Regulation of glycogen synthesis from glucose and gluconeogenic precursors by insulin in periportal and perivenous rat hepatocytes

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Glycogen synthesis in hepatocyte cultures is dependent on: (1) the nutritional state of the donor rat, (2) the acinar origin of the hepatocytes, (3) the concentrations of glucose and gluconeogenic precursors, and (4) insulin. High concentrations of glucose (15–25 mM) and gluconeogenic precursors (10 mM-lactate and 1 mM-pyruvate) had a synergistic effect on glycogen deposition in both periportal and perivenous hepatocytes. When hepatocytes were challenged with glucose, lactate and pyruvate in the absence of insulin, glycogen was deposited at a linear rate for 2 h and then reached a plateau. However, in the presence of insulin, the initial rate of glycogen deposition was increased (20–40 %) and glycogen deposition continued for more than 4 h. Consequently, insulin had a more marked effect on the glycogen accumulated in the cell after 4 h (100–200 % increase) than on the initial rate of glycogen deposition. Glycogen accumulation in hepatocyte cultures prepared from rats that were fasted for 24 h and then re-fed for 3 h before liver perfusion was 2-fold higher than in hepatocytes from rats fed *ad libitum* and 4-fold higher than in hepatocytes from fasted rats. The incorporation of [¹⁴C]lactate into glycogen was 2–4-fold higher in periportal than in perivenous hepatocytes in both the absence and the presence of insulin, whereas the incorporation of [¹⁴C]glucose into glycogen was similar in periportal and perivenous hepatocytes in the absence of insulin, but higher in perivenous hepatocytes in the presence of insulin. Rates of glycogen deposition in the combined presence of glucose and gluconeogenic precursors were similar in periportal and perivenous hepatocytes, whereas in the presence of glucose alone, rates of glycogen deposition paralleled the incorporation of [¹⁴C]glucose into glycogen and were higher in perivenous hepatocytes in the presence of insulin. It is concluded that periportal and perivenous hepatocytes utilize different substrates for glycogen synthesis, but differences between the two cell populations in the relative utilization of glucose and gluconeogenic precursors are dependent on the presence of insulin and on the nutritional state of the rat.

INTRODUCTION

It is controversial whether hepatic glycogen synthesis occurs mainly by a direct pathway involving phosphorylation of glucose and incorporation into glycogen, or by an indirect pathway from gluconeogenic precursors [1–6]. One hypothesis, based on the heterogeneous distribution of glucokinase and gluconeogenic enzymes within the liver acinus, proposes that the direct pathway may operate in the perivenous zone where there is a predominance of glucokinase, whereas the indirect pathway may occur predominantly in the periportal zone which has higher activities of gluconeogenic enzymes [7].

Studies using histochemical techniques have shown that during the fasted-to-fed transition, glycogen is heterogeneously distributed within the liver acinus, usually, but not always, with an increased distribution in the perivenous zone in the early stages of glycogen deposition [8–12] and a predominance in either the periportal or the perivenous zone in the late stages, depending on whether rats are maintained on a time-restricted feeding schedule or allowed to feed *ad libitum* [8]. Several factors may account for this acinar zonation of glycogen. One possibility is that hepatocytes in different zones utilize different substrates for glycogen synthesis, which may become available at different times

in the feeding cycle. Another possibility is that the mechanisms that control glycogen synthesis (endocrine or neural) differ in hepatocytes in different acinar zones.

Previous studies using hepatocytes isolated from the periportal or the perivenous zones of rat liver have shown that rates of gluconeogenesis are higher in periportal than in perivenous hepatocytes [13–16], and glycogen deposition during the incubation of hepatocyte suspensions with gluconeogenic precursors and glucose was also higher in periportal hepatocytes [13,15]. However, no attempt was made in these studies to distinguish between glycogen synthesis from glucose as compared with that from gluconeogenic precursors.

In this study, we examined whether periportal and perivenous hepatocytes express different rates of glycogen deposition and incorporation of labelled glucose and gluconeogenic precursors into glycogen when incubated with different concentrations of substrates, and whether the stimulation of glycogen deposition by insulin differs in the two cell populations. We used hepatocyte cultures because, unlike freshly isolated hepatocyte suspensions, they are not in a catabolic state of glycogen turnover and show net glycogen deposition from glucose as well as stimulation by insulin [17–20]. We demonstrate that when hepatocytes isolated from rats in different nutritional states are maintained in culture, they express differences in glycogen deposition which are related to

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the nutritional state. This suggests that hepatocyte cultures are a valid model with which to study mechanisms of glycogen synthesis in periportal and perivenous hepatocytes which reflect the situation *in vivo*.

MATERIALS AND METHODS

Materials

Digitonin was either from BDH Chemicals (Poole, Dorset, U.K.) or from Aldrich (Gillingham, Dorset, U.K.), pig insulin was from Sigma Chemical Co. (St Louis, MO, U.S.A.), culture media were from Northumbria Biologicals (Cramlington, Northumbria, U.K.), and multi-well culture dishes were from Corning (New York, NY, U.S.A.). [$U-^{14}C$]Lactate and [$U-^{14}C$]glucose were from Amersham International (Amersham, Bucks, U.K.) and [$3-^{14}C$]pyruvate was from New England Nuclear (Boston, MA, U.S.A.). Sources of other materials were as described previously [16,21].

Rats

Male rats of the Albino Wistar strain were used (body wt. 178 ± 5 g, mean $\pm$ S.E.M., $n = 52$). They were either allowed access to rat chow *ad libitum*, or deprived of food for 20–24 h before hepatocyte isolation; or, after food deprivation (20–24 h) they were allowed to feed (standard rat chow) for either 30 min or 180 min before hepatocyte isolation.

Hepatocyte isolation

Hepatocytes were isolated from either the periportal or the perivenous zone of rat liver by the digitonin/collagenase perfusion technique [13] with minor modifications [16,22], or from whole liver by conventional collagenase perfusion [23]. Only digitonin preparations which resulted in uniform destruction patterns of the liver acinus were used [24]. Cell yield and viability were as good as reported previously [16].

Enzyme activities in periportal and perivenous hepatocytes were determined as described previously [16,19]. There was a similar degree of heterogeneity of alanine aminotransferase between periportal and perivenous hepatocytes of rats fed *ad libitum* [periportal (PP), 157 ± 22 (12); perivenous (PV) 105 ± 16 (12); PP/PV, 1.49; means $\pm$ S.E.M. (n) munits/mg of protein]; fasted and re-fed for 30 min (periportal, 148 ± 12 (6); perivenous, 96 ± 5 (6); PP/PV, 1.54); and fasted and re-fed for 180 min [periportal, 157 ± 7 (6); perivenous, 107 ± 7 (6); PP/PV, 1.47]. The ratio of alanine aminotransferase/glutamate dehydrogenase ($\times 100$) between periportal and perivenous hepatocytes was also similar in the three nutritional states (*ad libitum*: periportal, 6.88 ± 0.5 ; perivenous, 3.8 ± 0.42 ; PP/PV, 1.8; fasted/re-fed-30 min: periportal 7.33 ± 0.61 ; perivenous 4.0 ± 0.36 ; PP/PV, 1.83; fasted/re-fed-180 min: periportal, 7.7 ± 0.20 ; perivenous, 4.2 ± 0.32 ; PP/PV, 1.83).

Hepatocyte culture

The hepatocytes were inoculated in minimum essential medium containing 5% (v/v) fetal bovine serum [21] in Corning multi-well plates at a cell density of 6.4×10^4 cells/cm² (6-well or 12-well plates were used for time-course experiments, and 24-well plates for other experiments). After cell attachment (4–7 h), the medium was replaced with serum-free minimum essential medium containing 10 nM-dexamethasone phosphate, and the

hepatocytes were cultured for 14–18 h [16]. Incubations for determination of glycogen deposition or incorporation of ^{14}C -labelled substrates into glycogen were started on termination of the 14–18 h culture.

Determination of glycogen deposition and incorporation of ^{14}C -labelled substrates into glycogen

The hepatocyte monolayers were incubated in fresh minimum essential medium (containing 5 mM-glucose) supplemented with the substrates indicated, without or with insulin (0.1–100 nM). The incubations were for between 30 and 240 min as indicated. In experiments using radiolabelled substrates, the specific activities were 0.5 Ci/mol for [$U-^{14}C$]lactate, 0.15–0.2 Ci/mol for [$3-^{14}C$]pyruvate and 1 μ Ci/ml for [$U-^{14}C$]glucose. All substrate and hormone conditions were in duplicate wells. Additional wells which did not receive a change of medium (time zero) or which received medium not supplemented with substrates were used for determination of cellular glycogen at the start of the incubation with substrates or during incubation without substrates.

On termination of the incubations, the medium was collected and the monolayers were rinsed rapidly twice with 150 mM-NaCl, extracted in 0.1 M-NaOH and frozen for later glycogen and protein analysis. Glycogen and protein were determined after sonication of the cell extract [25]. Protein was determined by an automated Lowry method [26]; glycogen was determined by digestion of an aliquot of the extract with amyloglucosidase and fluorimetric determination of glucose by the hexokinase/glucose-6-phosphate dehydrogenase method [25]. In experiments using ^{14}C -labelled substrates, the rest of the cell extract was used for glycogen precipitation [25]. The extract was deproteinized with trichloroacetic acid [final concentration 8% (w/v)] containing glycogen carrier (1 mg/sample). The glycogen in the supernatant was precipitated with ethanol [final concn. 63% (v/v)], the precipitate was redissolved in water and re-precipitated with ethanol, and the radioactivity was determined [25]. Lactate in the medium was determined enzymically [25].

Expression of results

Glycogen deposition was determined from the incremental increase in cellular glycogen (determined enzymically) during the incubation with substrates and is expressed as nmol of glucose units deposited as glycogen during the incubation/mg of cell protein. The incorporation of radiolabelled glucose, lactate or pyruvate into glycogen is expressed as nmol of substrate incorporated into glycogen during the incubation/mg of cell protein, using the specific activity of the substrate in the medium.

Comparisons between periportal and perivenous values were either by the unpaired Student's *t*-test or by 2-way Analysis of Variance [16]. Comparisons between different substrates in the same hepatocyte preparations were by the paired *t*-test.

RESULTS

Glycogen deposition from glucose and gluconeogenic precursors in periportal and perivenous hepatocytes from rats fed *ad libitum*

Periportal and perivenous hepatocytes isolated from rats fed *ad libitum* were cultured for 14 h in serum-free

Table 1. Glycogen deposition in periportal and perivenous hepatocytes isolated from rats fed *ad libitum*

Hepatocytes were cultured as described in the Materials and methods section. They were then incubated for 2 h in medium containing 5 mM-glucose and supplemented with the substrates indicated, without or with 10 nM-insulin. Glycogen deposition is expressed as nmol of glucose units deposited as glycogen/2 h per mg of cell protein. Values are means $\pm$ S.E.M. for six periportal and six perivenous preparations. All values with insulin are significantly higher than the controls ($P < 0.01$). Values of perivenous hepatocytes that are significantly different from the values of periportal hepatocytes are shown by * $P < 0.05$; ** $P < 0.01$.

Substrate additions (mM)	Acinar origin...	Glycogen deposition (nmol of glucose units/2 h per mg of protein)			
		Periportal		Perivenous	
		Control	Insulin	Control	Insulin
A. Lactate (10) + pyruvate (1)		8.8 $\pm$ 2.9	37 $\pm$ 7	3.3 $\pm$ 3.5	46 $\pm$ 4
B. Glucose (10)		75 $\pm$ 4	108 $\pm$ 13	89 $\pm$ 12	156 $\pm$ 18*
C. Glucose (10) + lactate (10) + pyruvate (1)		125 $\pm$ 11	215 $\pm$ 21	155 $\pm$ 11	255 $\pm$ 14
D. Glucose (20)		152 $\pm$ 10	207 $\pm$ 20	204 $\pm$ 12**	272 $\pm$ 21*
E. Glucose (20) + lactate (10) + pyruvate (1)		206 $\pm$ 17	310 $\pm$ 33	262 $\pm$ 18*	362 $\pm$ 25
C/(B + A)		1.49	1.48	1.68	1.26
E/(D + A)		1.28	1.27	1.26	1.14

medium as described in the Materials and methods section. Glycogen deposition was determined during a 2 h incubation with the substrate supplements shown in Table 1 without or with 10 nM-insulin. The glycogen content at the start of the incubation was similar in periportal and perivenous hepatocytes (56 ± 13 and 62 ± 27 nmol/mg of protein, means $\pm$ S.E.M., $n = 6$). Insulin caused a significant increase ($P < 0.01$ paired t test) in glycogen deposition with all substrates shown in Table 1, but the increase was greater in the presence of gluconeogenic precursors than with glucose alone (15 or 25 mM), particularly in periportal hepatocytes (Table 1). In both periportal and perivenous hepatocytes, rates of glycogen deposition in the combined presence of glucose supplement (10 or 20 mM) and lactate and pyruvate were higher than the sum of the rates with glucose alone and with lactate and pyruvate (in the presence of 5 mM-glucose), indicated by the ratios in the last two lines of Table 1. With 15 mM- or 25 mM-glucose, in the absence of gluconeogenic precursors, the incremental increase in glycogen deposition caused by insulin was greater in perivenous than in periportal hepatocytes (67–68 versus 33–36 nmol/mg of protein). Perivenous hepatocytes had significantly higher rates of glycogen deposition than periportal hepatocytes when incubated with 15 mM-glucose and insulin or with 25 mM-glucose without or with insulin. However, in the presence of lactate (10 mM), pyruvate (1 mM) and glucose (5 or 15 mM), there were no significant differences in rates of glycogen deposition between periportal and perivenous hepatocytes (Table 1).

We determined the dose-response to insulin (0.1–100 nM) of glycogen deposition in periportal and perivenous hepatocytes incubated with glucose, lactate and pyruvate (15, 10 and 1 mM respectively) for 30, 60 or 120 min. In the absence of insulin, rates of glycogen deposition were linear during 2 h (30, 60 and 120 min values: periportal, 30.5 ± 1.6 ; 65.2 ± 8.3 ; 125.6 ± 17.6 ; perivenous, 31.5 ± 3.3 ; 65.2 ± 8.7 ; 145.6 ± 9.8 nmol of glucose units deposited/time interval per mg of protein; means $\pm$

S.E.M., $n = 6$). The percentage stimulation of glycogen deposition by insulin (1 nM) was greater at 120 min (69–75 % increase) than at 30 and 60 min (24–35 % increase), and was similar in periportal (31, 35 and 75 %) and perivenous (24, 30 and 69 %) hepatocytes. The concentration of insulin causing half-maximal stimulation was 0.27 ± 0.02 nM for periportal hepatocytes and 0.13 ± 0.05 nM for perivenous hepatocytes (means $\pm$ S.E.M., $n = 6$ for 30, 60 and 120 min incubations).

Effects of nutritional state of the rat on the rates of glycogen deposition in hepatocyte cultures

Studies *in vivo* have shown that in the fasted-to-fed transition, hepatic glycogen synthesis increases rapidly during the first 3 h [27–29]. In order to examine whether rates of glycogen deposition in hepatocytes maintained in culture (for 16 h in serum-free medium) are related to the nutritional state of the rat, we compared the time courses (0–240 min) of glycogen deposition (during incubation with glucose, lactate and pyruvate, without or with insulin) in hepatocytes isolated from whole liver (by the conventional collagenase perfusion technique) of rats fed *ad libitum*, fasted for 24 h, or fasted (24 h) and then re-fed for 180 min.

At the start of the incubation, the glycogen content was higher in hepatocytes from fasted/re-fed rats (251 ± 74 nmol of glucose/mg of protein) than in hepatocytes from rats fed *ad libitum* (134 ± 33 nmol/mg) or fasted rats (110 ± 16 nmol/mg). Rates of glycogen deposition were highest in cells from fasted/re-fed rats and lowest in cells from fasted rats (Figs. 1a and b). In the absence of insulin, glycogen deposition reached a plateau after 2 h (Fig. 1a), whereas in the presence of insulin it continued to increase between 2 and 4 h (Fig. 1b). Consequently, the stimulation by insulin (relative to control cultures) increased progressively with time. After 120 min the stimulation by 100 nM-insulin was 68, 101 and 47 % for hepatocytes from *ad libitum* fed, fasted and fasted/re-fed rats respectively, and after 240 min the corresponding values were 180, 170 and 108 %; the

Fig. 1. Time course of glycogen deposition in hepatocytes isolated from rats in different nutritional states

Hepatocytes were isolated from whole liver by the conventional collagenase perfusion technique from rats fed *ad libitum* (●), fasted for 24 h (▲), or fasted for 24 h and re-fed for 180 min (■), and were then cultured for 16 h as described in the Materials and methods section. Glycogen deposition was determined during incubation in medium containing glucose (20 mM), lactate (10 mM) and pyruvate (1 mM) either without insulin (a) or with 100 nM-insulin (b), and is expressed as nmol of glucose units deposited as glycogen/mg of cell protein. Values are means $\pm$ S.E.M. for each of five preparations.

concentrations of insulin causing half-maximal stimulation were respectively 0.19, 0.13 and 0.09 nM. The rate of glycogen deposition in hepatocytes from fasted/re-fed rats during incubation with insulin (307 ± 33 nmol/h per mg of protein; 0–60 min) was similar to the rates observed *in vivo* in the fasted-to-re-fed transition [27]; and the glycogen that these cells accumulated after 4 h of incubation with substrates and insulin (initial + deposition during 4 h = 1359 ± 256 nmol of glucose units/mg of protein) was similar to the maximum hepatic glycogen concentration during meal feeding *in vivo* [27]. These findings indicate that hepatocytes isolated from rats in these nutritional states express different rates of glycogen deposition when maintained in culture for 16 h without insulin, and the rates in hepatocytes from rats that were fasted and re-fed for 180 min are similar to the maximal rates observed *in vivo* in the fasted-to-fed transition.

Hepatocytes from fasted rats re-fed for either 30 min or 180 min

Previous studies have shown that zonal differences in glycogen deposition occur mainly in the early stages after re-feeding [8–10], and when insulin-deficient rats are treated with insulin, structural changes in the endoplasmic reticulum are observed in the periportal zone only after 30 min but throughout the liver acinus after 60 min [30]. In the rest of this study we examined glycogen deposition from different substrates in periportal and perivenous hepatocytes isolated from rats fasted for 20 h and re-fed for either 30 min or 180 min before hepatocyte isolation to investigate whether hepatocytes from rats in these nutritional states express different mechanisms of glycogen synthesis.

While this work was in progress, Chen & Katz [15] reported that low concentrations of digitonin severely impair glycogen synthesis in hepatocyte suspensions without affecting rates of gluconeogenesis or cell viability. They suggested that glycogen synthesis in hepatocytes isolated by the digitonin-perfusion technique may be impaired by cell damage caused by digitonin [15]. In order to establish whether our periportal or perivenous hepatocyte cultures had been damaged by digitonin, we

compared rates of glycogen deposition in periportal and perivenous hepatocytes (isolated by the digitonin-perfusion technique) with hepatocytes isolated from whole liver without using digitonin, but using otherwise similar perfusion conditions, from fasted rats re-fed for 30 min. There was no significant difference in rates of glycogen deposition between the three hepatocyte populations during incubation with 15 mM-glucose, 10 mM-lactate and 1 mM-pyruvate either without or with 10 nM-insulin (periportal, 93 ± 12 ; periportal + insulin, 152 ± 17 ; perivenous, 91 ± 17 ; perivenous + insulin, 157 ± 20 ; total hepatocytes, 78 ± 14 ; + insulin, 158 ± 26 nmol of glucose units/2 h per mg of protein; means $\pm$ S.E.M., $n = 6, 6$ and 4 for periportal, perivenous and total hepatocytes respectively), indicating that rates of glycogen deposition were not affected by digitonin.

We determined the stimulation of glycogen deposition by insulin after incubation with 15 mM-glucose, 10 mM-lactate and 1 mM-pyruvate for between 30 and 240 min. In the absence of insulin, glycogen deposition in periportal and perivenous hepatocytes reached a plateau at about 2 h (Table 2) and the stimulation by insulin (expressed as a percentage of the controls) increased progressively with time (Table 2). In hepatocytes from fasted rats re-fed for 30 min, the stimulation by insulin at the later time points was greater in perivenous hepatocytes (Table 2 and Fig. 2), whereas in hepatocytes from fasted rats re-fed for 180 min, there was a delay in the stimulation by insulin in perivenous compared with periportal hepatocytes (Table 2). Since the effect of insulin on glycogen deposition increased progressively with time, in the rest of this study the effects of insulin on the incorporation of ^{14}C -labelled substrates into glycogen was determined during a 180 min incubation.

Incorporation of ^{14}C -labelled gluconeogenic precursors and glucose into glycogen

Having established that in hepatocytes from fasted/re-fed rats the overall rate of glycogen accumulation is dependent on the presence or absence of insulin, the acinar origin of the cells and the duration of re-feeding, we attempted to determine the relative glyconeogenic

Table 2. Time course of stimulation of glycogen deposition by insulin in periportal and perivenous hepatocytes

Hepatocytes isolated from fasted (20 h) rats that were re-fed for either 30 min or 180 min before hepatocyte isolation were cultured as described in the Materials and methods section and incubated in medium containing 15 mM-glucose, 10 mM-lactate and 1 mM-pyruvate either without or with insulin (10 or 100 nM) for 30, 60, 120 or 240 min. The glycogen deposition in the control incubations (without insulin) is expressed as nmol of glucose units deposited/time interval per mg of cell protein (means $\pm$ S.E.M.). The stimulation of glycogen deposition by insulin (mean of 10 nM and 100 nM) is expressed as a percentage of the respective control without insulin. * $P < 0.05$, 180 min re-feeding versus 30 min re-feeding. Values of n are given in parentheses for each group.

Nutritional state...	Glycogen deposition (nmol/per mg of protein)				Stimulation by insulin (%) of control)			
	Fasted/re-fed-30 min		Fasted/re-fed-180 min		Fasted/re-fed-30 min		Fasted/re-fed-180 min	
	Periportal (6)	Perivenous (6)	Periportal (5)	Perivenous (5)	Periportal (6)	Perivenous (6)	Periportal (5)	Perivenous (5)
30	25 $\pm$ 7	41 $\pm$ 7	30 $\pm$ 4	34 $\pm$ 9	36 $\pm$ 20	40 $\pm$ 13	53 $\pm$ 12	0 $\pm$ 12
60	53 $\pm$ 7	62 $\pm$ 11	89 $\pm$ 20	109 $\pm$ 15*	46 $\pm$ 13	49 $\pm$ 16	41 $\pm$ 5	-1 $\pm$ 6
120	93 $\pm$ 12	91 $\pm$ 7	229 $\pm$ 62	239 $\pm$ 70	84 $\pm$ 15	80 $\pm$ 7	57 $\pm$ 13	46 $\pm$ 7
240	86 $\pm$ 14	87 $\pm$ 21	282 $\pm$ 82*	247 $\pm$ 54*	152 $\pm$ 19	226 $\pm$ 28	102 $\pm$ 25	82 $\pm$ 13

Fig. 2. Dose-response to insulin of glycogen deposition in periportal and perivenous hepatocytes

Periportal (●) and perivenous (▼) hepatocyte cultures were isolated from fasted rats re-fed for 30 min. Glycogen deposition was determined during incubation for either 120 min (a) or 240 min (b) with glucose (15 mM), lactate (10 mM) and pyruvate (1 mM) at the concentrations of insulin indicated and is expressed as nmol of glucose units deposited as glycogen during either 120 min or 240 min per mg of cell protein. Values are means $\pm$ S.E.M. for each of six hepatocyte preparations.

contributions of glucose and lactate in periportal and perivenous hepatocytes from fasted rats re-fed for either 30 or 180 min during incubation either with glucose alone or with a combination of glucose and gluconeogenic precursors.

In hepatocytes from fasted rats re-fed for 30 min, the incorporation of [U- 14 C]lactate (5 mM) into glycogen during incubation with either 5 mM-glucose or 15 mM-glucose was between 2- and 4-fold higher in periportal than in perivenous cells, in the absence or the presence of insulin (Table 3). The incorporation of [14 C]glucose into glycogen was similar in periportal and perivenous hepatocytes in the absence of insulin but higher in perivenous cells ($P < 0.05$, 2-way Analysis of Variance)

in the presence of insulin. Rates of glycogen deposition showed similar trends as the incorporation of [14 C]glucose into glycogen (Table 3).

In hepatocytes from fasted rats re-fed for 180 min, there were similar differences between periportal and perivenous hepatocytes (3–5-fold) in the incorporation of [14 C]lactate into glycogen (as in cells from fasted rats re-fed for 30 min, Table 3) in the presence of both 5 mM-glucose (periportal, 3.7 ± 1.1 ; periportal+insulin, 13.1 ± 8.7 ; perivenous, 0.9 ± 0.4 ; perivenous+insulin, 2.4 ± 1.3 nmol of glucose units/3 h per mg) and 15 mM-glucose (periportal, 8.9 ± 2.5 ; periportal+insulin, 22.7 ± 10.9 ; perivenous, 2.9 ± 1.1 ; perivenous+insulin, 6.1 ± 2.9 nmol/3 h per mg). However, there was no

Table 3. Incorporation of [¹⁴C]lactate and [¹⁴C]glucose into glycogen in periportal and perivenous hepatocytes from fasted rats re-fed for 30 min

Periportal and perivenous hepatocyte cultures were incubated for 3 h in medium containing either [U-¹⁴C]lactate or [U-¹⁴C]glucose as indicated, without or with insulin (100 nM). The incorporation of ¹⁴C substrate into glycogen is expressed as nmol of substrate incorporated/3 h per mg of cell protein, and glycogen deposition is expressed as nmol of glucose units deposited/3 h per mg of protein. Values are means $\pm$ S.E.M. for six periportal and six perivenous preparations. The incorporation in the presence of insulin expressed as a percentage of the respective control is shown in parentheses. * $P < 0.05$ (2-way Analysis of Variance); periportal versus perivenous.

Substrates (mM)	Insulin (100 nM)	Incorporation of ¹⁴ C-substrate into glycogen (nmol of substrate/3 h per mg)			Glycogen deposition (nmol of glucose units/3 h per mg)	
		Periportal	Perivenous	PP/PV	Periportal	Perivenous
[¹⁴ C]Lactate (5) + glucose (5)	—	1.38 $\pm$ 0.18	0.55 $\pm$ 0.23*	2.5	—16 $\pm$ 4	—16 $\pm$ 7
[¹⁴ C]Lactate (5) + glucose (5)	+	5.05 $\pm$ 2.36 (366 %)	1.25 $\pm$ 0.36 (227 %)	4.0	12 $\pm$ 8	22 $\pm$ 9
[¹⁴ C]Lactate (5) + glucose (15)	—	4.00 $\pm$ 0.75	1.10 $\pm$ 0.27*	3.6	35 $\pm$ 11	47 $\pm$ 10
[¹⁴ C]Lactate (5) + glucose (15)	+	7.15 $\pm$ 3.6 (179 %)	3.40 $\pm$ 1.05 (309 %)	2.1	92 $\pm$ 11	149 $\pm$ 23*
[¹⁴ C]Glucose (15)	—	40.8 $\pm$ 9.6	42.8 $\pm$ 11.2	0.95	37 $\pm$ 14	43 $\pm$ 11
[¹⁴ C]Glucose (15)	+	75.2 $\pm$ 14.5 (184 %)	119.2 $\pm$ 19.3* (279 %)	0.63	69 $\pm$ 11	90 $\pm$ 26
[¹⁴ C]Glucose (15) + lactate (5)	—	46.2 $\pm$ 1.2	51.2 $\pm$ 13.0	0.90	46 $\pm$ 18	54 $\pm$ 10
[¹⁴ C]Glucose (15) + lactate (5)	+	95.2 $\pm$ 13.8 (206 %)	135.8 $\pm$ 23.4 (269 %)	0.70	116 $\pm$ 16	154 $\pm$ 20

difference in incorporation of [¹⁴C]-glucose between periportal and perivenous hepatocytes (periportal, 88 $\pm$ 20; periportal + insulin, 198 $\pm$ 40; perivenous, 79 $\pm$ 16; perivenous + insulin, 192 $\pm$ 40 nmol/3 h per mg). In one experiment where we examined the incorporation of [¹⁴C]pyruvate, we found similar differences between periportal and perivenous cells as for lactate, but at much higher rates of incorporation.

In the above studies (Table 3), the incorporation of [¹⁴C]lactate into glycogen was low in comparison with that of [¹⁴C]glucose or the amount of glycogen deposited, suggesting that glycogen accumulation could be accounted for mainly by the incorporation of [¹⁴C]glucose. We used lactate alone as opposed to lactate and pyruvate to avoid problems associated with dilution of [¹⁴C]lactate and lack of complete equilibration between lactate and pyruvate. We investigated the problem of the low incorporation of [¹⁴C]lactate by comparing the incorporation of equimolar concentrations (5 mM) of ¹⁴C-labelled lactate or pyruvate in the presence of different glucose concentrations (Table 4) and the incorporation of 5 mM-lactate and 0.5 mM-pyruvate added separately or in combination (Table 5).

The data in Table 4 show that the accumulation of glycogen was higher with 5 mM-pyruvate than with 5 mM-lactate at all glucose concentrations. The incorporation of 5 mM-[¹⁴C]pyruvate into glycogen was several-fold higher than the incorporation of [¹⁴C]lactate (20–50-fold in the presence of 5 mM-glucose and 10–20-fold in the presence of 15 mM-glucose), and was comparable to the rate of incorporation of 15 mM-[¹⁴C]glucose. The incorporation of [¹⁴C]lactate into glycogen was increased by increasing the glucose concentration, and by insulin, unlike the incorporation of [¹⁴C]pyruvate which was independent of the glucose concentration (Table 4).

Table 5 shows that the incorporation of 0.5 mM-[¹⁴C]pyruvate was higher than that of 5 mM-[¹⁴C]lactate when the lactate and pyruvate were added separately. In the combined presence of lactate (5 mM) and pyruvate (0.5 mM), the incorporation of [¹⁴C]lactate into glycogen was similar to the incorporation with lactate alone, but the incorporation of [¹⁴C]pyruvate (0.5 mM) was 4.7 times lower in the additional presence of 5 mM-lactate, suggesting partial (approx. 50 %) but not complete dilution of [¹⁴C]pyruvate label.

To examine whether other oxidized or reduced gluconeogenic substrates show similar differences in rates of glycogen deposition as pyruvate and lactate, we incubated hepatocyte cultures with either 10 mM-dihydroxyacetone or 10 mM-glycerol (oxidized and reduced substrates respectively, which enter the gluconeogenic pathway at the level of triose phosphate). In the absence of insulin, rates of glycogen deposition were higher with dihydroxyacetone than with glycerol in periportal and perivenous hepatocytes, but insulin caused a larger increase in glycogen deposition with glycerol than with dihydroxyacetone (Table 6). With dihydroxyacetone or glycerol as substrate supplements there was little difference in rates of glycogen deposition between hepatocytes from fasted rats re-fed for 30 min as compared with 180 min. This contrasts with glycogen deposition during incubation with glucose alone (10 or 35 mM), which was 2–3-fold higher in hepatocytes from fasted rats re-fed for 180 min as compared with 30 min (Table 6).

In the perfused liver [31] and in isolated hepatocyte suspensions [32], dihydroxyacetone is metabolized more rapidly than glycerol. The rate-limiting step for glycerol metabolism is the translocation of reducing equivalents from the cytosol to the mitochondria [33]. In the present study, rates of glucose formation were similar in

Table 4. Effects of glucose concentration on the incorporation of [U-¹⁴C]lactate and [3-¹⁴C]pyruvate into glycogen

Hepatocyte cultures prepared from rats in the nutritional states indicated were incubated for 3 h with either 5 mM-[U-¹⁴C]lactate or 5 mM-[3-¹⁴C]pyruvate and the concentrations of glucose and insulin shown. In experiments A and B, parallel incubations were performed with [U-¹⁴C]glucose and unlabelled lactate or pyruvate. Column (1) represents the incorporation of [¹⁴C]lactate or [¹⁴C]pyruvate into glycogen, and column (2) the incorporation of [¹⁴C]glucose into glycogen, both expressed as nmol of substrate incorporated/3 h per mg of protein; values are means of duplicate wells for the individual hepatocyte preparations. Column (3) values represent the net change in cellular glycogen during the incubation expressed as nmol of glucose units/3 h per mg of protein; values are means of duplicate wells for experiment C and of quadruplicate wells for experiments A and B (negative values indicate net degradation).

Experiment... State of rat...			A Fed ad libitum			B Fasted			C Fasted/180 min re-fed			
	Acinar origin...		Total			Periportal			Periportal		Perivenous	
	[Glucose] (mM)	Insulin (100 nM)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(3)	(1)	(3)
5 mM-[¹⁴ C]Lactate	5	—	0.8	0.8	—3	2.1	5.8	—11	3.7	—27	0.5	6
	5	+	2.7	11.3	26	3.6	7.6	—11	4.8	20	1.0	53
	15	—	2.7	32.3	48	6.4	49.6	44	7.8	63	1.8	180
	15	+	7.4	81.1	115	8.1	58.8	53	12.8	138	3.0	204
	30	—	5.0	134	132	7.9	105	114	9.8	213	3.3	389
	30	+	12.2	170	235	16.2	129	151	16.3	329	4.8	440
5 mM-[¹⁴ C]Pyruvate	5	—	19.5	4.8	15	108	10.2	18	124	48	73	18
	5	+	83.9	15.1	62	116	11.6	22	148	44	92	71
	15	—	46.8	41.6	61	98	73	83	120	168	76	269
	15	+	76.8	83.0	133	85	87	101	137	269	76	399
	30	—	36.9	125	156	84	106	131	112	402	51	507
	30	+	65.1	185	232	90	128	209	115	401	56	566

Table 5. Incorporation of [U-¹⁴C]lactate and [3-¹⁴C]pyruvate into glycogen

Hepatocyte cultures isolated from rats in the nutritional states indicated were incubated for 3 h with the substrates indicated without or with 100 nM-insulin. The incorporation of ¹⁴C-substrates into glycogen is expressed as nmol of substrate incorporated (determined from the specific activity in the medium)/3 h per mg of protein, and values represent means of duplicate wells in four individual hepatocyte preparations. Abbreviations: Lac, lactate; Glc, glucose; Pyr, pyruvate; PP, periportal; PV, perivenous.

[Substrate] (mM)	Insulin (100 nM)	State of rat... Acinar original...	Incorporation of ¹⁴ C-substrate into glycogen (nmol of substrate/3 h per mg)			
			Fed	Fasted	Fasted/180 min re-fed	
					PP	PV
[¹⁴ C]Lac (5) + Glu (15)	—		6.4	5.8	8.2	2.0
[¹⁴ C]Lac (5) + Glu (15)	+		9.2	10.5	13.9	2.5
[¹⁴ C]Pyr (0.5) + Glu (15)	—		14.1	9.4	18.9	6.6
[¹⁴ C]Pyr (0.5) + Glu (15)	+		21.1	15.8	22.6	7.0
[¹⁴ C]Lac (5) + Pyr (0.5) + Glu (15)	—		8.5	5.2	10.2	3.8
[¹⁴ C]Lac (5) + Pyr (0.5) + Glu (15)	+		11.6	10.0	16.7	3.9
Lac (5) + [¹⁴ C]Pyr (0.5) + Glu (15)	—		3.9	2.1	3.0	1.3
Lac (5) + [¹⁴ C]Pyr (0.5) + Glu (15)	+		5.1	2.7	4.8	1.8
Lac (5) + Pyr (0.5) + [¹⁴ C]Glu (15)	—			42	118	151
Lac (5) + Pyr (0.5) + [¹⁴ C]Glu (15)	+			58	174	184

incubations with dihydroxyacetone and glycerol (results not shown), but the rate of formation of lactate was 2.5–2.7 times higher in incubations with dihydroxyacetone (periportal, 834 ± 40; perivenous, 1085 ± 116 nmol of lactate/3 h per mg of protein; means ± S.E.M.; *n* = 6) than with glycerol (periportal 309 ± 46; perivenous,

428 ± 44) suggesting that dihydroxyacetone is metabolized more rapidly than glycerol in hepatocyte cultures also. Insulin increased (*P* < 0.05) lactate formation from glycerol (periportal, 528 ± 28; perivenous, 571 ± 63) but not from dihydroxyacetone (periportal 858 ± 40; perivenous, 1075 ± 111). Since insulin increased both gly-

Table 6. Glycogen deposition in hepatocytes from fasted rats re-fed for either 30 min or 180 min

Periportal and perivenous hepatocyte cultures were incubated for 3 h in medium containing 5 mM-glucose and the substrate supplements indicated without or with 100 nM-insulin. Rates of glycogen deposition are expressed as nmol of glucose units deposited as glycogen/3 h per mg of protein. Values are means $\pm$ S.E.M. for five to seven hepatocyte preparations. Significant differences (* $P < 0.05$, unpaired t test) between hepatocytes from fasted rats re-fed for 180 min as compared with 30 min are indicated.

Substrate (mM)	Insulin (100 nM)	Nutritional state... Acinar origin...	Glycogen deposition (nmol of glucose units/3 h per mg)			
			Fasted/re-fed for 30 min		Fasted/re-fed for 180 min	
			Periportal	Perivenous	Periportal	Perivenous
Dihydroxyacetone (10)	—		148 $\pm$ 22	218 $\pm$ 36	189 $\pm$ 24	190 $\pm$ 39
Dihydroxyacetone (10)	+		162 $\pm$ 22	256 $\pm$ 55	320 $\pm$ 68	241 $\pm$ 45
Glycerol (10)	—		86 $\pm$ 28	82 $\pm$ 10	107 $\pm$ 12	107 $\pm$ 27
Glycerol (10)	+		132 $\pm$ 22	150 $\pm$ 25	224 $\pm$ 53	179 $\pm$ 52
Glucose (+5)	—		12 $\pm$ 5	19 $\pm$ 7	51 $\pm$ 8*	45 $\pm$ 7*
Glucose (+5)	+		40 $\pm$ 12	48 $\pm$ 13	141 $\pm$ 26*	138 $\pm$ 21*
Glucose (+30)	—		128 $\pm$ 16	179 $\pm$ 18	364 $\pm$ 30*	379 $\pm$ 29*
Glucose (+30)	+		196 $\pm$ 16	288 $\pm$ 45	620 $\pm$ 77*	593 $\pm$ 83*

cogen deposition and lactate formation (but did not affect glucose formation) during incubation with glycerol, it can be inferred that insulin increased overall glycerol metabolism. If the reduced cytosolic NADH/NAD⁺ redox state limits glycerol metabolism [33], the effect of insulin on glycerol metabolism may be due to changes in the cytosolic redox state.

Effects of glucose concentration on glycogen deposition and lactate production

It is not known whether the gluconeogenic substrates which are incorporated into hepatic glycogen *in vivo* are derived from extrahepatic tissues or from the liver itself [6]. One hypothesis is that the perivenous zone of the liver converts glucose into lactate, which is released into the circulation and is subsequently converted to glycogen in the periportal zone. There is, however, little information on the relative rates of glucose conversion into glycogen and lactate by periportal and perivenous hepatocytes. We therefore examined the relationship between medium glucose concentration and glycogen deposition and formation of lactate in hepatocytes from fasted rats re-fed for either 30 or 180 min (Fig. 3). Insulin increased ($P < 0.05$, paired t test) lactate production and glycogen deposition in periportal and perivenous hepatocytes over a range of glucose concentrations (Fig. 3). In hepatocytes from fasted rats re-fed for 180 min as compared with 30 min, rates of glycogen deposition were between 2 and 3 times higher, but rates of lactate formation were only slightly higher (13–38%). Consequently, the rate of glycogen formation expressed as a percentage of glycogen and lactate formation was greater after 180 min than after 30 min of re-feeding.

In hepatocytes from fasted rats re-fed for 30 min, at high glucose concentrations (> 20 mM) the rates of both lactate formation and glycogen deposition were higher in perivenous than in periportal hepatocytes (27–47%). However, in hepatocytes isolated after 180 min of re-feeding there were no differences in rates of either lactate formation or glycogen deposition between periportal and perivenous cells (Fig. 3). We examined the effects of

various concentrations (0.2–6 mM) of fructose, which activates glucokinase [34], on the incorporation of [¹⁴C]glucose into glycogen in hepatocytes from fasted rats re-fed for 180 min. Although in the absence of fructose there was no difference between periportal and perivenous cells in incorporation of [¹⁴C]glucose (20 mM) into glycogen (periportal, 98 $\pm$ 28; +insulin, 158 $\pm$ 6; perivenous, 87 $\pm$ 20; +insulin, 160 $\pm$ 40 nmol/3 h per mg; means $\pm$ S.E.M.; $n = 3$), in the presence of 3 mM-fructose the incorporation of [¹⁴C]glucose was higher in perivenous hepatocytes (periportal, 151 $\pm$ 17; +insulin, 195 $\pm$ 14; perivenous, 188 $\pm$ 28; +insulin, 248 $\pm$ 43 nmol/3 h per mg). This indicates that rates of glucose incorporation into glycogen are submaximal in the absence of fructose and that fructose causes a greater stimulation in perivenous than in periportal hepatocytes.

DISCUSSION

The present investigation had three goals: first, to examine whether hepatocytes isolated from rats in different nutritional states express different rates of glycogen deposition when they are maintained in culture; second, to examine whether hepatocytes isolated from the periportal and the perivenous zones of the liver acinus synthesize glycogen by different mechanisms and/or express different rates of glycogen deposition; and third, to study the time course of the stimulation of glycogen deposition by insulin in periportal and perivenous hepatocytes from rats in different nutritional states.

Effects of nutritional state

Most studies on glycogen deposition in perfused liver or hepatocyte suspensions have used fasted rather than fed rats [35–37]. Hepatocytes from fasted rats have low levels of glycogen and show net glycogen deposition when incubated with high concentrations of gluconeogenic precursors, whereas cells from fed rats have a higher initial glycogen content and show either no net glycogen deposition, or net breakdown when incubated with physiological concentrations of substrates or with

Fig. 3. Effects of glucose concentration on glycogen deposition and lactate formation in periportal and perivenous hepatocytes

Periportal (P) and perivenous (V) hepatocyte cultures were isolated from fasted rats that had been re-fed for either 30 min (a and c) or 180 min (b and d) before hepatocyte isolation. They were incubated for 180 min with the concentrations of glucose indicated in either the absence of insulin (▨) or in the presence of 100 nM-insulin (■). (a) and (b) show lactate formation expressed as nmol/3 h per mg of protein, and (c) and (d) show glycogen deposition expressed as nmol of glucose units deposited/3 h per mg of protein. Values are means $\pm$ S.E.M. for each of six hepatocyte preparations.

glucose alone [36–43]. When hepatocytes are maintained in culture, they recover from their catabolic state of glycogen breakdown and synthesize glycogen from physiological concentrations of substrates [18,19]. Studies on glycogen synthesis in hepatocyte cultures have used either fed or fasted rats. Few studies, however, have compared rates of glycogen synthesis in hepatocyte cultures isolated from rats in different nutritional states [18]. In the present study, we observed three main differences in glycogen metabolism in hepatocyte cultures derived from different nutritional states. First, when challenged with the same concentrations of substrates, hepatocytes from fasted rats that were re-fed for 180 min had higher rates of glycogen synthesis and accumulated glycogen to a higher concentration than hepatocytes from either fasted rats or rats fed *ad libitum*. Second, when hepatocyte cultures were isolated from fasted rats that were re-fed for either 30 or 180 min and then incubated with either high concentrations of glucose or with dihydroxyacetone or glycerol, there were larger differences between the two stages of re-feeding in rates of glycogen synthesis from glucose than from the gluconeogenic precursors. Third, during incubation with a range of glucose concentrations, differences in rates of

glycogen deposition between nutritional states were not associated with parallel differences in rates of lactate formation, suggesting differences in the partitioning of flux between formation of lactate and glycogen in different nutritional states.

The finding that hepatocytes from fasted rats that were re-fed for 180 min had higher rates of glycogen synthesis from glucose but not from dihydroxyacetone compared with hepatocytes from fasted rats re-fed for 30 min suggests that in the fasted-to-re-fed transition, glycogen synthesis from glucose may increase more markedly than from gluconeogenic precursors. The lower rates of glucose metabolism in hepatocytes from fasted rats re-fed for 30 min may be due to a lower concentration and/or activity of glucokinase in the early compared with later stages of re-feeding. Since most studies on glycogen deposition in hepatocyte suspensions have used fasted rats [35–41], the low rates of glycogen synthesis from glucose in these studies may be due to the fasted state of the rat. In experiments on hepatocytes from fasted rats re-fed for 180 min we found higher rates of glucose conversion to glycogen in the presence of fructose, indicating that even in these cells, rates of glucose metabolism are submaximal. The effect of fructose may

in part be due to activation of glycogen synthase [44,45], but is probably also due to activation of glucokinase [34].

Insulin action on glycogen metabolism in hepatocyte cultures

The effect of insulin on glycogen synthesis in isolated liver preparations has been the subject of extensive debate [3,5,6,46]. Stimulation of glycogen synthesis by insulin in freshly isolated hepatocyte suspensions has been observed in some studies [39,40,47] but not others [41, 48], and on the basis of the latter it has been proposed that, *in vivo*, insulin may exert its acute effects on hepatic glycogen not directly but by suppression of secretion of glucagon and counteracting its catabolic action [3]. Effects of insulin on hepatocyte cultures are more widely documented [17–20,49]. These studies have examined mainly the effect of insulin on the incorporation of ^{14}C -labelled precursors into glycogen or on the cellular glycogen content after fixed time intervals. There is little information, however, on the effects of insulin on the time course of glycogen deposition in hepatocyte cultures [19]. The present study demonstrates that when hepatocytes are challenged with substrates in the absence of insulin, glycogen is deposited at a linear rate for about 2 h and then reaches a plateau, whereas in the presence of insulin, glycogen deposition continues for more than 4 h. Although insulin increases the initial rate of glycogen deposition (by 20–40%), it has a more marked effect on the total glycogen that accumulates in hepatocytes after 4 h. In a previous study using hepatocyte cultures, it was shown that the incorporation of [^{14}C]glucose into glycogen was linear during a 6 h time course in both the absence and the presence of insulin [49]. In experiments where we compared the time course of glycogen deposition (determined enzymically) and the incorporation of [^{14}C]glucose or ^{14}C -labelled gluconeogenic precursors into glycogen during 8 h, we found that in situations where a plateau of glycogen deposition is reached after 2 h, the incorporation of both [^{14}C]glucose and ^{14}C -labelled gluconeogenic substrates is linear (M. Peak & L. Agius, unpublished work). This suggests (1) that the ratio of incorporation of [^{14}C]glucose/ ^{14}C -gluconeogenic precursors is independent of time, and (2) that when glycogen deposition reaches a plateau, glycogen synthesis continues and is presumably balanced by an equal rate of degradation.

In a previous study in which the time course of glycogen deposition was examined at different glucose concentrations in glycogen-depleted hepatocyte cultures, a similar time course to that of the present study was obtained at low glucose concentrations (< 10 mM) [19], but at higher glucose concentrations (> 20 mM), the plateau in the control cultures was reached after a longer time interval. The shorter time for glycogen deposition to reach a plateau (in the absence of insulin) in the present study compared with the study by Fleig and co-workers [19] is probably due to two factors. First, in the present study the hepatocytes were not depleted of glycogen before the start of the time course, and the glycogen content at the start of the incubation with substrate was about 30% of the final concentration when the plateau was reached. Secondly, in our time-course studies we used a combination of glucose and gluconeogenic precursors, which results in a higher rate of glycogen deposition compared with glucose alone.

This increased rate may contribute to the shorter time interval for the cellular glycogen level to be attained. A further difference between the present study and that of Fleig and co-workers [19] is the use of dexamethasone during preculture in the present study, which would be expected to preserve the activity of glycogen synthase [17]. In preliminary studies in which we compared the time course of glycogen deposition with glucose (15 or 35 mM) without or with gluconeogenic precursors, we found that in the presence of glucose alone cellular glycogen reached a plateau after a longer time interval (> 4 h) than in the presence of gluconeogenic precursors (2 h). The present findings and those of Fleig and coworkers [19] suggest that the maximum level of glycogen that hepatocytes accumulate is a function of (1) the nutritional state of the donor rat (present study), (2) the glucose concentration [19], (3) the insulin concentration ([19] and present study), and (4) probably also the acinar origin of the hepatocytes. Since the effects of insulin on glycogen deposition varied with time and substrate, a comparative study of insulin action in periportal and perivenous hepatocytes necessitates a study of the time course. The results of time-course studies of insulin action on glycogen accumulation in periportal and perivenous hepatocytes from rats in different nutritional states suggest that the action of insulin may be either delayed (hepatocytes from fasted rats re-fed for 180 min) or sustained for longer (fasted rats re-fed for 30 min) in perivenous as compared with periportal hepatocytes. These differences may be due in part to differences in glycogen degradation between periportal and perivenous hepatocytes (D. Tosh & L. Agius, unpublished work).

The stimulation of glycogen synthesis by insulin was more marked in the presence of lactate and pyruvate than in the presence of glucose alone, and it was also more marked with glycerol than with dihydroxyacetone. Since the limiting step in the metabolism of glycerol is probably the transfer of reducing equivalents from the cytosol to the mitochondria [33], it may be inferred that insulin favours the metabolism of reduced substrates (glycerol and lactate) through an effect on cytosolic redox state.

Zonation of glycogen synthesis within the liver acinus

It has been proposed on the basis of the acinar zonation of gluconeogenic enzymes and glucokinase that hepatocytes in the periportal zone synthesize glycogen from gluconeogenic precursors, whereas hepatocytes in the perivenous zone synthesize glycogen from glucose by a direct pathway [7]. Histochemical studies have shown that there are differences in patterns of glycogen distribution in the liver acinus [8–12,50,51]. However, these studies provide only qualitative information of differences in glycogen metabolism in the periportal and perivenous zones. Chen & Katz [15] showed higher rates of glycogen deposition in periportal compared with perivenous hepatocyte suspensions from fasted rats during incubation with gluconeogenic substrates and glucose. Since freshly isolated hepatocyte suspensions have a limited capacity to utilize glucose for glycogen synthesis [5,6], the higher rates of glycogen deposition in the periportal hepatocytes probably reflects the higher gluconeogenic capacity of these cells [13–16]. In the present study, using hepatocyte cultures isolated either from rats fed *ad libitum* or from fasted rats that were re-

fed, we found similar rates of glycogen deposition in periportal and perivenous hepatocytes during incubation with glucose and gluconeogenic precursors, but higher rates of glycogen deposition in perivenous hepatocytes during incubation with glucose and insulin. The combination of high concentrations of glucose (15–25 mM) and gluconeogenic precursors had a synergistic effect on glycogen deposition in both periportal and perivenous hepatocytes, but the effect was more marked in periportal cells. The synergism between glucose and gluconeogenic precursors has been reported previously in hepatocytes isolated from whole liver [36,42], and is in part due to activation of glycogen synthase and inactivation of glycogen phosphorylase by glucose, thereby increasing the incorporation of gluconeogenic precursors into glycogen (Tables 3 and 4) and suppression of glycolysis by gluconeogenic precursors, and so increasing the partitioning of glucose towards glycogen formation. Despite similar rates of glycogen deposition in periportal and perivenous cells incubated with glucose and gluconeogenic precursors, the incorporation of [^{14}C]lactate into glycogen was between two and four times higher in periportal than in perivenous hepatocytes irrespective of the glucose concentration, the presence of insulin or the nutritional state of the rat. This finding supports the hypothesis that incorporation of gluconeogenic substrates into glycogen predominates in the periportal zone.

The incorporation of [^{14}C]glucose into glycogen presented a reciprocal distribution to that of the incorporation of ^{14}C -labelled gluconeogenic precursors only in a limited number of situations; namely, in the presence but not in the absence of insulin, and in some nutritional states but not others. This contrasts with the predominance of the incorporation of gluconeogenic precursors into glycogen, which was independent of the presence of insulin or the nutritional state of the rat, and suggests that the zonation of glycogen synthesis from glucose may change in different nutritional states. However, the studies with fructose suggest that data on glucose metabolism should be interpreted cautiously; first, because fructose increased glucose incorporation into glycogen suggesting that glucose metabolism is submaximal even in hepatocytes isolated after 3 h-refeeding, and secondly, because the stimulation by fructose was greater in perivenous than in periportal hepatocytes, suggesting that differences in glucose metabolism between periportal and perivenous hepatocytes (determined in the absence of fructose) may not reflect the maximal capacity of the two cell populations.

It has been suggested that after ingestion of glucose, the perivenous hepatocytes metabolize glucose into glycogen and lactate, and the latter, after recirculation, is converted to glycogen in periportal hepatocytes [7]. However, Chen & Katz found no evidence for heterogeneity of lactate formation [15]. In the present study, although there was a trend of increased lactate production by perivenous as compared with periportal hepatocytes (of fasted rats re-fed for 30 min) during incubation with increasing concentrations of glucose, this difference was small (perivenous/periportal ratio of 1.22) compared with differences in gluconeogenesis [13–16] and incorporation of gluconeogenic precursors into glycogen (2–4-fold) between the two cell populations, and supports the findings of Chen & Katz of lack of heterogeneity of glycolysis [15]. However, if glucokinase

is present in an inactive state in the absence of fructose [34], rates of glucose metabolism may not reflect the maximum capacity. The zonation of glucose metabolism in the acinus may be dependent on the degree of activation of glucokinase and may be maximal in situations associated with full activation of the enzyme.

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