The Development of Gluconeogenesis in Rat Liver

EXPERIMENTS *IN VIVO*

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1. The injection of substrate amounts of lactate into newborn rats produced an increase in the concentration of phosphoenolpyruvate in liver. Similar experiments with foetal rats showed no increase in phosphoenolpyruvate concentration although pyruvate formation was observed. 2. The administration of pyruvate to foetal rats was also without effect on the hepatic phosphoenolpyruvate concentration, although a 20-fold increase in this was observed when pyruvate was injected into newborn animals. 3. Analogous experiments with aspartate produced qualitatively similar differences between foetal and newborn rats. 4. When [^{14}C]-lactate, -pyruvate or -aspartate was injected into foetal or newborn rats incorporation of radioactivity into liver glucose was observed only in the newborn animals. 5. Lactate/pyruvate ratios of 21.3 in foetal liver and 13.5 in the livers of newborn rats indicated a relatively reduced environment in the cytosol of foetal liver. This difference in redox state was illustrated experimentally by a greater conversion of pyruvate into lactate and an increased formation of malate in foetal liver. 6. Although both the substrate-loading and tracer experiments indicated a block in gluconeogenesis in foetal liver at the stage of conversion of oxaloacetate into phosphoenolpyruvate, gluconeogenesis was also hindered by a highly reduced environment.

The importance of hepatic gluconeogenesis for the maintenance of blood glucose concentrations under conditions of starvation has been established by both isotopic tracer experiments *in vivo* and the measurement of adaptive changes in activities of key enzymes (Friedmann, Goodman & Weinhouse, 1965; Foster, Ray & Lardy, 1966). However, experiments *in vitro* with the developing rat liver have indicated that the appearance of the gluconeogenic pathway does not occur until after birth (Ballard & Oliver, 1963, 1965).

Concomitant with the demonstration of an active hepatic gluconeogenic pathway at birth, there is also a 25-fold increase in the activity of extra-mitochondrial phosphoenolpyruvate carboxykinase (EC 4.1.1.32) between term fetuses and 2-day-old animals (Ballard & Hanson, 1967). Inasmuch as all other enzymes involved in the conversion of pyruvate into glucose are present in foetal rat liver (Ballard & Hanson, 1967; Dawkins, 1966; Ballard & Oliver, 1962) it is implied that the appearance of this phosphoenolpyruvate carboxykinase in the cell cytosol is the initiating factor in the development of the functional metabolic pathway.

In this paper we have attempted to study the development of the gluconeogenic pathway *in vivo*.

In general, our findings support the experiments *in vitro* in that glucose synthesis from precursors such as pyruvate, lactate or aspartate cannot be detected in the foetal rat. Furthermore, results of injection of trace or load amounts of these precursors imply a block in the gluconeogenic sequence at the phosphoenolpyruvate carboxykinase reaction.

MATERIALS AND METHODS

Chemicals. DL-[3- ^{14}C]Lactate and [3- ^{14}C]pyruvate were purchased from New England Nuclear Corp., Boston, Mass., U.S.A.; DL-[3- ^{14}C]aspartate was from Mallinkrodt Nuclear Corp., Orlando, Fla., U.S.A.; NAD $^{+}$, NADH, and ATP were from P-L Laboratories, Milwaukee, Wis., U.S.A.; lactate dehydrogenase (EC 1.1.1.27), pyruvate kinase (EC 2.7.1.40), malate dehydrogenase (EC 1.1.1.37), aspartate aminotransferase (EC 2.6.1.1) and L-aspartate were from Boehringer (Mannheim) Corp., New York, N.Y., U.S.A.

Animals. Pregnant Wistar albino rats were obtained from Carworth Farms Inc., New City, N.Y., U.S.A. The foetuses were used when 21–22 days old and all newborn rats were 1–2 days after birth.

The technique used to maintain the foetal rats in an external environment was a modification of that used by Reade & Jenkin (1964). The pregnant rat was anaesthetized with ether and tied to a plastic board. Tracheotomy was performed and the animal was kept under light anaesthesia

with a mixture of oxygen and ether that was administered through the tracheal cannula. The pregnant rat was immersed in a bath in such a way that the abdomen was under a Ringer-Locke solution (Best & Taylor, 1943) maintained at 37°. A small incision in the abdominal wall was made and the uterus was spread out into the bath. The foetal rats were removed from the uterus by cutting through the uterine wall and the embryonic membranes but they were left connected to the mother through their umbilical cords. Each foetus was injected with 50 μ l. of a solution of 0.9% NaCl containing any of the tested compounds and the controls were injected with 0.9% NaCl only. At appropriate times the foetuses were cut off from the mother, their livers were excised and frozen immediately with liquid dichlorodifluoromethane.

For each determination of metabolites two livers were pooled and homogenized in 6% HClO₄ to give a final concentration of 4% HClO₄. The homogenates were centrifuged at 2° and the extracts neutralized with 4M-KHCO₃ to pH 6. The KClO₄ was removed by centrifugation at 2° and the supernatants were used for the measurements of pyruvate, phosphoenolpyruvate, lactate, aspartate and malate.

Assays of intermediates. Pyruvate was determined as described by Bücher, Czok, Lamprecht & Latzko (1965). After the addition of lactate dehydrogenase, the oxidation of NADH was followed at 340 nm. The subsequent addition of pyruvate kinase and ADP permitted the measurement of phosphoenolpyruvate as pyruvate (Czok & Eckert, 1965).

L-Lactate was measured by the method of Hohorst (1965a), aspartate as described by Pfeleiderer (1965), and malate as described by Hohorst (1965b).

Radioactivity experiments. Foetal rats were injected intraperitoneally with 50 μ l. of a solution of 0.9% NaCl containing 1 μ C of the appropriate labelled compound; controls were injected with 0.9% NaCl. Extracts were prepared as described above and were chromatographed on Dowex 1 (formate form). The elution from this ion-exchange resin was accomplished with a pH gradient of 0.6M-formic acid as described by Busch, Hurlbert & Potter (1952). Fractions (4 ml.) were collected and radioactivity was determined in 0.5 ml. portions by liquid-scintillation counting with Diotol (Herberg, 1953) as solvent. The chromatographic method separated glucose, aspartate, lactate, pyruvate and malate. Neutral and basic amino acids were eluted with glucose in the void volume. Separation of these amino acids was carried out with a Beckman model 120 amino acid analyser by the method of Moore, Spackman & Stein (1958).

RESULTS

Experiments with liver slices and direct enzyme assays have shown that the gluconeogenic pathway is functioning at minimal rates in the foetus (Ballard & Oliver, 1963, 1965) and suggested that a block in the pathway exists at the phosphoenolpyruvate carboxykinase step. To study the pathway *in vivo*, term foetuses were injected intraperitoneally with 200 μ moles of lactate/animal. The lactate concentration (Fig. 1) increased up to 15 min. and then decreased. A twofold increase in pyruvate concentration was observed but there was no change in the concentration of phosphoenol-

Fig. 1. Effects of injection of sodium lactate (200 μ moles/animal) on the concentration of lactate (●), pyruvate (○) and phosphoenolpyruvate (▲) in livers of (a) foetal or (b) 1-day-old rats. Values are the average of determinations in three experiments. Other details are given in the Materials and Methods section.

pyruvate. These unchanged concentrations of phosphoenolpyruvate suggest a possible block before phosphoenolpyruvate in the metabolic sequence, but do not exclude a high rate of the gluconeogenic pathway and thus a rapid removal of this intermediate. Term foetuses were therefore injected with [3-¹⁴C]lactate intraperitoneally, and at various times livers were excised, two or three were pooled and extracts were chromatographed on Dowex 1 (formate form). No radioactivity was incorporated into glucose from lactate (Table 1). Most of the radioactivity recovered in the liver was present as lactate, but some was in alanine, presumably owing to transamination of pyruvate. There was some conversion of lactate into aspartate and β -hydroxybutyrate.

At 5 min. after injection of 200 μ moles of lactate into each newborn rat the hepatic lactate concentration increased 25-fold and it reached a maximum at 30 min. (Fig. 1). Pyruvate and phosphoenolpyruvate concentrations were also increased. To compare the metabolism in the 1-day-old animal with that of the foetus we must consider the pool sizes of metabolites at both ages. As seen in Table 2, hepatic pyruvate, lactate, phosphoenolpyruvate, aspartate and malate concentrations were measured under normal conditions. No differences between foetal and newborn rats were found in the pyruvate, aspartate or phos-

Table 1. *Incorporation in vivo of [3-¹⁴C]lactate into liver metabolites*

Incorporation is expressed as percentage of the injected radioactivity recovered in the liver. Values are means $\pm$ S.E.M. for determinations in three experiments. Other details are given in the Materials and Methods section.

Age	Measurement	Time after injection of ¹⁴ C (min.)		
		10	25	45
21-day foetus	Percentage of radioactivity in liver	6.89 $\pm$ 2.00	3.97 $\pm$ 1.06	1.82 $\pm$ 0.60
	Percentage in liver as:			
	glucose	< 0.5	< 0.5	< 0.5
	alanine + asparagine + glutamine	11.4 $\pm$ 1.18	11.7 $\pm$ 1.69	22.9 $\pm$ 2.0
	aspartate + β -hydroxybutyrate	2.85 $\pm$ 0.48	4.10 $\pm$ 0.97	12.4 $\pm$ 2.4
	pyruvate	1.46 $\pm$ 0.67	0.73 $\pm$ 0.13	1.99 $\pm$ 0.02
	lactate	76.0 $\pm$ 5.7	76.3 $\pm$ 10.4	39.7 $\pm$ 6.9
1-day-old rat	Percentage of radioactivity in liver	2.27 $\pm$ 0.85	1.48 $\pm$ 0.26	0.91 $\pm$ 0.22
	Percentage in liver as:			
	glucose	15.4 $\pm$ 4.8	36.0 $\pm$ 5.5	46.3 $\pm$ 9.4
	alanine + asparagine + glutamine	1.30 $\pm$ 0.44	3.07 $\pm$ 0.47	4.30 $\pm$ 1.08
	aspartate + β -hydroxybutyrate	6.83 $\pm$ 4.38	7.67 $\pm$ 2.45	9.33 $\pm$ 2.34
	pyruvate	< 0.5	< 0.5	< 0.5
	lactate	59.0 $\pm$ 19.8	41.7 $\pm$ 15.6	22.7 $\pm$ 2.0

Table 2. *Metabolites in liver of foetal and newborn rats*

Values are from four experiments and are expressed as means $\pm$ S.E.M. The NAD⁺/NADH ratio was calculated from the lactate/pyruvate ratio as described by Williamson, Lund & Krebs (1967).

Intermediate	Concn. of metabolite (μ moles/g. wet wt.)	
	Term foetus	1-day-old rat
Pyruvate	0.053 $\pm$ 0.006	0.074 $\pm$ 0.017
Lactate	11.8 $\pm$ 2.2	1.00 $\pm$ 0.22
Phosphoenolpyruvate	0.020 $\pm$ 0.001	0.029 $\pm$ 0.001
Aspartate	0.48 $\pm$ 0.05	0.45 $\pm$ 0.14
Malate	0.75 $\pm$ 0.14	0.21 $\pm$ 0.03
Lactate/pyruvate ratio	213	13.5
NAD ⁺ /NADH ratio	42	667

phoenolpyruvate concentrations, but the lactate concentration in the foetus was 11.8 times that in the newborn rat. The malate concentration in livers of term foetuses was about 3.5 times that in those from 1-day-old animals. To take into account the differences in the size of lactate pools between foetal and newborn animals, radioactivity-injection experiments were carried out in newborn animals in the presence of 200 μ moles of unlabelled precursor. The lactate concentrations in the newborn were increased to values that were similar to those found in foetal liver (compare Figs. 1a and 1b). By 45 min. 46% of the recovered radioactivity in the liver was in glucose (Table 1). The incorporation into neutral and basic amino acids was lower than in the foetus, but considerable radioactivity was found in lactate.

Fig. 2. The effects of injection of sodium pyruvate (200 μ moles/animal) on the concentration of lactate (●), pyruvate (○) and phosphoenolpyruvate (▲) in livers of (a) foetal or (b) 1-day-old rats. Values are the average of determinations in three experiments. Other details are given in the Materials and Methods section.

To bypass a possible limiting step at the conversion of lactate into pyruvate, 100 μ moles of pyruvate were injected into foetuses and newborn animals, and concentrations of pyruvate, lactate and phosphoenolpyruvate measured. Although the concentration of pyruvate in foetal liver was increased more than 100-fold, there were no significant

Table 3. *Incorporation in vivo of [3-¹⁴C]pyruvate into liver metabolites*

Incorporation is expressed as percentage of the injected radioactivity recovered in the liver. Values are means $\pm$ s.e.m. for determinations in three experiments. Other details are given in the Materials and Methods section.

Age	Measurement	Time after injection of ¹⁴ C (min.)		
		10	25	45
21-day foetus	Percentage of radioactivity in liver	5.2 $\pm$ 0.3	3.6 $\pm$ 0.3	3.1 $\pm$ 0.4
	Percentage in liver as:			
	glucose	< 0.5	< 0.5	< 0.5
	alanine + asparagine	6.2 $\pm$ 1.3	8.1 $\pm$ 4.7	9.8 $\pm$ 6.7
	aspartate + β -hydroxybutyrate	7.0 $\pm$ 0.6	11.0 $\pm$ 0.4	12.7 $\pm$ 3.0
	lactate	32.5 $\pm$ 10.6	31.4 $\pm$ 11.4	20.3 $\pm$ 7.2
1-day-old rat	pyruvate	0.96 $\pm$ 0.20	1.4 $\pm$ 0.85	< 0.5
	Percentage of radioactivity in liver	8.2 $\pm$ 0.3	4.6 $\pm$ 0.8	3.2 $\pm$ 0.5
	Percentage in liver as:			
	glucose	42.3 $\pm$ 4.3	47.3 $\pm$ 6.3	47.6 $\pm$ 7.7
	alanine + asparagine	3.7 $\pm$ 0.3	4.1 $\pm$ 0.6	4.1 $\pm$ 0.7
	aspartate + β -hydroxybutyrate	23.0 $\pm$ 3.0	18.0 $\pm$ 4.6	21.7 $\pm$ 8.8
	lactate	7.4 $\pm$ 1.2	3.2 $\pm$ 0.7	2.9 $\pm$ 1.6
	pyruvate	1.9 $\pm$ 0.2	1.2 $\pm$ 0.2	< 0.5

changes in concentrations of phosphoenolpyruvate (Fig. 2). A limiting step at the lactate dehydrogenase reaction is therefore unlikely in foetal liver. In addition, the experiment eliminates the possibility of pyruvate kinase-catalysed formation of phosphoenolpyruvate. In the newborn animal, however, a 100-fold increase in pyruvate concentration was accompanied by a 20-fold increase in that of phosphoenolpyruvate. This increase in phosphoenolpyruvate concentration suggests that in the newborn rat there is no block in the gluconeogenic pathway before phosphoenolpyruvate.

To study the overall pathway from pyruvate, 1 μ c of [3-¹⁴C]pyruvate was injected into each foetus and the appearance of radioactivity in glucose was followed against time. No radioactivity was incorporated into glucose up to 45 min. (Table 3). About 40% of the pyruvate was converted into lactate. On the other hand, when the newborn rat was injected with [3-¹⁴C]pyruvate 50% of the radioactivity in the liver was found in glucose (Table 3) but little radioactivity was recovered in lactate. No carrier pyruvate was injected since the concentrations of pyruvate in the livers of fetuses and newborn rats were similar. The difference between the percentages of pyruvate converted into lactate in the foetus and the newborn rat may be attributed to the differences in the respective NAD⁺/NADH ratios in the cytoplasm (see Table 2).

To investigate the possibility of a block at the pyruvate carboxylase reaction, aspartate was used as a gluconeogenic precursor. After each foetus was injected with 100 μ moles of aspartate, aspartate concentration increased 25-fold in the liver. No significant increase in pyruvate concentration was

Fig. 3. Effects of injection of sodium aspartate (100 μ moles/animal) on the concentration of aspartate (■), pyruvate (○), phosphoenolpyruvate (▲) and malate (□) in livers of (a) foetal or (b) 1-day-old rats. Values are the average of determinations in three experiments. Other details are given in the Materials and Methods section.

observed, since pyruvate is probably not normally a metabolic product of aspartate. Phosphoenolpyruvate concentrations did not change, in agreement with the existence of a metabolic block at phosphoenolpyruvate carboxykinase. If pyruvate

Table 4. *Incorporation in vivo of [3-¹⁴C]aspartate into liver metabolites*

Incorporation is expressed as percentage of the injected radioactivity recovered in the liver. Values are means $\pm$ s.e.m. for determinations in three experiments. Other details are given in the Materials and Methods section.

Age	Measurement	Time after injection of ¹⁴ C (min.)		
		10	25	45
21-day foetus	Percentage of radioactivity in liver	4.8 $\pm$ 0.3	2.9 $\pm$ 1.3	2.6 $\pm$ 1.3
	Percentage in liver as:			
	glucose	< 0.5	< 0.5	< 0.5
	asparagine + glutamine	1.9 $\pm$ 0.1	7.4 $\pm$ 1.8	12.7 $\pm$ 3.8
	lactate	2.20 $\pm$ 0.45	7.1 $\pm$ 2.1	8.7 $\pm$ 3.5
	malate	18.9 $\pm$ 4.6	17.5 $\pm$ 4.3	11.8 $\pm$ 3.1
	pyruvate	1.5 $\pm$ 0.2	2.25 $\pm$ 0.55	2.45 $\pm$ 1.35
	aspartate	63.7 $\pm$ 8.2	36.8 $\pm$ 12.3	29.50 $\pm$ 11.9
1-day-old rat	Percentage of radioactivity in liver	1.95 $\pm$ 0.55	1.30 $\pm$ 0.10	1.45 $\pm$ 0.25
	Percentage in liver as:			
	glucose	34.0 $\pm$ 4.0	39.0 $\pm$ 4.0	45.0 $\pm$ 1.0
	asparagine + glutamine	1.95 $\pm$ 1.0	2.05 $\pm$ 0.95	2.7 $\pm$ 1.3
	lactate	3.55 $\pm$ 0.55	4.75 $\pm$ 0.35	3.80 $\pm$ 1.20
	malate	4.3 $\pm$ 3.7	1.4 $\pm$ 0.8	0.70 $\pm$ 0.20
	pyruvate	2.80 $\pm$ 0.40	1.40 $\pm$ 0.20	< 0.5
	aspartate	34.70 $\pm$ 6.30	32.50 $\pm$ 6.50	17.0 $\pm$ 1.0

carboxylase rather than phosphoenolpyruvate carboxykinase were rate-limiting, an increase in the concentration of phosphoenolpyruvate would be expected for gluconeogenesis with aspartate as substrate. When the newborn rat was injected with aspartate, the 15-fold increase in aspartate concentration was accompanied by a substantial increase in that of phosphoenolpyruvate (Fig. 3). Unlike the situation in the foetus, this injection of large amounts of aspartate caused increases in the concentrations of malate and pyruvate.

When [3-¹⁴C]aspartate was injected into foetal rats (Table 4), no incorporation into glucose was detected, whereas in the newborn rat 40% of the radioactivity in the liver was present as glucose. Table 4 also shows that the rate of aspartate metabolism in the liver of the foetus is lower than in that of the newborn. In addition, a block in the further metabolism of aspartate and malate can be inferred from the time-dependent changes in the radioactivity of malate. Although some radioactivity was recovered as malate in livers from newborn animals 10 min. after injection of [3-¹⁴C]-aspartate, this radioactivity occurred only transiently, but in the foetal liver 12% of the radioactivity found in the liver was present as malate 45 min. after injection. In all radioactivity-injection experiments, the radioactivity in livers of foetuses that had not been injected with ¹⁴C was negligible.

DISCUSSION

With the appearance of the gluconeogenic pathway at birth, phosphoenolpyruvate carboxykinase activity increases 25-fold in the extramitochondrial

fraction of liver (Ballard & Hanson, 1967). Yeung & Oliver (1967b) have shown a similar increase in the activity of phosphoenolpyruvate carboxykinase in prematurely delivered rats, but if the birth process is delayed so is the appearance of phosphoenolpyruvate carboxykinase activity. It can be concluded that birth in some way causes a stimulation of the enzyme activity at the time that the overall gluconeogenic pathway can first be detected.

The information available on the appearance of the gluconeogenic pathway during development comes from studies *in vitro* with liver slices (Ballard & Oliver, 1963, 1965; Yeung & Oliver, 1967a). It has been observed, however, that liver slices show decreased rates of gluconeogenesis from all precursors except glycerol compared with the rates observed in perfused livers (Ross, Hems & Krebs, 1967; Krebs, Notten & Hems, 1966). Furthermore, the rates of gluconeogenesis observed in perfused rat livers are within the same range as those determined from studies on whole animals (Holt, Schmidt, Feldmann & Hallmann, 1961; Baker, Shipley, Clark, Incefy & Skinner, 1961). For these reasons we attempted to study the gluconeogenic pathway *in vivo*. Technical problems, such as the cannulation of the extremely thin umbilical veins of the foetus, made the use of perfused foetuses or perfused foetal livers impossible. As a result, whole animals were used for the studies *in vivo*. We tried to maintain the foetuses in a state that is similar to that normally present *in vivo*. To this end, a minimum anaesthesia was used to limit hypoxia and the foetuses were maintained at 37° in a medium that approximated to the physiological fluid.

Possible control of gluconeogenesis by redox environment. In their measurements of gluconeogenesis in liver slices from foetal and newborn rats, Ballard & Oliver (1963, 1965) incubated the tissue under an atmosphere of $O_2 + CO_2$ (95:5). This atmosphere has been found advantageous for the penetration of oxygen into the inner cells of the liver slice, but it must be considered that this oxygen-rich environment might permit erroneous conclusions if oxygen is a limiting factor in gluconeogenesis *in vivo*. As transfer of oxygen from the maternal haemoglobin to the foetal haemoglobin in the placenta might be limiting *in vivo*, it is conceivable that the foetus could be in a relatively anoxic condition caused by low oxygen supply. This possible hypoxia may be a cause of the relatively reduced environment in the cytosol that is indicated by the lactate/pyruvate ratios reported for the foetal liver both in this paper and by Hornbrook, Burch & Lowry (1965). As the rapid removal of NADH at both the lactate dehydrogenase and cytosol malate dehydrogenase steps is necessary for the conversion of lactate into oxaloacetate, the reduced environment in foetal liver will probably hinder gluconeogenesis, at least from lactate. This suggestion has also been put forward to explain the differential effects of administration of ethanol on conversion of pyruvate and lactate into glucose (Kreisberg, 1967; Exton & Park, 1968; Forsander, Rih, Salaspuro & Maenp, 1965).

Control of the gluconeogenic pathway by the development of phosphoenolpyruvate carboxykinase activity in the liver cytosol. Notwithstanding the possible contribution of the redox state to the control of gluconeogenesis at the stage of conversion of lactate into oxaloacetate, the evidence is compelling that the initiation of the overall pathway is caused by the synthesis of cytosol phosphoenolpyruvate carboxykinase. However, a mitochondrial phosphoenolpyruvate carboxykinase activity is also present in foetal rat liver (Ballard & Hanson, 1967). The use of substrate amounts of lactate, pyruvate or aspartate to study the possible synthesis *in vivo* of glucose indicates that (1) an increase in phosphoenolpyruvate concentration does not occur in foetal rat liver, and (2) the mitochondrial phosphoenolpyruvate carboxykinase is not involved in the conversion of oxaloacetate into phosphoenolpyruvate as part of the gluconeogenic sequence in foetal rat liver. It has been suggested (Ballard & Hanson, 1967) that this mitochondrial phosphoenolpyruvate carboxykinase is involved in lipogenesis, a pathway that is very active in foetal liver. Both in newborn animals, as shown in this paper, and in adult rats, as described by Hornbrook *et al.* (1965), the injection of gluconeogenic substrates causes a considerable increase in the concentration of phosphoenolpyruvate.

The injection of isotopically labelled precursors of glucose in foetal rats clearly illustrates the lack of glucose synthesis *in vivo* from either lactate, pyruvate or aspartate. Freedman & Nemeth (1961) found that pyruvate carboxylation was low in foetal liver but much greater in the livers of newborn guinea pigs, a result consistent with low or negligible rates of gluconeogenesis in the foetal animals. In conflict with our conclusion that gluconeogenesis is absent *in vivo* in foetal rat liver, Jacquot, Kretchmer, Tsuboi, Taylor & McNamara (1961) found considerable incorporation of $[^{14}C]$ glycine into glycogen of foetal livers. However, these authors injected the radioactive isotope into the mother rather than directly into the foetuses, and we presume that glycine was converted into glucose before passage across the placenta. Furthermore, as both cytosol and mitochondrial aspartate aminotransferase activities are present in foetal rat liver (Hanson & Ballard, 1968) the lack of conversion of aspartate into glucose excludes a control of gluconeogenesis before phosphoenolpyruvate carboxykinase. In the newborn rat, on the other hand, glucose synthesis from all precursors is rapid, so that the appearance of phosphoenolpyruvate carboxykinase completes the development of the gluconeogenic pathway.

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