PERINATAL DEVELOPMENT OF THE METABOLIC ZONATION OF HAMSTER LIVER PARENCHYMA

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1. Introduction

Adult liver catalyzes the antagonistic processes gluconeogenesis and glycolysis [1]. The heterogeneous distribution of glucose-6-phosphatase (G6Pase) over the liver parenchyma of the rat [2,3] and mouse [4] was interpreted to indicate a metabolic zonation of the liver lobule with respect to carbohydrate metabolism [5,6]. In the G6Pase-rich periportal zone glucose should be formed from gluconeogenesis and glycogenolysis. In the G6Pase-poor pericentral zone glycolysis may be the predominant process [7]. This metabolic zonation is influenced by changes of the nutritional state [5]. Prenatal liver parenchyma appears to catalyze only glycolysis [8,9], it should thus not be metabolically zonated. It was, therefore, of interest to study the perinatal development of the metabolic zonation and to try to correlate it with the drastic alteration of the nutritional state occurring with birth and during the following neonatal period.

In the present study the following results have been obtained: (1) G6Pase is absent from prenatal liver. It appears rapidly in a homogeneous distribution with birth. From the 3rd or 4th postnatal day the zonation of G6Pase begins to develop; it is completed around the 12th day. (2) Glycogen storage begins 3 days before birth; it reaches a maximum with a homogeneous distribution at birth. Glycogen breakdown occurs rapidly during the first postnatal days predominantly in the G6Pase-rich zones. (3) Both the development of the zonation of G6Pase and of glycogen can be correlated to the perinatal switch from carbohydrate- to protein- plus fat- and back to carbohydrate-rich nutrition.

2. Methods

Syrian goldhamsters (80–120 g) were fed a carbohydrate-rich pelleted diet (Aufzuchtfutter 7010, Altromin, D-4937 Lage) ad libitum and maintained in a 12 h day–night rhythm. The copulations were performed at 7 p.m.; birth then occurred during the morning hours of the 16th day post coitum (p.c.) [10]. All animals were killed between 9 and 11 a.m. by decapitation and exsanguinated. Fetuses were obtained by Caesarean section after anesthesia of the mother animal with 60 mg sodium pentobarbital per kg body weight. For biochemical determinations the livers were removed and stored in liquid nitrogen; for histochemical investigations the whole body of fetuses and neonatal (up to 5th days of age) animals were frozen in liquid nitrogen after decapitation. Of older animals only the livers were frozen.

The preparation of cryostat sections of the livers or of whole animals across the liver, the histochemical and the biochemical methods have previously been described [5].

3. Results

During the last 2 days before term a rapid and continuous increase of glycogen content from 0.3 g/100 g liver to more than 9 g/100 g around birth took place (fig.1). Histochemically glycogen-rich hepatocytes were homogeneously distributed over the whole liver parenchyma (fig.2a). Glycogen was stored before birth (fig.2b) as fast as it was degraded immediately after birth. This depletion of glycogen initially

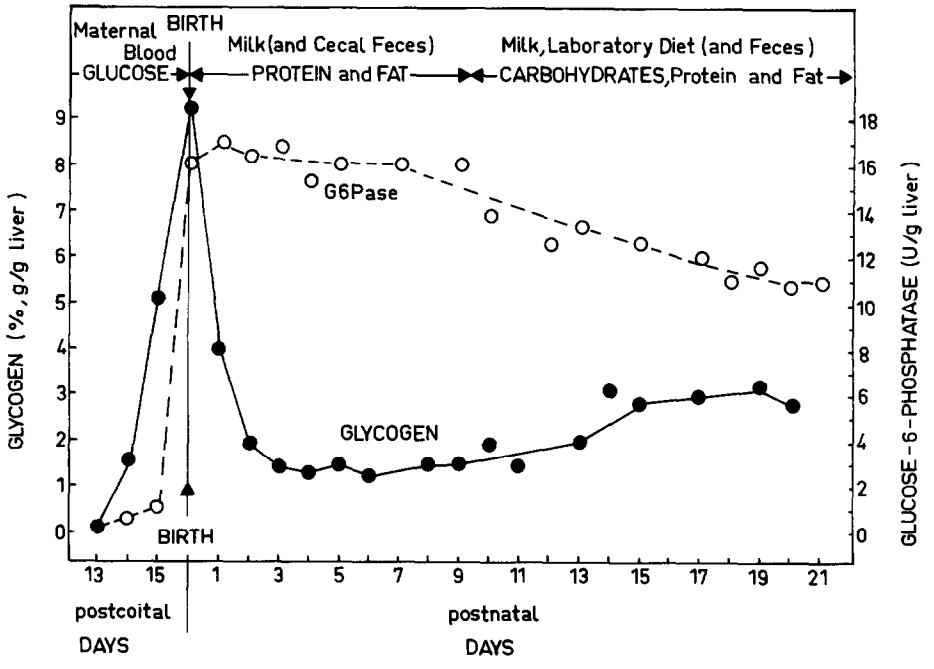

Fig.1. Levels of glucose-6-phosphatase and glycogen in prenatal and postnatal hamster livers (U/g = μmol/min × g wet weight). The points represent the average of two or three determinations.

appeared to be homogeneous over the liver acinus. On the second and third postnatal day it continued in the periportal zone 1 (and 2) while it was not demonstrable in the perivenous zone 3 (fig.2c). At the same time the differentiation of the functional structure of the liver acini began to be visible. Between the 3rd and 10th day of life the described heterogeneous glycogen distribution in the liver parenchyma and the biochemically determined glycogen content of about 1.5 g/100 g liver remained nearly unchanged. From the 10th or 11th day on the glycogen content increased again slowly to adult values around 4 g/100 g liver. Histochemically an intense storage of glycogen was visible in the periportal zone 1 and 2 and only a small or no additional accumulation in the previously glycogen-rich perivenous zone 3 (fig.2d).

Glucose-6-phosphatase increased rapidly during the last day before term from 0.5 U/g liver to 17 U/g and showed a maximum at the first postnatal day (fig.1). During its first prenatal appearance G6Pase activity appeared transitorily to be more intense around the afferent vessels (fig.2e); this initial uneven distribution was followed by a homogeneous increase of enzyme activity (fig.2f). In contrast to the glycogen content. G6Pase showed only a slow decrease during the postnatal period, which was slightly accelerated after the 9th postnatal day. At the end of the 4th week of life the G6Pase activity reached the adult values of 8 U/g liver. Histochemically G6Pase increased in the periportal zone 1 (and 2) of the acinus from the 3rd postnatal day on, while reciprocally the activity in the perivenous zone 3 decreased (fig.2g). Thus also G6Pase began to be heterogeneously distributed over the liver acinus at the same time as glycogen. The metabolic zonation which is found in adult liver (fig.2h) was established around the 12th day a periportal G6Pase-rich zone, where during the normal daily feeding rhythm glycogen is stored and depleted and a pericentral G6Pase-poor zone, where glycogen metabolism is apparently less active.

4. Discussion

It was shown in this communication that – as judged from the G6Pase and glycogen distribution over

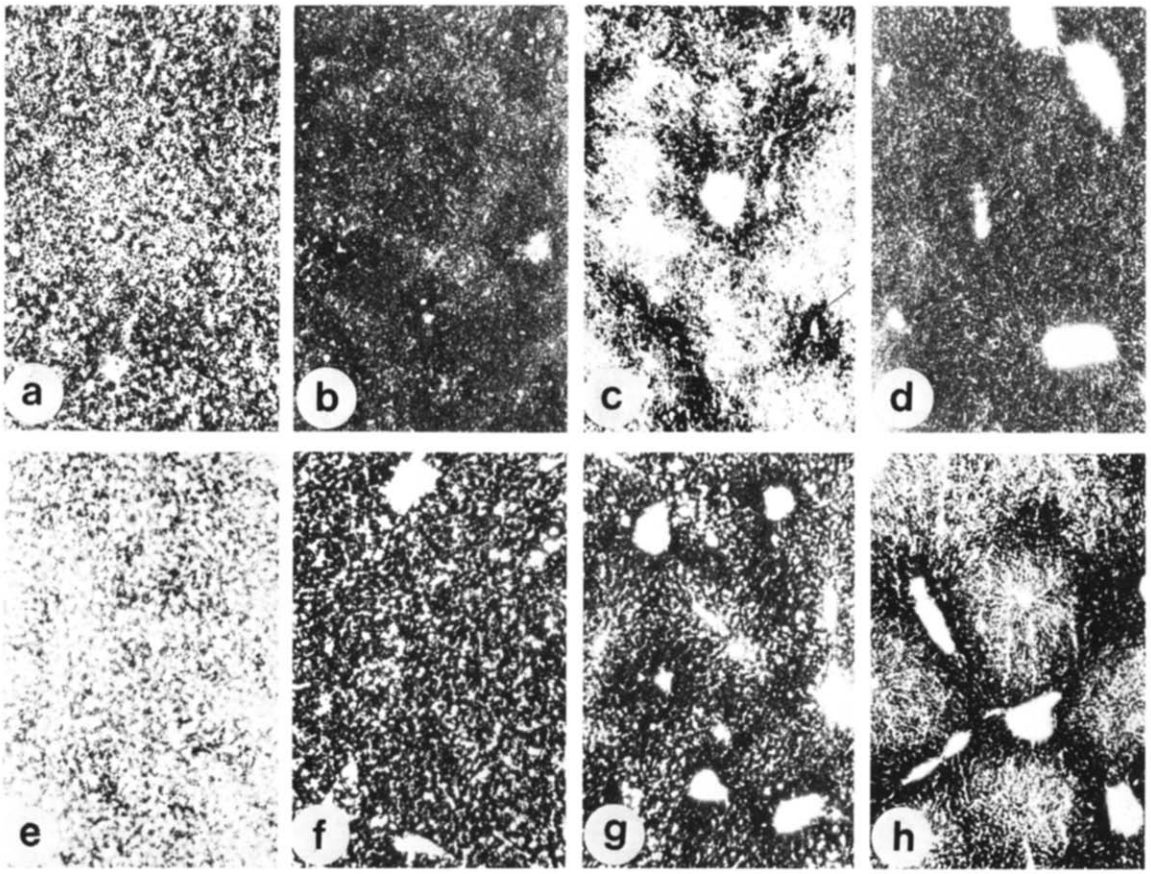

Fig.2. Distribution of glucose-6-phosphatase and of glycogen over the liver parenchyma of hamsters during perinatal development. All photographs 25:1. (a) Low glycogen level two days before birth, (b) high glycogen level homogeneously distributed at birth, (c) glycogen depletion in zone 1 and 2 four days after birth, (d) glycogen repletion in zone 1 and 2 eighteen days after birth. (e) First appearance of glucose-6-phosphatase one day before birth, (f) high glucose-6-phosphatase level homogeneously distributed at birth, (g) glucose-6-phosphatase begins to be heterogeneously distributed four days after birth, higher levels in zone 1 and 2, (h) high glucose-6-phosphatase in zone 1 (and 2) and low levels in zone 3 eighteen days after birth.

the parenchyma – the metabolic zonation of the 'bifunctional*' adult liver parenchyma into glycogenic and glycolytic hepatocytes develops from a 'unifunctional' glycolytic state of the organ before birth via another probably also 'unifunctional' glucogenic state with birth. The development of the metabolic zonation may be viewed on the background of the two major physiological changes occurring with birth: the change of the hemodynamics causing the differentiation of the anatomical structure of the liver acinus and the change of nutrients [9].

With the appearance of portal fields and efferent venules after birth, steep gradients of oxygen, substrate and hormones may be formed for the first time. With the switch from maternal blood to maternal milk after birth and later to laboratory diets as nutrient sources, different energy substrates have to be utilized. Both the gradients and the different energy substrates should be important factors causing the metabolic zonation.

*In this context the terms uni- and bifunctional are used only with respect to carbohydrate metabolism; they are therefore always given in parentheses.

A good correlation appears to exist between this development and the alteration of the nutritional state (fig.1). The fetal liver utilizes glucose from the maternal blood [11] as main energy substrate and thus is 'unifunctional' glycolytic: glycogen and the glucogenic enzyme G6Pase are neither present nor necessary (figs.1 and 2a, e) [8].

With birth the glucose infusion by the maternal blood is suddenly interrupted. The liver now has the function to maintain the glucose supply by glucogenolysis until other energy substrates are offered by the fat- and protein-rich but carbohydrate-poor maternal milk. During suckling glucose production by gluconeogenesis seems to be necessary for the energy supply of erythrocytes and the central nervous system. Therefore the liver should be 'unifunctional' glucogenic. Indeed G6Pase is high (fig.1) and during the first postnatal days uniformly distributed (fig.2b, f).

At the same time the liver structure with periportal fields and acini develops under the influence of the postnatal hemodynamic state. The appearing gradient of oxygen (pO_2) from the afferent portal vessel to the efferent vein may influence strongly the development of metabolic zonation starting around the 3rd postnatal day preparing the organ for the next nutritional change to come. The G6Pase-rich periportal and the G6Pase-poor perivenous zone become demonstrable (fig.2c, g).

Gradually during the second week of life the liver is offered again more glucose besides amino acids and fatty acids due to the increased uptake of high carbohydrate laboratory food besides maternal milk [12]. The organ now has the dual function of a glucostat. It maintains the glucose supply via gluconeogenesis and glycogenolysis, when the glucose uptake from the intestine is low, and it removes glucose, when the uptake is high, via glycolysis (liponeogenesis) and glycogen synthesis. The organ should be 'bifunctional' glucogenic and glycolytic, and as this may be of regulatory advantage it should also be metabolically zonated. In fact, the uneven distribution of G6Pase that began to develop at the 3rd day is now completed; G6Pase is predominantly located in the periportal zone 1 and 2 (fig.2h).

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