0031-3998/88/2302-0224$02.00/0
PEDIATRIC RESEARCH
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Vol. 23, No. 2, 1988
Printed in U.S.A.

Premature Appearance of Gluconeogenesis and Fatty Acid Oxidation in the Liver of the Postterm Rabbit Fetus

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ABSTRACT. The metabolic consequences of a prolonged gestation (35 *vs* 32 days) have been studied in the rabbit fetus. Gestation was prolonged by daily subcutaneous injections of progesterone (1.5 mg·kg^{-1}) from day 28 to 34. In control animals, progesterone was injected from day 25 or 28 to day 31 of gestation. When the capacities for gluconeogenesis and fatty acid oxidation, measured on isolated hepatocytes, are normally low in the term control fetus and increase only within the first 24 h after birth, these capacities appear high in the postmature fetus. The rate of glucose production from lactate is 4-fold higher in the postmature fetus than in the normal term fetus. The rate of ketone body production from oleate is also 5-fold higher in the postmature fetus, which results from a switch on of the partition of oleate into esterification and oxidation: 8% of [1-^{14}C]oleate is oxidized in term fetus hepatocytes, but 34% in postmature fetus hepatocytes. As a similar rate of lipogenesis takes place in both stages, this metabolic change could be explained by a 5-fold lower sensitivity of carnitine palmitoyltransferase I to the inhibition by malonyl-coenzyme A. Postmaturity decreases plasma insulin concentrations by 45% and increases plasma glucagon concentrations by 50% which, in turn, induces a 3-fold decrease in the plasma insulin:glucagon molar ratio. As previously shown in fasted or diabetic adult rat, this hormonal change might be a likely candidate for an enhancement of gluconeogenic and ketogenic capacity in the liver of the postterm rabbit fetus. (*Pediatr Res* 23: 224–228, 1988)

Abbreviation

CoA, coenzyme A

During pregnancy, the fetus receives a continuous supply of nutrients, principally glucose and amino acids for its growth and its oxidative metabolism which makes unnecessary the development of metabolic pathways as those involved in endogenous glucose production or free fatty acid oxidation (1). Previous studies performed in rabbit hepatocytes have shown that gluconeogenesis from lactate or oleate oxidation and ketogenesis proceed to a very low rate in term fetus and increase during the first day after birth (2, 3). The postnatal emergence of hepatic gluconeogenesis and fatty acid oxidation correspond to a shifting of specific limiting steps that have been well described. The emergence of hepatic gluconeogenesis in the newborn rabbit may result from a rapid fall in hepatic fructose 2,6-bisphosphate concentration (4) and from an increase in the activity of cytosolic phosphoenolpyruvate carboxykinase (2). Moreover, it has been shown that the postnatal increase in mitochondrial adenine nucleotide content stimulates pyruvate carboxylation causing a rapid increase in the rate of hepatic gluconeogenesis (5, 6). The development of hepatic fatty acid oxidation in the newborn rabbit is linked to modifications of the channeling of fatty acids in the liver (3) associated with profound changes in the characteristics of carnitine palmitoyltransferase I (7). It has been suggested that the postnatal increase in plasma glucagon concomitantly with the fall in plasma insulin which characterize many species including the rabbit (1, 8), are involved in the induction of hepatic gluconeogenesis and ketogenesis (9, 10).

Previous observations in the rat have shown that prolongation of gestation for 2 days induces changes in the circulating pancreatic hormone concentrations in the postmature fetus similar to those observed in the term newborn (11, 12). Nevertheless, the metabolic consequences of these hormonal alterations in the liver of the postmature fetus have not been investigated.

The purpose herein was to study the changes in pancreatic hormone concentrations in the plasma of postmature rabbit fetus and to measure the capacity for gluconeogenesis and fatty acid oxidation in isolated hepatocytes from postmature fetuses.

MATERIALS AND METHODS

Animals. Does from the New-Zealand White strain were fed a pelleted laboratory food *ad libitum* (% of energy: 33% protein, 55% carbohydrate, 12% fat) and had free access to water. Term in this strain is 32 days postcoitum. Gestation was prolonged by daily subcutaneous injections of progesterone (Roussel Uclaf, Romainville, France, 1.5 mg·kg^{-1}) from day 28 to 34. In preliminary control experiments, progesterone was injected from day 25 or 28 to day 31 of gestation. Fetuses were delivered by cesarean section either in the morning of day 32 (term fetus) or of day 35 (postmature fetus) and weighed. The isolation of hepatocytes or mitochondria was performed within 15 min after delivery or 24 h after birth on day 32 in newborns maintained unfed in an humidified incubator at 36° C (70% of relative humidity). Because the parameters measured in hepatocytes isolated from control term fetuses (fatty acid oxidation, gluconeogenesis from lactate) are identical whatever the gestational age at the first progesterone injection (day 25 or 28), fetuses born from mothers injected from day 28 of gestation were used as control animals.

Blood and liver sampling. Blood from one or two fetuses in each litter was sampled after cutting axillary vessels and was collected in heparinized tubes cooled in ice. Aliquots of whole blood were immediately deproteinized in ice-cold 6% (w/v)

Received July 31, 1987; accepted October 14, 1987.
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Supported in part by Grant CRE 867010 from the Institut National de la Santé et de la Recherche Médicale.

perchloric acid. Glucose, lactate, acetoacetate, and β-hydroxybutyrate were determined on neutralized perchloric filtrates. Plasma obtained after centrifugation at 4° C was stored at −20° C until insulin and glucagon determinations. Livers were removed immediately after blood sampling, frozen in liquid nitrogen, and stored at −20° C until analysis of glycogen and triacylglycerol concentrations.

Isolation and incubation of hepatocytes. Hepatocytes from three to four newborns were prepared by the reverse perfusion technique as described previously (2). Isolated hepatocytes (5×10^6) were incubated for 60 min in a shaking-water bath at 37° C in a final volume of 2 ml Krebs Henseleit bicarbonate buffer (pH 7.4). Before sealing, the flaskes were gassed with O_2:CO_2 (19:1) for 30 s.

Measurement of the rate of gluconeogenesis. Hepatocytes were incubated without addition of exogenous substrate for the determination of the rate of endogenous glucose production, or in the presence of lactate or dihydroxyacetone at a final concentration of 10 mmol/liter or of galactose at a final concentration of 5 mmol/liter. The incubations were ended by adding 0.25 ml perchloric acid 40% (v/v). Glucose, lactate, and pyruvate were determined in the neutralized perchloric extracts. Net glucose production for each substrate corresponds to the rate of glucose production measured in the presence of substrate minus the rate of glucose production without the substrate.

Metabolism of [1-^{14}C]oleate. Hepatocytes were incubated in the absence of exogenous substrate for the determination of the rate of endogenous ketone body production, or in the presence of 0.35 μCi of [1-^{14}C]oleate with oleate (1 mmol/liter) bound to 2% dialyzed albumin (fraction V, fatty acid free) plus L-carnitine (1 mmol/liter). For the measurement of oleate oxidation and ketogenesis, incubations were ended by adding 0.25 ml perchloric acid (40% v/v) as described previously (3). For the study of oleate esterification, incubations were ended by centrifugation for 30 s at $3000 \times g$ to separate cells from the medium. Cell lipids were extracted by 4 ml of chloroform:methanol (2:1 v/v) and an aliquot of this extract was separated by thin-layer chromatography as previously described (3).

Measurement of the rate of lipogenesis. Hepatocytes were preincubated for 30 min in the absence or in the presence of oleate (1 mmol/liter) and L-carnitine (1 mmol/liter) bound to dialyzed albumin. Then, 250 μCi of ^3H_2O were added and hepatocytes were further incubated for 30 min. The incubations were ended by centrifugation for 30 s at $5000 \times g$. Labeled fatty acids were extracted from the cell pellet as described by Stansbie *et al.* (13).

Isolation and incubation of liver mitochondria. Mitochondria from the livers of one or two newborns were isolated as described previously (7).

Assay of carnitine palmitoyltransferase I. CPT I activity was assayed at 30° C as described previously (7). Freshly isolated mitochondria (0.2 mg/ml) were first preincubated in the presence of 80 μmol/liter palmitoyl-CoA bound to 1% albumin for 3 min alone or in the presence of raising concentrations of malonyl-CoA from 0.01 to 150 μmol/liter and then further incubated 6 min in the presence of (2 μCi/μmol) [methyl-^{3}H]L-carnitine and L-carnitine 1 mmol/liter. The [^{3}H]palmitoylcarnitine formed was measured in the *n*-butanol extract as described by Bremer (14).

Analytical methods. Glucose, lactate, pyruvate, acetoacetate, and β-hydroxybutyrate were measured by using enzymic methods (15). Plasma insulin was measured by radioimmunoassay as described previously (16). Plasma glucagon was determined using the biodata glucagon kit (Biodata Laboratoire, Serono, Italy). Liver glycogen was assayed using α-amylo 1–4, 1–6 glucosidase according to Roehring and Allred (17). Liver triacylglycerol concentrations were determined as described by Le Marchand *et al.* (18). Proteins were determined by the method of Lowry *et al.* (19) using bovine serum albumin as standard.

Chemicals. All enzymes, substrates and cofactors were obtained from Boehringer Corp. (Meylan, France). Fatty acid free albumin, oleate, L-carnitine, and palmitoyl-CoA were purchased from Sigma (St. Louis, MO). ^3H_2O, [1-^{14}C]oleate and [methyl-^{3}H]L-carnitine were obtained from the Radiochemical Center Amersham (Bucks, England).

Statistics. Results are expressed as means ± SEM. Statistical analysis were performed using the Student's unpaired *t* test (two tailed).

RESULTS

The prolongation of normal gestation by 3 days produces fetuses with a higher body weight at delivery: 66 ± 2 g ($n = 20$) than in term control fetuses: 51 ± 2 g ($n = 16$) without alteration in the number of fetuses per litter (seven to nine). Fetal blood and liver parameters and the changes associated with prolonged gestation are shown in Table 1. In postmature fetuses, the liver glycogen and triacylglycerol concentrations are significantly decreased, by 60 and 40%, respectively. Blood glucose and ketone body concentrations are not different in both groups. In contrast, blood lactate concentration, which is already high in term fetuses, further increases (+22%) in postmature fetuses. Postmaturity is associated with a decrease in plasma insulin level and an increase in plasma glucagon level which result in a 3-fold decrease in the plasma insulin:glucagon molar ratio.

As shown in Table 2, the rates of endogenous glucose production in hepatocytes isolated from rabbit fetus depend on the gestation length. In hepatocytes from term fetuses, the rate of endogenous glucose production is high and markedly decreases in hepatocytes isolated 24 h after birth. In contrast, the rate of endogenous glucose production in postmature fetus is much lower. The net production of glucose from lactate or dihydroxyacetone is very low in fetal hepatocytes isolated after a normal gestation and it increases 6-fold during the first 24 h after birth. In contrast, gluconeogenesis from galactose is already well developed in term fetus. The rates of gluconeogenesis from dihydroxyacetone or galactose in hepatocytes isolated from postmature fetus are very similar to the rates measured in 24-h-old newborns, whereas the rate of gluconeogenesis from lactate remains slightly lower than the value obtained in 24-h-old newborn hepatocytes.

Table 1. *Effect of postmaturity on rabbit fetal liver glycogen and triglyceride concentrations, and metabolite or pancreatic hormone concentrations**

	Term fetus	Postmature fetus
Liver energy stores (mg/g)		
Glycogen	37 ± 5	14 ± 3†
Triacylglycerol	115 ± 16	68 ± 8†
Blood metabolites (mmol/liter)		
Glucose	3.93 ± 0.17	3.37 ± 0.40
Lactate	6.24 ± 0.38	8.10 ± 0.40†
Total ketone bodies	0.08 ± 0.01	0.14 ± 0.02
Plasma hormones		
Insulin (μU/ml)	83 ± 9	46 ± 5†
Glucagon (pg/ml)	193 ± 28	288 ± 30‡
Molar ratio I:G	11.1 ± 2.2	3.9 ± 0.4†

* Liver from term fetus (32 days) or postmature fetus (35 days) were removed and immediately stored at −20° C until assay. Blood of one or two fetuses per litter was immediately sampled from axillary vessels. Glucose, total ketone body (acetoacetate + β-hydroxybutyrate) and lactate concentrations were assayed on the whole blood, while insulin and glucagon were determined on plasma fraction. Values are means ± SEM of 8–16 samples (blood or liver) performed in duplicate.

† $p < 0.01$ when compared with term fetus.

‡ $p < 0.05$ when compared with term fetus.

Table 2. *Effect of postmaturity on endogenous glucose production and gluconeogenesis from lactate, dihydroxyacetone, or galactose in isolated hepatocytes from term fetus (32 days) postmature fetus (35 days) or 24-h-old rabbit born on day 32 of gestation**

Animals	Endogenous glucose production	Net glucose production ($nmol \cdot h^{-1} \cdot 10^6$ cells^{-1}) from		
		Lactate	Dihydroxyacetone	Galactose
Term fetus	361 ± 58	30 ± 13	32 ± 8	150 ± 21
24-h-old newborn	18 ± 4†	196 ± 15†	191 ± 19†	240 ± 41‡
Postmature fetus	87 ± 24†	114 ± 15†	221 ± 22†	243 ± 28‡

* Hepatocytes were incubated for 1 h at 37° C in the absence or in the presence of lactate 10 mM, dihydroxyacetone 10 mM, or galactose 5 mM. Values are means ± SEM of six experiments performed in duplicate. Net glucose production is obtained by substracting endogenous glucose production.

† $p < 0.01$ when compared with term fetus.

‡ $p < 0.05$ when compared with term fetus.

The rates of lactate plus pyruvate production from dihydroxyacetone in isolated hepatocytes have also been measured. The apparent amount of dihydroxyacetone metabolized is 3-fold higher in postmature than in term fetus (Table 3). Furthermore, the rate of lactate plus pyruvate production from dihydroxyacetone, as expressed in percentage of the amount of dihydroxyacetone metabolized, is higher in normal term fetus whereas the relative rate of glucose production predominates in postmature fetus. It is noteworthy that the endogenous production of lactate plus pyruvate (*i.e.* without addition of exogenous precursor) is higher in hepatocytes from term fetus (303 ± 71 $nmol \cdot h^{-1} \cdot 10^6$ cells^{-1}, $n = 8$) than in postmature fetus (52 ± 15 $nmol \cdot h^{-1} \cdot 10^6$ cells^{-1}, $n = 9$).

As shown in Table 4, the rates of ketone body production from endogenous sources or from oleate are very low in hepatocytes isolated from term fetus and markedly increase within the first day after birth. The rate of hepatic lipogenesis decreases by 95% between birth and 24 h after birth. In postmature fetus, the rate of endogenous ketogenesis or the rate of ketogenesis in the presence of oleate is 4- to 5-fold higher than in the term fetus. However, this enhanced capacity for ketogenesis remains slightly lower than the capacity measured in hepatocytes from 24-h-old newborns. It is noteworthy that this increased capacity for ketogenesis in hepatocytes from postmature fetuses is observed despite a significant higher rate of lipogenesis measured from endogenous sources or in the presence of oleate (Table 4).

The metabolic fate of [1-^{14}C]oleate in the presence of exogenous oleate (1 mmol/liter) is presented in Table 5. Total [1-^{14}C] oleate metabolized is similar both in term and postmature fetuses and in 24-h-old rabbits allowing comparisons of the metabolic distribution of labeled oleate into CO_2, acid-soluble products, or triacylglycerols. In term fetus, only 8% of [1-^{14}C]oleate is oxidized while 92% is esterified. This distribution is profoundly modified in 24-h-old newborn where oleate oxidation is increased 6-fold and oleate esterification is decreased by 40%. This metabolic distribution is also modified in postmature fetus inasmuch as oleate oxidation is increased 4-fold and oleate esterification is decreased by 26%, values slightly lower than the corresponding values observed in 24-h-old newborn hepatocytes.

The activity of CPT I and its inhibition by malonyl-CoA have been determined in freshly isolated mitochondria (Table 6). In all experiments, the CPT I activity is completely inhibited by a high concentration of malonyl-CoA (0.15 mmol/liter), suggesting that the activity of CPT II does not interfere with the measurement of CPT I activity. CPT I activity is similar in term or postmature fetus but is lower than in liver mitochondria of 24-h-old newborn rabbit. The concentration of malonyl-CoA necessary to suppress the activity of CPT I by 50% (IC_{50}) is 30-fold lower in term fetus than in 24-h-old newborn. Nevertheless IC_{50} for malonyl-CoA increases 4-fold in mitochondria of postmature fetus compared with term fetus.

Table 3. *Rates of glucose and lactate + pyruvate production from dihydroxyacetone in isolated hepatocytes from term fetus (32 days) or postmature rabbit fetus (35 days)**

	Term fetus	Postmature fetus
Amount of dihydroxyacetone metabolized† ($nmol \cdot h^{-1} \cdot 10^6$ cells^{-1})	219 ± 77	696 ± 64‡
% glucose	31 ± 6	66 ± 2‡
% lactate + pyruvate	69 ± 6	34 ± 2‡

* Hepatocytes were incubated for 1 h at 37° C in the presence of dihydroxyacetone 10 mM. Values are means ± SEM of six experiments performed in duplicate.

† Calculated as [2 × (glucose produced$\cdot h^{-1} \cdot 10^6$ cells^{-1}) + (lactate + pyruvate produced$\cdot h \cdot^{-1} 10^6$ cells^{-1})].

‡ $p < 0.01$ when compared with term fetus.

Table 4. *Rate of ketone body production and lipogenesis in isolated hepatocytes from term (32 days) or postmature fetus (35 days) and 24-h-old rabbit born on day 32 of gestation**

Animals	Ketogenesis ($nmol \cdot h^{-1} \cdot 10^6$ cells^{-1})		Lipogenesis ($nmol \cdot 30\ min^{-1} \cdot 10^6$ cells^{-1})	
	Endogenous	+ Oleate	Endogenous	+ Oleate
Term fetus	18 ± 12	26 ± 9	16 ± 1	8 ± 3
24-h-old newborn	206 ± 9†	236 ± 18†	1 ± 1†	Undetectable
Postmature fetus	68 ± 11‡	138 ± 8†	8 ± 1†	9 ± 1

* Hepatocytes were incubated for 1 h at 37° C in the absence or in the presence of oleate 1 mM + carnitine 1 mM. Lipogenesis was measured from tritiated water (250 µCi/flask) added at 30 min. Values are means ± SEM of six experiments performed in duplicate.

† $p < 0.01$ when compared with term fetus.

‡ $p < 0.05$ when compared with term fetus.

DISCUSSION

The present data demonstrate that the capacities for hepatic gluconeogenesis and fatty acid oxidation are significantly increased during postmaturity in the rabbit fetus, whereas these capacities are normally low in the term fetus and increase only within the first 24 h after birth (2, 3). As gestation was prolonged in the rabbit by daily injections of progesterone, it was necessary to discard an eventual effect of this hormone on the fetal liver metabolism. Term control fetuses were obtained from mother injected with progesterone from the same stage of gestation, *i.e.* during 4 days before normal term. In these term fetuses, hepatic glycogen and triglyceride concentrations are high as previously reported (3, 8). Term fetuses also exhibit high plasma insulin and low plasma glucagon levels as already shown (8). Moreover, despite the progesterone treatment undergone by the doe, the metabolic capacity of the liver of term fetus is unaffected. Active glycolysis and lipogenesis are concomitant with a low capacity for gluconeogenesis and for fatty acid oxidation. Moreover, term control fetuses obtained from mother injected with progesterone during an identical period of 7 days (*i.e.* from day 25 to 31) are also characterized by a low hepatic capacity for gluconeogenesis and for fatty acid oxidation (data not shown).

Table 5. *Effect of postmaturity on [1-^{14}C]oleate metabolism in isolated hepatocytes from term (32 days) or postmature fetus (35 days) and 24-h-old rabbit born on day 32 of gestation**

Animals	[1-^{14}C]oleate metabolized (dpm·10^3·h^{-1}·10^6 cells^{-1})	Metabolic distribution of [1-^{14}C]oleate metabolized (%)		
		CO_2	Acid-soluble products	Triacylglycerols
Term fetus	29.2 ± 4.4	3.2 ± 0.4	4.8 ± 1.9	92.0 ± 2.3
24-h-old newborn	29.8 ± 3.8	2.2 ± 0.4	45.0 ± 3.6†	52.8 ± 4.0†
Postmature fetus	31.6 ± 1.6	6.4 ± 0.7†	27.7 ± 1.9†	65.9 ± 2.1†

* Hepatocytes were incubated for 1 h at 37° C in the presence of [1-^{14}C]oleate 1 mM (0.35 μCi/flask) bound to 2% fat-free albumin. Carnitine 1 mM was added in each experiment. The term "oleate metabolized" represents the sum of dpm recovered in each fraction, *i.e.* acid-soluble products, CO_2 and triacylglycerols. Values are mean ± SEM of six experiments performed in duplicate.

† $p < 0.01$ when compared with term fetus.

Table 6. *Effect of postmaturity on hepatic carnitine palmitoyltransferase I (CPT I) activity and sensitivity to malonyl-CoA**

Animals	CPT I activity [nmol·min^{-1}·(mg protein)$^{-1}$]	IC_{50} for inhibition by malonyl-CoA (μM)
Term fetus	0.89 ± 0.13	0.03 ± 0.01
24-h-old newborn	1.87 ± 0.13†	0.75 ± 0.10‡
Postmature fetus	0.96 ± 0.07	0.12 ± 0.02‡

* Liver mitochondria were isolated from term (32 days) or postmature fetus (35 days) and from 24-h-old rabbit born on day 32 of gestation. CPT I activity was measured in the presence of palmitoyl-CoA 80 μM and [methyl-^{3}H]carnitine 1 mM (2 μCi/μmol). The term "IC_{50}" refers to the concentration of malonyl-CoA giving 50% inhibition of CPT I activity. Values are means ± SEM of six determinations performed in duplicate.

† $p < 0.05$ when compared with term fetus.

‡ $p < 0.01$ when compared with term fetus.

These metabolic characteristics of the fetal liver are profoundly modified during postmaturity. Hepatocytes isolated from rabbit postmature fetus display high rates of glucose production from various substrates. Inasmuch as lactate, dihydroxyacetone, and galactose enter the gluconeogenic pathway at different levels, this study allows to check the rate-limiting steps in the development of this metabolic pathway in the postmature rabbit (4). With galactose as a substrate, a maximal rate of glucose production, when compared with 24-h-old newborns, is present in the hepatocytes of postmature fetus, which underlines that the futile cycle between glucose-6-phosphate and glucose is displaced toward glucose synthesis. The rates of glucose and of lactate plus pyruvate production from dihydroxyacetone may reflect the apparent amount of dihydroxyacetone used as C_3 units and its partitioning into the pathways of gluconeogenesis and glycolysis. The present data suggest that the high rate of glucose production from dihydroxyacetone in the postmature fetus arises primarily from a reduced glycolytic flux through inhibition of phosphofructokinase and pyruvate kinase. A similar shift of glycolysis and gluconeogenesis is also achieved in hepatocytes isolated from 24-h-old newborn rabbits. This is consistent with a decrease in the hepatic fructose 2,6-bisphosphate concentration (4), secondary to the drop in the hepatic glycogen concentration (Tables 1 and 2). Although the maximal rate of glucose production in the presence of lactate is not yet reached in the hepatocytes isolated from postmature fetus, the data show a high capacity for gluconeogenesis from this precursor which enters the pathway below phosphoenolpyruvate (Table 2), suggesting a high flux through cytosolic phosphoenolpyruvate carboxykinase (2). In agreement with this, the activity of cytosolic phosphoenolpyruvate carboxykinase is markedly increased in the liver of postmature rat fetus (20–23). However, the fact that the rate of glucose production from lactate is not maximal in the postmature fetus could be due to a lower tissue oxygenation as suggested by other experiments (5, 6, 24).

The rates of ketogenesis from endogenous sources or from oleate are markedly increased in the hepatocytes isolated from postmature rabbit fetus but did not reach the maximal capacity observed in 24-h-old newborn hepatocytes. This result does not agree with previous data obtained in liver slices of the postmature rabbit fetus showing similar rates of ketone body production from palmitate to the term fetus (25). The emergence of ketogenic capacity in the liver of postmature fetus corresponds to an increased fatty acid oxidation at the expense of fatty acid esterification. Postmaturity induces in the fetal liver a maturation of the fatty acid oxidation pathway that normally appears few hours after birth in the term fetus (3). Such a reciprocal relationship between esterification and oxidation of long-chain fatty acids has also been observed in the adult rat during the fed-starved transition and it seems now well established that the principal site of control of hepatic fatty acid oxidation is located at the level of the carnitine palmitoyltransferase I, which catalyses the entry of fatty acids into mitochondria (10, 26, 27). More precisely, it has been shown that malonyl-CoA, the first committed intermediate in the conversion of carbohydrate to fat, is a potent inhibitor for carnitine palmitoyltransferase I (28). Low malonyl-CoA concentrations are probably present in the liver of postmature fetus as suggested by the low hepatic glycogen concentration and the low rates of endogenous glycolysis and lipogenesis. This decrease in lipogenic capacity is likely the consequence of the fall in lipogenic enzyme activities because addition of lactate plus pyruvate to isolated hepatocytes from postmature rabbit fetus fails to increase the rate of lipogenesis (data not shown). Nevertheless, the difference in the rate of lipogenesis between term and postmature fetus cannot explain the large increase in ketone body production in the presence of oleate because it does not further decrease the rate of lipogenesis in the postmature fetus (see Table 4). The decrease in the sensitivity of carnitine palmitoyltransferase I to malonyl-CoA in the liver mitochondria of postmature fetus could contribute to the increased capacity for fatty acid oxidation, as it occurred after birth (7) or in adult rat during starvation (26, 27). Now, the present experiments underline that postmature rabbit fetuses continue to grow during a 3-day period after normal term without marked change in the fetal mortality. As previously shown in this species (29–31) or in the rat (11, 32, 33) this suggests that postmature fetus is in the fed state and that the placental transfer of nutrients and oxygen is sufficient to sustain fetal growth. Recently, a significant increase in the coefficient of oxygen and glucose extraction has been reported in the postterm rabbit (31). However, a lower placental transfer of fatty acids in the postterm rabbit cannot be excluded, as suggested by other data (34–36).

Postmaturity induces clear-cut changes in the concentrations of plasma insulin and glucagon which leads to a lower insulin:glucagon molar ratio in the postmature rabbit fetus. This change in fetal hormonal environment extends previous observations performed in the rat (11, 20, 21). These hormonal changes during postmaturity are similar, although less pronounced, than those occurring during the postnatal period in the rabbit (8). In adult rats, metabolic states (starvation, diabetes)

associated with a low plasma insulin:glucagon ratio or glucagon treatment induce a liver glycogen depletion and high rates of gluconeogenesis from lactate or dihydroxyacetone (37). Moreover, it has been shown that injection of glucagon or cAMP to fetal rats *in utero* causes a marked induction of mRNA for phosphoenolpyruvate carboxykinase which suggests a regulation of phosphoenolpyruvate carboxykinase synthesis by this hormone (9). Conversely, newborn rats of diabetic mothers exhibit a high plasma insulin:glucagon ratio associated with an impaired development of gluconeogenesis after birth (38, 39). Similarly, a decrease in the plasma insulin:glucagon molar ratio must favor a low lipogenic rate and a high rate of fatty acid oxidation (25, 40–42). Moreover, recent data suggest that glucagon stimulates ketogenesis by early activation of the activity of carnitine palmitoyltransferase I probably by phosphorylation through a cAMP-dependent protein kinase (43, 44). The factors responsible for the changes in plasma pancreatic hormones in the postmature rabbit fetus are unknown. However, a low plasma insulin:glucagon molar ratio might be a likely candidate for an enhancement of gluconeogenic and ketogenic capacity in fetal rabbit liver during postmaturity.

Acknowledgments. The authors thank B. Morel (Institut National de la Recherche Agronomique, Jouy-en-Josas) and G. Orain for their assistance in taking care of pregnant rabbits and I. Coquelet and M. Fernandez for the careful preparation of the manuscript.

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