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# Zonation of glycogen and glucose syntheses, but not glycolysis, in rat liver*

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We have investigated the cause of defective glycogen synthesis in hepatocyte preparations enriched with cells from the periportal or perivenous zones obtained by the methods of Lindros & Penttila [Biochem. J. (1985) **228**, 757–760] and of Quistorff [Biochem. J. (1985) **229**, 221–226]. A modified procedure which yields hepatocytes capable of consistent rates of glycogen synthesis is described, and the rates of glucose and glycogen syntheses and of glycolysis in hepatocytes from the two zones are compared. Glycogen synthesis in cells was greatly impaired by very low concentrations (0.01–0.05 mg/ml) of digitonin, which had little effect on glucose and protein syntheses and Trypan Blue exclusion. Cells exposed to such low concentrations of digitonin lose all their synthetic capacity and ability to exclude Trypan Blue when incubated with EGTA, which does not affect cells not exposed to digitonin. With a modified procedure based on this phenomenon, our study reveals that hepatocyte preparations enriched with cells from the periportal zone synthesized glucose from lactate and alanine at rates twice those by cells from the perivenous zone, whereas the rate of glycogen synthesis from $C_3$ precursors in periportal cells was 4 times that in the perivenous preparations. With substrates entering the pathway at the triose phosphate level, gluconeogenesis in periportal-cell preparations was 20 % higher, and glycogen synthesis was twice that in perivenous preparations. Glycolysis was studied by the formation of $^3HOH$ from [2-$^3$H]glucose, the yield of lactate, and the conversion of [$^{14}$C]glucose into [$^{14}$C]lactate. In cell preparations from both zones glycolysis by all criteria was negligible at 10 mM-glucose, but was substantial at higher concentrations. However, there was no difference between the zones. We confirm that the capacities for glucose and glycogen syntheses in periportal cells are higher than in perivenous cells, but that at physiological glucose concentrations there is negligible glycolysis in liver parenchyma in both zones. The metabolic pattern in the perivenous cells is not glycolytic.

## INTRODUCTION

Mounting evidence from several laboratories indicates that the enzymic profile, and by implication the metabolic function, of liver parenchyma cells is zonated according to its location in the acinus (for reviews, see Thurman *et al.*, 1986; Jungermann, 1986). Generally the population proximal to the afferent vessels (periportal zone) has been shown to contain higher amounts of the gluconeogenic and ureagenic enzymes, whereas those from areas adjacent to the efferent vessels (perivenous zone) contain higher amounts of lipogenic and glycolytic enzymes. Jungermann and co-workers have proposed that cells in the periportal zone are predominantly gluconeogenic and those in the perivenous zone predominantly glycolytic. Moreover, they proposed that glycogen is formed from $C_3$ precursors in the periportal zone, but predominantly from glucose in the perivenous zone (Jungermann, 1986). The technique of cell isolation by use of collagenase has been adapted to livers in which one of the acinar zones had been destroyed by digitonin infusion (Lindros & Penttila, 1985; Quistorff, 1985), thus allowing direct measurement of various metabolic activities in cells isolated from the two zones. In agreement with earlier predictions, gluconeogenesis and ureagenesis in cells from the periportal zone were found to be higher than in those from the perivenous zone (Quistorff, 1985; Quistorff & Grunnett, 1986). However, glycogen synthesis in these preparations was low and poorly reproducible, and glycolysis was not studied. We decided to examine these aspects.

## MATERIALS AND METHODS

Male rats of the Sprague–Dawley strain (250–300 g) were either fed *ad libitum* or starved overnight, and all animals were anaesthetized by peritoneal injection of pentobarbital (100 mg/kg) before surgery.

Hepatocyte preparation from the whole liver was by a modification of the method of Berry & Friend (1969) as described previously (Katz *et al.*, 1975). The procedure for preparation of hepatocytes from selected zones follows the basic steps introduced by Lindros & Penttila (1985) and by Quistorff (1985), but with modifications. After loose ties were placed around the inferior vena cava above the right kidney and around the portal vein, the portal vein was cannulated. The vena cava was then severed below the right kidney, and perfusion (20 ml/min) was commenced with Krebs–Henseleit $Ca^{2+}$-free buffer containing 5 mM-pyruvate, 5 mM-glucose and 0.5 mM-EGTA, equilibrated with $O_2/CO_2$ (19:1). The chest cavity was then opened for cannulation of the superior vena cava, and the ties around the inferior vena cava were securely fastened. For preparation of perivenous cells, the perfusion was continued for another 2 min.

Abbreviation used: DDW, defatted dry wt.

* Dedicated to Professor Henry A. Lardy for his 71st birthday.

Perfusion was then stopped momentarily, and about 2 ml of digitonin (10 mg/ml) was injected through the cannula of the portal vein. The liver was then perfused with the EGTA-containing buffer in the retrograde direction for 18 min at about 30 ml/min, during which the eluate emerging from the portal vein was discarded. The liver was then flushed with buffer containing no EGTA, but otherwise the same components, for about 3 min to remove EGTA, which inhibits collagenase digestion. The liver was then perfused *in situ* (antegrade direction) with 200 ml of recirculating Krebs–Henseleit buffer ($Ca^{2+}$-free) containing 5 mM-glucose, 5 mM-pyruvate and collagenase (0.35 mg/ml). $CaCl_2$ (5 $\mu$mol) was added to the medium to chelate any remaining EGTA. After 10 min, during which the perfusion medium turned turbid, the liver was flushed with the $Ca^{2+}$-free Krebs–Henseleit buffer and transferred to a perfusion apparatus, and the collagenase digestion was continued for 20 min further with another 200 ml of the collagenase buffer. The liver was then dispersed in Krebs–Henseleit buffer and filtered through nylon mesh, and the suspension was centrifuged at 600 *g* for 1 min. Cell debris and dead cells which form a white layer at the top of the pellet were removed, and the darker lower layer was washed repeatedly by resuspension, in fresh buffer containing 2.5 mM-$Ca^{2+}$ but without glucose and pyruvate, and centrifugation. The yield was 2–3 ml of packed cells from a 10 g liver.

For preparation of periportal cells, the procedure was the same except for the following: after cannulation, the liver was perfused for 2 min in the retrograde direction before about 2.5 ml of digitonin (10 mg/ml) was injected through the cannula of the vena cava. EGTA-containing buffer was then flushed through the liver via the portal vein, and chased with buffer containing no EGTA. The first 10 min perfusion of collagenase was in the retrograde direction and the second 20 min perfusion was in the antegrade direction.

### Incubations

Hepatocytes (30 mg wet wt./ml) were incubated in Krebs–Henseleit buffer supplemented with various substrates, as described in the Figure legends. When indicated, $Ca^{2+}$ was replaced with 0.5 mM-EGTA. $HClO_4$ was added to terminate incubations.

### Isolation of labelled compounds

Detritation of [2-$^3$H]glucose was measured as described by Katz *et al.* (1975). Briefly, deproteinized $HClO_4$ extracts were neutralized with KOH and the supernatants passed through three resin columns: Dowex-1 ($H^+$ form; 50–100 mesh) at the top; Dowex-50 (acetate form; 100–200 mesh) in the middle; and Dowex-50 (borate form; 100–200 mesh) at the bottom. The eluate contains $^3H_2O$. Labelled lactate was eluted from the middle column with 2 M-acetic acid.

### Incorporation of [$^{14}$C]leucine into proteins

A portion of cells incubated with [$^{14}$C]leucine and substrate was precipitated with $HClO_4$. The precipitate was washed three times further with 5% $HClO_4$, and dissolved in NaOH for radioactivity counting.

### Analysis

Glucose, lactate and glycogen production was measured as previously described (Katz *et al.*, 1976). Total alanine aminotransferase was measured in freeze–thawed cell suspensions by published methods (Bergmeyer & Gawehn, 1979). For phosphoenolpyruvate carboxykinase approx. 0.4 ml of packed cells was frozen in 2 ml of buffer containing 0.2 M-sucrose, 50 mM-Hepes, pH 7.5, and 3 mg of dithiothreitol. The thawed homogenate was then centrifuged at 12500 *g* for 15 min, and the enzyme activity of the supernatant was measured as described by Bentle & Lardy (1976), with $Mn^{2+}$ as the activating cation. For glucokinase and glutamine synthetase, 0.4 ml of packed cells was frozen with 2 ml of a solution comprising 150 mM-KCl, 50 mM-Hepes, pH 7.5, and 3 mg of dithiothreitol. The sample was thawed, homogenized and centrifuged as described above. Glucokinase was measured at 37 °C with 2 units of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*, in 50 mM-Hepes, pH 7.5, 7.5 mM-$MgCl_2$, 100 mM-KCl, 1 mM-$NAD^+$, 2.5 mM-dithiothreitol and 100 mM-glucose. The reaction was initated by adding ATP to 5 mM. Low-$K_m$ hexokinase was measured as for glucokinase, except the glucose concentration was 0.5 mM. Blanks omitting ATP were performed. Low-$K_m$ hexokinase activity was not significant in all cell preparations. Glutamine synthetase was measured as described by Vorhaben *et al.* (1973). Protein was determined with the biuret reagent.

### Materials

Digitonin was purchased from ICN Biochemicals, Plainview, NY, U.S.A. [U-$^{14}$C]Glucose, [2-$^3$H]glucose and [U-$^{14}$C]leucine were from NEN Research Products, Boston, MA, U.S.A. The [$^3$H]glucose was purified as described by Katz *et al.* (1975) before use. All other reagents were purchased from Sigma, St. Louis, MO, U.S.A., or Boehringer Mannheim Biochemicals, Indianapolis, IN, U.S.A.

## RESULTS AND DISCUSSION

Other investigators as well as ourselves observed a variable and often greatly impaired capacity of periportal and perivenous cells isolated by the published method to synthesize glycogen. We thus examined the effect of digitonin, the agent employed to destroy selectively the unwanted zone in the liver before cell isolation, on glucose and glycogen synthesis.

Low concentrations of digitonin (0.01–0.05 mg/ml) had little effect on protein synthesis as measured by the incorporation of [$^{14}$C]leucine into proteins, and on the synthesis of glucose from lactate, alanine or dihydroxyacetone (Fig. 1). In contrast, glycogen synthesis from these substrates was decreased by 30–35% by 0.01 mg of digitonin/ml and by nearly 70% at 0.02 mg/ml. Similar inhibitions were observed when glycogen synthesis was activated by mercaptopicolinate (Okajima & Katz, 1979) instead of glutamine. The presence of leucine, which enhances the stimulation of glycogen synthesis by glutamine (Chen & Lardy, 1985), did not affect the sensitivity of hepatocyte glycogen synthesis to digitonin (results not shown). The inhibition of glycogen synthesis by low concentrations of digitonin requires only very brief exposure. A 70% inhibition of glycogen synthesis was observed in cells suspended in 0.02 mg of digitonin/ml for 15 s, followed by repeated washings with buffer.

Since the action of digitonin is believed to reside in its

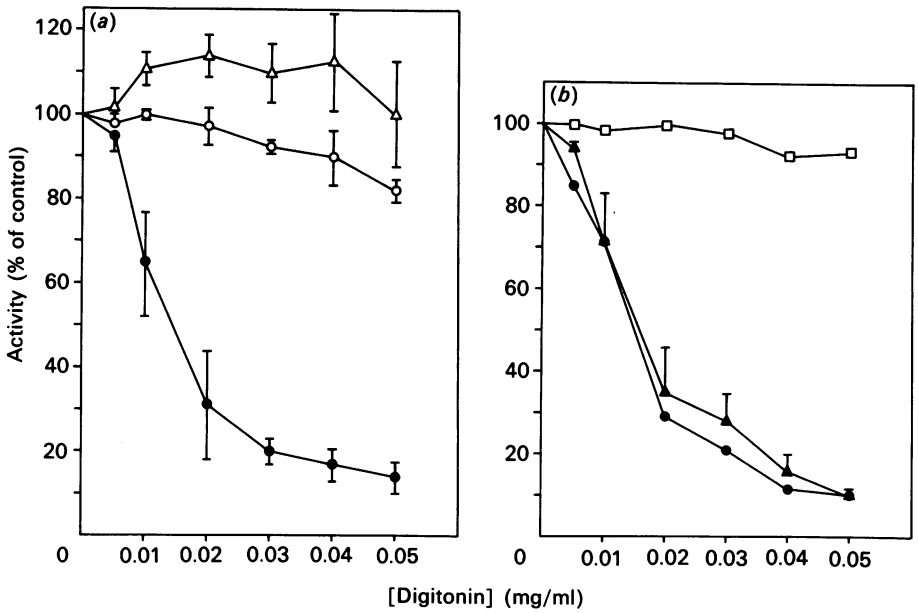


**Fig. 1. Effect of digitonin on gluconeogenesis, glycogen synthesis and protein synthesis in hepatocytes isolated from starved rats**

Hepatocytes were incubated with the following substrates for 30 min for measurement of gluconeogenesis: 10 mM-alanine (△; *a*), control rate 13.8±0.8 μmol/h per 100 mg DDW; 20 mM-lactate plus 2 mM-pyruvate (○; *a*), control rate 33.6±0.8 μmol/h per 100 mg DDW. For glycogen synthesis, hepatocytes were incubated with the following: 20 mM-lactate, 2 mM-pyruvate, 10 mM-alanine plus 10 mM-glucose (●; *a*), control rate 13.5±3 μmol/h per 100 mg DDW; 20 mM-dihydroxyacetone, 10 mM-glutamine plus 10 mM-glucose (●; *b*), control rate 20.8±0.8 μmol/h per 100 mg DDW; 20 mM-dihydroxyacetone, 0.5 mM-3-mercaptopicolinate plus 10 mM-glucose (▲; *b*), control rate 14.5±3 μmol/h per 100 mg DDW. For protein synthesis, hepatocytes were incubated with 20 mM-dihydroxyacetone, 10 mM-glutamine, 10 mM-glucose and 0.1 μCi of [U-$^{14}$C]leucine (tracer amounts) (□; *b*). Results for glycogen synthesis are expressed as glucose equivalents.

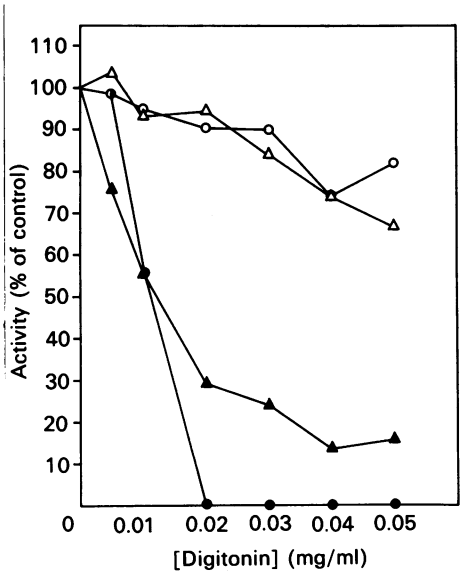


**Fig. 2. Effect of EGTA on glycogenolysis and Trypan Blue exclusion by hepatocytes incubated with digitonin**

Hepatocytes isolated from fed rats were incubated with various concentrations of digitonin in medium containing 2.5 mM-$Ca^{2+}$ (○, △) or 0.5 mM-EGTA (●, ▲), for 20 min. A sample was pipetted into $HClO_4$ for determination of glycogenolysis (▲, control rate 5.8 μmol of glucose/20 min per 100 mg DDW; △, control rate 8.1 μmol of glucose/20 min per 100 mg), and another was used for estimation of cell viability by Trypan Blue exclusion (●, control 84% viable; ○, control 85% viable). Results are averages of two experiments.

ability to complex with cell-membrane cholesterol, a possible reason for the effect on glycogen synthesis is the permeabilization of the cell to extracellular $Ca^{2+}$, which would activate glycogen phosphorylase to promote glycogen breakdown and inhibit glycogen synthesis. We thus examined the effect of EGTA on glycogenolysis in hepatocytes isolated from fed rats. Fig. 2 shows that in the presence of 2.5 mM-$Ca^{2+}$ digitonin did not promote glycogen breakdown. Indeed, glycogenolysis in these cells appeared to be inhibited to a greater extent by digitonin than was gluconeogenesis in cells isolated from starved rats (cf. Fig. 1). Surprisingly, the ability of low concentrations of digitonin to inhibit glycogenolysis was greatly increased when cells were incubated with 0.5 mM-EGTA in $Ca^{2+}$-free medium. This phenomenon was accompanied by permeabilization of the cell membrane and cell death, as evidenced by Trypan Blue exclusion (Fig. 2). Cells isolated from rats starved for 24 h were similarly susceptible to digitonin plus EGTA; gluconeogenesis from dihydroxyacetone was strongly inhibited, and the cells did not exclude Trypan Blue under such circumstances (Fig. 3). In the absence of digitonin, the rate of gluconeogenesis from 10 mM-dihydroxyacetone was 30% lower in the EGTA medium than in the $Ca^{2+}$ medium, but this decrease was not accompanied by any change in Trypan Blue exclusion by the cells. Similar results were observed when pyruvate and glucose was substituted for dihydroxyacetone as substrate (results not shown).

It appears that low concentrations of digitonin selectively inhibit the ability of cells to synthesize glycogen, while having little effect on protein synthesis,

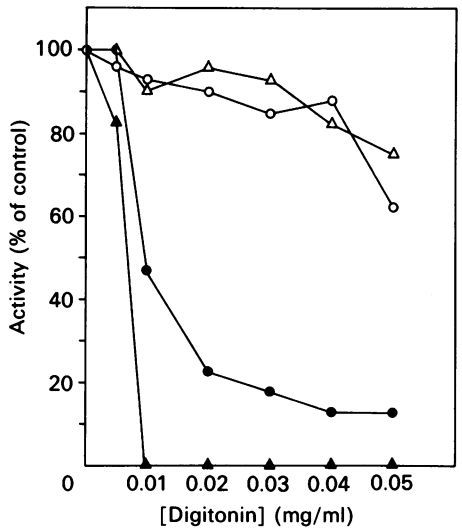


**Fig. 3. Effect of EGTA on Trypan Blue exclusion and gluconeogenesis from dihydroxyacetone in hepatocytes incubated with digitonin**

Hepatocytes isolated from rats starved for 24 h were incubated with 10 mM-dihydroxyacetone and the indicated concentrations of digitonin in medium containing 2.5 mM-$Ca^{2+}$ (○, △) or 0.5 mM-EGTA (●, ▲) for 30 min. Control rates of glucose synthesis were 49 μmol/h per 100 mg DDW (○), and 34 μmol/h per 100 mg DDW (●). Trypan Blue exclusion (▲, △) was estimated by light microscopy.

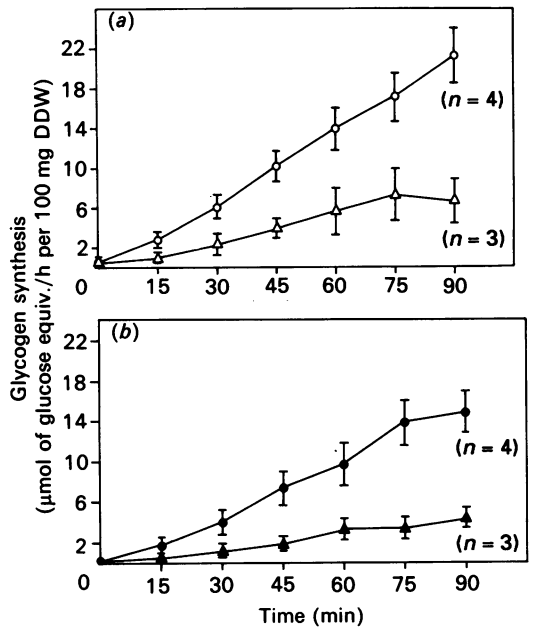


**Fig. 4. Glycogen synthesis by hepatocytes isolated from the periportal and perivenous regions**

Hepatocytes isolated from the periportal (●, ○) and perivenous (▲, △) regions of livers of 24 h-starved rats were incubated with 20 mM-dihydroxyacetone, 10 mM-glutamine and 10 mM-glucose (○, △) or with 20 mM-lactate, 2 mM-pyruvate, 10 mM-glutamine and 10 mM-glucose (●, ▲). Incubations were stopped at the indicated times and glycogen was measured as described in the text. Results are means ± S.E.M. for *n* experiments.

**Table 1. Enzyme activities in periportal and perivenous hepatocytes**

Hepatocytes were isolated from starved rats, and the enzyme activities measured as described in the text. Results (nmol/min per mg of protein) are the means ± S.E.M. for the numbers of cell preparations in parentheses.

| Enzyme | Periportal | Perivenous |
|---|---|---|
| Alanine aminotransferase | 100 ± 5 (8) | 65 ± 3 (9) |
| Glucokinase | 20 ± 3 (7) | 32 ± 5 (9) |
| Glutamine synthetase | < 2 | 35 ± 7 (7) |
| Phosphoenolpyruvate carboxykinase | 150 ± 15 (4) | 83 ± 9 (5) |

gluconeogenesis and Trypan Blue exclusion. The extraction of digitonin during its perfusion through the liver appears to be fairly efficient; for example, when perfused with a 6 mg/ml digitonin solution at 20 ml/min, the liver removed 92% of the compound from the first fractions that passed through (Quistorff *et al.*, 1985). The extraction is expected to be even higher with the slower flow rate (7 ml/min) used in the published method for zonal cell preparation (Quistorff, 1985). However, as shown in Fig. 1, even a greater than 99% extraction would result in the remaining zone being exposed to digitonin at concentrations high enough to inhibit glycogen synthesis. When $Ca^{2+}$ in the medium is replaced by EGTA, these partially defective cells die. We have exploited this feature to decrease the number of partially defective cells in order to determine the capacity of periportal and perivenous cells to synthesize glycogen. Thus we decreased the volume of digitonin used to destroy the unwanted zone of the liver and followed the digitonin pulse with a perfusion of buffer containing EGTA from the opposite direction, as described in the Materials and methods section.

Fig. 4 shows that with 20 mM-dihydroxyacetone, 10 mM-glutamine plus 10 mM-glucose in the medium, glycogen synthesis proceeded at a rate of 14 μmol of glucose equivalent/h per 100 mg defatted dry wt. (DDW) in cells isolated from the periportal zone, compared with 6 μmol/h per 100 mg DDW in cells from the perivenous zone. With 20 mM-lactate, 2 mM-pyruvate, 10 mM-glutamine plus 10 mM-glucose, the difference in the rate of glycogen synthesis between periportal and perivenous cells is even more evident; the rate for periportal cells was 12 μmol of glucose equivalent/h per 100 mg DDW, whereas that for perivenous cells was less than 3 μmol/h per 100 mg DDW. Very low rates of glycogen synthesis were obtained with only 10 mM-glutamine and 10 mM-glucose in the medium.

The enzymic profile of hepatocytes isolated from 24 h-starved rats by the above method is shown in Table 1. Of the enzymes measured, the largest difference in specific activity between the two zones is found for glutamine synthetase (Gebhardt & Mecke, 1983), for which little or no activity was detected in the periportal cell preparations. Alanine aminotransferase and phosphoenolpyruvate carboxykinase activities were higher in the periportal zone, and glucokinase activity was higher in the perivenous preparations. Hexokinase was virtually absent in all cell preparations indicating the absence of sinusoidal cells.

**Table 2. Gluconeogenesis in hepatocytes isolated from the periportal or perivenous regions of livers of fasted rats**

Cells were incubated with substrates for 30 min. Results ($\mu$mol/h per 100 mg DDW) are means ± S.E.M. for three or four experiments.

| Substrate | Gluconeogenesis | |
|---|---|---|
| | Periportal | Perivenous |
| 10 mM-Lactate + 1 mM-pyruvate | 34 ± 2 | 20 ± 2 |
| 10 mM-Dihydroxyacetone | 61 ± 1 | 49 ± 3 |
| 10 mM-Alanine | 16 ± 1 | 8 ± 1 |
| 10 mM-Glycerol | 41 ± 2 | 33 ± 2 |

The difference in gluconeogenic capacity of periportal and perivenous hepatocytes is shown in Table 2. Of the substrates tested, a periportal/perivenous ratio of 2:1 was found for substrates entering the pathway before the step catalysed by phosphoenolpyruvate carboxykinase. Gluconeogenesis from precursors entering the pathway at the triose phosphate step was only 20% lower in the perivenous-enriched preparations as compared with the periportal preparations.

Our study thus confirms the conclusion of others on the prevalence of gluconeogenesis in the periportal zone, and reveals a larger difference between the zones for glycogen synthesis as compared with the corresponding difference for gluconeogenesis. This indicates the zonation of one or more enzymes affecting the interconversion of glucose 6-phosphate and glycogen. Our results are in broad agreement with those of Richards & Potter (1980), which indicate that glycogen repletion during the re-feeding period occurs faster in the periportal zone, but are at odds with a report (Jepson *et al.*, 1987) indicating the contrary.

It has been repeatedly shown that isolated hepatocytes as well as the perfused liver have a very limited capacity to utilize glucose at physiological concentrations, in spite of the presence of active glucokinase. This has been accounted for by the glucose-6-phosphatase activity, which exceeds glucose phosphorylation at physiological glucose concentrations. Also, it is well established that the rate of glycogen synthesis from glucose as sole substrate, even at high concentrations, is very low. The higher activity of glucokinase in the perivenous zone may thus suggest greater glucose utilization and glycolysis in these cells. Indeed, Jungermann (1986) and co-workers believe these cells to be predominantly glycolytic. We thus examined the cells for their capacity to phosphorylate glucose and for production of lactate from glucose by these criteria: the yield of $^3$HOH from [2-$^3$H]-glucose, the net yield of lactate from glucose, and the conversion of [$^{14}$C]glucose into [$^{14}$C]lactate. As shown in Table 3, lactate production by cells incubated with up to 10 mM-glucose was negligible, even though detritiation of [2-$^3$H]-glucose, which measures glucose phosphorylation, was fairly extensive. Substantial amounts of lactate production from glucose occurred only at glucose concentrations of 20 mM and higher, when lactate production was one-third the rate of glucose phosphorylation. Production of [$^{14}$C]lactate from [$^{14}$C]glucose was somewhat higher in perivenous-cell preparations compared with periportal preparations, but the difference was not statistically significant. The presence of 10 mM-glutamine in the incubation medium did not affect conversion of [2-$^3$H]glucose into $^3$HOH, or [$^{14}$C]lactate production from [$^{14}$C]glucose, but production of unlabelled lactate, presumably from glutamine, was increased greatly in the periportal-cell preparations, compared with a marginal increase in perivenous-cell preparations. This difference reflects the greater amounts of aminotransferase and phosphoenolpyruvate carboxykinase in the periportal cells. The calculated amount of lactate produced from glutamine increased from 3 to 8 $\mu$mol/min per 100 mg DDW in periportal cells when glucose in the medium was increased from 5 to 30 mM. This was probably due to diversion of glutamine carbon atoms from gluconeogenesis to lactate production.

### Role of glucokinase in liver

It is now established that glycogen carbon is derived partly from gluconeogenic precursors (indirect pathway) and partly from glucose (direct pathway). The direct

**Table 3. Detritiation of [2-$^3$H]glucose and lactate production in periportal and perivenous hepatocytes**

Hepatocytes isolated from starved rats were incubated with the indicated substrates for 1 h; 0.1 $\mu$Ci of [U-$^{14}$C]glucose and 0.2 $\mu$Ci of [2-$^3$H]glucose were present in all incubations. Results ($\mu$mol/h per 100 mg DDW) are means ± S.E.M.

| Substrate | Periportal cells ($n$ = 3) | | | Perivenous cells ($n$ = 4) | | |
|---|---|---|---|---|---|---|
| | $^3$HOH production | [$^{14}$C]Lactate production | Total lactate production | $^3$HOH production | [$^{14}$C]lactate production | Total lactate production |
| 5 mM-Glucose | 3.0 ± 0.4 | 0.13 ± 0.09 | 0.7 ± 0.3 | 2.9 ± 0.5 | 0.39 ± 0.18 | 0.2 ± 0.1 |
| 10 mM-Glucose | 7.9 ± 1.1 | 0.21 ± 0.11 | 0.8 ± 0.1 | 8.1 ± 1.4 | 1.15 ± 0.60 | 1.4 ± 0.9 |
| 20 mM-Glucose | 18.3 ± 2.1 | 6.26 ± 0.66 | 6.5 ± 0.4 | 20.5 ± 2.4 | 7.88 ± 2.19 | 9.2 ± 2.1 |
| 30 mM-Glucose | 27.4 ± 3.2 | 11.10 ± 1.56 | 11.8 ± 0.8 | 27.9 ± 4.2 | 13.53 ± 4.52 | 15.3 ± 3.4 |
| 10 mM-Glutamine plus: | | | | | | |
| 5 mM-Glucose | 3.2 ± 0.5 | 0.15 ± 0.11 | 3.0 ± 1.3 | 3.0 ± 0.2 | 0.20 ± 0.06 | 0.8 ± 0.5 |
| 10 mM-Glucose | 9.2 ± 1.5 | 0.89 ± 0.46 | 6.6 ± 0.7 | 9.2 ± 1.3 | 1.94 ± 0.76 | 3.0 ± 0.8 |
| 20 mM-Glucose | 21.4 ± 3.1 | 6.71 ± 1.07 | 13.9 ± 2.0 | 21.5 ± 2.8 | 9.71 ± 2.57 | 11.5 ± 2.1 |
| 30 mM-Glucose | 29.4 ± 4.5 | 11.89 ± 3.45 | 19.9 ± 2.0 | 30.0 ± 4.0 | 16.05 ± 3.35 | 16.7 ± 2.7 |

incorporation of glucose into glycogen would depend on glucose phosphorylation. Several investigators reported concurrent net synthesis of glucose and of lactate in the liver *in vivo* (Davis *et al.*, 1987). Jungermann (1986) proposed that the synthesis of glucose and glycogen from $C_3$ precursors predominates in the 'gluconeogenic' periportal hepatocytes, whereas simultaneously glucose uptake, lactate production and incorporation of glucose into glycogen predominate in the 'glycolytic' perivenous zone. At present, the evidence for substantial net uptake of glucose by the perivenous zone of the liver is controversial. Bartels *et al.* (1987) showed deposition of glycogen in only the perivenous zone when liver was perfused with glucose, but in the periportal zone when perfused with lactate. On the other hand, Anundi *et al.* (1987) found that when liver was perfused *in situ* with $N_2/CO_2$ (19:1), and either 20 mM-glucose or -fructose, lactate production from glucose was 7 $\mu$mol/h per g, the same as with no substrate, compared with 182 $\mu$mol/h per g with the same concentration of fructose. Fructose counteracted the lethal effect of anoxia on liver cells, whereas glucose was without effect. Our own experiments show no enhanced glycolysis in perivenous cells, in spite of the higher activity of glucokinase in this zone. The physiological function of glucokinase thus appears obscure. We suggest here that the role of glucokinase is as a regulator of glucose and glycogen synthesis through a futile cycle between glucose 6-phosphate and glucose. At physiological glucose concentrations, the combined action of phosphorylation and dephosphorylation will determine the net rate of glucose synthesis, and will affect the flux of glucose 6-phosphate to UDP-glucose and glycogen. This regulation may operate via the intracellular concentration of glucose 6-phosphate, as suggested by Hue & Hers (1974).

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