Pediatr. Res. *17:* 224–229 (1983)

Development of Hepatic Fatty Acid Oxidation and Ketogenesis in the Newborn Guinea Pig

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Summary

The capacity for ketone synthesis by the liver is low for at least 8 h after delivery in newborn human infants. The present studies define developmental changes in hepatic ketogenesis in the newborn guinea pig with particular emphasis on whether there is a delay in development after birth and the site of developmental change. Studies in fresh liver homogenates indicated that the capacity to synthesize ketones from the oxidation of long-chain fatty acid (1 m*M* oleate plus 0.5 m*M* L-carnitine) was very low in term-fetal (5.4 ± 0.4) and newborn pups less than 12 h old (7.7 ± 1) compared to pups 24 or more h old (24–26) and adult guinea pigs (17.8 ± 0.3 μM/h/g). In contrast to the newborn rat, no developmental changes were observed in the carnitine content of tissues (liver, heart, skeletal muscle) or plasma in newborn guinea pigs. The developmental limitation in ketogenesis in the newborn guinea pig was not due to low activity of the enzymes of the hydroxymethylglutaryl-CoA pathway. More rapid rates of ketone production were found with 0.5 m*M* palmityl-carnitine in term-fetal (13.4 ± 1.7) and newborn pups less than 12 hours old (17.8–21 μM/h/g), suggesting that part of the limitation in ketone synthesis in the newborn guinea pig resides at the step of fatty-acyl carnitine formation on the outer aspect of the inner mitochondrial membrane. The activity of this carnitine palmityl transferase (CPT_1) was low in term fetal liver (1.0 ± 0.03 μM/h/g), remained low for 5–10 h after delivery, and then rose to 13 ± 0.7 μM/h/g at 24–48 h of age.

Evidence for at least two additional sites of developmental change in fatty acid oxidation was found in studies of ketogenesis from short-(butyrate)- and medium-(octanoate)-chain fatty acids. (1) In term-fetuses, ketone production from butyrate (17.8 ± 1.5 μM/h/g) was only half that found after 24–36 h of age (36 ± 3.8 μM/h/g), suggesting a limitation in the intramitochondrial beta-oxidation capacity. (2) In adult guinea pigs and in pups 8–10 days old, ketone synthesis from octanoate was 40–45 μM/h/g compared to 4.1 ± .8 μM/h/g in term-fetuses. At 24–36 h old, carnitine-stimulated ketone production from octanoate from 2.4 ± 2.1 to 37 ± 0.9 μM/h/g. This third site of developmental change in hepatic ketogenesis may involve the intramitochondrial activating step for medium-chain fatty acids.

Abbreviations

AcAc, acetoacetate
BOB, beta-hydroxybutyrate
CPT, carnitine palmityl transferase
CPT_1, component of CPT associated with outer aspect of inner mitochondrial membrane
HMG, hydroxymethylglutaryl

We recently reported evidence which suggests that the capacity for ketone synthesis by the liver is very low for at least 8 h after delivery in newborn human infants (20). A restricted capacity for hepatic ketogenesis might be an important limitation in the ability of the neonate to fast because ketone production from endogenous fatty acids not only provides the energy needed for hepatic gluconeogenesis, but also provides a major alternative fuel for brain metabolism when glucose is in short supply. There have been reports by several groups of investigators, primarily in the newborn rat, which support the possibility of major developmental changes in ketogenesis around the time of birth (2, 3, 9, 10, 18, 22, 27, 28). But there are important conflicts among these reports, which leave uncertain both the timing of developmental change and the site in the pathway of fatty acid oxidation at which development occurs. In addition, it is not known whether the newborn rat is an appropriate model for the development of ketogenesis in the human newborn because other aspects of perinatal fat metabolism appear to be dissimilar between the two species. In contrast to the newborn rat, newborn infants have very large deposits of fat at birth (24), are fed a milk much lower in fat (16) and carnitine (14), and do not develop hyperketonemia during the suckling period (1).

The present studies were carried out to define developmental changes in hepatic ketogenesis in the newborn guinea pig. This species was chosen because it resembles the human newborn in having large stores of fat at birth (10% of body weight) and consumes a milk low in fat (4% by volume) (16). Because of the conflicting results of studies in the newborn rat model, particular emphasis was placed on determining whether there is a delay in development after birth and the site of this developmental change.

MATERIALS AND METHODS

Animals. Albino guinea pigs were purchased from Perfection Breeders (Douglassville, PA) and housed in the small animal quarters at The Children's Hospital of Philadelphia. To obtain newborn pups and fetuses for study, nontimed pregnant guinea pigs were purchased 1–3 weeks before their estimated due dates and either allowed to deliver spontaneously or were delivered by caesarian section. Caesarian-sections were performed after stunning and decapitation of uanesthesized dams and the fetuses were rapidly delivered and resuscitated. Near term gestation (65 days) was verified from fetal weights (85–100 g) (7). Spontaneously delivered pups were left with their dams. Fetuses and newborn pups were maintained in an incubator at 33°C. Animals were sacrificed by decapitation, and livers were rapidly excised and weighed for the studies described below. In some studies, samples were also taken of cardiac ventricle muscle, gastrocnemius muscle, and plasma.

Rates of ketogenesis in liver homogenates. The method described by Krebs (8) was used to determine the rate of ketone synthesis from fatty acids in whole liver homogenates. Liver was homogenized at 4°C with a Teflon pestle homogenizer in assay buffer containing only potassium phosphate, KCl, $MgCl_2$, and EDTA. Incubations were carried out in duplicate in a shaking incubator at 37°C in 10 mM potassium phosphate buffer (pH 7.4), 137 mM KCl, 10 mM alpha-ketoglutarate, 1 mM $MgCl_2$, 1 mM ATP, 0.05 mM coenzyme-A, 0.5 mM EDTA, and 1% fatty acid-free bovine

serum albumin. The final volume was 2.5 ml and the final homogenate concentration was 4% (w/v). Fatty acids were complexed to bovine serum albumin and added to the incubation mixture at concentrations which gave maximal rates of ketone production. Where used, L-carnitine was added at 0.5 mM final concentration; this gave maximal rates of ketogenesis from oleate. Incubations were stopped after 30 min with 0.2 ml 40% perchloric acid; the supernatant was neutralized and assayed in duplicate for beta-hydroxybutyrate (BOB) and acetoacetate (AcAc) spectrophotometrically with BOB dehydrogenase (23).

In preliminary studies, linear rates of ketone production were obtained for 60–90 min with liver from adult guinea pigs. Rates were proportional to final homogenate concentrations ranging from 1–8%. Rates of total ketone production from 1 mM oleate and ratios of BOB:AcAc were similar to those reported in perfused liver from fasted guinea pigs (19). As discussed by Krebs (8), the use of alpha-ketoglutarate as oxidizable substrate in the homogenate system maximizes the flow of acetyl-CoA towards ketone synthesis rather than into the tricarboxylic acid cycle by maintaining ATP inhibition of citrate synthase. Studies with labeled palmitate confirmed that CO_2 production accounted for less than 5% and ketone production for more than 95% of the fatty acid oxidized; therefore, ketone production in these experiments essentially represents total fatty acid beta-oxidation.

Hydroxymethylglutaryl CoA (HMG-CoA) synthase (EC 4.1.3.5) activity. The rate-limiting step in ketone synthesis from acetyl-CoA was measured using a modification of the coupled enzyme assay described by Williamson, *et al.* (25). This assay follows the rate of AcAc produced from acetyl-CoA by the combined action of tissue AcAc-CoA thiolase, HMG-CoA synthase, and HMG-CoA lyase. Liver was homogenized in 2 mM HEPES (pH 7.4), 250 mM sucrose, 0.1 mM EDTA and 0.5 mM dithiothreitol. Mitochondria were lysed with 0.5% (w/v) Triton X-100. Incubations were carried out in triplicate for 2 min at 30°C with 0.1 ml final volume in 100 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 0.5 mM [1-^{14}C] acetyl-CoA (960,000 cpm/μM) and 0.25 mg of tissue. The reaction was stopped with 0.4 ml 62.5% citric acid. The [^{14}C] incorporated into the carboxyl carbon of AcAc at the synthase step was released as [$^{14}CO_2$] by stoppering the tubes and adding another 0.4 ml of 62.5% citric acid containing 3.6 g aniline/6 ml. After 1 h at 37°C, the [$^{14}CO_2$] was trapped in Hyamine in plastic center wells and counted in a liquid scintillation counter.

Preliminary studies showed that the reaction rate was linear with time and proportional to the amount of tissue from 0.025–0.5 mg. The identification of AcAc as product was established by demonstrating that enzymatic conversion of AcAc to BOB in the product with excess NADH and BOB dehydrogenase reduced the [$^{14}CO_2$] released by aniline-citrate decarboxylation to the level of the blank. Identical values for enzyme activity were also obtained when the reaction was done on a larger scale and the AcAc produced was assayed by the enzymatic spectrophotometric method. In accord with the results of Williamson, *et al.* (25), 95% of the HMG-CoA synthase activity with the radioisotopic assay was associated with the particulate (mitochondrial) fraction of liver homogenates.

Carnitine palmityl-transferase (CPT) (EC 2.3.1.21) activity. The "forward" assay used measures the incorporation of [^{3}H]-carnitine and palmityl-CoA into palmityl-[^{3}H]-carnitine. Liver was homogenized at 4°C in 10 volumes of 2 mM HEPES (pH 7.4), 200 mM D-mannitol, 70 mM sucrose, and 0.5% bovine serum albumin. Reactions were carried out in duplicate in a final volume of 1 ml at 37°C in 12.5 mM Tris-HCl (pH 7.4), 137 mM KCl, 3.1 mM KCN, 6.2 mM reduced glutathione, 0.25 mM palmityl-CoA, 0.1 mM L-carnitine, luCi D, L-[methyl-^{3}H] carnitine, and 2% bovine serum albumin. The reaction was started by adding 0.1 ml homogenate and stopped after 2 min with 1 ml 1.2 N HCl. Labeled palmityl-carnitine was extracted with 1 ml *n*-butanol. After centrifuging, an 0.8 ml aliquot of the butanol phase was transferred to a tube containing 4.0 ml water saturated with butanol. This tube was centrifuged and 0.5 ml of the butanol phase removed for liquid scintillation counting. The component of CPT activity associated with the outer aspect of the inner mitochondrial membrane (CPT_I) (6) was determined by using freshly-prepared whole liver homogenates. Total CPT activity was determined in whole homogenates freeze-thawed three times.

The above reaction conditions were similar to those used by McGarry *et al.* (12), except that 37°C instead of 25°C was used and the concentration of palmityl-CoA was increased from 0.05 to 0.25 mM, which gave nearer maximal CPT_I velocity. To confirm that the assay with fresh tissue reflected primarily CPT_I activity, the inhibition of activity by malonyl-CoA was also determined. Preliminary experiments indicated that 20 μM malonyl-CoA gave maximal inhibition of both CPT_I and total CPT activity in liver from adult guinea pigs.

AcAc-CoA thiolase (EC 2.3.1.9) activity. The activity of AcAc-CoA thiolase was measured spectrophotometrically using a chart recorder to determine initial rate (13). The assay mixture consisted of 100 mM Tris-HCl, pH 8.1, 25 mM $MgCl_2$, 50 mM KCl, 0.05 mM CoA, 0.1 mM AcAc-CoA, and 0.1 mg tissue. The decrease in optical density at 303 nm was followed at 30°C and the rate calculated assuming a molar extinction coefficient of 16.9×10^3.

HMG-CoA lyase (EC 4.1.3.4) activity. The activity of HMG-CoA lyase was measured spectrophotometrically by determining initial reaction rates at 37°C (21). The reaction mixture consisted of 200 mM Tris-HCl, pH 8.1, 10 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM L-malate, 1.4 mM NAD, 0.05 mM NADH, 1 μg citrate condensing enzyme, 5 μg malate dehydrogenase, 0.1 mg of tissue, and 0.7 mM HMG-CoA. The increase in NADH was followed at 340 nm.

Carnitine. Plasma and tissue carnitine levels were determined by the radioenzymatic assay described by McGarry and Foster (11) using the modifications suggested by Seccombe *et al.* (17) to measure both free and total (free plus esterified) carnitine in plasma. Total carnitine in homogenates of tissue samples was measured in the same way as total plasma carnitine by hydrolyzing the sample with $Ba(OH)_2$ for 1 h at 37°C before precipitation with $ZnSO_4$ to release esterified carnitine.

Materials. Fatty acids, fatty acyl-carnitine and CoA derivatives, L-carnitine, and malonyl-CoA were purchased from Sigma Chemical Company, St. Louis, MO. Carnitine acetyl-transferase, BOB dehydrogenase, malate dehydrogenase, citrate condensing enzyme, acetyl-CoA, coenzyme-A and ATP were from Boehringer Mannheim Biochemicals, Indianapolis, IN. D,L-[methyl-^{3}H]-carnitine was from Amersham Corporation, Arlington Heights, IL and [1-^{14}C]-acetyl-CoA from New England Nuclear, Boston, MA.

RESULTS

Figure 1 shows the changes in the *in vitro* capacity for ketogenesis from the long-chain fatty acid, oleate, by liver homogenates from term-fetal pups, newborn pups of different ages, and adult guinea pigs. At all ages, maximum ketogenesis required supplemental L-carnitine (0.5 mM). The results indicate that the capacity to synthesize ketones from the oxidation of long-chain fatty acid is poorly developed in both the late fetal and early postnatal period. The capacity for ketogenesis from oleate plus carnitine was low in term-fetuses and in newborn pups less than 12 h old (5.4 ± 0.4 and 7.7 ± 1 μM/h/g). By 24–36 h of age, the maximal rate of ketogenesis had risen 3–4-fold and remained in the range of 24–26 μM/h/g through the first 10 days after delivery. Rates of ketone synthesis in adult guinea pigs (17.8 ± 0.3 μM/h/g) were slightly lower than in newborn pups over 24 h old, but were more than twice as high as the values found in term fetal and newborn pups less than 12 h of age.

Studies in rats (15, 26) have suggested that fatty acid oxidation may be limited in the fetal and newborn period by a deficient supply of carnitine in liver and muscle tissue. Because the measurement of rates of ketogenesis in homogenates (Fig. 1) does not depend on endogenous carnitine content, levels of carnitine were measured in several tissues from fetal, newborn, and adult guinea pigs. As shown in Table 1, carnitine levels in plasma, heart, and skeletal muscle from term-fetal guinea pigs were similar to the

values found in older newborn pups and adults. Liver carnitine concentration was actually higher in term-fetuses and 1–2-day-old pups than in adult guinea pigs. In contrast to the newborn rat, carnitine does not appear to be a limiting factor for either hepatic or muscle fatty acid oxidation in the guinea pig.

In order to ensure that the low rates of ketone production found in fetal and newly born guinea pigs were not due to a limitation in AcAc formation from acetyl-CoA, the activities of the enzymes of the HMG-CoA pathway were determined in fetal, newborn, and adult guinea pig liver (Table 2). The activity of AcAc-CoA thiolase was one-third lower in term-fetuses than in 1–10-day-old pups. But values in term fetuses and adults were similar, suggesting that AcAc-CoA thiolase activity was not responsible for limiting ketone production. The activity of HMG-CoA lyase in fetal and newborn liver was about half that found in liver from adult guinea pigs; however, the overall activity of the pathway, as determined by the coupled enzyme assay of HMG-CoA synthase, was similar at all ages.

The above experiments suggested that the site of developmental change in hepatic ketogenesis in the guinea pig may lie in the steps of fatty acid transport into mitochondria and/or in the beta-oxidation of fatty acids. To identify the location of developmental change, further measurements were made of rates of ketone production by whole liver homogenates from substrates which enter the fatty acid oxidation pathway at different points. Preliminary experiments in adult guinea pig liver established that maximal rates of ketone production were obtained with the concentrations of substrates shown in Table 3. The developmental changes in rates of ketone synthesis from these substrates are shown in Table 3. Table 4 shows the results of studies in single pups from a litter observed during spontaneous delivery and who were then fasted from 1–16 h before sacrifice. In term-fetal liver it was found that, among the long-chain acyl substrates, palmityl-carnitine supported much higher rates of ketogenesis than either palmitate or palmityl-CoA plus carnitine (Table 3). In guinea pig pups, 24 h of age or older, and in adult guinea pigs rates of ketogenesis were similar among all three of these substrates. This suggests that part of the limitation in ketone synthesis from long-chain fatty acids in the fetus and newborn guinea pig pup resides at the step of fatty-acyl carnitine formation on the outer aspect of the inner mitochondrial membrane (CPT_1). As shown in Table 4, there appeared to be a delay in development at this step for at least 6 h after delivery and then a rapid increase between 6–16 h of age. By 24–36 h of age, ketone synthesis from long-chain fatty acids was fully developed (Table 3).

Although the rate of ketogenesis from palmityl-carnitine in fetal and early newborn pups was higher than from other long-chain substrates, it did not reach the values found in older pups and adult guinea pigs (Tables 3 and 4). This was also the case with the short-chain fatty acid, butyrate. In term-fetuses, ketone production from butyrate was only about half the values found after 24–36 h of age. The same was true in five spontaneously-delivered pups less than 12 h old where ketone production from butyrate was 14.6 ± 0.7 and 13.5 ± 0.9 μM/h/g, with and without 0.5 mM carnitine, respectively (data not shown). At all ages, ketogenesis from butyrate was carnitine-independent, which is consistent with the concept that short-chain fatty acids enter mitochondria directly and are then activated in the mitochondrial matrix. These observations suggest the existence of a second site of developmental

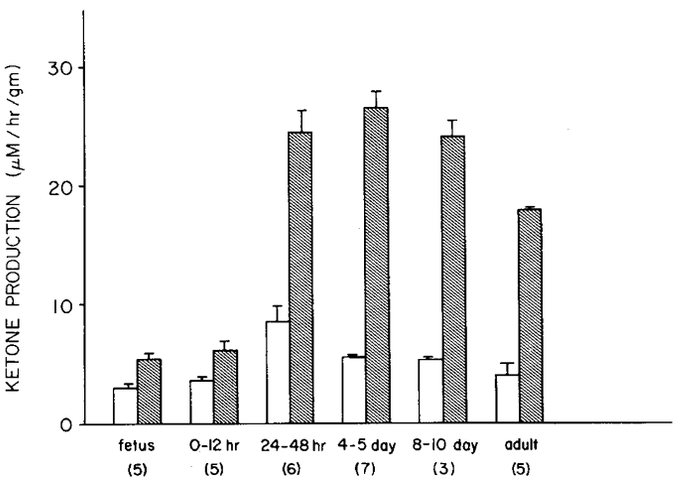

Fig. 1. Rate of total ketone synthesis from 1 mM oleate, with (*hatched bars*) or without (*open bars*) 0.5 mM carnitine, by whole liver homogenates from term-fetal guinea pigs, newborn pups of different ages, and adult guinea pigs. Experiments were carried out as described in "Materials and Methods." Bars show mean ± S.E. of duplicate measurements in the number of animals indicated in brackets.

Table 2. *Activities of the enzymes of the hepatic HMG-CoA pathway in guinea pigs of different ages (μM/min/g) (mean ± S.E.)*

	Term-fetus	1–10 day old	Adult
AcAc-CoA thiolase	36 ±1.3 (6)[1]	48 ± 3 (6)[1]	33, 41 (2)[1]
HMG-CoA lyase	9.0 ± 0.4 (6)	14.3 ± 4 (6)	22, 25 (2)
HMG-CoA synthase	2.3 ± 0.1 (6)	2.9 ± 0.15 (6)	1.8, 1.5 (2)

[1] Figures in parentheses indicate number of animals.

Table 1. *Carnitine concentration in liver, cardiac ventricle, and gastrocnemius muscle (nM/g), and in plasma (μM/liter) from guinea pigs of different ages (mean ± S.E.)*[1]

				Plasma	
	Liver	Heart	Gastrocnemius	Free	Total
Term-fetus	930 ± 68 (11)	1260 ± 39 (4)	600 ± 31 (4)	31 ± 1 (6)	44 ± 1.8 (6)
12 h	1050 ± 200 (5)	1370 ± 120 (5)	580 ± 43 (5)		
24–36 h	1020 ± 130 (3)	1330,1220 (2)	700,715 (2)		
48–72 h	340 ± 48 (4)	1820 ± 160 (4)	990 ± 7 (3)	33 ± 3.6 (5)	50 ± 4 (5)
8–10 day	470 ± 39 (6)	1540 ± 110 (5)	860 ± 127 (4)		
Adult	450 ± 55 (7)	1470 ± 111 (3)	730 ± 43 (3)	26 ± 4 (4)	34 ± 5 (4)

[1] Figure in parentheses indicate number of animals.

Table 3. *Rates of ketone production (μM/h/g) from long-, medium-, and short-chain fatty acid substrates in liver homogenates from guinea pigs of different ages (mean ± S.E.)*

Substrate	Term-fetus	24–36 h	8–10 days	Adult
None	2.0 ± 0.56 (4)[1]	7.0 ± 0.5 (3)[1]	3.0, 2.6 (2)[1]	5.4, 6.8 (2)[2]
Palmitate 1 mM	2.6 ± 0.56 (4)	7.2 ± 0.3 (3)	5.6, 4.5 (2)	6.7 ± 0.4 (3)
Palmitate 1 mM + carnitine 0.5 mM	2.3 ± 0.4 (4)	24 ± 0.3 (3)	17, 28 (2)	27 ± 1.3 (3)
Palmityl-CoA 0.5 mM + carnitine 0.5 mM	2.3 ± 0.8 (4)	27 ± 1.2 (3)	25, 27 (2)	20 ± 1.1 (3)
Palmityl-carnitine 0.5 mM	13.4 ± 1.7 (4)	33 ± 1.8 (3)	31, 32 (2)	32 ± 4.2 (3)
Octanoate 2 mM	4.1 ± 0.8 (4)	2.4 ± 2.1 (3)	40 ± 2.5 (5)	45 ± 1.4 (3)
Octanoate 2 mM + carnitine 0.5 mM	4.2 ± 0.7 (4)	37 ± 0.9 (3)	39 ± 2.6 (5)	46 ± 1.8 (3)
Octanoyl-CoA 2 mM + carnitine 0.5 mM	3.8 ± 0.6 (4)	36 ± 1 (3)	34, 42 (2)	42 ± 1.9 (3)
Octanoyl-carnitine 2 mM	20.3 ± 2.2 (4)	46 ± 1.1 (3)	48, 51 (2)	51 ± 2.6 (3)
Butyrate 10 mM	17.8 ± 1.5 (5)	36 ± 3.8 (4)	48 ± 2.6 (5)	45.2 ± 1.6 (4)
Butyrate 10 mM + carnitine 0.5 mM	18.6 ± 0.6 (5)	42 ± 3.4 (4)	48 ± 2.5 (5)	46 ± 1.8 (4)

[1] Figures in parentheses indicate number of animals.

Table 4. *Rates of ketone production (μM/h/g) from long-, medium-, and short-chain fatty acid substrates in liver homogenates from one litter of newborn guinea pigs fasted 1–16 h after birth*

	1 h	2 h	6 h	16 h
None	2.4	2.6	3.0	7.2
Palmitate 1 mM	4.5	4.0	3.4	6.2
Palmitate 1 mM + carnitine 0.5 mM	5.8	4.8	6.8	20.6
Palmityl-CoA 0.5 mM + carnitine 0.5 mM	4.3	4.9	7.9	17.8
Palmityl-carnitine 0.5 mM	21.0	18.0	17.8	23.5
Octanoate 2 mM	6.1	6.2	5.7	8.6
Octanoate 2 mM + carnitine 0.5 mM	6.1	5.8	5.2	12.0
Octanoyl-CoA 2 mM + carnitine 0.5 mM	7.8	8.4	12.2	22.3
Octanoyl-carnitine 2 mM	17.5	17.0	18.8	30.6

change in fatty acid oxidation in guinea pig liver in addition to the CPT_1 step. As shown in Table 3, ketogenesis from both butyrate and palmityl-carnitine was fully mature by 24–36 h after birth.

The data shown in Table 3 indicate the possible presence of still a third site of developmental change in fatty acid oxidation which affects only medium-chain fatty acids. In adult guinea pigs and in pups 8–10 days of age, ketone synthesis from octanoate was rapid and carnitine-independent; however, in term-fetuses, only the carnitine ester of octanoate supported very rapid rates of ketogenesis. At 24–36 h of age, carnitine was required for the oxidation of octanoate. A similar carnitine-dependence for ketone synthesis from octanoate was also seen in younger newborn pups (Table 4) and in groups of pups 48–72 h old (data not shown). At 4–5 days of age, the adult pattern was found with rates of ketogenesis from octanoate equal to 32 ± 2.5 μM/h/g without added carnitine and 35 ± 2.6 μM/h/g in the presence of 0.5 mM carnitine (n = 7) (data not shown). These observations suggest that the intramitochondrial activating step for medium-chain fatty acids is not developed at birth and is not acquired until approximately 4 days of age.

The above studies in whole liver homogenates suggested that CPT_1 may be a major site of developmental change in the capacity of the liver to oxidize long-chain fatty acids. To confirm this, the developmental changes in CPT_1 and total CPT activities were examined. Table 5 shows the activity of CPT in fresh (CPT_1) and freeze-thawed (total CPT) liver from guinea pigs of various ages. The % inhibition of activity by 20 μM malonyl-CoA was determined to verify that CPT activity in fresh homogenate was primarily due to CPT_1. Data were also obtained on term-fetuses during fasts of 5, 10, and 24 h after caesarian delivery. In both premature and term-fetuses, CPT_1 activity was only 10–25% of values seen in fed adult animals. In fed newborn pups, CPT_1 activity had risen to 3 times adult values by 1–2 days of age, remained elevated during the suckling period, and appeared to be declining at 12 days of age. In contrast to CPT_1, total CPT activity at term was 65% of fed adult values, suggesting that CPT_2 activity was well-developed before birth.

In the groups of term-fetuses which were fasted after delivery, CPT_1 activity had risen at 10 and 24 h to levels seen in 1–2-day-old animals. Although CPT_1 activity was slightly increased at 5 h, this value appears to be falsely elevated by disruption of mitochondria because only 25% was inhibited by malonyl-CoA. The timing of the increase in CPT_1 activity, therefore, appeared to correlate closely with the increase in ketogenic capacity noted in Table 4.

DISCUSSION

These studies indicate that the ability of the liver to synthesize ketones from the beta-oxidation of fatty acids by the liver is not developed at the time of birth in the guinea pig. In spontaneously-delivered pups, there was a delay of 6–12 h after birth before the pathway of ketogenesis began to develop. After this period of delay, development occurred rapidly and by 24–36 h of age, *in vitro* rates of ketogenesis from long-chain fatty acids were slightly greater than values found in adult liver. The most important site of developmental change for ketone synthesis from long-chain fatty acids appeared to be the activity of CPT_1, which initiates the transport of these substrates into mitochondria. The activity of this enzyme remained low for several hours after delivery and then increased 20-fold by 24 h of age.

The hypothesis that the step catalyzed by CPT_1 plays a major role in the development of capacity for hepatic ketogenesis is supported by several studies of the development of fatty acid

Table 5. *Developmental changes in carnitine palmityl-transferase activity in fresh (CPT_1) and freeze-thawed (total CPT) guinea pig liver (μM/h/g) (mean ± S.E.)*

					% inhibition by mal-CoA	
	n	CPT_1	Total CPT	Delta	CPT_1	Total CPT
Fetus (50 day)	2	0.57, 0.47	7.3, 6.5	6.7, 6.0	67, 64	10, 27
Term-fetus						
1-h fasted	5	1.0 ± 0.03	12 ± 0.37	11 ± 0.35	45 ± 1.6	6 ± 1.4
5-h fasted	2	2.7, 2.3	12, 15	9.3, 13	25, 25	8, 10
10-h fasted	2	12, 14	16, 19	4.1, 4.3	64, 74	13, 26
24-h fasted	3	18 ± 0.5	27 ± 0.7	8.8 ± 1.2	68 ± 3	25 ± 0.3
24–48 h old	5	13 ± 0.7	29 ± 1.4	15 ± 1.2	73 ± 4	6 ± 1.4
4–6 day old	4	10.5 ± 0.2	25 ± 0.52	14 ± 0.6	79 ± 1.5	22 ± 1.9
12 day old	2	6, 14	24, 28	18, 15	64, 80	15, 24
Adult	3	4.6 ± 0.7	19 ± 0.9	13 ± 1.4	63 ± 2.6	14 ± 2.6

oxidation in the rat newborn model. Three groups (2, 3, 28) have reported a marked increase in liver CPT activity in the rat around the time of birth, comparable to that found in the guinea pig. In addition, Wittles and Bressler (26) reported that CPT activity increased around the time of birth in rat heart, suggesting that development at this step of fatty acid oxidation may occur in other organs as well as liver. Some of the data on the newborn rat, however, are not consistent with a central role for CPT_1 activity in development. Warshaw (22) reported that CPT activity rose only gradually after birth, not reaching adult values until 20 days of age. Shah and Bailey (18) reported major developmental changes in HMG-CoA synthase activity in the rat. These latter results differ from those of Lee and Fritz (9) and the present data (Table 2) in which no changes in HMG-CoA synthase activity were seen. The functional significance of changes in CPT activity to development of fatty acid oxidation in the rat has also been questioned by Yeh and Zee (28) who found impaired respiratory control in isolated mitochondria from fetal rat liver. In studies to be reported elsewhere (5), however, we have found normal respiratory control in fetal guinea liver mitochondria. Although the pattern of development of fatty acid oxidation in the rat remains unclear, it seems likely that maturation of CPT_1 activity plays an important role similar to that found in the present study in the guinea pig.

Although the present data emphasize that a developmental change in CPT_1 activity plays a major role in the maturation of hepatic fatty acid oxidation, the results of experiments with liver homogenates indicate that there may be at least two additional areas of development of this pathway. In homogenates, increased rates of ketogenesis were achieved in the newborn with butyrate and palmityl-carnitine, both of which bypass the CPT_1 step (Tables 3 and 4). But even with these substrates, rates of ketogenesis in the fetus and newborn were one-third to one-half lower than in older pups and adults. Similar findings in fetal rat liver were reported by Lee and Fritz (9). This suggests that there may be a second site of developmental change in the intramitochondrial pathway of fatty acid oxidation, which is common to a variety of fatty acid substrates. This finding appears not to be due to differences in number of mitochondria or the amount of hematopoietic tissue in fetal guinea pig liver because we have found similar results with isolated mitochondria (5). The nature of this second site of development is not known, but could involve a step in the beta-oxidation sequence itself or the electron transport chain. The experiments with liver homogenates also suggest a third site of developmental change which is specific for the oxidation of medium-chain fatty acids. Medium-chain fatty acid oxidation is generally considered not to be dependent on carnitine, because these acids can enter mitochondria in the unesterified form and be activated to acyl-CoA esters within the matrix space. In newborn guinea pigs, however, the mitochondrial activation of octanoate appeared to be limited and did not mature until 3–5 days of age (Table 3). Foster and Bailey (3) have reported developmental increases in the activity of the mitochondrial ATP-dependent medium-chain acyl-CoA synthetase in newborn rat liver, which are compatible with this finding. The observation that ketogenesis from octanoate was partially carnitine-dependent between 1–3 days of age may be due to the fact that CPT_1 (or a specific medium-chain transferase) development is completed before the intramitochondrial activation path matures.

The present data suggest that the role of carnitine in the development of fatty acid oxidation is very different in the rat and guinea pig newborn. Fetal rats have low levels of carnitine in liver, heart, and skeletal muscle and it has been suggested that this may limit fatty acid oxidation in the newborn period. Robles-Valdez, *et al.* (15) have obtained evidence that the postnatal increase in tissue carnitine levels in the rat may depend on the high concentrations of carnitine supplied by rat milk. Hahn (4) has recently reported that rates of carnitine synthesis are low in fetal rat liver. In contrast to the rat, however, the present results clearly indicate that there is no deficiency of tissue carnitine in either heart, skeletal muscle, or liver in the newborn guinea pig. In *in vivo* studies not presented in this report, we have found that term-fetal guinea pigs fasted for 24 h after caesarian delivery are able to elevate plasma ketones to levels seen in fasted adult guinea pigs (BOB, 2 mM/liter). Thus, a requirement for dietary carnitine in the postnatal development of fatty acid oxidation may be a feature which is unique to the rat. It is tempting to speculate that the apparent dependence on exogenous carnitine in the newborn rat might be related to its unusually low stores of fat at birth and the unusually high fat content of its diet during the suckling period.

The observation that hepatic ketogenesis is not fully developed at birth and does not become functional for at least 6–12 h after delivery in the guinea pig may have important clinical implications. We have reported that newborn infants who become hypoglycemic during the first 8 h after birth have low levels of plasma ketones despite elevated levels of free fatty acids (1). These data suggest that the human newborn has a delay in maturation of hepatic fatty acid oxidation similar to that found in the newborn guinea pig. The present data (Tables 4 and 5) indicate that feeding is not required for the maturation of either ketogenesis or CPT_1 activity. Further studies are necessary to define other factors which might control development of hepatic ketogenesis, including the possible effects of hormonal signals and gestational maturity, and whether other organs, such as heart and skeletal muscle, share in this developmental limitation in the oxidation of fatty acids.

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30. This research was supported in part by NIH grants AM 13518, RR 00240, and NS 17752.
31. The authors thank Susan M. Anderson for assistance in preparing this manuscript and Dr. Albert I. Winegrad for many helpful discussions.
32. Received for publication March 26, 1982.
33. Accepted for publication June 23, 1982.

0031-3998/83/1703-0224$02.00/0

Printed in U. S. A.