The Effect of Premature Delivery on the Development of Gluconeogenic Enzymes in the Guinea Pig

BRIAN H. ROBINSON⁽²⁸⁾ AND JOS OEI

Departments of Paediatrics and Biochemistry, University of Toronto, and Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada

Extract

The activities of key gluconeogenic enzymes in the livers of newborn guinea pigs were monitored as a function of time following birth either vaginally at term or prematurely by cesarian section at 62 days of gestation. The activity of hepatic glucose-6-phosphatase rose dramatically from $1.40 \pm 0.26 \mu\text{mol/min/g}$ at birth to a maximum of $6.8 \pm 0.9 \mu\text{mol/min/g}$ at 24 hr in prematurely delivered animals although there was little significant change in activity in full term animals. The activity of hepatic fructose-1,6-diphosphatase and mitochondrial phosphoenolpyruvate carboxykinase changed little over the first 3 days of life in either full term or premature animals. Cytosolic phosphoenolpyruvate carboxykinase, on the other hand, had low activity at birth being $0.11 \pm 0.03 \mu\text{mol/min/g}$ in full term and $0.06 \pm 0.04 \mu\text{mol/min/g}$ in premature animals rising to values of 0.71 ± 0.06 and $1.12 \pm 0.12 \mu\text{mol/min/g}$, respectively, at 24 hr of life. Pyruvate carboxylase activities in the premature animals remained significantly lower than those in full term animals in the first 72 hr of life. Transient hypoglycemia was evident in the prematurely delivered animals, but not in the full term animals, the blood glucose values being $82 \pm 7 \text{ mg/100 ml}$ for the full term animals and $20 \pm 8 \text{ mg/100 ml}$ for the premature infants at 2 hr of life.

Speculation

Premature delivery of the newborn guinea pig leads to neonatal hypoglycemia which is due to a combination of deficient hepatic glycogen and compromised gluconeogenic enzyme activity.

In a recent publication, we were able to show that the full term newborn guinea pig did not develop postnatal hypoglycemia as is seen in the newborn rat (17). It was postulated at that time that the presence of mitochondrial phosphoenolpyruvate carboxykinase at birth in these animals enabled the gluconeogenic pathway to function adequately, compared with the situation in the rat where there is a virtual absence of phosphoenolpyruvate carboxykinase in the mitochondrial compartment (2, 20). Both of these species have low activities of cytosolic phosphoenolpyruvate carboxykinase at birth (3, 12, 17), a situation which in the rat has been thought to be responsible for the low rates of gluconeogenesis seen in these animals (3). However, induction of cytosolic phosphoenolpyruvate carboxykinase by prenatal treatment with progesterone failed to prevent postnatal hypoglycemia in newborn rats (12). In this communication we investigate the effect of premature delivery on the glycemic status, on the development of gluconeogenic enzymes, and on the gluconeogenic capacity of newborn guinea pigs.

MATERIALS AND METHODS

CHEMICALS

Special chemicals, intermediates, and coenzymes were obtained from the Sigma Chemical Co., St. Louis, Mo. Enzymes were

purchased from Boehringer-Mannheim Corporation (Quebec City). Radioactive intermediates were obtained from Amersham-Searle (Toronto).

ANIMALS

Pregnant guinea pigs were purchased from High Oak Ranch, Ltd., Toronto, as timed pregnancies at 60 days of gestation. In the control group, birth was allowed to proceed naturally at term (65-68 days) and the newborn animals remained with the mother until the time they were killed. Another group of neonates was delivered at 62 days of gestation by cesarian section under light anesthesia; the mother was killed postoperatively. The newborn animals were given immediately to foster mothers who had given birth naturally 1 or 2 days previously and had been deprived of their offspring. It was judged by stomach contents and by observation that this second group were fed by their foster mothers. Both groups were observed to be feeding and drinking independently of the mothers by 3 days postpartum.

ENZYME ASSAYS

Samples of liver taken at time of death were blotted, weighed, and then homogenized in 10 volumes of an ice-cold buffer containing 0.25 M sucrose, 5 mM Tris-HCl, and 0.1 mM EGTA, pH 7.4. The resulting homogenate was centrifuged at $600 \times g$ for 10 min to remove cell debris and the supernatant from this step was then centrifuged at $12,000 \times g$ for 10 min to give fractions containing (1) the mitochondrial pellet and (2) the microsomes plus the soluble components of the cells. Fructose-1,6-diphosphatase was measured by the method of Pontremoli (14), glucose-6-phosphatase by the method of Swanson (21), and pyruvate carboxylase by the method of Crabtree *et al.* (7). Phosphoenolpyruvate carboxykinase was measured by the method of Roobol and Alleyne (18), mitochondria being solubilized for the determination by the addition of 0.1% Triton X-100 to the assay system. Phosphoenolpyruvate carboxykinase activity is not affected by the presence of Triton X-100 (16). All activities were measured at 37°. Blood glucose was measured by the method of Slein (19) in blood samples collected at the time of death and liver glycogen by the same method after alkaline extraction of the tissue followed by alcohol precipitation and hydrolysis of glycogen into glucose.

RADIOISOTOPE INCORPORATION EXPERIMENTS

Animals matched for weight and glycemic state were injected intraperitoneally at the time following birth stated with a combined dose of 2 μCi L-[U-¹⁴C]lactic acid and 2 μCi D-[2-³H]glucose. At 10-min intervals, after injection, the animals were killed and 1-2 ml whole blood were collected and added to an equal volume of 0.6 N perchloric acid (PCA). The perchloric acid extract (0.5 ml) was added to ion exchange columns of overlaid equal bed volumes of anion (Bio-Rad AG1-X2) and cation (Amberlite IR-120) exchange resin as described by Peng *et al.* (13) which, on

elution with water, gave a good separation of glucose from other metabolites. [^{14}C]Glucose was estimated in the eluant by counting the ^{14}C radioactivity in the [^3H , ^{14}C]glucose eluant peak with dual channel counting using a Beckman LS 355 liquid scintillation spectrometer. [^3H]Glucose was determined in PCA samples which had been dried under a stream of N_2 , rehydrated, and counted using dual channel liquid scintillation spectrometry.

The above dual ion exchange resin system was routinely used to remove ^{14}C -labeled alanine, pyruvate, and lactate for the samples, while glucose was eluted, since label from lactate might be distributed among these other three carbon moieties in plasma. However, thin layer chromatography of blood PCA extracts on cellulose in butanol-water-pyridine (1:1:1) and ethanol-ammonia-water (80:4:16) gave only two detectable peaks of radioactivity, one with the same mobility as that of glucose and the other with that of L-lactate. [^{14}C]Lactate was thus estimated in blood PCA extract samples by subtracting [^{14}C]glucose from the total ^{14}C in the sample at any point in time. L-Lactate was estimated independently in these same extracts by the enzyme spectrophotometric method of Wieland (25).

RESULTS

BLOOD GLUCOSE LEVELS

The blood glucose levels in full term neonates showed an increase from a mean of 80.9 ± 1.9 mg/100 ml to 131 ± 3 mg/100 ml over the first 72 hr of life with no value lower than 82 mg/100 ml in between (Fig. 1). In contrast, the prematurely delivered neonates have a somewhat lower blood glucose at birth, 51.5 ± 5.1 mg/100 ml, which falls to a minimum 19.7 ± 8.4 mg/100 ml at 2 hr of life to rise out of the hypoglycemic range by 12 hr of life. During this period, the hepatic glycogen levels decreased dramatically in both groups, the net glycogen utilization being much greater in the full term animals. Significant recovery of hepatic glycogen reserves was also evident by three days of life in the full term animals (Fig. 2).

ENZYMATIC ACTIVITY

Whereas the activity of hepatic fructose-1,6-diphosphatase activity changed little over the first 3 days of life in either full term or premature animals (Fig. 3), the activity of glucose-6-phosphatase rose dramatically from 1.40 ± 0.26 $\mu\text{mol}/\text{min}/\text{g}$ at birth to a maximum of 6.8 ± 0.9 $\mu\text{mol}/\text{min}/\text{g}$ at 24 hr in prematurely delivered animals, although again there was little significant change in activity in full term animals (Fig. 4). Little or no significant change was seen in the activity of mitochondrial phosphoenolpyruvate carboxykinase over the first 3 days of life in either full term or prematurely delivered guinea pigs (Fig. 5). Cytosolic phosphoenolpyruvate carboxykinase, on the other hand, has low activity at birth in both groups of neonates and shows a

Fig. 1. Blood glucose levels in full term (○—○) and premature (●—●) guinea pigs over the first 72 hr of life. Values are the means of six determinations $\pm$ SEM.

Fig. 2. Liver glycogen in full term and prematurely delivered guinea pigs over the first 72 hr of life. Values are the means of six determinations $\pm$ SEM. F.T.: full term; C.S.: cesarian section.

Fig. 3. Hepatic fructose-1,6-diphosphatase activity in full term and prematurely delivered guinea pigs over the first 72 hr of life. Values are the means of six determinations $\pm$ SEM. F.T.: full term; C.S.: cesarian section.

Fig. 4. Hepatic glucose-6-phosphatase activity in full term and prematurely delivered guinea pigs over the first 72 hr of life. Values are the means of six determinations $\pm$ SEM. F.T.: full term; C.S.: cesarian section.

steady increase over the first 24 hr of life, the increase being more pronounced in the premature animals to give a value of 1.12 ± 0.12 at 24 hr compared with 0.71 ± 0.06 $\mu\text{mol}/\text{min}/\text{g}$ in full term animals (Fig. 6). Pyruvate carboxylase activity in prematurely delivered animals was some 53% of the activity seen in full term animals at birth and in both full term and premature animals underwent a steady rise in the first 3 days of life to achieve activities of 1.70 ± 0.37 in the premature compared with 2.43 ± 0.05 $\mu\text{mol}/\text{min}/\text{g}$ in the full term animal (Fig. 7). Mitochond-

drial protein was not significantly different in the two groups when expressed as milligrams per g liver.

RADIOISOTOPE INCORPORATION EXPERIMENTS

The injection of dual isotopic labels of D-[2- ^3H]glucose and L-[U- ^{14}C]lactate was designed to give an estimate of the rate of incorporation of lactate into glucose. Figure 8 shows the result of such an experiment performed with prematurely delivered animals 5 min postnatally and the combined results of four such experiments for each time period shown in Table 1. Extrapolation of the [^3H]glucose specific activity to zero time on a semilogarithmic plot (23, 24, 26) gives a mean glucose space of 60.8 ml/100 g body weight. By monitoring the [^{14}C]glucose specific activity with time and knowing the glucose space it was calculated that the peak incorporation of lactate into glucose at 20 min represented 15.5% of the total [^{14}C]lactate injected at time zero. This figure rose to 32.4% at 3 days of life (Table 1). In contrast, the full term animals showed a high [^{14}C]lactate incorporation into glucose at birth which was virtually unchanged at 3 days of life. Glucose space decreased by some 33% in both full term and premature animals by 3 days of life. Incorporation of [^{14}C]lactate into hepatic

Fig. 5. Hepatic mitochondrial phosphoenolpyruvate carboxykinase activity in full term and prematurely delivered guinea pigs over the first 72 hr of life. Values are the means of six determinations $\pm$ SEM. F.T.: full term; C.S. cesarian section. PEPCK: phosphoenolpyruvate carboxykinase.

Fig. 6. Hepatic cytosolic phosphoenolpyruvate carboxykinase activity in full term and prematurely delivered guinea pigs over the first 72 hr of life. Values are the means of six determinations $\pm$ SEM. F.T.: full term; C.S.: cesarian section. PEPCK: phosphoenolpyruvate carboxykinase.

Fig. 7. Hepatic pyruvate carboxylase activity in full term and prematurely delivered guinea pigs over the first 72 hr of life. Values are the means of six determinations $\pm$ SEM. F.T.: full term; C.S.: cesarian section.

Fig. 8. Time course of specific activity of [^{14}C]lactate (●—●), [^3H]glucose (○—○), and [^{14}C]glucose (□—□) after injection of [U- ^{14}C]lactate and [2- ^3H]glucose in newborn prematurely delivered guinea pigs. Experiments were carried out as described under *Methods*.

glycogen was initially measured in these experiments but found to constitute less than 1% of the total [^{14}C]lactate administered.

DISCUSSION

Hypoglycemia in the newborn period in the human population of neonates is encountered most commonly among those individuals having a low birth weight due to premature delivery or interuterine growth retardation (6). In contrast, the full term newborn laboratory rat develops hypoglycemia over the first few hours of life, and this has been variously attributed to hypoxia (12) or to the low activities of the key gluconeogenic enzymes in the liver, especially the enzyme phosphoenolpyruvate carboxykinase (2, 3, 9). In the newborn guinea pig neonatal hypoglycemia was seen only in the prematurely delivered animals. The activities of gluconeogenic enzymes at birth show significant differences only in glucose-6-phosphatase and in pyruvate carboxylase, which, especially in the case of the latter enzyme, may compromise the adequate working of the gluconeogenic pathway. The difference seen in the available reserves of hepatic glycogen between premature and full term animals may have some bearing on the hypoglycemic status of the former group. Hepatic glycogen in the rat falls rapidly after birth (9) as it was observed to do in the guinea pig both in the full term and premature animals. However, calculation of the amount of glucose utilized during the first 4 hr of life from the available glycogen stores gives a rate of 2.70 $\mu\text{mol}/\text{min}/100$ g body weight for the full term but only 1.15

Table 1. [^3H]Glucose space, blood lactate, and [^{14}C]lactate incorporation into glucose in newborn guinea pigs¹

	Cesarian section		Full term	
	At birth	3 days	At birth	3 days
Glucose space (ml/100 g body weight)	60.8 $\pm$ 2.8	40.6 $\pm$ 3.1	69.0 $\pm$ 2.4	46.0 $\pm$ 3.1
Blood lactate (mM)	3.19 $\pm$ 0.39	2.76 $\pm$ 0.28	3.60 $\pm$ 0.58	2.34 $\pm$ 0.29
Peak [^{14}C]lactate incorporation (%) into glucose of total injected	15.5 $\pm$ 2.1	32.4 $\pm$ 3.3	31.6 $\pm$ 3.2	35.2 $\pm$ 4.1

¹ Each experiment was carried out as described for Figure 8, using guinea pigs at birth or at 3 days of age. The results shown are the means of results from four experiments $\pm$ SEM.

$\mu\text{mol}/\text{min}/100\text{ g}$ body weight for the premature animals. This must be a contributory factor to the hypoglycemia seen in the prematurely delivered animals. Assay of total phosphorylase activity at birth in these two groups at birth showed no significant difference, although we were not able to measure in this series of experiments the proportion of active to inactive phosphorylase.

The possibility that hypoglycemia could result from the low activity of cytosolic phosphoenolpyruvate carboxykinase seen at birth in the newborn rat and the applicability of this argument to the situation in man has been a subject for some comment (1, 4, 17). The fact that the mitochondrial complement of phosphoenolpyruvate carboxykinase shows adequate activity at birth in the guinea pig (1, 17) may give reason to suppose the low cytosolic phosphoenolpyruvate carboxykinase in the animal at birth does not compromise the activity of the gluconeogenic pathway. Since the disposition of phosphoenolpyruvate carboxykinase is similar in man and guinea pig, being equally active in both cytosolic and mitochondrial compartments in the adult, the guinea pig may be a better model for the human neonatal situation. Our results here suggest that in the immediate postnatal period in which hypoglycemia is experienced by the premature animals, the phosphoenolpyruvate carboxykinase activities differ little between the two groups until 12 hr postpartum, when the prematurely delivered animals show a significantly greater activity than the full term animals, possibly because of induction by a combined high glucagon to insulin ratio and cortisol level experienced during the period of hypoglycemia (8, 10).

The information from the radioisotope experiments could possibly give information about the glucose space, the rate of glucose utilization, and the rate of lactate incorporation into glucose. The rate of detritiation of [^3H]glucose gives a measurement of the rate of glucose utilization since any metabolism of glucose as far as hexose 6-phosphate isomerase leads to detritiation. However, calculated rates may seriously overestimate the actual glucose utilization rate because of "futile cycling" which leads to detritiation in the liver and kidney (5, 11). Both full term and premature animals show very large glucose spaces at birth compared with that of 3 days of life, a situation which is unexpected as glucose levels are generally higher at the 3-day period. Stimulation of glucose transport and utilization by hypoxia as described by Randle (15) may be responsible for this.

The peak incorporation of [^{14}C]lactate into the glucose pool is lower at birth in the premature animals (15.5%) compared with the full term animals (31.6%), there being little difference at 3 days of life. This contrasts with data from the rat which shows very little [^{14}C]lactate incorporation into glucose in the first few hours of life (<3%) (12, 23, 24). We conclude from our studies that the hypoglycemia seen in prematurely delivered animals stems from (1) an insufficient store of hepatic glycogen and (2) a slower rate of functioning of the gluconeogenic pathway in these animals. One possible problem with this model is the unquantitated effect which alimentionation may be having on glycemic status in these animals since we cannot be sure that premature animals receive adequate milk from their foster mothers. Experiments now under way to examine the effect of starvation on full term neonates indicates that food deprivation has little effect on glycemic status in the first

24 hr of life. It is therefore unlikely that differential alimentionation has a great effect on glycemic status in this experimental model.

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2,3-Diphosphoglycerate newborn
hemoglobin oxygen hemoglobin affinity

The Adaptation of the Fetal Red Cells of Newborn Lambs to Extrauterine Life: The Role of 2,3-Diphosphoglycerate and Adult Hemoglobin

H. BARD,^(1,2) J.-C. FOURON, A. M. GROTHE, M. A. SOUKINI, AND A. CORNET

Perinatal Service and Research Center of Hôpital Sainte-Justine, Department of Pediatrics, University of Montreal, Montreal, Quebec, Canada

Extract

The purpose of this study was to determine the interrelationship of the rise and fall of 2,3-diphosphoglycerate (DPG) with the increase in adult hemoglobin and the decrease in red cell oxygen hemoglobin affinity after birth in normal lambs. It was found that the mean maximum DPG level was 26.71 ± 4.98 mol/g Hb at 7.5 ± 1.1 days. At the same time the mean P_{50} and adult hemoglobin level was 27.0 ± 1.4 mm Hg and $31.1 \pm 11.1\%$, respectively. In the individual lambs, the level of their maximum DPG correlated inversely with the amount of adult hemoglobin ($r = -0.77$, $P < 0.05$). Once the DPG began to decrease, there was an inverse correlation between the DPG and the adult hemoglobin present in the red cell ($r = 0.68$, $P < 0.001$). It appeared that the rise in DPG postnatally is only a compensatory mechanism until an adequate amount of adult hemoglobin is present. This fact was borne out by the second part of the study in which exchange transfusions with adult red cells were performed on five newborn lambs during the first 24 hr after birth and aborted the rise in DPG.

Speculation

The adaptation from the *in utero* to the extrauterine environment requires a rapid reduction in red cell oxygen affinity. The decrease in oxygen affinity is achieved by a transitory postnatal increase in intraerythrocyte DPG.

During *in utero* life the role of high affinity fetal blood is to provide a physiologic advantage that facilitates oxygen transport across the placenta. After birth a more efficient extraction of oxygen by the tissues is needed which is accomplished by the lowered affinity of adult blood.

It was Battaglia *et al.* (1) who demonstrated in newborn lambs that pH across the red cell and DPG concentration within the red cell both increase as there is a marked decrease in oxygen affinity compared with fetal blood. Similar postnatal changes in human red cells, although much less pronounced, have been described by Versmold *et al.* (6).

In order to further the understanding of the great transitory increase in DPG during the first weeks of life in the newborn lamb, the following studies were planned: (1) to follow sequentially in the same animal the rise and fall of DPG, the percentage of adult and fetal hemoglobin along with changes of the oxygen dissociation curve; and (2) to repeat the above study after having provided the newborn animal with an adult oxygen hemoglobin dissociation curve within hours after birth.

MATERIALS AND METHODS

Mixed breed newborn lambs (Dorset and Suffolk) were used in this study. The experiment was divided up into two sections. The first part was to sample postnatally at 3- to 5 day intervals via the jugular vein normal newborn lambs and to correlate their erythrocyte DPG, P_{50} , and proportion of adult and fetal hemoglobin with postnatal age.

Erythrocyte DPG concentration was determined on fresh heparinized blood according to the method of Keitt (5) and expressed as micromoles per g Hb. The reagents were obtained from Sigma Chemical Company (7).

The P_{50} was determined by gas mixing tonometry using a IL 217 blood gas laboratory (8) (213 blood gas analyzer, 237 tonometer, 208 gas mixing system, and 182 co-oximeter). The P_{50} values were expressed at a temperature of 37° , pH 7.40, and $p\text{CO}_2$ of 40 mm Hg.

Samples of fresh blood were equilibrated in a tonometer for 30 min at 37° with gas mixtures containing 40 mm Hg (± 0.05 mm Hg) CO_2 and varying proportions of O_2 and N_2 . The tonometer permitted an equilibration and sampling of successive aliquots of blood at different O_2 tensions. Measurement of O_2 saturation, pH, and PO_2 provided the information for plotting the O_2 dissociation curve and P_{50} . PO_2 was always converted to pH 7.4 using a Bohr effect of 0.46 (4).

The oxygen saturation obtained by use of the co-oximeter was checked by determining the oxygen contents of blood samples equilibrated in the tonometer by use of the Lex- O_2 -Con (9).

The percentage of adult and fetal hemoglobin was obtained by