The Development of Glucose Metabolism in Infants of Diabetic Mothers

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The concentrations of plasma glucose, lactate and phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32) were studied in caesarian-delivered newborn rats of diabetic mothers (IDM) and normal mothers, at time-intervals up to 6 hr after delivery. Glucose concentrations in plasma of cord blood of IDM were significantly higher than those in normal newborn rats. The glucose concentration of normal newborn rats decreased markedly during 1 hr after delivery and thereafter increased gradually. However, the glucose concentration of IDM decreased to a minimum at 4 hr after delivery and thereafter increased. Lactate concentration in plasma of cord blood was high at delivery. Plasma lactate concentration of normal newborn rats decreased rapidly during 2 hr after delivery. However, plasma lactate concentration of IDM increased during 1 hr after delivery and thereafter decreased markedly over the next 5 hr. Hepatic soluble PEPCK activity in caesarian delivered rats was low at birth. The activity of normal infants increased after a lag of 2 hr whereas the activity of IDM increased after a lag of 4 hr. The concentrations of plasma glucose and hepatic PEPCK activity were measured as a function of time after the administration of glucose (5 g/kg body weight) to caesarian-delivered newborn rats. The glucose concentration increased to a maximum at 2 hr after administration and decreased markedly over the next 2 hr. The development of enzyme activity was delayed in administered rats. The glucose concentration and hepatic PEPCK activity were measured as a function of time after the intraperitoneal injection of insulin (250 mU/rat) into caesarian-delivered rats from diabetic pregnant rats. The injection of insulin decreased plasma glucose concentration in newborn rats. The development of PEPCK in IDM was advanced by the injection of glucagon.

At birth supply of maternal glucose terminates and hypoglycemia occurs in newborn infants until gluconeogenesis initiates. Frequently severe hypoglycemia develops in infants of diabetic mothers (IDM) (McCann et al. 1966; Martin et al. 1975). It appears that hyperinsulinism in IDM caused this severe hypoglycemia (Rosita 1973). It has been also suggested that hypoglycemia immediately after
birth appears to be due to the high rate of glucose utilization (Snell and Walker 1973). So the rate of glucose production determines the seriousness of hypoglycemia at that time (Saheki et al. 1979). Therefore the development of gluconeogenic pathway in the immediate postnatal period (Ballard and Oliver 1963; Yeung and Oliver 1967) may be important to recovery from neonatal hypoglycemia. Kalahan et al. (1977) reported that the glucose production rate in infants of diabetic mother was lower than that of normal infants. In the present paper, we investigated the plasma glucose concentrations and the activity of hepatic phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32) in the newborn rats of diabetic pregnant rats to elucidate the effects of maternal diabetes on the development of gluconeogenesis in the newborn rats.

**MATERIALS and METHODS**

**Animals.** Virgin female Wistar rats weighing 250±50 g were divided in two groups. One group was injected intraperitoneally with streptozotocin (45 mg/kg body weight, Sigma Chemical Co., Lot no. 31P-0130) dissolved in citrate buffer (0.01 M, pH 4.0). Normal and diabetic virginal rats were mated and the presence of vaginal plugs and sperm was used to confirm and designate day 0 of pregnancy. Pregnant rats (21 day of gestation) were sacrificed by decapitation after slight ether anesthesia and the fetuses removed by caesarian section. The fetuses of normal and diabetic pregnant rats were maintained in an incubator at 37°C without feeding until the initiation of the study (0 to 6 hr after delivery). Over 6 hr after delivery fetuses were placed with a lactating foster-mother until the initiation of the study.

**Injection.** Glucose (5 g/kg body weight) dissolved in 50 μl of water was administered orally by tube feeding. Control group was administered water alone. 250 mU of insulin (Sigma Chemical Co., 25.5 IU/mg) or 50 μg of glucagon (Novo Industri A/S, Denmark, 1 USP/mg) dissolved in 0.9% NaCl was injected intraperitoneally by using a microsyringe into caesarian-delivered newborn rats. Control group was injected with 0.9% NaCl alone.

**Analysis of glucose, lactate and insulin in plasma.** Rats were killed at intervals by decapitation and blood samples were collected. The blood was heparinized and centrifuged immediately. Plasma glucose was measured by a glucose oxidase method (Glucose C-Test, Wako Pure Chemical Industries, Osaka). Plasma was deproteinized with HClO₄ and the L-lactate concentration was measured by the method of Hohorst (1963). Plasma insulin was measured by RIA using porcine insulin standard and antiserum directed against porcine insulin (Insulin-Riakit, Dainabo Co., Tokyo).

**PEPCK assay.** Liver was homogenized in a buffered iso-osmotic medium containing sucrose (0.2 M), triethanolamine (20 mM), glutathione (1 mM) and EDTA (1 mM) at pH 7.5 by using a coaxial homogenizer with Teflon pestle. The homogenate was centrifuged at 105,000 × g for 30 min. PEPCK was assayed by the method of Chang and Lane (1966). The incubation mixture contains imidazole chloride (100 mM pH 6.6), MnCl₂ (2 mM), phosphoenolpyruvate (15 mM), KHCO₃ (50 mM), 2 μCi of NaH¹⁴CO₃ (Amersham Corp., 0.1 mCi/mmol), 2 units of malate dehydrogenase, dithiothreitol (1 mM), NADH (2.5 mM) and enzyme (10 μl of supernatant). The assay was carried out for 20 min at 37°C, and the reactions were stopped by the addition of trichloroacetic acid to final concentration of 4% (w/v). After each reaction the tube was gassed with CO₂ for 3 min. The incorporation of [¹⁴C]bicarbonate into oxaloacetate was measured on a scintillation counter (Pakard Instrument, Co., Tricarb model 3385). Protein concentration was determined by the method of Lowry et al. (1951).
RESULTS and DISCUSSION

Plasma glucose concentrations and weight changes in the normal and diabetic gestational rats are shown in Table 1. Plasma glucose concentrations of diabetic rats were higher than those of normal rats. Table 2 shows litter size, body weights and concentrations of plasma glucose and insulin in 21-day fetuses from normal and diabetic pregnant rats. There was no difference in body weight between the normal fetuses and fetuses of diabetic pregnant rats (IDM). IDM had higher plasma glucose and IRI (immunoreactive insulin) levels as compared with normal fetuses.

Fig. 1 shows the time-course change in plasma glucose and lactate concentrations in caesarian-delivered normal fetuses and IDM after delivery. Glucose concentrations of normal fetuses decreased during 1 hr after delivery and thereafter increased, whereas those of IDM were high at delivery and decreased during 4 hr after delivery, and thereafter began to increase. Therefore the development of hypoglycemia and recovery from the hypoglycemia were about 2 hr late in IDM than in normal rats. Then plasma glucose gradually increased till 48 hr of age in suckled newborn rats (Fig. 1b). These changes of plasma glucose were the same in both groups. McCann et al. (1966) observed that plasma glucose concentration of IDM was substantially lower than that of normal infants in human in the first few hours of life. However, we could not find the significant difference between

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<tr>
<th>Table 1. Body weights and plasma glucose in normal and diabetic rats</th>
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<td><strong>Body weight (g)</strong></td>
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<td>Days of gestation</td>
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<td>Normal (n=5)</td>
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<td>Diabetes (n=6)</td>
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Values are means±s.d.  *Significantly different from normal group: p < 0.005.

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<tr>
<th>Table 2. Body weights, litter size, plasma glucose and plasma insulin in 21 day fetuses of normal and diabetic pregnant rats</th>
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<td><strong>Subject</strong></td>
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<td>Normal</td>
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Values are means±s.d. with the number of observations in parentheses.
*†Significantly different from normal group; †p<0.005, †p<0.025. IDM, infant of diabetic mother.
the normal newborn rats and IDM in the minimum level of glucose concentration. The difference in these findings may be due to the difference in species. Lactate concentrations of normal newborn rats decreased rapidly during 2 hr after delivery, whereas those of IDM increased during 1 hr after delivery and thereafter decreased (Fig. 1c). Immediately after birth plasma lactate seems to be a major source of glucose synthesis (Snell and Walker 1973). Consequently it is thought that the fall of plasma lactate concentration after birth was due to the initiation of glucose formation. In the present study, plasma lactate concentration remains at high level during 2 hours after delivery in IDM. This may imply that the initiation of glucose formation was delayed in IDM.

The gluconeogenic pathway develops immediately after birth. Ballard and Oliver (1963) described that cytosolic PEPCK activity increases 25 fold during the first 48 hr after delivery but the activities of other gluconeogenic enzymes such as pyruvate carboxylase, glucose 6-phosphatase and fructose 1, 6-bisphosphatase increase only 2-3.5 fold. As a result, the appearance of the cytosolic PEPCK at birth may be involved in the rapid increase in overall gluconeogenesis at this stage (Ballard and Hanson 1967). Hepatic cytosolic PEPCK activity was measured in
Fig. 2. PEPCK activity as a function of age in the livers of caesarian-delivered rats. The points represent (a) 21 day fetuses from normal (○) and diabetic (■) pregnant rats, and (b) suckled newborn rats from normal (□) and diabetic (■) pregnant rats. Each point represents the mean of five determinations and vertical bar shows ± s.d. *Significantly different from normal group; p < 0.005.

Fig. 3. Plasma glucose (a) and PEPCK activity (b) in 21 day fetuses after the administration of glucose. The points represent the oral administration of glucose in water (5 g/kg body wt) (■) and water alone (○) into caesarian delivered newborn rats. Each point represents the mean of four determinations and vertical bar shows ± s.d. *†Significantly different from water alone group; * p < 0.005, †p < 0.01.
newborn rats from normal and diabetic pregnant rats (Fig. 2). The enzyme activity of normal newborn rats increased after a lag of 2 hr, while the activity of IDM increased after a lag of 4 hr. That is, the development of PEPCK in IDM delayed about 2 hr compared to normal fetuses. Thereafter PEPCK activity increased rapidly till 24 hr and gradually from 24 hr to 48 hr in suckling newborn rats (Fig. 2b). There was no difference in these changes of PEPCK activity between IDM and normal newborn rats.

These results suggested that the development of gluconeogenesis was slow in IDM. Then, to elucidate the mechanism of the delayed development of PEPCK in IDM, administration of glucose, glucagon or insulin into caesarian-delivered newborn rats (21th day of gestation) were performed. Fig. 3 shows the time-course changes in plasma glucose concentration and PEPCK activity after glucose administration into caesarian-delivered normal newborn rats. Glucose concentration in administered group increased to a maximum 2 hr after administration and decreased over the next 2 hr period. The development of PEPCK activity in administered group was late as compared with control group. It has been demonstrated that glucose represses PEPCK and Yeung and Oliver (1968) also

Fig. 4. Plasma glucose (a) and PEPCK activity (b) in 21 day fetuses after the injection of glucagon. 21 day fetuses from diabetic pregnant rats were injected intraperitoneally with glucagon (50 μg/rat) in 0.9% NaCl (●) or with 0.9% NaCl alone (○). Each point represents the mean of four determinations and vertical bar shows ± s.d. *Significantly different from 0.9% NaCl alone group; p <0.005.
reported that the injection of glucose 2.5 hr after delivery leads to repression of PEPCK. So it is possible that hyperglycemia in IDM at delivery contributes to the delay in the development of PEPCK activity.

Caesarian-delivered newborn rats from diabetic pregnant rats at 21 th day of gestation (IDM) were injected with glucagon and their plasma glucose concentration and PEPCK activity were measured (Fig. 4). It has been suggested that PEPCK was induced by glucagon. We also found that PEPCK activity in glucagon treated IDM began to increase earlier than those in untreated IDM. So, it showed that PEPCK activity increased responding to the glucagon in the face of hyperglycemia at 2 hr after delivery in IDM. Therefore it appears that the hyperglycemia contributes to the delay in the development of PEPCK through the inhibition of glucagon release immediately after birth in IDM. There was no difference in changes of plasma glucose concentrations between treated and untreated IDM.

Plasma glucose concentration and PEPCK activity were measured as a function of time after the injection of insulin into caesarian-delivered normal newborn rats (Fig. 5). Glucose concentration in insulin injected group decreased and remained at low level to 6 hr after injection. The injection of insulin leads
to a severe hypoglycemia. PEPCK activity in injected group increased after a lag of 2 hr after birth as well as those in control group. The injection of insulin did not prevent the development of PEPCK activity in caesarian-delivered newborn rats. It has been suggested that both insulin and glucose are required for PEPCK deinduction, since glucose or insulin alone is not sufficient to decrease the rate of PEPCK translation (Tilghman et al. 1974). The finding in this experiment is consistent with this suggestion. However, it is uncertain whether hyperinsulinism in IDM at delivery has no effect on the development of PEPCK.

Studies presented here showed that the development of PEPCK and the recovery from the hypoglycemia were late in IDM at birth. Hyperglycemia in IDM at birth may be one of the factors of the delay in the development of PEPCK through the inhibition of glucagon release.

References