HETEROGENEOUS DISTRIBUTION OF GLUCOSE-6-PHOSPHATASE IN MICRODISSECTED PERIPORTAL AND PERIVENOUS RAT LIVER TISSUE

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1. Introduction

Liver and kidney catalyze glycolysis as well as gluconeogenesis [1–3]. In the rat nephron these two antagonistic processes are spatially separated between proximal and distal tubules, as could be demonstrated by studies of enzyme activities in microdissected kidney tissue [4–6]. In rat liver parenchyma the two pathways might also be catalyzed in different cells, which would best explain the simultaneous catalysis of glycolysis and gluconeogenesis observed [7,8]. Histochemical studies of liver parenchyma showed a heterogeneous distribution of glucose-6-phosphatase (G6Pase). It was proposed that in the G6Pase-rich periportal* zone glucose formation by gluconeogenesis and glycogenolysis should be catalyzed, while in the G6Pase-poor perivenous* zone glycolysis may be the predominant process [9].

Histochemical enzyme determinations mostly provide only a qualitative or at best a semiquantitative information. Therefore, a quantification of histochemical findings appeared desirable. In the present investigation G6Pase was quantitatively determined by direct enzyme measurement in periportal and perivenous liver tissue separated by microdissection.

It was found that the G6Pase activity of fed animals was about 2.3-fold higher and of fasted animals about 1.7-fold higher in zone 1 than in zone 3. This finding supports the theory of a 'metabolic zonation' of liver parenchyma into functionally different hepatocytes [7–9].

*Periportal = around the terminal portal vessels, i.e., mainly zone 1 of the liver acinus.
Perivenous (pericentral) = around the central vein, i.e., mainly zone 3 of the liver acinus [10]

2. Methods

Enzymes, coenzymes and substrates were from Boehringer GmbH, D 68 Mannheim; all other chemicals were reagent grade and obtained from Merck AG, D 61 Darmstadt. Radioactive substrates were supplied by Amersham Buchler GmbH, D 33 Braunschweig. [U-14C]Glucose-6-phosphate and [U-14C]fructose-6-phosphate were purified by chromatography on Whatman No. 3 paper with pyridine/butanol/water (1:1:1).

Female Wistar rats (150–180 g) were maintained on a 12 h day–night rhythm. Livers were removed from the abdominal cavity after a short perfusion with 0.9% NaCl solution during anesthesia with 60 mg sodium pentobarbital/kg body wt. For histochemical G6Pase determination and microdissection 20 μm sections were prepared as described [9]. Lyophilization, microdissection and determination of sample dry weight were carried out according to Lowry and Passonneau [11]. Parallel sections with histochemical demonstration of G6Pase [12] and succinate-dehydrogenase [13] were used for identification periportal and perivenous zones. Homogenates of fresh liver (1:500) or of lyophilized liver sections (1:2000) were prepared in a glass homogenizer with 40 mM sodium cacodylate buffer, pH 6.5. Endoplasmic reticulum was isolated by the method of de Duve et al. [14].

G6Pase Activity was assayed by colorimetrical determination of phosphate release [15] or by radiochemical measurement of [14C]glucose production from D-[U-14C]glucose-6-phosphate. Specificity tests were performed similarly with D-[U-14C]fructose-6-phosphate and [U-14C]glycerol-3-phosphate as sub-

strates. The incubation of 0.2–2.5 μg tissue dry wt
or equivalent amounts of homogenate was performed
at 30°C in 30 μl 40 mM sodium cacodylate buffer,
pH 6.5, containing 1 mM radioactive substrate
(8×10^6 dpm/μmol) and 0.1% bovine serum albumin.
The reaction was stopped in the cold by addition of
30 μl 1.2 N perchloric adic. After centrifugation 50
μl of supernatant were neutralized by addition of 28
μl 1.2 N potassium bicarbonate and precipitated
potassium perchlorate was removed. Separation of
glucose and glucose-6-phosphate, of fructose and
fructose-6-phosphate and of glycerol and glycerol-3-
phosphate was achieved by chromatography on 0.5 g
Dowex-1 $\times$ 8-formate. Neutralized supernatant, 70
μl, were applied and washed into small columns
(0.4 cm diam., 0.75 ml vol.) with 2 ml water. From the
corresponding effluent, 1.5 ml were collected as
fraction 1 and 0.5 ml as fraction 2. Elution was con-
tinued in 1 ml portions with 0.4 M sodium formate
as eluent. Glucose, fructose and glycerol appeared in
fraction 1, glycose-6-phosphate, fructose-6-phosphate
and glycerol-3-phosphate in fractions 3, 4 and 5 and
lactate in fractions 3 and 4. The fractions were mixed
with 10 ml Bray scintillator [16] and counted
directly. Protein determination was performed accord-
ing to Lowry et al. [17].

3. Results

3.1. The assay of glucose-6-phosphatase

Glucose-6-phosphatase activity is normally tested
by measuring the release of phosphate. Since the
sensitivity of the colorimetrical phosphate assay [15]
is not sufficient for the determination of glucose-6-
phosphatase in microdissected liver tissue, a radio-
chemical enzyme assay was developed. A substrate
concentration of 1 mM [U-^{14}C]glucose-6-phosphate,
which yielded 70% of the maximal enzyme activity
[18], was chosen, since it gave suitable blank to test
ratios. [^{14}C]Glucose formed by enzyme catalysis
was essentially not further metabolized. During an
incubation of 1 mM [U-^{14}C]glucose instead of
[U-^{14}C]glucose-6-phosphate with homogenate corre-
sponding to 1-2 μg liver dry wt only 0.1–0.5 μmol
glucose/min $\times$ g liver disappeared, which was con-
siderably less than 5% of the average G6Pase activity
(table 1).

Table 1
Comparison of liver glucose-6-phosphatase in fresh and
lyophilized and microdissected tissue

	Enzyme activity (μmol/min/g dry wt)
Homogenate of fresh tissue	12.4 ± 0.74 (3)
Homogenate of lyophilized tissue	13.1 ± 0.64 (3)
Average of two micro-dissected periportal and two perivenous tissue structures	14.4 (2 + 2)

Mean value ± standard deviation. Radiochemical test.
Number of determinations in parentheses.

Glucose was formed from glucose-6-phosphate
linearly with time for at least 35 min in an incubation
with homogenate of fresh or lyophilized liver
corresponding to 2 μg dry wt (fig.1). Glucose produc-
tion was also linearly correlated with the amount of
incubated liver homogenate between 0.25 μg and
2.5 μg dry wt/incubation tube (fig.1). Protein- as well
as time-dependences were also demonstrable if dis-
sected periportal liver tissue was incubated. Within
the same liver the enzyme activity in homogenates of
fresh and of lyophilized tissue was in the same range
as the average activity of two dissected periportal and
two perivenous tissue structures; this demonstrated,
that there was no loss of G6Pase activity during the
microdissection procedure (table 1).

The specificity of the radiochemical assay was the
same as that described for the colorimetrical G6Pase
test [19]. [U-^{14}C]Fructose-6-phosphate and [U-^{14}C]-
glycerol-3-phosphate were cleaved in homogenates
with relative (G6P 100%) activities of 61% and 4.4%
respectively and in endoplasmic reticulum prepara-
tions with relative activities of 20.3% and 2.7%
(table 2). In homogenates the relatively active hydro-
lysis of fructose-6-phosphate seemed to be catalyzed
mainly after isomerization to glucose-6-phosphate
by the hexose-6-phosphate-isomerase of the prepara-
tion [20]. In the isolated reticulum hydrolysis of
fructose-6-phosphate seems to represent a genuine
unspecificity of G6Pase [19]. The pH-optimum of
the radiochemical assay was around 6.5 with only
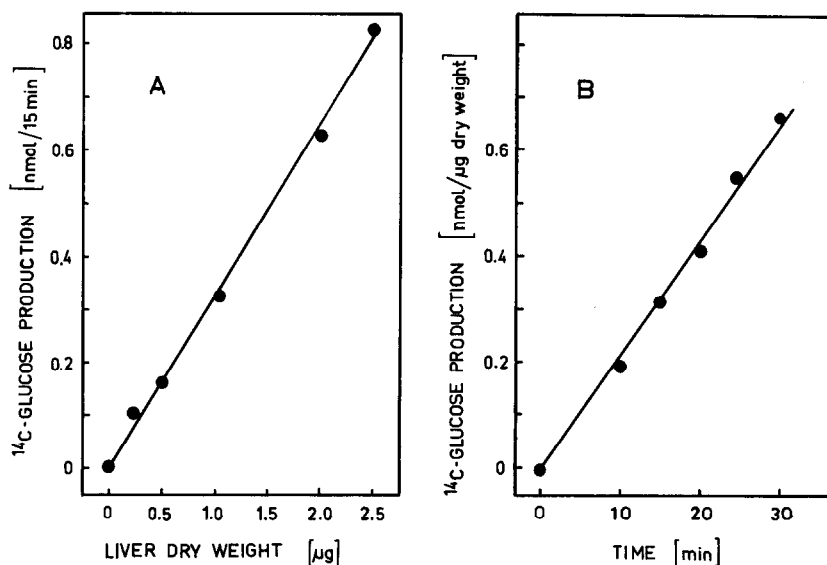20% activity, at pH 4.8 and 19%, at pH 9.8. G6P-

Fig.1. (A) Protein- and (B) time-dependence of glucose formation from glucose-6-phosphate in rat liver homogenate. Sodium cacodylate 40 mM, pH 6.5, [U-^{14}C]glucose-6-phosphate 1 mM (8 X 10^6 dpm/μmol), bovine serum albumin 0.1%, rat liver homogenate corresponding to dry wt 0.25–2.5 μg (A) 2 μg (B), respectively. Incubation vol. 30 μl. The incubation at 30°C was stopped after 15 min (A) or after the indicated time (B) by addition of 30 μl of 1.2 N perchloric acid. Separation of glucose and glucose-6-phosphate was performed by chromatography of the neutralized supernatant as described under Methods.

Hydrolysis by unspecific acid or alkaline phosphatases could thus have been of minor importance only.

3.2. *The distribution of glucose-6-phosphatase in dissected periportal and perivenous liver tissue*

Having established the assay and shown, that it was sensitive and specific enough for enzyme determination in less than 1 μg liver dry wt, G6Pase was measured in 0.2–1 μg microdissected periportal and perivenous liver tissue. The specific G6Pase activity varied from one liver to the other (table 3). The ratio of periportal to perivenous activities was on the

Table 2
Specificity of the radiochemical glucose-6-phosphatase assay

Substrate (1 mM)	Phosphatase activity			
	Homogenate		Endoplasmic reticulum	
	(μmol/min/g dry weight)	(%)	(μmol/min/g protein)	(%)
Glc[a]-6-P, pH 6.5	23.1	100	59.6	100
pH 4.8	4.7	20.6		
pH 9.8	4.4	19.0		
Frc[b]-6-P, pH 6.5	14.1	61.0	12.0	20.3
Gll[c]-3-P, pH 6.5	1.0	4.4	1.6	2.7

[a] Glc = Glucose
[b] Frc = Fructose
[c] Gll = Glycerol

Table 3
Glucose-6-phosphatase activity in periportal and perivenous liver tissue

Liver		Enzyme activity (μmol/min/g dry wt)				
		Periportal	Perivenous	P	Periportal / Perivenous
Fed	1[a]	21.9 ± 4.0 (3)	8.5 ± 0.6 (3)	<0.01	2.58
	2[a]	12.6 ± 5.1 (3)	3.4 ± 0.1 (2)	<0.05	3.70
	3[a]	20.3 ± 5.0 (3)	5.9 ± 5.0 (2)	<0.025	3.44
	4	19.1 ± 1.1 (2)	7.6 ± 3.5 (4)	<0.01	2.52
	5	11.3 ± 1.0 (3)	8.1 ± 2.1 (2)	<0.1	1.39
	6	14.1 ± 4.4 (3)	6.3 ± 2.5 (3)	<0.05	2.24
	7	11.4 ± 1.5 (4)	6.6 ± 0.7 (4)	<0.0025	1.73
	8	14.7 ± 1.4 (3)	5.4 ± 0.8 (3)	<0.0005	2.72
	1–8	15.33 ± 4.94 (24)	6.75 ± 2.54 (23)	<0.0005	2.27
Fasted	9[a]	51.6 ± 17.3 (4)	22.2 ± 5.5 (3)	<0.025	2.32
	10	33.9 ± 4.4 (4)	19.2 ± 5.3 (4)	<0.005	1.77
	11	20.9 ± 4.7 (4)	14.2 ± 1.4 (4)	<0.025	1.47
	12	22.0 ± 2.5 (4)	14.3 ± 0.9 (7)	<0.0005	1.54
	13	27.4 ± 2.8 (6)	23.0 ± 5.5 (5)	<0.1	1.19
(24 h)	9–13	30.81 ± 13.12 (22)	18.06 ± 5.29 (23)	<0.0005	1.71

[a] Values from earlier experiments [25]

Mean value ± standard deviation. Number of determinations in parentheses. Statistics: Student's t-test.

average 2.27 in fed rats and 1.71 in fasted animals (table 3). After 24 h starvation G6Pase activity rose as expected [9] two-fold in the periportal and 2.6-fold in the perivenous zone (table 3). This further indicates the specificity of the G6Pase determination.

4. Discussion

In this communication it was shown by means of microdissection techniques and of a new radioactive enzyme assay of high sensitivity, that in fed rats G6Pase had an approx. 2.3-fold higher activity in periportal than in perivenous areas. Other enzymes of carbohydrate metabolism also exhibit similar periportal/perivenous activity ratios. The gluconeogenic phosphoenolpyruvate carboxykinase showed a ratio of 2.88:1, the glycolytic pyruvate kinase of 1:2.28 [21], and the glutamate–pyruvate-transaminase, which can be classified in liver as glucogenic, a ratio of 2.78:1 [22] or 5:1 [23], respectively. All these

ratios are decreased in the fasted state. These results, which had been expected on the basis of previous histochemical observations [9,24] are consistent with the proposed metabolic zonation of liver parenchyma into periportal glucogenic and perivenous mainly glycolytic zones [7–9]. In liver, therefore, gluconeogenesis and glycolysis seem to be located in different parenchymal cells in a manner similar to the spatial separation of the two processes in kidney [4–6].

The activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase was higher in proximal than in distal tubules; the difference was 8-fold in fed animals [4,5]. Reciprocally the glycolytic enzymes showed higher activities in distal than in proximal tubules; in the fed state the ratio observed was 12 for hexokinase, 7 for phosphofructokinase and 30 for pyruvate kinase [4,6]. Compared with these data, the 2.3-fold difference in G6Pase activity between periportal and perivenous liver tissue seems to be relatively low. This may be due to physiological differences between liver and kidney.

In kidney the metabolic zonation along the tubule must be static. The size of each zone, i.e., the metabolic capacity of the nephrocytes, is irreversibly determined by the microarchitecture of the nephron. The capacity may only be increased or decreased with changing physiological conditions such as starvation or acidosis [4]. The enzyme outfit of the different zones can therefore be expected to be almost unifunctional with respect to carbohydrate metabolism. In liver, however, the zonation appears to be dynamic [9]. The size of the proposed zones, i.e., the metabolic capacity of hepatocytes within the liver acinus may reversibly change as a function of feeding or starvation. The enzyme pattern of the various zones in liver seems therefore to be not as strictly unifunctional as in kidney.

Although the periportal/perivenous activity ratios so far found may be relatively low, they should nevertheless be functionally significant. If the ratio for a glucogenic enzyme is 2.3:1 and for a glycolytic enzyme 1:2.3 the overall enzyme outfit for gluco-genesis and glycolysis in the two zones is different by a factor of about 5. Differences of this order of magnitude should not be metabolically meaningless. If, as can be expected under some conditions, all three irreversible steps of gluconeogenesis and glyco-lysis contribute to the rate-limitation, it is well feasible that this difference factor of the zones may even further be increased. Therefore, the findings of the present study can be regarded as an important support for the hypothesis of a 'Metabolic Zonation' of liver parenchyma.

Acknowledgements

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