Role of Carbohydrates in Energy Balance of Prematurely and Term Born Rats

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Speculation

This study shows that during the early neonatal period the fuel consumption and the energy production is relatively low. This could be related to a slow operating Kreb's cycle. This, in turn, could be a protective mechanism of the organs that allows them to get through the hypoxic phase of birth without damage and explains the greater resistance of the newborn to hypoxia.

Birth is a major metabolic event in the course of development, characterized by 1) an abrupt arrest of energy supply of maternal origin to the newborn and 2) an increase of the caloric requirements because new physiologic functions have to be supported (thermoregulation; respiratory movements, etc. . . .). In order to cope with this new situation, the newborn must be provided with sources of energy; these are either exogenous (alimentary supply) or endogenous (stores of high energy compounds accumulated during fetal life). During the first hr of life, the newborn is not fed and must exclusively rely upon his own reserves. This is true for prematures as well as for full term animals. As far as full term animals are concerned, there is some information about their metabolism after birth, but it is rare for prematures. It is commonly stated that in the early neonatal period of the rat no other stores but carbohydrates are available. Indeed, gluconeogenesis is not fully operative as yet (26) and this is attributed to the absence of the key enzyme phosphoenol pyruvate carboxykinase (25, 27).

On the other hand, mobilization of lipid stores seems possible because after birth a rise in serum of NEFA (9, 17, 22) as well as of glycerol (9, 18, 19) was observed. Oxidization of lipids also is possible in the rat: Lockwood and Bailey (13) have shown *in vitro*, that labeled palmitate can be oxidized to CO₂ by liver slices from the newborn rat. However, the relative importance of this pathway *in vivo* has not been evaluated. Therefore, a careful appraisal of newborn's carbohydrate stores seems of particular interest to gain insight into the metabolic balance of the newborn rat.

Some authors have studied glycogen stores (3, 9, 20-22), but these investigations have been limited to certain organs, mostly the liver. Total glycogen stores have but occasionally been evaluated (22). Some studies are dealing with blood glucose levels (2, 4, 5, 9, 10, 22, 23, 25, 27) but no consistent results are offered: either hypoglycemia or normoglycemia is mentioned. Only a few investigations (6, 9, 22) have studied lactic acid concentrations in the rat, although this parameter has extensively been investigated in human newborns. In preliminary experiments, De Meyer *et al.* (6) have shown that the levels of lactic acid at birth in premature as well as in term animals are nearly ten times higher than in adult rats. Normalization of this lactacidemia takes place within the first 2 hr of life. These data have been confirmed for term animals by Girard *et al.* (9) and by Snell and Walker (22). In the full term animal, much information is available on neonatal metabolism but the data are of limited value because one important parameter, namely CO₂ production has usually not been investigated. Therefore, the purpose of our present work was to compare prematurely born and term rats as to their energy metabolism during the first 6 hr of life. The specific aim was to perform an accurate deter-

mination of the animal's fuel stores and metabolites such as glycogen, glucose, lactic acid, and CO₂ production.

MATERIALS AND METHODS

ANIMAL

Rats of Wistar Strain bred in our laboratory for 20 yr were used. Virgin animals were put in cages with males at 2 PM one day; the next day at 11 AM they were separated from the males and kept in individual cages; this day was considered as day 0 for calculation of gestational age. Rats were killed by decapitation on day 20 (prematures) or 21 (full term animals), a few hr before spontaneous birth, and the fetuses quickly removed from uterine horns. The fetuses were kept with their placentae attached in a warm environment until they were eventually injected with different substrates through the umbilical vein. Fetuses were then placed immediately in individual incubators at 32°C (unless otherwise specified) with continuous warm and humidified 100% oxygen flow. Only healthy looking newborns were included in this study; any animal showing signs of cyanosis or looking lethargic was discarded. In a few experiments, fetuses on days 17, 18, or 19 of gestation were used. The animals prematurely delivered on day 20 were divided into two groups: the first contained animals whose body weight was between 2.4-4.0 g, the second contained animals with a body weight above 4 g.

ASSAYS

Blood samples of 10 µl obtained by decapitation were deproteinized with 0.10 ml HClO₄ (0.33 M), centrifuged and the glucose concentration was measured in the supernatant by the glucose oxydase method of Huggett and Nixon (12). Lactic acid was assayed in a 50 µl blood sample deproteinized with 100 µl HClO₄ (0.6 M). The lactate dehydrogenase method of Hohorst (11) was employed.

Citrate concentration was measured by the method of Moellering and Gruber (14). HClO₄ (0.6 M) was used for deproteinizing. The method was adapted to 50 µl volume (16).

Glycogen in the whole fetus as well as in isolated organs was assayed by the anthron method of Morris (15), after dissolution in hot 30% KOH and subsequent precipitation with cold ethanol.

Incubators consisted of cylindric glass tubes measuring 5 cm in length and 2 cm in diameter. They were closed at both ends with rubber stoppers through which at one side a plastic tube permitted the entry of warm and humidified 100% oxygen and at the other side a plastic tube served as a outlet of the gas.

For measurements of CO₂ production, the gas at the outlet of the incubation chamber was allowed to bubble through 5 ml of a 1 M NaOH solution.

RESULTS

GLYCOGEN CONTENT

In Table 1, values of glycogen are reported in the whole fetus and in the liver, at different ages. The concentration of glycogen

in the liver increases nearly 100-fold between day 17 and day 21, while the extrahepatic glycogen was raised only about 6-fold. However, it still accounted for nearly half of the glycogen at the end of gestation and, therefore, must be considered as an important store for energy supply.

Table 2 gives the glycogen content of individual organs, expressed in mg/g weight, but for comparative purposes the amount of glycogen in each organ is calculated also per g body weight and as the percent of total body glycogen. It appears from these data that nearly 50% of the glycogen stores were located outside the liver, lung, and heart.

The behaviour of the "total" and "liver" glycogen content over the first 6 hr of life is shown in detail in Figure 1. These data indicate that 1) glycogen was mobilized from extrahepatic tissues as well as from the liver and that the disappearance rate was faster in the former than in the latter; 2) mobilization of glycogen seems to occur with some delay in small prematures (D 20, 2.4-4.0 g).

Figure 2 shows the concentration of glycogen in different organs

in relation to gestational age. By "brown fat", we mean the two fatty lobes easily detectable in the interscapular space. Although this is but a small part of the total brown fat present in the animal, we considered it metabolically to be representative of all brown fat.

GLYCEMIA

Figure 3 shows the behaviour of the blood glucose level in the early neonatal period of animals born at different gestational ages. In the left upper corner are the values for the three different animal groups. Statistical significance was calculated by the Student's *t* test for different intervals. For animals of day 19, only a few values were available because of the high mortality rate. Nevertheless, these values show that glycemia at birth on day 19 is still lower than on day 20. Glucose space was calculated after an iv injection of a known amount of 50% glucose solution and measurement of the blood glucose increment. The values were

Table 1. Total glycogen and liver glycogen in the fetal rat (mean $\pm$ SD)

	Day 16	Day 17	Day 18	Day 19	Day 20			Day 21
					2.4-4.0g	4.0g		
Body wt (g)	0.439 $\pm$ 0.022	0.799 $\pm$ 0.104	1.189 $\pm$ 0.074	2.094 $\pm$ 0.206	3.054 $\pm$ 0.306	4.685 $\pm$ 0.287		4.839 $\pm$ 0.248
<i>n</i>	7	12	18	25	25	13		9
Total glycogen (mg/fetus)	1.659 $\pm$ 0.143	3.832 $\pm$ 0.621	7.672 $\pm$ 3.660	12.232 $\pm$ 3.357	32.661 $\pm$ 8.032	50.497 $\pm$ 4.587		45.438 $\pm$ 7.698
Liver glycogen (mg/fetus)	0.016 $\pm$ 0.003	0.035 $\pm$ 0.012	0.210 $\pm$ 0.097	3.103 $\pm$ 1.717	13.664 $\pm$ 3.849	27.381 $\pm$ 2.514		22.471 $\pm$ 4.450
Liver glycogen (mg/g liver)	0.536 $\pm$ 0.095	0.612 $\pm$ 0.173	2.400 $\pm$ 1.052	18.972 $\pm$ 9.860	57.263 $\pm$ 12.457	83.632 $\pm$ 8.091		67.615 $\pm$ 13.785
Extrahepatic glycogen (mg/fetus)	1.643	3.797	7.462	9.129	18.997	23.116		22.970
Extrahepatic glycogen (mg/g liver)								
% of total glycogen in liver	0.964	0.913	2.73	25.368	41.836	54.223		49.454

Fig. 1.

304.9 $\mu\text{l/g}$ body wt and 340.6 $\mu\text{l/g}$ body wt, respectively, for animals of day 20 weighing 2, 4–4.0 g, and for animals of day 20 weighing more than 4.0 g. When compared with glycogen stores, blood glucose appears to represent only a small fraction of the total carbohydrate stores (nearly 1/35th).

LACTIC ACID

Blood lactate levels are given in Figure 4. The levels are high at birth and fall very abruptly. The ability to remove lactic acid from the bloodstream is directly related to the degree of maturation.

Table 2. Glycogen content of different organs in term and premature rats (mean $\pm$ SD)

	Day 20 (2.4–4.0g)			Day 20 (4.0g)			Day 21		
	mg/g organ	mg/g body wt	% of total glycogen	mg/g organ	mg/g body wt	% of total glycogen	mg/g organ	mg/g body wt	% of total glycogen
Liver	51.07 ± 16.00	4.48 ± 0.96	47.56	80.32 ± 12.29	5.11 ± 0.74	46.67	67.62 ± 13.79	4.64 ± 0.90	49.47
Lung	24.23 ± 4.90	0.67 ± 0.13	7.11	13.03 ± 3.47	0.32 ± 0.13	2.92	2.80 ± 2.37	0.10 ± 0.9	1.07
Heart	24.24 ± 3.82	0.14 ± 0.02	1.49	28.27 ± 6.65	0.16 ± 0.04	1.46	21.85 ± 7.89	0.14 ± 0.03	1.49
Brown fat	14.61 ± 3.83			12.80 ± 1.84			6.62 ± 4.34		
Total		9.42 ± 3.29	100		10.95 ± 0.85	100		9.38 ± 1.52	100
Not accounted for other items			43.84			48.95			47.97

Fig. 2.

Fig. 3.

Fig. 4.

CITRATE

CO₂ PRODUCTION

Figure 5 shows the concentration of citrate in blood. Similar to lactic acid, the values are high at birth and decrease progressively to adult levels in approximately 2 hr.

Figure 6 shows the cumulative CO₂ production curves for day 20 and day 21. The production seems linear with a change of the slope at 135 min. The mean production rate for the first 135 min

was 14.0 and 16.2 $\mu\text{l/min/g}$ body wt, respectively, for day 20 and day 21 animals. For the period from 135 to 240 min, the production rate amounted to 28.0 and 23.2 $\mu\text{l/min/g}$ body wt for day 20 and day 21 animals.

DISCUSSION

In term animals, the adaptation of carbohydrate metabolism to extrauterine life has been fairly well investigated (for review see Snell and Walker (22) and Girard *et al.* (9)).

Our data show that premature animals too are able to regulate their glucose metabolism.

The course of the blood sugar curve shows four characteristic features. First, prematures have lower blood glucose values than do term animals. Second, all groups show a slight increase in blood glucose between 15 and 30 min after birth. Third, this increase is followed by a fall, reaching values as low as 40 mg/dl at the 120 min. This value is nearly the same for all groups. Lastly, approximately 3 hr after birth, a secondary raise occurs; the values observed in the three groups of animals being nearly the same.

The data suggest that although the glucose levels at birth differ according to gestational age, a homeostatic mechanism is operational even in prematures keeping glycemia within relatively narrow limits. It should be mentioned that we did not observe in our experimental series the very low blood glucose values of 20 mg/dl observed in several other laboratories (5, 9, 22). The reason why neither prematures nor term animals were severely hypoglycemic is likely related to the temperature in our incubation chamber, which was 32°C, this being close to the natural nest temperature.

Girard *et al.* (9) have shown in term rats that hypoglycemia is severe if the animals are maintained at 37°C. Our experiments in prematures (Figure 7) indicate that the regulation of glycemia was temperature dependent in that lower blood glucose values were seen at 37°C than at 32°C.

Our experiments have demonstrated for the first time that premature animals are capable of mobilizing glycogen. Whereas this was known for term animals (3, 9, 22), it was only suspected from the results of the experiments conducted by Devos and Hers (7), who has shown that anesthesia in the mother induced a marked glycogen reduction in the fetal liver 1 and 2 days before birth. Similar results were obtained by us after administration of 0.5 U insulin to the mother (unpublished data). These data showing that intrauterine glycogenolysis is possible at least 24 hr before birth, are in keeping with our own data in the prematurely born rat. As far as the inducing factor of glycogenolysis is concerned, hypoglycemia, fall in lactic acid and the raise of the

Table 3. Metabolic features of new born rat during the first 6 hr of life

Glycogen disappearing from stores	5.6 mg/g body wt
CO ₂ produced from glycogen	4.20 ml/g body wt
Total CO ₂ produced	6.80 ml/g body wt
CO ₂ from noncarbohydrate source	2.60 ml/g body wt
O ₂ consumed for glycogen metabolism	4.20 ml/g body wt
Total O ₂ consumption (Taylor)	7.20 ml/g body wt
O ₂ consumed by noncarbohydrate sources	3.00 ml/g body wt
RQ of noncarbohydrate part of fuel	2.60/3.00 = 0.87

Fig. 5.

Fig. 6.

EFFECT OF BREEDING TEMPERATURE ON GLYCEMIA OF NEW-BORN RATS

Fig. 7.

oxygen tension, all changes occurring in blood almost simultaneously at birth have to be considered and their role as glycogenolytic signals remains to be elucidated.

We have shown that extrahepatic glycogen is preferentially mobilized at birth. This glycogen can not join the glucose pool in the blood, but by being metabolized *in situ*, it can have a sparing effect on blood glucose, and by that can contribute to the maintenance of normoglycemia. The role of extrahepatic glycogen in this context has not been emphasized previously. Even in human neonatal physiology, extrahepatic glycogen is but seldom considered as an important factor in glucose homeostasis. It would be interesting to know the modifications of these stores in different pathologic conditions.

The amount of glycogen mobilized (hepatic and extrahepatic) during the first 6 hr of life was nearly the same in all three experimental groups, namely 5.6 mg/g body wt. This amount can not account for all the metabolic needs of the newborn and it must be assumed that sources different from carbohydrates are utilized as well. If no other source but glycogen would be available, the amount of CO₂ production would be 4.2 ml/g body wt/6 hr. Because the total production of CO₂ was in our experiments close to 6.8 ml/g body wt/6 hr, a substantial amount, 2.6 ml/g body wt/6 hr of CO₂, was probably derived from metabolic fuels other than carbohydrate.

According to Taylor (24), the oxygen consumption of the newborn rat is 7.20 ml/g body wt/6 hr. After subtracting from this amount 4.20 ml/g body wt/6 hr for carbohydrate oxidation, 3 ml/g body wt/6 hr is left. This volume is close to 2.6 ml/g body wt/6 hr of CO₂ claimed by us to be produced from non carbohydrate sources. The RQ of this portion is $2.6/3.00 = 0.87$ and suggests protein breakdown for caloric supply (cfr, Table 3). It is estimated by us that the amount of protein utilized corresponds to approximately 3.2 mg/g body wt/6 hr.

Thus, our data permit the following calculation of caloric production: 1) 5.6 mg glucose yield 22.4 cal/g/6 hr; 2) 3.2 mg protein yield 12.9 cal/g/6 hr; this means a total of 35.2 cal/g/6 hr or about 140 cal/g/24 hr. According to Benedict's curve extrapolated for animals of 4 g(1), a supply of 400–500 cal/g/24 hr is required. This is 3 to 4 times more than what is observed in our experiments, suggesting a slow metabolic rate in the early first postnatal hr. This observation is important enough to direct care for further direct calorimetric investigation in order to ascertain that in the early hours the metabolic expenditure is indeed reduced.

It is also of interest to comment on the glycogen content in different organs as shown in Figure 2. In these organs, the total amount of glycogen is low and its contribution in maintaining glucose homeostasis is reduced. However the concentration in respect to the organ weight is high. This glycogen plays probably an important role in the processes of differentiation. Postnatally the concentration of glycogen in these organs increases first and decreases afterwards at a rate specific for each organ.

At birth, lacticidemia is high in the premature as well as in the term animal. It is still not known why lactate levels are high *in utero* and what enables the newborn to lower the level quickly to adult values. The high lactate levels are associated with high citrate levels suggesting the involvement of the Krebs cycle. The role played by the high concentration of lactic acid in blood should be investigated and could eventually be one of the major factors preventing glycogenolysis *in utero*. The low energy production in the early hours suggest also a slow operating rate of the Krebs cycle. High citrate blood values and slow CO₂ production argue in the same sense. This may perhaps be related to modifi-

cations in the activity of the mitochondria. It may be suggested that by lowering the mitochondrial activity, the cells reduce their energy needs and thereby protect themselves against disturbances in oxygen supply particular to the period of birth. It is noteworthy that the majority of our parameters of study show alterations within the first 120 min, as if this were a period of rapid adaptation. Such a metabolic adaptation stress may explain the high mortality observed in our experiments. Thus, a thorough understanding of this early phase of extrauterine life is of great clinical importance.

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