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Adenine Nucleotide Concentrations in Liver of Fetal Rats. Neonatal Changes in the Premature Newborn

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Changes in ATP, ADP and AMP concentrations in the liver in term and preterm newborns during the perinatal period have been studied. Fetal liver ATP concentrations decreased during the penultimate day of gestation and remained low during the last day of pregnancy. ATP concentrations sharply increased in the liver of term newborns immediately after delivery, remaining unchanged after 1 h of extrauterine life. High ATP concentrations were observed in the liver of preterm newborns at delivery without changes within the early postnatal period. No significant changes in liver ADP and AMP concentrations were observed in either group throughout the observation period. Energy charge was higher in preterm liver at delivery but similar values were reached in term liver after 30 min of extrauterine life. These results suggest that the energy stage of the preterm liver is high at delivery without changes during the early postnatal period.

Transition to extrauterine life means the onset of breathing which presumably increases the oxygen supply to the tissues. Some evidence suggests that oxidative metabolism may play an important role in the utilization of the main metabolic fuels in the early newborn (5, 8). Therefore, it has been found (9) that liver mitochondria shows very low respiratory control ratio and osmotic activity at birth, but these parameters substantially increase after 1-2 h of extrauterine life. This result has been interpreted as the postnatal assembly —«hypercontraction»— of the inner mitochondrial membrane leading to a coupled active mitochondria (9). In addition, the «maturation» of the mitochondrial membrane may be brought about *in vitro* (9) by the incubation of mitochon-

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dria in the presence of ATP. Consequently, it has been suggested (9) that the increase of ATP concentration which occurs immediately after delivery may account for the assembly of the inner mitochondrial membrane.

In previous papers we have studied some aspects of the energy metabolism in preterm newborns during the presuckling period (5, 6). We have found that the decrease of cytoplasmic NADH/NAD redox state which occurred in the liver of term newborns immediately after delivery was delayed in preterm newborns (5). This suggests that the preterm newborn suffers decreased oxygen availability during the first hour of extrauterine life. Alternatively, these results may be explained by the immaturity of the mitochondrial function due to premature delivery which would prevent proper utilization of the available oxygen.

Since adenine nucleotide concentrations may be an indicator of the maturity of the mitochondrial function, the present research was designed to study the time courses of liver ATP, ADP and AMP concentrations in term and preterm newborn rats during the first two hours after delivery. The time course of adenine nucleotide concentrations in fetal liver during the last two days of gestation has been also studied.

Materials and Methods

Reagents. Substrates, coenzymes and enzymes were obtained from Boehringer (Mannheim, FRG) or Sigma. Standard analytical grade laboratory reagents were purchased from Merck or Sigma.

Animals. Albino Wistar rats, fed on a stock laboratory diet (carbohydrate 49.5 %, protein 21.4 %, fat 3.8 %, and added salts and vitamins), and of known gestational age were used for the experiments. Females were caged overnight with males and conception was considered to occur the next morning. Pregnancy was confirmed at day 19 of gestation. On day 21 or day 22 gestation the rats were sacrificed by cervical dislocation without anaesthesia and the fetuses were delivered by rapid hysterectomy (about 3 min). The newborns were carefully wiped and the umbilical cords tied and cut. All these operations were carried out in a Perspex cabin al 37° C. The newborns were weighed and those which presented low weight for the average of the litter were discarded. Neonates weighing 3.9 ± 0.1 or 5.5 ± 0.1 g (mean $\pm$ SEM) were considered as «preterm» and «term» ras, respectively. The newborn animals were maintained in a humid crib at 37° C without feeding for the duration of the experiments. To prevent the effects of hypothermia (4), the enviromental temperature of the newborn was carefully controlled and kept at 37° C. Owing to the rapid postmortem changes in the adenine nucleotide concentrations, the livers of the first two fetuses still attached to the mother were frozen in less than 3s using Wollenberger clamps precoolled in liquid N_2 (11). These samples were considered the fetal and zero time levels of adenine nucleotides in all experiments. At various time intervals after delivery the livers of the neonates were freeze-clamped (3s) without exsanguination of the animal. Frozen livers were pulverized in a precooled mortar and extracted with 6 % perchloric acid avoiding thawing of the liver powder during operation.

Analytical Methods. ATP was determined by the reactions catalyzed by hexokinase and glucose-6-phosphate dehydrogenase according to LAMPRECHT and TRAUTSCHOLD (7). ADP and AMP were assayed by the reactions catalyzed by myokinase, pyruvate kinase and lactate dehydrogenase according to ADAM (1).

Results are shown as the mean $\pm$ S.E.M.

Statistical analysis was carried out by the student's *t* test.

Results

Fetal liver ATP concentrations (fig. 1, left) sharply decreased ($p < 0.05$) during the penultimate day of gestation remaining mostly unchanged during the last day of the gestational period. No significant changes in fetal liver ADP and AMP concentrations were observed during the last two days of gestation. Therefore, the decrease of ATP concentration resulted in a parallel decrease of total nucleotide concentrations ($p < 0.05$). Liver ATP concentrations increased considerably ($p < 0.0005$) in term newborns (fig. 1, right) during the first hour of extrauterine life, while no significant changes were observed during the second hour. Liver ADP and AMP concentrations remained mostly unchanged in term newborns throughout the first two hours after delivery. The increase of ATP concentrations resulted in a parallel increase of total nucleotide concentration reaching the values observed at the beginning of the 21st day of gestation. No significant changes in ATP, ADP, AMP and total nucleotide concentrations were observed in preterm rats during the first two hours after delivery (fig. 1, right). It is noteworthy that liver nucleotide concentrations observed in preterm newborn at birth were maintained during the first two hours of extrauterine life. Thus, preterm newborns were delivered at the beginning of the 21st day of gestation i.e. before fetal liver ATP concentrations had decreased.

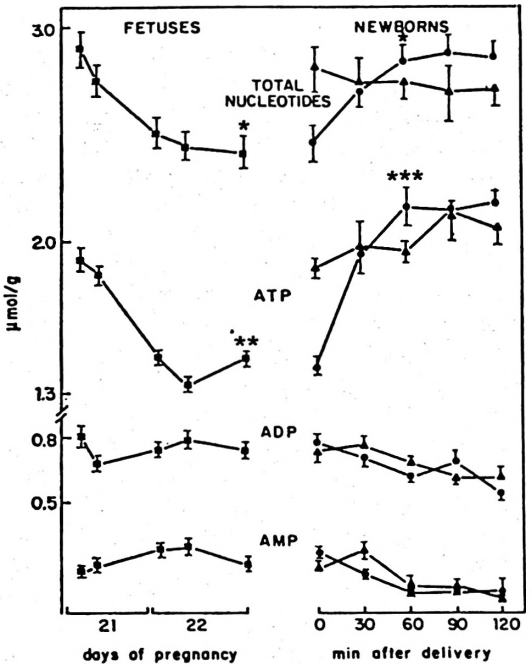

Fig. 1. *Liver adenine nucleotide concentrations during the perinatal period. Liver adenine nucleotide concentrations were measured in fetuses (—■—) and term (—●—) and preterm (—▲—) newborns delivered by caesarian section.*

Newborns from pregnant rats on day 22 («term») or day 21 («preterm») were maintained in a humid crib at 37° C without feeding for the duration of the experiments. At appropiate times, livers were removed without exsanguination of the animals and immediately freezeclamped. Nucleotide concentrations are expressed in micromols per g of liver wet weight and are means of 6 to 8 observations ± S.E.M. *$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$ compaired to their respective initial values.

Table I shows the ratio of liver ATP/ADP concentrations and the energy charge of term and preterm newborns during the first hours after delivery. The ATP/ADP concentration ratio approximately doubled in term newborns during the first hour after delivery. Preterm newborns showed higher ATP/ADP ratios at delivery but very litle increase was seen during the observation period. It is interesting that the initial ATP/ADP ratio observed in preterm newborns was reached by term newborns within the first 30 min after delivery. The energy charge (3) of term newborns at delivery was lower than that found in preterm newborns. The balance between synthesis and utilization of ATP

Table I. *Liver energy state of term and preterm newborn rats during the first two hours after delivery.*

	Time elapsed from delivery (min)				
	0	30	60	90	120
TERM					
ATP/ADP	1.84	2.73	3.48	3.15	4.04
Energy charge *	0.73	0.81	0.86	0.85	0.87
PRETERM					
ATP/ADP	2.54	2.59	2.87	3.45	3.38
Energy charge *	0.80	0.78	0.83	0.85	0.86

* Energy charge = 1/2 2(ATP) + (ADP) / (AMP) + (ADP) + + (ATP) according to (3). For the experimental conditions see legend of figure 1. Values are calculated from the data depicted in figure 1.

— energy charge = 0.85 — was reached by both groups of newborns during the first hour after delivery (table I).

It should be mentioned that the supply of pure oxygen during the first two hours after delivery does not make any significant change in liver adenine nucleotide concentrations or in ATP/ADP ratio and energy charge (results not shown).

Discussion

In spite of the interpretation discrepancy of the increase in the respiratory control ratio and of adenine nucleotide concentrations which occur in newborn liver mitochondria immediately after delivery (2, 9), it seems clear that liver ATP concentrations may reflect the rate of mitochondrial respiration at birth. If so, the rate of respiration increased in the liver of term newborns immediately after delivery as indicated by the sharp increase observed in ATP concentrations (fig. 1). In addition, the increase in the ATP concentration closely coincided with the decrease in the cytoplasmic NADH/NAD ratio observed in the neonatal liver immediately after delivery (5). Consequently, the increase in the ATP concentration seems to be related to the increase of oxygen availability due to the onset of breathing, rather than to a rapid maturation of the mitochondrial function. Thus, the increase of ATP concentrations was initiated very early after delivery (fig. 1), when it was unlikely that the maturation of the mitochondrial function, brought about by the increase of intramitochondrial ATP concentrations (9) and/or by the effect of hormones (2, 10) would be attained.

In contrast to term newborns, preterm newborns showed high ATP concentrations at delivery without dramatic changes throughout the observation period (fig. 1). These results may suggest that the respiratory rate of preterm liver was enough to maintain high ATP concentrations. However, these results give no information concerning the oxygen availability of the preterm liver. Thus, it was not possible to observe the expected rise of ATP concentrations which were already high at delivery. Therefore, these results suggest that liver adenine nucleotide concentrations may not be an indicator of oxygen availability in these circumstances. This suggestion is consistent with the observation that the supply of pure oxygen to term and preterm newborns during the first two hours after delivery did not make any significant change in liver adenine nucleotide concentrations (results not shown).

On the other hand, if it is assumed that high ATP concentrations may be the trigger for the maturation of newborn liver mitochondria (9), we can suggest that «hypercontraction» of the inner mitochondrial membrane takes place in preterm liver immediately after delivery while an hour delay for the effect should be expected in term liver i.e. before initial ATP concentrations of preterm liver were reached. Alternatively, if the increase of ATP concentrations is a consequence of the onset of mitochondrial ADP-depend-

ent respiration (2) our results are insufficient to decide definitely about the maturity of the preterm liver mitochondria. Consequently, further work has to be done to establish an indicator of mitochondrial maturity *in vivo* where the oxygen availability was not artefactual.

In conclusion, our results suggest that the efficiency of preterm liver mitochondrial function may not be limited by the immaturity of the mitochondrial machinery. If so, oxygen availability may play a major role in the efficiency of preterm liver mitochondria during the early extrauterine life.

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Resumen

Se estudian los cambios de las concentraciones hepáticas de nucleótidos adenílicos durante el período perinatal en neonatos a término y prematuros. La concentración de ATP disminuye durante los dos últimos días de vida intrauterina. Tras el parto, las concentraciones de ATP en neonatos a término aumentan durante la primera hora de vida extrauterina. En neonatos prematuros, las concentraciones de ATP son superiores a las de neonatos a término y no cambian durante las dos primeras horas de vida extrauterina. En el momento del parto, la «carga energética» es superior en neonatos prematuros que en neonatos a término. Sin embargo, 30 minutos después del parto la «carga energética» se iguala en ambos grupos. Estos resultados sugieren que el estado energético del hígado es mayor en el neonato prematuro de rata en el momento del parto y no sufre cambios significativos durante el período postnatal inmediato.

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