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Glycogen synthesis from pyruvate in the periportal and from glucose in the perivenous zone in perfused livers from fasted rats

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The isolated liver of 24 h fasted rats was perfused in a non-recirculating manner in the orthograde or retrograde direction with media containing glucose and/or gluconeogenic precursors. Glycogen formation was determined biochemically and demonstrated histochemically. With glucose as the only exogenous substrate glycogen was formed exclusively in the perivenous area during both orthograde and retrograde perfusion. With gluconeogenic precursors as the exogenous substrates glycogen was deposited in the periportal zone during orthograde perfusion and in the intermediate zone during retrograde perfusion. Supply of glucose and gluconeogenic substrates initiated glycogen synthesis only in the upstream region, i.e. in the periportal zone during orthograde and in the perivenous zone during retrograde perfusion. This localization of glycogen synthesis was probably due to an unavoidable, insufficient oxygen supply of the respective downstream area. In general, the results confirm the hypothesis that periportal and perivenous glycogen was synthesized from different substrates.

Glycogen synthesis; Gluconeogenesis; Glucose paradox; Liver perfusion; Hepatocyte heterogeneity; Metabolic zonation

1. INTRODUCTION

It has been demonstrated by different groups that in livers from fasted rats glycogen is synthesized both via a direct pathway from glucose and via an indirect pathway from gluconeogenic precursors such as lactate and alanine [1-3]. The key enzymes of glycolysis and gluconeogenesis are distributed heterogeneously in the liver acinus. According to the model of metabolic zonation the gluconeogenic pathway is predominantly localized in the periportal area whereas glycolysis occurs mainly in the perivenous area. Consequently, the model assumes that periportal glycogen is synthesized via the indirect route from C_3 compounds and that perivenous glycogen is formed directly from glucose [4,5]. This concept is supported by the present investigation using the isolated perfused liver from fasted rats. A preliminary report of some of the findings has appeared previously [6].

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2. MATERIALS AND METHODS

2.1. *Materials*

All reagents were of analytical grade and were obtained from commercial sources. Enzymes were purchased from Boehringer (Mannheim), Sigma (Taufkirchen) and Merck (Darmstadt); parafuchsin hydrochloride from Fluka (Neu-Ulm), *p*-nitroblue tetrazolium from Serva (Heidelberg) and bovine serum albumin from Boehringer (Mannheim). Mercaptopicolinic acid was a gift from Smith Kline & French Laboratories (Philadelphia, USA).

2.2. *Animals*

Male Wistar rats (140-180 g before the 24 h fast)

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from Winkelmann (Borchen) were maintained on a 12 h day-night rhythm with free access to water and food (standard diet 1320 from Altromin, Lage). For all experiments the animals were fasted for 24 h. Anaesthesia was performed by intraperitonal injection of pentobarbital (60 mg · kg body wt^{-1}).

2.3. *Liver perfusion*

The liver was perfused in situ in a non-recirculating fashion in the orthograde and retrograde direction with a Krebs-Henseleit-bicarbonate buffer. Three different media were used: medium 1 contained 30 mM glucose and 0.2% bovine serum albumin, medium 2 contained 2 mM lactate, 0.2 mM pyruvate, 2 mM glutamine, 2 mM ornithine, 0.5 mM ammonium chloride and 0.2% bovine serum albumin and medium 3 contained both 30 mM glucose and the substrates of medium 2. The medium was equilibrated with 95% (v/v) O_2 and 5% (v/v) CO_2. In every experiment the liver was perfused for 1 h with a flow of about 5 $ml \cdot min^{-1} \cdot g^{-1}$ and samples of the perfusate were taken every 10 min. At time 0, 30 and 60 min tissue samples (150–250 mg) were cut off and immediately frozen in isopentane at −30°C.

2.4. *Histochemistry*

Air-dried 8 μm cryostat liver sections were used throughout. Succinate dehydrogenase (SDH, EC 1.3.99.1) was demonstrated using succinate and *p*-nitroblue tetrazolium as substrates [7]. Glycogen was demonstrated by periodic acid Schiff base (PAS) staining [7]. Controls were immersed in aqueous solutions of amyloglucosidase (EC 3.2.1.3) for 2 h at room temperature prior to PAS staining and no glycogen was then to be detected.

2.5. *Determination of substrate balance*

All substrates in the perfusate were measured with standard methods [8]: glucose with glucose dehydrogenase (EC 1.1.1.47), lactate with lactate dehydrogenase (EC 1.1.1.27) and glutamate pyruvate transaminase (EC 2.6.1.2), pyruvate with lactate dehydrogenase and glutamine with glutaminase (EC 3.5.1.2) and glutamate dehydrogenase (EC 1.4.1.3). Substrate balance in $\mu mol \cdot min^{-1} \cdot g\ liver^{-1}$ is given by (concentration in hepatic vein − concentration in portal vein in $\mu mol \cdot ml^{-1}$) · flow in $ml \cdot min^{-1} \cdot g\ liver^{-1}$. Metabolic rates were determined as the means of the substrate balance measured at 20, 30, 40, 50 and 60 min of perfusion. With glucose the results can only be regarded as an indication of a trend, since the hepatovenous-portal concentration gradients at the high glucose concentrations and at the high flow rates, that had to be used, are the differences of two large numbers and thus ipso facto cannot be measured with high accuracy.

2.6. *Determination of glycogen synthesis*

Tissue samples were homogenized for 30 s with an Ultra-Turrax (Kunkel, Staufen) in ice-cold 0.9% NaCl and then inactivated by addition of perchloric acid to a final concentration of 0.9 M. Glycogen was hydrolyzed with amyloglucosidase at pH 4.8 and free glucose was measured with glucose dehydrogenase [8]. Rates of glycogen synthesis were determined from the differences of glycogen content at 30 and 60 min of perfusion.

3. RESULTS

3.1. *Overall glycogen synthesis*

The rate of glycogen synthesis from different substrates was measured and compared to the rate of net uptake of the exogenous substrates, i.e. the balance of uptake or output of glucose, lactate, pyruvate and glutamine (cf. section 3.2). With glucose as the only exogenous substrate glycogen synthesis and net substrate uptake were highest during orthograde and clearly lower during retrograde perfusion (table 1). In both orthograde and retrograde perfusions about 30% of the glucose taken up was utilized for glycogen synthesis.

During ortho- and retrograde perfusions with gluconeogenic precursors as the sole exogenous substrates glycogen deposition was relatively low, probably due to the absence of glucose as an important activator (not substrate) of glycogen synthesis (table 1). Comparison of glycogen formation with net substrate uptake might indicate that not only exogenous but also endogenous substrates could have been used for glycogen formation; however, the differences were not significant.

During ortho- and retrograde perfusions with both glucose and gluconeogenic precursors glycogen formation was clearly increased again in comparison to perfusions with only gluconeogenic precursors; yet it reached the values observed with

Table 1

Glycogen synthesis and net substrate uptake in perfused livers from 24 h-fasted rats

Exogenous substrate	Glycogen synthesis (μmol $C_6 \cdot min^{-1} \cdot g\ liver^{-1}$)	Net substrate uptake (μmol $C_6 \cdot min^{-1} \cdot g\ liver^{-1}$)
Orthograde perfusion		
Glucose	0.70 ± 0.16 (4)	1.70 ± 1.36 (4)[a]
Lactate, pyruvate, glutamine	0.11 ± 0.05 (5)	0.05 ± 0.06 (5)
Glucose plus lactate, pyruvate, glutamine	0.22 ± 0.04 (5)	1.13 ± 0.31 (5)[a]
Retrograde perfusion		
Glucose	0.21 ± 0.03 (4)	0.52 ± 0.31 (4)[a]
Lactate, pyruvate, glutamine	0.03 ± 0.02 (4)	0.01 ± 0.04 (4)
Glucose plus lactate, pyruvate, glutamine	0.19 ± 0.06 (4)	0.34 ± 0.27 (4)[a]

Livers were perfused, and glycogen content as well as net substrate uptake, i.e. the balance of uptake or output of glucose, lactate, pyruvate and glutamine (cf. fig.1), were determined as described in section 2. Values are means ± SE of the number of experiments given in parentheses. [a] Due to the inevitable inaccuracy of the determination of the glucose balance at high glucose concentrations (see section 2), the results have a high SE and can thus be regarded only as an indication of a trend

glucose as substrate only during retrograde but not orthograde perfusions (table 1). Apparently, glycogen formation from glucose was inhibited by the presence of gluconeogenic substrates in the medium during orthograde flow.

3.2. *Substrate balance*

In the presence of 30 mM glucose about 2 and 0.8 μmol glucose $\cdot min^{-1} \cdot g\ liver^{-1}$ were taken up during ortho- and retrograde perfusion, respectively. During ortho- and retrograde perfusion 20 and 30%, respectively, of the glucose taken up were released as lactate; pyruvate release was negligible (fig.1). When livers were perfused with gluconeogenic substrates about 0.7 and 0.4 μmol glucose $\cdot min^{-1} \cdot g\ liver^{-1}$ were released during ortho- and retrograde perfusion, respectively. The amount of glucose released was similar to the sum of lactate, pyruvate and glutamine taken up (fig.1).

In the presence of both glucose and gluconeogenic precursors glucose was taken up at a rate of about 0.4 μmol $\cdot min^{-1} \cdot g\ liver^{-1}$ during orthograde perfusion, while there was essentially no net uptake or release during retrograde perfusion. Yet, a net uptake of lactate, pyruvate and glutamine was observed independent of the direction of flow (fig.1). In general, net substrate uptake was higher

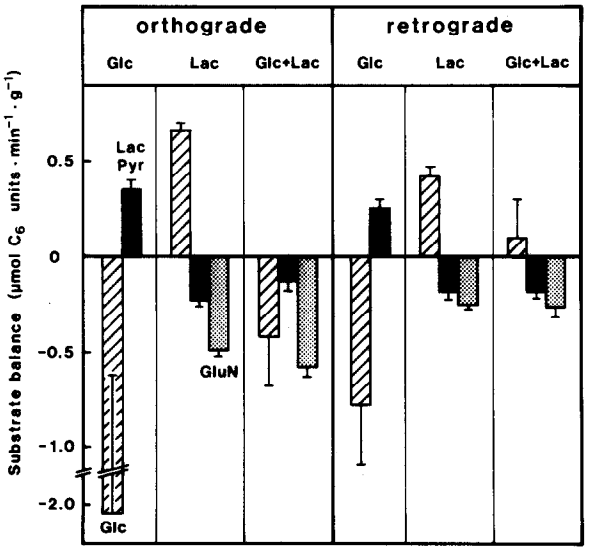

Fig.1. Substrate balance during ortho- and retrograde perfusion of livers from 24 h-fasted rats. The substrates offered are indicated on top of each column: Glc, 30 mM glucose; Lac, 2 mM lactate, 0.2 mM pyruvate and 2 mM glutamine. Values are means ± SE of 4 (columns 1,4–6) or 5 (columns 2,3) experiments. Positive values, output; negative values, uptake. The glucose balance at high glucose concentrations can only be regarded as an indication of a trend due to the inevitable inaccuracy of its determination (see section 2).

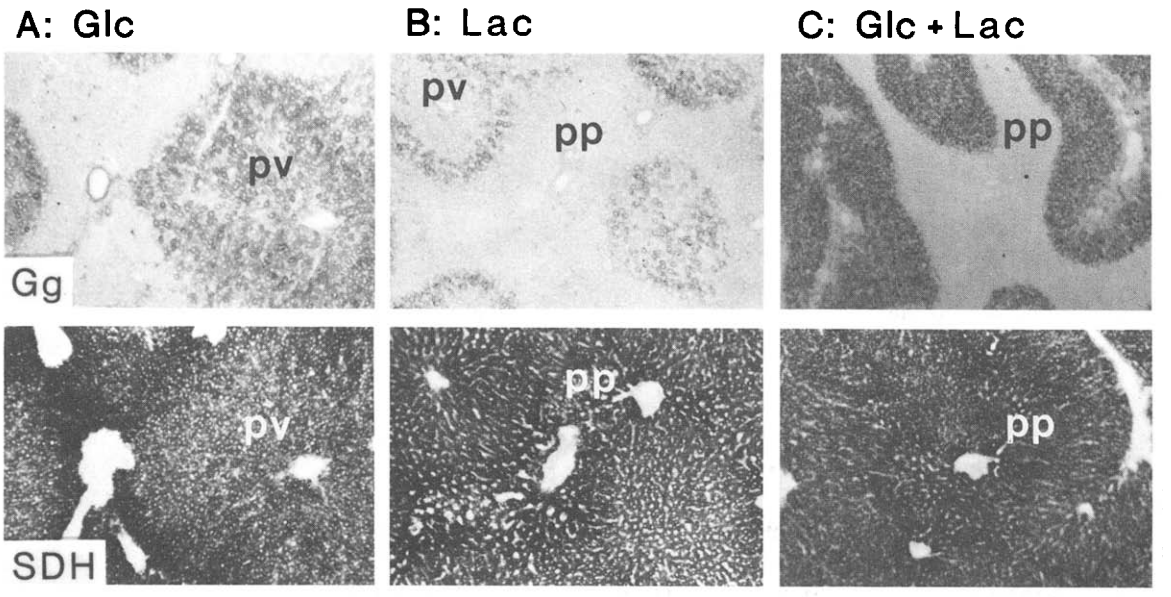

Fig.2. Distribution of glycogen in the parenchyma of livers from 24 h-fasted rats after orthograde perfusion. The substrates are indicated above each column: Glc, 30 mM glucose; Lac, 2 mM lactate, 0.2 mM pyruvate plus 2 mM glutamine. Glycogen (Gg) and for comparison succinate dehydrogenase (SDH) were demonstrated histochemically after 1 h of perfusion (dark precipitates); pp, periportal; pv, perivenous. Qualitatively identical results were obtained with 10 mM glucose or with 2 mM lactate and 0.2 mM pyruvate without glutamine as substrates.

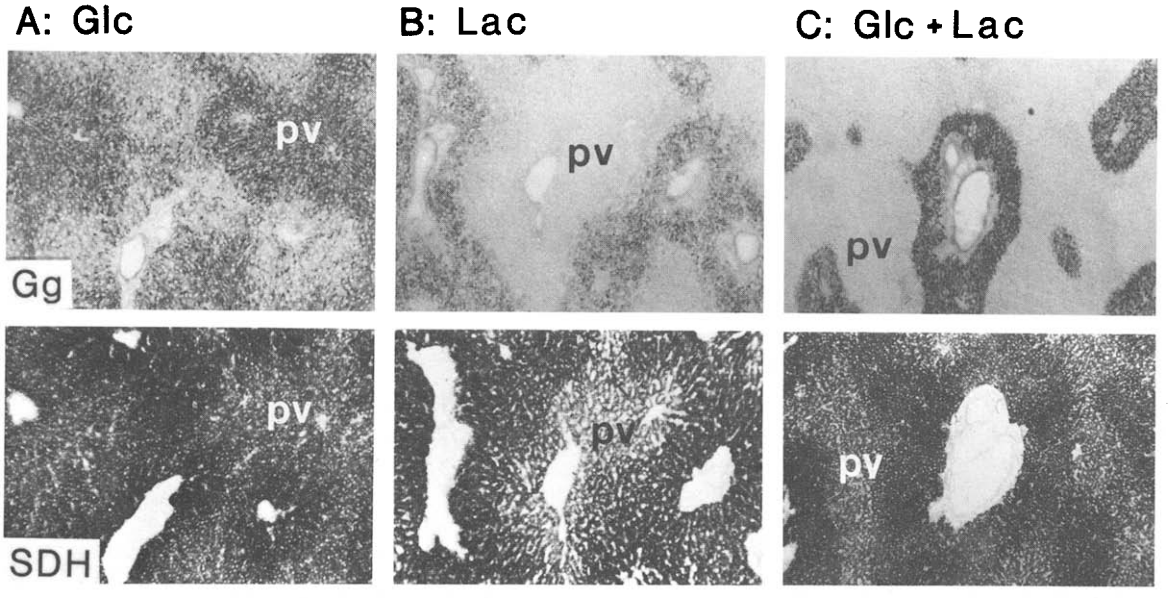

Fig.3. Distribution of glycogen in the parenchyma of livers from 24 h-fasted rats after retrograde perfusion. For further explanation see fig.2.

during ortho- than during retrograde perfusion (fig.1; cf. table 1).

3.3. *Glycogen synthesis in the periportal or perivenous zone*

Glycogen and succinate dehydrogenase as a marker enzyme of periportal hepatocytes were demonstrated histochemically in parallel sections at the beginning and after 30 and 60 min of perfusion. After a 24 h fast the liver of most of the rats was free of glycogen so that the pattern that was to be seen at the end of the experiment (figs 2,3) could be assigned to glycogen synthesis during the perfusion. Experiments in which glycogen was to be detected at zero time were rejected.

In the presence of glucose as the only exogenous substrate glycogen deposition occurred exclusively in the perivenous area. The localization of newly formed glycogen in this zone was independent of the flow direction (figs 2A,3A). In the presence of gluconeogenic precursors as exogenous substrates glycogen was deposited in periportal cells during orthograde perfusion (fig.2B), but was formed only in the intermediate zone during retrograde perfusion (fig.3B).

Unexpected results were obtained when both glucose and gluconeogenic compounds were offered. In spite of substrate availability for glycogen synthesis in both areas, glycogen deposition was limited to the periportal zone during orthograde perfusion (fig.2C) and to the perivenous zone when the liver was perfused in the retrograde direction (fig.3C). Thus, glycogen was synthesized only in the upstream region whereas the downstream zone remained glycogen-free.

3.4. *Glycogen synthesis in the presence of mercaptopicolinic acid*

Glycogen formation was measured in the presence of glucose or gluconeogenic precursors when phospho*enol*pyruvate carboxykinase (EC 4.1.1.32), a key enzyme of gluconeogenesis, was inhibited by 100 μM mercaptopicolinic acid [9,10]. Glycogen synthesis and its deposition in the perivenous zone were not inhibited, when glucose was the substrate. These results indicate that glycogen in the perivenous zone was formed via the direct pathway from glucose. When gluconeogenic precursors were offered, glycogen synthesis and its storage in the periportal zone were clearly reduced to less than one-third in parallel to glucose output. These results indicate that glycogen in the periportal zone was formed via the indirect pathway from gluconeogenic substrates, part of which came from endogenous sources circumventing the phospho*enol*pyruvate carboxykinase step, e.g. glycerol from triglycerides.

4. DISCUSSION

In this work, the rate (table 1) and the parenchymal localization (figs 2,3) of glycogen synthesis from different substrates were studied in the isolated perfused liver of fasted rats. The results support the concept that glycogen in the periportal zone is formed via gluconeogenesis whereas glycogen in the perivenous zone is synthesized from glucose as predicted by the model of metabolic zonation [4,5].

4.1. *Rates of glycogen synthesis*

It is well known that the isolated perfused liver is not a satisfactory model for the study of glycogen synthesis [11]; the reasons for this are poorly understood. During recirculating perfusions with erythrocyte-containing media offering physiological postprandial levels of about 10–12 mM glucose and varying levels of gluconeogenic substrates, glycogen was synthesized with or without insulin or acetylcholine at rates between 0.04 and 0.4 μmol $C_6 \cdot min^{-1} \cdot g\ liver^{-1}$ [12–15]; only with unphysiologically high levels of 20–30 mM glucose were higher rates in the range of up to 0.8 μmol $C_6 \cdot min^{-1} \cdot g^{-1}$ observed [12,15]. During non-recirculating perfusions with erythrocyte-free media and 20 mM concentrations of glucose rates of glycogen synthesis did not exceed 0.025 μmol $C_6 \cdot min^{-1} \cdot g^{-1}$ [16]. These rates are clearly lower than those of 0.95 μmol $C_6 \cdot min^{-1} \cdot g^{-1}$ observed in vivo during a normal light-dark feeding rhythm [17] or of 0.9–1.5 μmol $C_6 \cdot min^{-1} \cdot g^{-1}$ found upon refeeding after 24 h starvation [15,18–20]. Although the isolated perfused liver is not as efficient in synthesizing glycogen as the organ in vivo, it had to be chosen as the experimental model, since it alone allows the study of both the rate and parenchymal localization of glycogen synthesis as a function of a defined substrate supply. In contrast to previous studies [11–15], except for [16], the livers were perfused without erythrocytes in a non-recirculating manner in order to avoid the accumulation of carbon substrates such as glucose or

lactate, pyruvate and amino acids. Glucose was offered at the hyperphysiological concentration of 30 mM, since this was found to be optimal by previous workers [12]. Lactate and pyruvate were supplied at the physiological levels of 2 and 0.2 mM, respectively. Glutamine as a putative activator of glycogen synthesis [21] was added at 2 mM. Under the conditions used the rates of glycogen synthesis varied considerably depending on the substrate used (table 1); they were, however, in the range expected from previous work.

4.2. *Localization of glycogen synthesis from glucose*

With glucose as the sole exogenous substrate glycogen was formed in the perivenous zone during both ortho- and retrograde perfusion (figs 2A,3A). The perivenous localization of glycogen synthesis is in line with the predominance of glucokinase in the perivenous and of glucose-6-phosphatase in the periportal zone; the glucokinase to glucose-6-phosphatase ratio is 6-times higher in the perivenous than in the periportal region [4,5]. If the glucose/glucose 6-phosphate cycle is regulated only by substrate availability, as is widely believed ([22] cf., however [23]), the capacity for net glucose uptake is clearly higher in the perivenous than in the periportal area. Thus, using the enzyme distribution and the measured substrate concentrations under various physiological states during a normal day-night rhythm, it has been shown that only the perivenous zone should be able to catalyze a net glucose uptake [17].

4.3. *Localization of glycogen synthesis from gluconeogenic substrates*

With lactate, pyruvate and glutamine as the exogenous substrates glycogen was deposited in the periportal zone during orthograde perfusion and only in a small intermediate zone during retrograde perfusion (figs 2B,3B). The periportal localization of glycogen formation during orthograde perfusion is in accord with the predominance of the gluconeogenic phospho*enol*pyruvate carboxykinase and fructose-1,6-bisphosphatase in the periportal zone and of the glycolytic pyruvate kinase type L in the perivenous zone [4,5]. Thus, the capacity for net gluconeogenesis is clearly higher in the periportal than in the perivenous area.

The intermediate localization of glycogen synthesis during retrograde perfusion was therefore unexpected. The absence of glycogen formation in the periportal zone was not due to an inhibition of gluconeogenesis per se, since glucose was formed from lactate, pyruvate and glutamine (fig.1), but apparently to an inhibition of glycogen synthesis from glucose 6-phosphate. The possibility was considered that glycogen synthesis requires a better oxygenation of the liver than gluconeogenesis per se and that this better oxygenation in the presence of lactate, pyruvate and glutamine, which have easy access to the citrate cycle and thus oxidative metabolism, can be maintained during perfusion with erythrocyte-free media only in the upstream region. This assumption is supported by the observation (not shown) that glycogen was formed from lactate, pyruvate and glutamine during retrograde perfusions throughout the periportal zone when the media contained erythrocytes (20%, v/v). Further evidence for an inadequate oxygenation of the liver in the usual perfusions with erythrocyte-free buffers is provided by the following findings: (i) O_2 uptake was higher in perfusions with than in those without erythrocytes at an O_2 delivery of about 12 and 4 $\mu mol \cdot min^{-1} \cdot g^{-1}$, respectively [24]; (ii) glucose and oxygen metabolism may be different in perfusions with 90 and 45% blood, at an O_2 delivery of about 20 and 10 $\mu mol \cdot min^{-1} \cdot g^{-1}$, respectively [15,25].

4.4. *Localization of glycogen synthesis from glucose and gluconeogenic substrates*

With glucose and lactate, pyruvate, glutamine as exogenous substrates glycogen was formed in the periportal zone during orthograde, but in the perivenous zone during retrograde perfusions (figs 2C,3C). If glycogen were formed via gluconeogenesis in the periportal and via glucokinase from glucose in the perivenous zone, a homogeneous distribution of glycogen synthesis would have been expected. The most plausible explanation for the formation of glycogen only in the upstream zone, i.e. periportal during orthograde and perivenous during retrograde perfusions, is again the inadequate oxygenation of the organ during perfusions with erythrocyte-free media. This view is supported by the finding (not shown) that glycogen synthesis from glucose plus lactate, pyruvate, glutamine was distributed nearly homogeneously in the parenchyma when the media contained erythrocytes (20%, v/v).

4.5. *Conclusion*

The present finding that glycogen is formed from glucose in the perivenous and from gluconeogenic substrates in the periportal zone would explain a number of apparently contradictory findings. If C_3 substrates are indeed major precursors for glycogen synthesis [9,10,12,18,26], they should be taken up net by the liver during the absorptive phase and be released in the periphery, mainly from skeletal muscle. This apparently is not the case; after a glucose load the liver releases net lactate and muscle takes it up [27,28]. Thus, it is obvious that the lactate (pyruvate), which serves as a major substrate for hepatic glycogen synthesis, must be formed from glucose metabolism in the liver itself, i.e. glycolysis, which gives rise to the formation of C_3 substrates, and gluconeogenesis, which utilizes C_3 substrates to form glycogen, must be active simultaneously [2,4,5]. In accord with the model of metabolic zonation perivenous hepatocytes would take up glucose from the ingested food, incorporate some of the glucose into glycogen, but degrade the major part of the glucose to lactate. This lactate is subsequently released into the circulation, from which it eventually reaches the periportal hepatocytes and is taken up for glycogen synthesis via gluconeogenesis. This model requires that the liver has a sufficient capacity for net glucose uptake and phosphorylation via glucokinase. Adequate net glucose uptake is observed, when the liver is offered a glucose concentration gradient, portal concentration higher than arterial [29,30], in line with the demonstration of sufficient glucokinase capacity ([31,32] cf., however [19]).

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