm$ S.E.M. for four to six experiments.

Substrate	Additions	Days after birth . . .	Ketone bodies (nmol/10 min per mg of protein)			
			0 (term)	1	7	Adult
None	None		1.3 $\pm$ 0.5	2.5 $\pm$ 0.4	1.4 $\pm$ 0.5	0.4 $\pm$ 0.2
Octanoate	ADP		1.0 $\pm$ 0.6	6.3 $\pm$ 1.3	14.1 $\pm$ 1.8	20.8 $\pm$ 2.4
Octanoate	ATP		13.3 $\pm$ 1.1	40.9 $\pm$ 4.9	84.7 $\pm$ 8.5	47.7 $\pm$ 5.2
Octanoate	ATP + CoA + carnitine		18.3 $\pm$ 2.4	41.8 $\pm$ 5.2	36.5 $\pm$ 4.0	22.0 $\pm$ 2.2
Octanoyl-CoA	ATP + CoA + carnitine		13.4 $\pm$ 1.8	31.9 $\pm$ 4.9	15.5 $\pm$ 2.3	18.3 $\pm$ 2.4
Octanoylcarnitine	ATP		50.1 $\pm$ 8.7	59.4 $\pm$ 12.2	58.3 $\pm$ 6.4	32.6 $\pm$ 2.8

Fig. 1. Activities of fatty acyl-CoA synthetases in developing guinea-pig liver

Particulate fractions were prepared from 10% homogenates by centrifugation at 100000 g_{av} for 1 h. The fractions were freeze-thawed and used for enzyme assays as described in the Materials and methods section. The values plotted are means $\pm$ S.E.M. (vertical bars) for six to eight samples

activities were measured in particulate fractions obtained by centrifuging whole liver homogenates at 100000 g_{av} . The developmental profile for these activities from foetus to adulthood is illustrated in Fig. 1. With octanoate as substrate, the enzyme activity expressed per mg of protein was slightly lower in the foetal liver than in the newborn (4.4 ± 0.4 versus 6.3 ± 0.7 nmol/min). This activity increased by 45–50% from birth to 12 h after birth and remained relatively unchanged until 2 days after birth. A subsequent increase was noticeable during the suckling period, reaching peak values at 10 days of age. Similar changes were also seen with palmitate as substrate, except that the increase in the first 24 h after birth was more pronounced. These profiles were the same whether the activities were expressed per mg of protein or per g of liver. However, they represent total activities and may not be strictly representative of mitochondrial activities. Long-chain and medium-chain fatty acids are believed to be converted into acyl-CoA by the acyl-CoA synthetases on the mitochondrial outer and endoplasmic-reticulum membranes, whereas short-chain and also medium-chain fatty acids are converted into their CoA derivatives in the mitochondrial matrix (Aas, 1971). We therefore considered it important to determine acyl-CoA synthetase activities associated only with mitochondria. With only mitochondrial preparations this study therefore included assays of the GTP-dependent medium-chain synthetase activity. Octanoate was used as the substrate for this assay as well as for assaying total mitochondrial (membrane- plus matrix-associated)

per point. Fatty acyl-CoA synthetases (EC 6.2.1.2 and EC 6.2.1.3) were measured with 3 mM-octanoate (○) and 2 mM-palmitate (●) respectively. The assay time was 30 min. Abbreviations: F, foetus; T, newborn at term; A, adult.

Table 3. *Developmental changes in the activities of guinea-pig liver mitochondrial long-, medium- and short-chain fatty acyl-CoA synthetases*

Mitochondria were prepared as described in the Materials and methods section and frozen (-80°C) and thawed twice. Total mitochondrial fatty acyl-CoA synthetase activities were determined with this preparation. In some experiments, fatty acyl-CoA synthetase activity was determined in the mitochondrial fraction before initial freezing. This activity was taken to represent that associated with the mitochondrial outer membrane and was subtracted from the total activity to obtain matrix-associated activity. No GTP-dependent activity was detected in the intact mitochondria, therefore all measured GTP-dependent octanoyl-CoA synthetase activity reflects activity in the matrix. At 12 and 48 h, respectively, ATP-dependent octanoyl-CoA synthetase activities in the mitochondrial-outer-membrane fraction were $9.9 \pm 4.0\%$ ($n = 3$) and $32.3 \pm 2.7\%$ ($n = 4$) of the total mitochondrial activity. Values given are means $\pm$ s.e.m. for four to six animals. Where s.e.m. is not indicated, the number of animals was less than four. Abbreviation: n.d., not determined. Enzyme activities are expressed as nmol/min per mg of protein.

Time after birth (h)	Palmitoyl-CoA synthetase	ATP-dependent octanoyl-CoA synthetase	GTP-dependent octanoyl-CoA synthetase	ATP-dependent butyryl-CoA synthetase
0	2.76 ± 0.11	5.03 ± 2.44	0.037	n.d.
6	2.50 ± 0.36	8.03 ± 0.86	0.025	n.d.
12	8.02 ± 1.62	8.81 ± 1.85	0.146 ± 0.046	0.828 ± 0.199
24	5.03 ± 0.82	8.44 ± 2.48	0.180 ± 0.069	0.745 ± 0.208
48	6.54 ± 0.67	8.27 ± 1.97	0.107 ± 0.034	0.954 ± 0.375
Adult	n.d.	8.48 ± 1.09	0.192 ± 0.017	n.d.

ATP-dependent medium-chain acyl-CoA synthetase activity. The results, summarized in Table 3, support the data in Fig. 1 in that maximal activities for all three enzymes were attained within 12 h *post partum*. The matrix-located GTP-dependent medium-chain acyl-CoA synthetase was the least active of the three. Its activity, at all age groups examined, was about two orders of magnitude lower than the total mitochondrial (membrane- plus matrix-associated) ATP-dependent medium-chain acyl-CoA synthetase activity. As shown in Table 3, the GTP-dependent activity was barely measurable in mitochondria from animals aged less than 12 h. At 12 h most (>90%, $n = 3$ experiments) of the ATP-dependent medium-chain acyl-CoA synthetase activity was associated with the mitochondrial outer membrane. This distribution is reminiscent of the localization data by Aas (1971). However, at 48 h, up to 30% of the total activity was associated with the mitochondrial outer membrane. Long-chain fatty acyl-CoA synthetase is not known to be present in the mitochondrial matrix. Therefore palmitoyl-CoA synthetase activities shown in Table 3 represent long-chain acyl-CoA synthetase activity associated with the mitochondrial outer membrane. As expected, these values are less than the total tissue activity seen in high-speed particulate fractions (Fig. 1), which includes the microsomal fraction. Short-chain acyl-CoA synthetase activity was also assayed in the frozen-thawed mitochondria with $[1-^{14}\text{C}]$ butyrate and ATP as substrates. Although assays were not done in samples from animals aged less than 12 h, the measured activities were identical at 12 and 48 h and were one order of magnitude lower than

ATP-dependent medium-chain acyl-CoA synthetase activities measured in the same samples.

Carnitine acyltransferase is considered to be a rate-limiting enzyme for long-chain fatty acid oxidation in mitochondria (Warshaw, 1979a; McGarry & Foster, 1980). The developmental profile for its activity is shown in Fig. 2. A doubling of the activity within 24 h of birth was evident with palmitoyl-CoA as substrate, and no further increase was discerned within 24 h after birth and the activity remained virtually unchanged thereafter. With octanoyl-CoA as substrate, the increase in activity was 1.5-fold in 24 h. Earlier time points were not examined. A doubling of palmitoylcarnitine transferase activity between birth and 12 h of age has been reported in pig liver mitochondria (Bieber *et al.*, 1973).

To extend the findings of the experiments with mitochondrial preparations to an intact cell preparation, isolated hepatocytes prepared from animals of different postnatal ages were used to study β -oxidation and ketogenesis, with fatty acids of various chain lengths. Fatty acid oxidation was measured as the formation of $^{14}\text{CO}_2$ as well as acid-soluble radioactive products from $1-^{14}\text{C}$ -labelled fatty acid (McGarry *et al.*, 1978b). In our experiments, as in many others with guinea-pig liver preparations (Jomain-Baum & Hanson, 1975), acetoacetate was the major ketone body produced and, because the amount of 3-hydroxybutyrate produced was too low to be accurately measured, only acetoacetate values are reported in Table 4. We found that little or no oxidation of any fatty acid occurred in hepatocytes prepared immediately after birth (Table 4). By the end of 24 h, however,

Fig. 2. Activities of carnitine acyltransferase in developing guinea-pig liver

Isolated mitochondria were frozen at 80°C , and thawed and diluted 1:10 before assay. The enzyme was assayed for 2 min in the presence of palmitoyl-CoA (●) or octanoyl-CoA (○) by the incorporation of [methyl- ^3H]carnitine into acylcarnitine. The values plotted are means $\pm$ S.E.M. (vertical bars) for five samples per point. Abbreviations: T, newborn at term; A, adult.

maximal rates of $^{14}\text{CO}_2$ production and acetoacetate were achieved for all fatty acids tested. At 7–10 days after birth, the values were the same as at 24 h and therefore have not been included in Table 4. At 6 h of age, however, the rates of production of $^{14}\text{CO}_2$, acid-soluble products and acetoacetate were 40–50% of those at 24 h, and at 12 h of age these rates had already reached the 24 h values. For comparison, similar parameters for adult hepatocytes are indicated for only octanoate and palmitate. The results obtained with oleate (results not shown) were similar to those for palmitate. Except for acid-soluble products and acetoacetate values for the long-chain fatty acid, the results are not significantly different from those for newborns aged 24 h. Taken together with Fig. 1 and Table 3, these data suggest that the capacity for β -oxidation and ketogenesis develops maximally in this species during the first 6–12 h after birth.

Discussion

Milk is the predominant, if not the sole, diet of most newborns, and more than 50% of their dietary energy is derived from this fat-rich source. Because of the critical role played by fatty acids, which are the preferred substrates in tissues such as heart, renal cortex and intestine (Warshaw, 1979a), several

Table 4. Fatty acid utilization by hepatocytes isolated from newborn, suckling and adult guinea pigs

Hepatocytes (30–60 mg wet wt.) were incubated with appropriate fatty acid (0.4 mM) for 10 min, at 37°C , in 2 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 35 mg of fatty acid-free bovine serum albumin. The molar ratio of fatty acid to albumin was 0.65. Results are expressed as nmol of products formed/min per g wet wt. of cells. Acetoacetate was measured enzymically. Values are means $\pm$ S.E.M. for four to six experiments. S.E.M. values were not calculated for less than three measurements. Abbreviations: T, newborn at term; n.d., not detectable.

Substrate	Time after birth (h)	Metabolites measured		
		$^{14}\text{CO}_2$ (nmol/min per g)	Acid-soluble products (nmol/min per g)	Acetoacetate ($\mu\text{mol}/10$ min per g)
Butyrate	T	<1.0	10.2	n.d.
	6	12.5 ± 1.3	67.7 ± 16.8	3.4 ± 0.1
	12	21.3 ± 2.1	103.5 ± 6.3	5.4 ± 1.3
	24	25.2 ± 1.1	129.9 ± 9.2	5.4 ± 0.7
Octanoate	T	1.0	14.8 ± 5.4	n.d.
	6	6.2 ± 1.3	67.5 ± 14.2	2.9 ± 0.3
	12	12.8 ± 0.9	168.8 ± 17.2	6.8 ± 0.4
	24	17.0 ± 1.7	192.0 ± 24.0	6.5 ± 0.3
	48	17.8 ± 1.9	159.6 ± 36.4	5.4 ± 0.5
	Adult	28.4 ± 1.7	216.3 ± 31.6	6.2 ± 0.3
Laurate	T	<1.0	12.0	n.d.
	6	7.3	96.2	2.3
	12	13.9 ± 1.6	124.4 ± 6.5	6.0 ± 0.7
	24	18.1 ± 3.1	177.8 ± 16.6	6.4 ± 0.4
Palmitate	T	<1.0	5.3 ± 3.1	n.d.
	6	6.3 ± 1.0	86.9 ± 15.0	2.4 ± 0.2
	12	13.0 ± 1.0	109.0 ± 5.1	5.7 ± 0.4
	24	17.4 ± 1.4	103.9 ± 17.3	6.9 ± 0.6
	48	26.3 ± 2.8	98.7 ± 22.1	3.4 ± 0.4
	Adult	20.0 ± 1.8	38.2 ± 3.9	1.9 ± 0.1

investigators have been interested in the development of fatty acid metabolism in newborn animals, particularly the rat. Published studies on the oxidation of fatty acids by tissues of newborn rats have focused on the long-chain fatty acid palmitate (for review see Warshaw, 1979a). Several years ago Bailey & Lockwood (1973) stressed the need for more detailed studies on the enzymes of degradation of fatty acids with various chain lengths.

As mentioned in the introduction, milk of many species of animals contains significant proportions of medium-chain fatty acids, which are available to the suckling animal. In the present paper an attempt has been made to study the development of mitochondrial fatty acid oxidation in guinea-pig liver with reference to short-, medium- and long-chain fatty acids. This species is especially appropriate for this study because the liver of its newborn is rich in fat deposited late in the gestation period (Bohmer *et al.*, 1972; Bohmer & Havel, 1975; Jones, 1976). As reported by Jones (1976), the foetal guinea-pig liver, through which all umbilical-vein blood flows, removes most of the short- and long-chain fatty acids passing through it. The results of the present study with octanoylcarnitine as substrate show that the pathways of β -oxidation and ketogenesis in liver mitochondria of the newborn guinea pig are functional and do not limit oxidation of activated fatty acids. Therefore the fact that free octanoate is not utilized strongly indicates that it is not activated in these preparations.

Fatty acid-activating enzymes for both octanoate and palmitate are present only at very low concentrations in liver of 50–55-day fetuses and in the newborn animal. The use of isolated intact hepatocytes confirmed the observations with mitochondria that newborn guinea-pig liver has little ability to oxidize short-, medium- or long-chain fatty acids. A comparison of the data in Fig. 1, and Tables 3 and 4, indicates that the increase in fatty acid utilization was concomitant with the increase in fatty acid-activating enzymes, although the magnitude of the changes in enzyme activity was less than that in fatty acid oxidation. We conclude from these studies that hepatocytes from this species develop the ability for maximal oxidation of fatty acids in the first 6–12 h of life.

The regulation of fatty acid oxidation and ketogenesis during development is poorly understood. However, considerable insight can be gained from studies which clearly delineate, in neonatal liver, the time course of activation and oxidation of fatty acids of various chain lengths. In the liver of starved adult rats, for example, a reciprocal relationship between glycogen content and fatty acid oxidation seems to exist in which the depletion of glycogen and increase in carnitine content appear to trigger fatty acid oxidation and ketogenesis

(McGarry *et al.*, 1978a). Furthermore, it has been postulated that lipolysis in subcutaneous adipose tissue in newborns may be dependent on the breakdown of glycogen in the adipocyte (Novak & Monkus, 1972), with glucose oxidation being necessary for local ATP synthesis and generation of substrates for esterification of fatty acids which do not otherwise undergo oxidation. It might also be speculated that in the neonatal liver, breakdown of glycogen and increase in carnitine concentration (Yeh & Zee, 1979) may trigger fatty acid oxidation. In this respect it is noteworthy that activation of glycogenolysis in liver of newborn guinea pigs begins at about 4 h after birth, and by 7–8 h the hepatic glycogen concentrations have fallen to minimal values (Kawai & Arinze, 1981). Since short- or medium-chain fatty acids do not need carnitine to enter mitochondria, changes in carnitine concentrations cannot be responsible for the post-natal initiation of oxidation of these fatty acids. Presumably the activities of fatty acid-activating enzymes or carnitine acyltransferases alone are not the only factors important in the initiation of fatty acid oxidation in liver of newborn mammals. Peroxisomal contributions should be further avenues for study. The present study provides the groundwork on which further studies on the factors which control the appearance and regulation of fatty acid oxidation and ketogenesis during neonatal development can be based.

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