FUNCTIONAL HETEROGENEITY OF RAT LIVER PARENCHYMA AND OF ISOLATED HEPATOCYTES*

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1. Introduction

It has been observed recently that rat hepatocyte suspensions catalyze glycolysis and gluconeogenesis simultaneously [1]. The simultaneous catalysis of the two antagonistic processes may occur in one and the same hepatocyte or, as has been proposed [1], in two different types of hepatocytes, one catalyzing glycolysis the other gluconeogenesis. The key enzyme for the differentiation of such two hypothetical types of hepatocytes is glucose-6-phosphatase; only the cells possessing this enzyme will be able to release glucose and hence be "gluconeogenic".

In the present study it is shown that in the rat liver parenchyma zones with high and low activities of glucose-6-phosphatase, glycogen synthase and glycogen phosphorylase can be differentiated and that this differentiation is preserved in isolated single hepatocytes. The glucose-6-phosphatase-rich zone is always located around the portal branches; its relative size is subject to dietary changes. The observed doubling of the glucose-6-phosphatase level in the liver upon starvation appears to be due to both an increase in the number of glucose-6-phosphatase-rich liver cells and a rise in enzyme level within the already enzyme-rich hepatocytes.

2. Methods

All chemicals were reagent grade. Enzymes, coenzymes and substrates were from Böhringer GmbH, D 68 Mannheim, except amyloglucosidase, which was supplied by Merck AG, D 61 Darmstadt.

Female Wistar rats (150–200 g) were maintained in a 12 hr day–night rhythm on ad libitum feeding until about 1 hr before the experiment (fed animals; 09.00 hr) or 8, 24 and 36 hr before the experiment (fasted animals; 16.00 hr, 09.00 hr and 21.00 hr next day). The animals were anesthetized by intraperitoneal injection of 60 mg sodium nembutal per kg body weight. Livers were exsanguinated in situ by the non-recirculating pre-perfusion routinely used for isolating hepatocytes. For biochemical analyses livers were then removed and frozen in liquid N_2. Glycogen was determined after amyloglucosidase hydrolysis enzymatically [2]. Glucose-6-phosphatase was assayed as reported [3] DNA was measured with the indole·HCl reagent [4].

For histochemical purposes the papilliform lobe was ligated, cut off and frozen in liquid N_2. From the remainder of the same liver single hepatocytes were prepared by the method of Berry and Friend [5] and frozen equally. The frozen liver pieces and hepatocyte pellets were brought together into the cryostat (Kryotom WKF). Native cryostat sections of 20 μm were made at -20°C and thawed on coverslips. Glucose-6-phosphatase was demonstrated by precipitation of phosphate, liberated from glucose-6-phosphate at pH 6.7, with lead [6]. Glycogen synthase was detected by staining glycogen, which was newly formed from UDP-glucose as substrate in the presence of glucose-6-phosphate as activator at pH 7.4, with the PAS reaction [7]. Glycogen phosphorylase was located by staining glycogen, which was newly synthesized from glucose-1-phosphate as substrate at pH 6.8, with Lugol's solution [8]. Liver glycogen was demonstrated with the PAS reaction [9]. All specimens were imme-

* Dedicated to Professor Dr. F. Timm on the occasion of his 80th birthday.

diately photographed at the end of the histochemical assays. The border between the areas with high and low or no activity of G6Pase was arbitrarily set on the photographs. The relative size of the areas was roughly estimated by planimetry.

3. Results

Glucose-6-phosphatase, glycogen synthase and glycogen phosphorylase were found to be unevenly distributed in the liver parenchyma of fed rats. Glucose-6-phosphatase activity was high in the periportal area (i.e. zone 1 of the liver acinus [10]) and low or absent around the central vein (i.e. zone 2 and 3). Glycogen synthase and glycogen phosphorylase were also restricted to the periportal zone (fig.1). Glycogen was demonstrable in all hepatocytes; a slightly higher level being sometimes observed in the periportal area. These findings suggest that in a given dietary state there exists within the liver parenchyma a biochemical heterogeneity, which is probably 'induced' by the different environment of the cells with respect to the supply of oxygen, substrates and hormones [10]. The apparent adaptation of the cells to the different environment within the parenchyma was found to be stable at least during the preparation of single hepatocytes. Cells with 'high' levels of the enzymes studied can clearly be distinguished from cells with low levels or no apparent activity at all (fig.1). Thus also the widely used 'homogeneous' liver cell suspensions [5,11–13] may have to be regarded as biochemically heterogeneous.

Since the apparently different types of hepatocytes should still have the same genetic potential, the possibility was considered that their state of adaptation might reversibly change in response to the different substrate and hormone supply during absorptive and postabsorptive periods. Therefore the distribution of the enzymes and of glycogen in the liver parenchyma was also studied in fasted rats. Glycogen synthase was found after 24 hr of starvation only and with very low activity in the periportal region, whereas glycogen phosphorylase activity increased in the periportal zone 1 and became demonstrable also within the intermediate zone 2 and sometimes also in the perivenous zone 3.

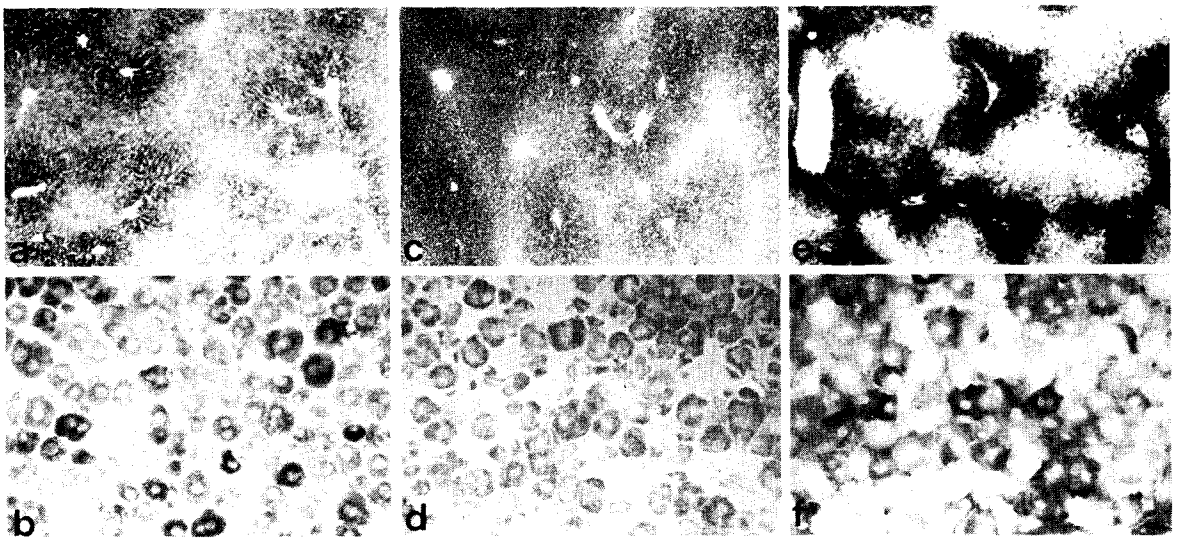

Fig.1. Uneven distribution of glucose-6-phosphatase (G6Pase), glycogen synthase (GgS) and glycogen phosphorylase (GgP) over the liver parenchyma of a fed rat and over a single hepatocyte population of the same animal. a) G6Pase in liver parenchyma, 25 : 1, main activity in the periportal zone 1; b) G6Pase in a hepatocyte suspension, 160 : 1, unequal distribution; c) glycogen synthase in liver parenchyma, 25 : 1; main activity in the periportal zone 1; d) glycogen synthase in a hepatocyte suspension, 160 : 1, unequal distribution; e) glycogen phosphorylase in liver parenchyma, 25 : 1, main activity in the periportal zone 1; f) glycogen phosphorylase in a hepatocyte suspension, 160 : 1; unequal distribution.

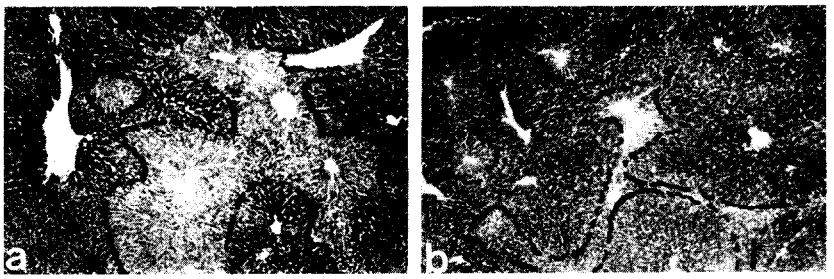

Fig.2. Distribution of glucose-6-phosphatase over the rat liver lobule in dependence of the dietary state. a) 8 hr, b) 36 hr of starvation. Boundaries between zones of high and low activity are marked with dotted lines.

After 8 hr of starvation glycogen was clearly diminished only in zone 1; after 24 hr it was no longer detectable in any of the 3 zones. The parenchymal distribution of glucose-6-phosphatase was not changed after 8 hr of starvation; yet after 24 hr a high activity was not only found in zone 1 – as in the fed state – but also in zone 2 and after 36 hr even in larger portions of zone 3. The glucose-6-phosphatase-rich zone often appeared slightly more active in the fasted than in the fed liver (fig.1).

Finally the biochemical activity of glucose-6-phosphatase was determined in livers of fed and fasted animals in order to support the histochemical findings. In agreement with a more recent report [4] but in contrast to an earlier observation [14] it was found that the level of the enzyme was increased 2 fold during the transition from the fed to the 24 hr fasted state (table 1). As control data the liver weight relative to the body weight and the glycogen level were recorded. A rough estimate of the relative size of the glucose-6-phosphatase-rich zone revealed that it occupied about 40–60% of the liver parenchyma in the fed animal and about 75–85% in 24 hr fasted animal. A comparison of the biochemical and histochemical data would suggest that the observed doubling of glucose-6-phosphatase activity upon starvation is due to both an increase in the number of enzyme-rich cells and to a rise in enzyme level within the already enzyme-rich cells. The first phenomenon seems to be more important (table 1).

4. Discussion

It was shown in this communication that glucose-6-phosphatase (G6Pase), glycogen synthase, glycogen phosphorylase, and glycogen are unevenly distributed in rat liver parenchyma as well as in isolated hepatocytes. It is impossible at present to decide whether the activity-poor cells contain much less or no molecules of these enzymes as such or only of their active forms. Whatever the case may be, the present results show that hepatocytes from one and the same physiological state have a different outfit of active enzymes and of cell constituents and thus probably different metabolic functions.

The key enzyme for a functional differentiation is G6Pase, since only cells containing the enzyme can release glucose. The G6Pase-rich zone is localized around the afferent vessels; it is enlarged upon starvation. An uneven distribution of G6Pase was first observed in mice [15] and later also in rats [16–18]; since this could not be confirmed in one report [19] a reinvestigation was called for [20]. A study of the dependence of G6Pase distribution on the dietary state did not appear to be available. It is well feasible now that different dietary and hormone states might have been the cause for the conflicting results previously reported.

Functionally the G6Pase-rich cells could be the 'gluconeogenic' and the G6Pase-poor or -negative cells the 'glycolytic' hepatocytes as proposed [1]; the intermediary cells or cells 'in transition' may be difficult to classify functionally at present. Zonation of the liver parenchyma into 'gluconeogenic' and 'glycolytic' hepatocytes implicates that the antagonistic enzymes phosphofructokinase (PFK) and fructosediphosphatase (FDPase) as well as pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK) should be predominantly localized in the assumed functionally different cells. The proposal of metabolic zones in the

Table 1
Comparison of the biochemical and histochemical activity of glucose-6-phosphatase in livers from fed and fasted rats.

Dietary state	Liver	Glycogen	G6Pase					
			Level	Content		Staining intensity		
	g/100 g bw	μmol/g lww	U/g lww	U/mg DNA	U/100 bw	zone 1	zone 2	zone 3
fed (n=4)	3.35 ± 0.13	279 ± 46	4.45 ± 0.38	2.07 ± 0.08	14.7 ± 1.6	++ ↓ +++	– ↓ +	– ↓ +
fasted 24 hrs (n=4)	2.75 ± 0.17	4 ± 2	8.70 ± 0.92	3.26 ± 0.36	24.0 ± 2.7	++ ↓ ++++	++ ↓ +++	– ↓ ++

lww = liver wet weight; bw = body weight; U = 1 μmol/min; values given are means ± S.D.

liver is strengthened by the observation that during the diurnal fast glycogen is degraded at first in the periportal G6Pase rich cells.

However, the presence of G6Pase is only a necessary but not a sufficient condition for a cell to be classified as gluconeogenic; FDPase and PEPCK would be required too. Since the parenchymal distributions of FDPase and PEPCK are not known, one cannot distinguish between two possibilities: a) If upon starvation not only G6Pase but also FDPase and PEPCK would spread over the parenchyma, the number of gluconeogenic cells would be increased and that of the glycolytic cells decreased. b) If upon fasting only G6Pase rather than FDPase or PEPCK would spread over the parenchyma, the number of gluconeogenic cells would stay unchanged but that of the effectively glycolytic cells would be decreased since the presence of G6Pase in the normally glycolytic cells might reduce or prevent utilization of glucose.

Metabolic zonation of the liver parenchyma with respect to gluconeogenesis and glycolysis may be regarded as a 'one big futile cycle' model in contradistinction to the usual 'three small futile cycles' model. The switch from net glycolysis to net gluconeogenesis would be dependent on the relative saturation of the single processes by their respective substrates and on the relative size of the metabolic zone.

The proposed model would circumvent the major problem encountered with the usual model, namely explanation of the regulation of the small futile cycles between fructose-6-phosphate and fructose-1,6-diphosphate or between phosphoenolpyruvate and pyruvate. In liver the concentration changes of effectors such as ATP, AMP, citrate or fructosediphosphate occur in the wrong direction [21]; they cannot explain the switch. This is in contrast to yeast, in which at least with fructose diphosphate a 100 fold change in the right direction is observed [22]. The proposed model does not appear to be energetically very favorable, nor does the usual model, but it would equally well account for the energy cost of gluconeogenesis observed experimentally [23,24]. However, it presents clear regulatory advantages: a) The liver can immediately respond in both metabolic directions to a sudden change of the substrate supply in an autoregulatory manner, independent of hormonal control; this behavior would be expected if the liver were an independent metabolitostat. b) The liver can adapt, dependent on hormonal control, to longer lasting physiological situations by changing the ratio of 'gluconeogenic' to glycolytic' cells. Thus regulatory potential appears to have its price, 'waste of energy' [25]. The 'big futile cycle' may be a utile one.

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