ON THE DEVELOPMENT OF PHOSPHOENOLPYRUVATE CARBOXYKINASE AND GLUCONEOGENESIS IN GUINEA PIG LIVER

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SUMMARY: The activity of cytosol phosphoenolpyruvate carboxykinase is negligible in near term fetal guinea pig liver but the mitochondrial form of the enzyme is present in amounts comparable to that found in the adult. If Cesarean delivered newborns are examined 24 hours after delivery, the activity of the mitochondrial enzyme remains unchanged whereas the cytosol activity almost equals that in the mitochondria. Livers taken from near term fetuses and perfused immediately after Cesarean section synthesize glucose from lactate, pyruvate, and glycerol, and from lactate-pyruvate mixtures.

Complex metabolic changes take place during the perinatal period in mammals such as the appearance, for the first time, of various functional metabolic pathways. Developmental studies of gluconeogenesis have focused primarily on a single specie, the rat, in which the capacity for gluconeogenesis is virtually absent during fetal life and is acquired only after birth (1-4). This acquisition coincides with an abrupt decline in fatty acid synthesis from glucose and with the postnatal induction of cytosol phosphoenolpyruvate carboxykinase[2] (EC 4.1.1.32) (5), the predominant form of this enzyme in rat liver. The induction of PEPCK in neonatal and adult rat liver, is influenced by a variety of hormonal and dietary factors (6-11) and its initial formation during development appears to be part of an intricate mechanism that triggers the initiation of hepatic gluconeogenesis at birth (12-14).

In contrast to the rat, relatively little is known concerning the initiation and development of gluconeogenesis in other animals. In addition to the cytosol enzyme, many mammalian species have a mitochondrial PEPCK which is not inducible (15) and which has been shown to make significant contribution to overall glucose

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[2]Abbreviation, PEPCK: phosphoenolpyruvate carboxykinase.

production (16, 17). This paper describes the presence of PEPCK in fetal guinea pig liver mitochondria and shows that perfused fetal guinea pig livers can synthesize glucose from appropriate substrates.

MATERIALS AND METHODS: Hartley strain pregnant guinea pigs were obtained 10 - 15 days prior to term from Hilltop Farms Inc., Scottdale, Pennsylvania. About 2 - 5 days prior to normal delivery, fetuses were removed by Cesarean section under light ether anesthesia. Livers from these animals were perfused immediately after delivery using the non-recirculating system described elsewhere (16). Perfused livers were in the range of 2 to 5 g. Perfusate flow was maintained at 8-15 ml/min and carefully controlled to avoid disruption of the liver by hydrostatic pressure. Glucose in the perfusate was measured as described previously (16). Litter mates not perfused immediately after Cesarean section were placed in a Humidicrib at 37° until used for other experiments. For enzyme measurements livers were homogenized in 0.25 M sucrose (18). PEPCK activity was measured by the ^{14}C-bicarbonate fixation assay as described by Ballard and Hanson (5). One unit of activity equals the incorporation of 1 umole of $NaH^{14}CO_3$/min at 37°. Glutamate dehydrogenase activity was measured at 25° in the direction of NADH oxidation by the method of Schmidt (19) in the presence of 2 umoles of ADP per assay tube. One unit of activity is defined as the oxidation of 1 umole of NADH/min at 25°.

RESULTS AND DISCUSSION: The activity of PEPCK in fetal guinea pig liver cytosol was 0.7 unit/g and was not substantially altered when measured in livers perfused for up to 2 hours immediately following Cesarean delivery of the fetuses (Table 1). When corrected for mitochondrial damage and contamination of the cytosol fraction by the use of glutamate dehydrogenase as a mitochondrial marker, it is apparent that fetal guinea pig liver, like the rat liver (5), virtually lacks cytosol PEPCK. In contrast, PEPCK activity was present in fetal guinea pig liver mitochondria in amounts comparable to that found in the adult. For comparison the distribution pattern found in the adult liver by Garber and Hanson (20) has been included in Table 1.

In parallel experiments litter mates were housed in a Humidicrib at 35-37° and the pattern of distribution of hepatic PEPCK was examined 24 hours later. The results of such experiments (Table 1) show that the cytosolic activity which was originally negligible in the fetal tissue equalled that in the mitochondrial fraction 24 hours post delivery. It should be pointed out that except for water, these newborns had no access to food and therefore the cytosol activity noted reflects the initial postnatal emergence of the enzyme plus any inductive effect of starvation. It has been shown repeatedly that this form of the enzyme increases

Table 1

Phosphoenolpyruvate Carboxykinase and Glutamate Dehydrogenase Activities in Liver of Fetal, Newborn and Adult Guinea Pigs

Source	Cytosol PEPCK	Cytosol GDH[a]	Mitochondria PEPCK	Mitochondria GDH
		Units/g wet weight		
Fetus (non-perfused)	0.72 ± 0.10	15.1 ± 3.0	5.35 ± 0.6	171 ± 20
(perfused)	0.81 ± 0.25	17.9 ± 3.3	5.3 ± 0.2	214 ± 34
Newborn (fasted, 24 hr)	7.3 ± 0.7	16.6 ± 1.3	7.1 ± 0.4	235 ± 34
Adult[b] (fed)	1.8		5.1	
(fasted, 48 hrs)	4.1		5.5	

Values for the fetal animals are the mean ± S.E. for 6 livers for PEPCK and 3 for GDH. Perfused fetal livers were the same as those used for the studies in figs. 1 and 2. Values for newborn animals are the mean ± S.E. for 5 livers.
a. Glutamate dehydrogenase

b. Taken from Ref. 20 for comparison.

in activity during starvation (6-8, 20). The results obtained with livers of 24 hour-old newborns resemble those obtained with starved adult guinea pigs in which hepatic PEPCK is almost equally distributed between the mitochondria and the cytosol (20).

The overall gluconeogenic capacity of fetal tissue must be assessed not only by measurement of gluconeogenic enzyme activity but also by determining the ability of the tissue to synthesize glucose from suitable substrates. When fetal guinea pig livers were perfused in the presence of gluconeogenic substrates immediately after delivery of the fetuses, they synthesized substantial amounts of glucose at low perfusate concentrations of pyruvate, lactate, and glycerol (Fig. 1). At 2 mM substrate concentration, for example, these livers synthesized 0.3 and 0.4 umole of glucose/min/g wet weight from lactate and glycerol, respectively. The

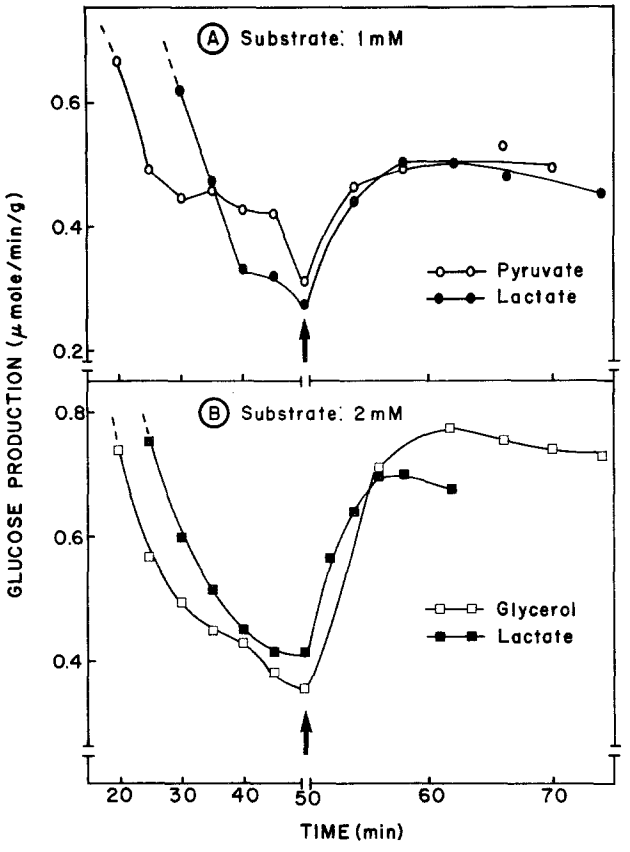

FIG. 1. Gluconeogenesis by isolated perfused livers from fetal guinea pigs. Livers were perfused in a non-recirculating fashion with Krebs-Ringer Bicarbonate buffer, pH 7.4, at 30-32°. After a 50 min wash-out to reduce the level of endogenous glucose release, the substrates were introduced into the perfusion system at the points indicated by the arrows and maintained at 1 (A) or 2 mM (B). Each point in A represents results from 3 livers. The glycerol and lactate curves in B were from 5 and 4 experiments respectively.

high levels of glucose during the wash-out period (Figs. 1 and 2) reflect the high glycogen content in fetal livers.

Fig. 2 shows the results of other perfusions with lactate-pyruvate mixture as substrate. In these experiments the rates were only marginally higher than those in Fig. 1 although the concentration of lactate in the mixture was 10 mM and were about equal to those observed in the perfused adult liver (17, 21). Slightly higher rates were obtained in livers from 24 hour-old newborns. Under

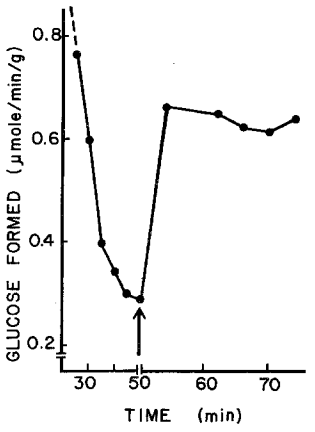

FIG. 2. Glucose production from lactate-pyruvate substrate mixture by fetal guinea pig livers. Livers were perfused as indicated in Fig. 1. The lactate-pyruvate mixture was a physiological ratio of lactate to pyruvate of 30:1 (20). The concentration of lactate in the mixture was 10 mM. The substrate was introduced at 50 min as indicated by the arrow.

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our experimental conditions perfusion does not alter the activity of PEPCK (see Table 1). If the activity data in Table 1 is corrected for mitochondrial damage it is apparent that the fetal guinea pig livers were synthesizing significant amounts of glucose (Figs. 1 and 2) in the virtual absence of cytosol PEPCK. Thus unlike the rat, fetuses from this species possess the capacity for gluconeogenesis.

In a recent abstract Jones and Ashton (22) reported the presence of pyruvate carboxylase and fructose-1,6-diphosphatase in fetal guinea pig liver several days before the end of gestation. In view of the results of the present study and of an earlier report (23) of measurable amounts of glucose-6-phosphatase in fetal guinea pigs 3-5 days prior to term, one must conclude that fetal guinea pig liver, unlike that of the rat (1,4), acquires the capacity for gluconeogenesis before birth. Whether this capacity is expressed *in utero* is not known. Recently Warnes *et al.* (24) showed that fetal lambs have susbtantial activities of gluconeogenic enzymes including PEPCK but do not synthesize glucose or glycogen from isotopically labeled lactate *in vivo*. They suggest that the highly oxidized redox states in fetal liver might be limiting gluconeogenesis *in utero*. Presently there

are no measurements of oxidation-reduction states in fetal liver of any species other than the rat. Our current working hypothesis is that the initiation and regulatin of gluconeogenesis in the perinatal period in species which have mitochondrial PEPCK is perhaps much more complicated than that hypothesized for the developing rat.

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REFERENCES

1. Ballard, F.J., and Oliver, I.T. (1963) Biochim. Biophys. Acta 71, 578-588.
2. Ballard, F. J., and Hanson, R.W. (1967) Biochem. J. 102, 952-958.
3. Yeung, D., and Oliver, I.T. (1967) Biochem. J. 103, 744-748.
4. Phillipides, H., and Ballard, F.J. (1967) Biochem. J. 113, 651-657.
5. Ballard, F.J., and Hanson, R.W. (1967) Biochem. J. 104, 866-871.
6. Shrago, E., Lardy, H.A., Nordlie, R.C., and Foster, D.O. (1963) J. Biol. Chem. 238, 3188-3192.
7. Ballard, F.J., and Hanson, R.M. (1967) J. Biol Chem. 242, 2746-2750.
8. Yeung, D., and Oliver, I.T. (1968) Biochemistry 7, 3231-3239.
9. Wicks, W.D., Lewis, W., and McKibbin, J.B. (1972) Biochim. Biophys, Acta 264, 177-185.
10. Treadow, B.R., and Khairallah, E.A. (1972) Nature (New Biol.) 239, 131-133.
11. Nagai, K., and Kagawa, H. (1972) J. Biochem. (Tokyo) 71, 125-131.
12. Greengard, O. In: Biochemical Actions of Hormones (G. Litwack ed.) Academic Press, NY. 1970. p. 53-87.
13. Ballard, F.J. (1971)Biochem. J. 121, 169-178.
14. Hanson, R. W., Reshelf, L., and Ballard, F.J. (1975) Federation Proc. 34, 166-171.
15. Hanson, R.W., and Garber, A.J. (1972) Am. J. Clin. Nutr. 25, 1010-1021.
16. Arinze, I.J., Garber, A.J., and Hanson, R.W. (1973) J. Biol. Chem. 248, 2266-2274.
17. Arinze, I.J., and Hanson, R.W. (1973) F.E.B.S. Lett 31, 280-283.
18. Garber, A.J., and Ballard, F.J. (1969) J. Biol. Chem 244, 4696-4703.
19. Schmidt, E. (1965) Methods in Enzymatic Analysis 2nd. ed. Academic Press, N.Y. p. 752-756.
20. Garber, A.J., and Hanson, R.W. (1971) J. Biol. Chem. 246, 589-598.
21. Ogata, K., Jomain-Baum, M., and Hanson, R.W. (1974) Biochem. J. 144, 49-57.
22. Jones, C.K., and Ashton, I.K. (1972) Biochem. J. 130, 23p-24p.
23. Nemeth, A. (1954) J. Biol. Chem. 208, 773-776.
24. Warnes D., Ballard F.J., and Seamark, R.F. (1974) J. Reprod. Fert. 36, 471-472.