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Enhancement of glycogen concentrations in primary cultures of rat hepatocytes exposed to glucose and fructose

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Glycogen synthesis in isolated hepatocytes can occur from glucose both by a direct mechanism and by an indirect process in which glucose is first metabolized to C_3 intermediates before use for glycogenesis via gluconeogenesis. We studied the incorporation into glycogen of glucose and the gluconeogenic substrate, fructose, in primary cultures of hepatocytes from fasted rats. In the presence of insulin, both glucose and fructose promoted net deposition of glycogen; however, fructose carbon was incorporated into glycogen to a greater extent than that from glucose. When glucose and fructose were administered simultaneously, the glycogenic utilization of glucose was stimulated 2–3-fold, and that of fructose was increased by about 50%. At constant hexose concentrations, the total incorporation of carbon, and the total accumulation of glycogen mass, from glucose and fructose when present together exceeded that from either substrate alone. Fructose did not change the relative proportion of glucose carbon incorporated into glycogen via the indirect (gluconeogenic) mechanism. The synergism of glucose and fructose in glycogen synthesis in isolated rat hepatocytes in primary culture appears to result from a decrease in the rate of degradation of newly deposited glycogen, owing to (i) decreased amount of phosphorylase *a* mediated by glucose and (ii) non-covalent inhibition of residual phosphorylase activity by some intermediate arising from the metabolism of fructose, presumably fructose 1-phosphate.

INTRODUCTION

The efficiency of hepatic glycogen synthesis has been considered to be a major determinant of glucose tolerance; a long-standing concept holds that, after ingestion of a carbohydrate or mixed meal, a large fraction of the glucose is taken up by the liver and converted into glycogen by a direct process which involves the sequence glucose → glucose 1-phosphate → glucose 6-phosphate → UDP-glucose → glycogen. This concept is not completely certain, however, since (i) estimates of the hepatic uptake of glucose after a glucose load are controversial, ranging from 25% [1,2] to more than 60% [3,4] of the administered sugar, (ii) gluconeogenic substrates appear to play an important role in glycogen deposition [5,6], and (iii) recent proposals state that the major carbon source for hepatic glycogen synthesis during the immediate postprandial period is not glucose but rather C_3 intermediates (presumably lactate) formed during an initial extrahepatic catabolism of the ingested glucose [7–9].

Isolated hepatocytes in short-term primary culture have been considered to reflect accurately the intrinsic metabolic characteristics of the liver at the cellular level. We [10] and others [11] have demonstrated that isolated rat hepatocytes maintained in primary culture are able to synthesize glycogen from glucose by both a direct pathway and an indirect mechanism that uses gluconeogenic precursors. This latter route accounts for 60–70% of the flux of carbon into glycogen during the early fasted-to-refed transition *in vivo* [7,8]. The relative contributions of the two pathways to overall glycogen synthesis vary in hepatocytes in primary culture, and depend on conditions such as the nutritional status of the animal from which the cells are isolated. The direct route is emphasized in cells isolated from fed rats [10], whereas the indirect mechanism appears to predominate in hepatocytes from fasted animals [11]. Nonetheless, these data indicate that glycogen carbon which traverses the indirect pathway can be derived from glucose which has undergone catabolism to at least the triose phosphate level within the hepatocyte itself [10,11]; therefore, extrahepatic catabolism of glucose is not a prerequisite for glycogen synthesis. We have also noted that exogenous lactate is a poor precursor for glycogen synthesis in cultured hepatocytes [10]. Glucose uptake by the hepatocyte may thus provide a major carbon source for hepatic glycogen synthesis. However, little is known concerning the possible interactions and co-ordinate regulation of the two pathways for hepatocyte glycogen synthesis. Elevated rates of glycogen synthesis in isolated hepatocytes [12] and in perfused liver [13] have been observed when glucose and fructose are administered simultaneously. We have undertaken a series of studies with substrates presumed to traverse one glycogenic pathway predominantly in order to investigate the contributions of the dual glycogenic routes to overall glycogen synthesis. We report here studies of the incorporation into glycogen of the gluconeogenic substrate fructose, and its substantial stimulatory effect on the concomitant glycogenic utilization of glucose, in rat hepatocytes maintained in primary culture.

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MATERIALS AND METHODS

Materials

Fetal-calf serum and Leibovitz L-15 powdered medium were from GIBCO. L-15 medium with galactose omitted was prepared from the individual components as listed [14]. Collagenase (300–400 units/mg) was obtained from either Sigma or Boehringer–Mannheim. Hepes, bovine serum albumin and fructose 1-phosphate (sodium salt) were from the latter supplier. [U-^{14}C]glucose and [U-^{14}C]fructose were from ICN, [3-^{3}H]glucose was obtained from Amersham, and 2-deoxy[G-^{3}H]glucose was a product of New England Nuclear. All other reagents not specifically listed were obtained from local suppliers and were of analytical grade or better.

Methods

Hepatocytes were prepared from 200–250 g Sprague–Dawley rats which had been allowed free access to water but had been deprived of food for 24 h before use. The animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and hepatocytes were prepared by non-recirculating perfusion via the portal vein. Cell preparations were always obtained between 10:00 and 11:00 h. Sterile technique was maintained throughout all manipulations. The livers were first perfused with 60 ml of Ca^{2+}-free Hanks basal salt solution and then with 120 ml of L-15 medium containing 25 mM-Hepes (pH 7.4), 10 mM-glucose and 0.5% collagenase, over a period of about 30 min. The livers were excised, gently combed into L-15 medium containing 0.5% collagenase and then filtered through nylon mesh (150 μm). The isolated cells were washed three times at 4 °C by centrifugation for 10 min at 50 *g* and subsequent resuspension in L-15 medium without collagenase. The final cell pellet was suspended in L-15 medium containing 10% fetal-calf serum. The viability of the cells was over 90%, as judged by the exclusion of Trypan Blue. The cells were plated in 35 mm-diam. collagen-coated plastic tissue-culture dishes at a density of 1.5×10^6 cells/dish, in L-15 medium containing 25 mM-Hepes (pH 7.4) and 10% fetal-calf serum. After 4 h at 37 °C under a humidified-air atmosphere to allow cell attachment, the medium was changed to a serum- and glucose-free medium consisting of L-15 medium (modified to omit galactose) containing 25 mM-Hepes (pH 7.4), 1% bovine serum albumin and 0.1 μM-insulin. After an additional 20 h culture at 37 °C under a humidified-air atmosphere, the medium was removed, and the cells washed with Dulbecco's phosphate-buffered saline (GIBCO), and used for experimental purposes.

Experimental incubations were performed at 37 °C in Krebs–Ringer bicarbonate (pH 7.4) containing 1% bovine serum albumin with or without 0.1 μM-insulin and other additions as indicated. The incubation medium was thoroughly gassed with O_2/CO_2 (19:1) before addition to the washed cells. Experimental incubations were conducted in a humidified atmosphere of air/CO_2 (19:1). After suitable periods of incubation, the medium was aspirated and the cells were washed several times with ice-cold phosphate-buffered saline and then dissolved in 1 M-NaOH. Samples of these cell solutions were analysed for protein by the method of Lowry *et al.* [15]. Another portion of the cell solution, after the addition of oyster glycogen as carrier, was mixed with 2 vol. of ethanol and left for several hours at 4 °C. The glycogen pellet obtained after centrifugation was dissolved in water, and the glycogen was again precipitated with ethanol. After centrifugation, this pellet was redissolved in water, and samples of this aqueous solution were measured for radioactivity. A third portion of the alkaline cell solution was used for the determination of glycogen mass: the solution was heated at 100 °C for 30 min to destroy free glucose, cooled to room temperature, and then acidified with HCl (final concn. 1 M). This acidified solution was heated at 100 °C for 60 min to hydrolyse the glycogen. After neutralization, samples of this solution were used for the determination of glucose by a fluorimetric procedure using hexokinase/glucose-6-phosphate dehydrogenase [16].

Glucose 6-phosphate was measured in neutralized $HClO_4$ extracts of cells by a similar procedure, with the omission of hexokinase [16]. Glycogen synthase was measured as described by Thomas *et al.* [17], and phosphorylase *a* was measured in the direction of glycogen degradation as described by Lowry *et al.* [18], or in the direction of glycogen synthesis as described by Gilboe *et al.* [19]. Statistical analyses made use of analysis of variance (with least significant difference) or Student's *t* test, as appropriate. Curve fitting was performed with Asystant software programs (MacMillan Software Co.).

RESULTS

Hepatocytes isolated from starved rats and maintained in primary culture with medium lacking both glucose and serum responded to the administration of glucose, in the presence of insulin, with net deposition of glycogen (Table 1). This accumulation of glycogen occurred at low concentrations of glucose (5–10 mM), and in the absence of exogenous gluconeogenic precursors. In the experiments reported in Table 1, the ratio of nmol of [^{14}C]-glucose to nmol of glucosyl units in newly deposited glycogen was about 1, indicating that glucose was the sole source of glycogenic carbon in these cells. Similar data were obtained when [^{14}C]fructose was used as glycogenic precursor. These data are not surprising, given that glucose or fructose was the sole exogenous carbon source in these incubations. It might therefore be concluded that the contributions to glycogen synthesis of the intracellular gluconeogenic substrate pools is negligible under our incubation conditions. The concomitant administration of unlabelled fructose with [^{14}C]glucose resulted in a substantial increase in both the incorporation of radioactivity into glycogen and in the accumulation of glycogen mass as compared with that noted from glucose alone. The ratio of [^{14}C]glycogen to net glycogen accumulation was 0.4–0.5, indicating that approximately half the glycogen was derived from glucose.

The addition of 5 mM-fructose to incubation media containing different concentrations of [U-^{14}C]glucose consistently resulted in 2–3-fold increases in the incorporation of radioactivity from glucose into glycogen compared with that seen with glucose alone (Fig. 1). This stimulatory effect of fructose was noted both in the absence and in the presence of insulin, and was maximal at 5 mM-fructose; the addition of fructose at concentrations above 5 mM failed to enhance further the use of [^{14}C]glucose for glycogen synthesis (Fig. 1). The

Table 1. Glycogenic utilization of [^{14}C]glucose and [^{14}C]fructose in primary cultures of hepatocytes

Incubations were performed in Krebs–Ringer bicarbonate/1 %albumin/0.1 μM-insulin with additions as listed for 3 h at 37 °C. Each value is the mean ± S.E.M. for five or six dishes of cells. Net glycogen deposition was calculated by subtraction of the mean of the initial glycogen content (47.5 ± 1.9 nmol of glucosyl units/mg of cell protein; $n = 9$; determined from identical dishes of cells not exposed to incubation with sugars) from the final content of glycogen in each individual dish of cells after incubation with the labelled sugars. * Significantly different ($P < 0.005$) from the initial glycogen content determined in identical dishes of cells not exposed to the incubation conditions (47.5 ± 1.9 nmol of glucosyl units/mg of cell protein). † Not significantly different from 1 ($P > 0.05$). ‡ Significantly different from 1 ($P < 0.005$).

Incubation	^{14}C substrate converted into glycogen (nmol/mg of cell protein)	Total glycogen deposited (nmol of glucosyl units)	Ratio ^{14}C incorporation / net glucosyl units deposited
Expt. 1			
5 mM-[^{14}C]Glucose	18.2 ± 0.9	66.6 ± 2.0*	1.0 ± 0.10†
10 mM-[^{14}C]Glucose	53.8 ± 3.2	94.6 ± 14.0*	1.13 ± 0.18†
5 mM-[^{14}C]Glucose + 5 mM-fructose	42.5 ± 2.6	155.4 ± 18.2*	0.44 ± 0.08‡
Expt. 2			
5 mM-[^{14}C]Fructose	42.1 ± 2.4	96.2 ± 4.5*	0.90 ± 0.12†
10 mM-[^{14}C]Fructose	137.6 ± 7.4	178.9 ± 14.3*	1.13 ± 0.20†
5 mM-[^{14}C]Fructose + 5 mM-glucose	64.8 ± 2.9	235.0 ± 13.5*	0.36 ± 0.04‡

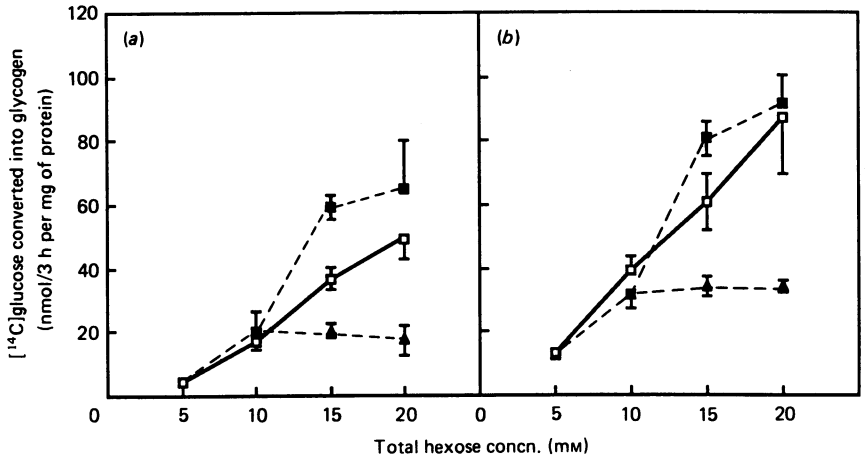

Fig. 1. Effect of fructose on the glycogenic utilization of [U-^{14}C]glucose in primary cultures of hepatocytes from fasted rats

□, With different concentrations of [U-^{14}C]glucose alone; ■, with 5 mM-fructose plus different concentrations of [U-^{14}C]glucose; ▲, with 5 mM-[U-^{14}C] glucose plus different concentrations of fructose. (*a*) Incubations in the absence of insulin. (*b*) Incubations in the presence of 0.1 μM-insulin. Each point is the mean ± S.D. for five dishes of cells.

addition of 5 mM-glucose to incubation media containing [U-^{14}C]fructose resulted in a stimulation of approx. 50 % of the incorporation of radioactivity from fructose into glycogen. This stimulation was noted only when fructose was present at 5 mM (Fig. 2); at concentrations of fructose greater than 5 mM, glucose was unable to enhance the glycogenic utilization of fructose. At constant hexose concentrations, the total incorporation of ^{14}C into glycogen, as well as the total accumulation of glycogen mass, from fructose and glucose when present together generally exceeded that observed when either substrate was present alone.

^{3}H from [3-^{3}H]glucose is lost during catabolism of glucose to the triose level [20]; thus the ratio of ^{3}H/^{14}C in glycogen relative to that of [3-^{3}H],[U-^{14}C]glucose initially present in the incubation medium can be used to estimate the contributions of the direct and the indirect glycogenic pathways to overall glycogen synthesis [7–11]. On exposure to [3-^{3}H], [U-^{14}C]glucose, the ^{3}H/^{14}C relative ratio in glycogen formed in cells which had been isolated from starved rats and cultured in the absence of glucose was 0.3–0.4 (Table 2). Since under our experimental conditions the carbon in the newly synthesized glycogen is derived almost entirely from exogenously administered glucose (Table 1), this indicates a significant degree of catabolism of glucose to at least the triose level before the incorporation of the glucose carbon into glycogen. The addition of fructose to the incubation medium resulted in an enhancement of the incorporation of glucose carbon into glycogen; however, fructose had no apparent effect

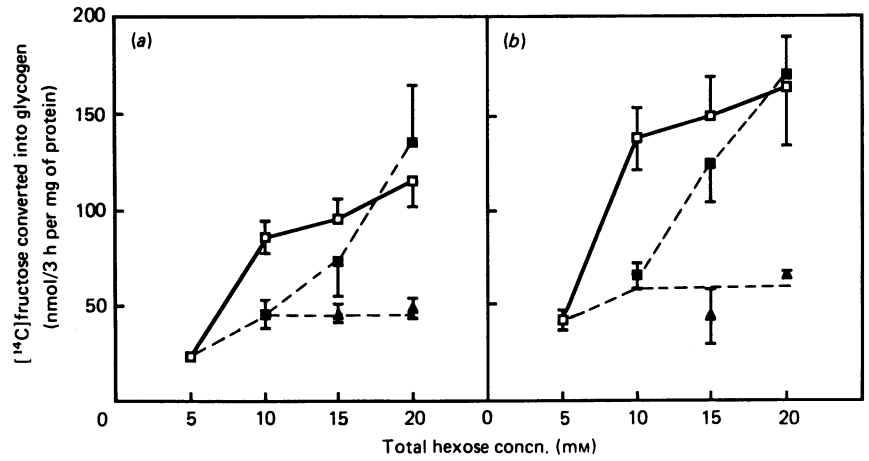

Fig. 2. Effect of glucose on the glycogenic utilization of [U-^{14}C]fructose in primary cultures of rat hepatocytes from fasted rats

□, With different concentrations of [U-^{14}C]fructose alone; ■, with 5 mM-glucose plus different concentrations of [U-^{14}C]fructose; ▲, with 5 mM-[U-^{14}C]fructose plus different concentrations of glucose. (*a*) Incubations in the absence of insulin. (*b*) Incubations in the presence of 0.1 μM-insulin. Each point is the mean ± S.D. for five dishes of cells.

Table 2. Effect of fructose on the incorporation of [3-^{3}H],[U-^{14}C]glucose into glycogen in primary cultures of hepatocytes from starved rats

Incubations were performed in Krebs-Ringer bicarbonate/1 % albumin with additions as indicated for 3 h at 37 °C. Each value is the mean ± S.E.M. for five or six dishes of cells. † Significantly different ($P < 0.005$) from the amount of glycogen (12.9 ± 0.8 μg/mg of cell protein) in cells before incubation.

Incubation	[^{14}C]Glucose in glycogen (nmol/mg of cell protein)	Total glycogen (μg/mg of cell protein)	^{3}H/^{14}C relative ratio in glycogen*
No insulin			
5 mM-Glucose	4.9 ± 0.2	13.5 ± 1.8	0.365 ± 0.005
10 mM-Glucose	17.4 ± 1.2	28.5 ± 1.5†	0.367 ± 0.006
5 mM-Glucose + 5 mM-fructose	19.8 ± 0.6	24.8 ± 0.3†	0.379 ± 0.007
+ 0.1 μM-insulin			
5 mM-Glucose	24.1 ± 0.6	18.0 ± 0.9†	0.346 ± 0.008
10 mM-Glucose	69.0 ± 3.1	25.9 ± 0.7†	0.352 ± 0.004
5 mM-Glucose + 5 mM-fructose	45.8 ± 1.6	27.2 ± 2.3†	0.364 ± 0.002

* Ratio of ^{3}H/^{14}C in glycogen relative to that of the glucose initially in the incubation medium.

on the ^{3}H/^{14}C relative ratio in the newly synthesized glycogen (Table 2). It therefore appears that fructose is able to enhance the use of glucose for glycogen synthesis not only by the indirect pathway (that path that fructose itself must traverse for glycogenesis), but also by the direct mechanism. This is further evident in the substantial enhancement by fructose of the incorporation of 2-deoxyglucose into glycogen (Table 3). The direct pathway is the only route by which 2-deoxyglucose can be incorporated into glycogen [10].

Studies on the mechanism of fructose enhancement of the use of glucose for glycogen synthesis

Incubation of the rat hepatocytes maintained in primary culture with 10 mM-glucose and 5 mM-fructose did not affect, as compared with similar incubations with 15 mM-glucose or 15 mM-fructose alone, either the amount of 'active' glycogen synthase (enzyme activity in absence of glucose 6-phosphate) or intrahepatocyte concentrations of glucose 6-phosphate (Table 4). Although it has been suggested that activation of glycogen synthase may play a role in increased glycogen deposition in response to fructose [21], others consider this not to be the case [13]. Our data tend to support the latter view. In our cells, the activity of phosphorylase *a* was decreased; this decrease was greater in incubations containing glucose than in those with fructose alone. Addition of fructose 1-phosphate (final concn. 10 mM) in assays of glycogen synthase in cell extracts had no effect on the activity of the enzyme either in the absence or in the presence of the effector glucose 6-phosphate. However, the ability of phosphorylase *a* in these extracts to degrade glycogen was dramatically inhibited by fructose 1-phosphate (Fig. 3). This phosphorylated intermediate has been shown to be a competitive inhibitor of

Table 3. Effect of fructose on the incorporation of 2-deoxyglucose into glycogen in primary cultures of hepatocytes from fasted rats

Data are means ± S.E.M. for six dishes of cells. All values are normalized with respect to mg of cell protein. Incubations in this experiment were carried out in Krebs–Ringer bicarbonate/1% bovine serum albumin/0.1 μM-insulin for 3 h. Cells were incubated with the indicated concentrations of 2-deoxy[G-^{3}H]glucose in the absence or presence of 5 mM-[U-^{14}C]fructose. Amounts of the respective sugars in glycogen were calculated from the extent of ^{3}H and ^{14}C labelling of the glycogen, and the specific radioactivities of the administered sugars. * Significantly different ($P < 0.001$) from control in the absence of 2-deoxyglucose.

Concn. of 2-deoxyglucose in incubation (mM)	2-Deoxyglucose in glycogen (nmol)		Fructose in glycogen (nmol)
	−fructose	+fructose (5 mM)	
0	–	–	57.9 ± 2.4
5	1.5 ± 0.1	7.6 ± 0.9	85.6 ± 3.7*
10	2.5 ± 0.2	18.6 ± 1.7	90.6 ± 4.4*
15	4.0 ± 0.2	18.7 ± 2.2	77.1 ± 3.0*

Table 4. Effect of incubation of cultured hepatocytes with glucose and fructose on various glycogenic parameters

Incubations were performed for 2 h in Krebs–Ringer bicarbonate containing 1% bovine serum albumin and the additions as listed below. Cells were harvested by scraping into incubation buffer [20 mM-Tris (pH 7.9, 4 °C) containing 50 mM-KF, 2 mM-EDTA and 2 mM-dithiothreitol] and frozen on solid CO_2. After thawing, the cells were homogenized with a Polytron (setting 8) for 2 min, then centrifuged at 12000 *g* for 15 min. Portions of the supernatant were used for the assays below as described in the Materials and methods section. '%I' refers to the enzyme activity in the absence of glucose 6-phosphate.

Incubation	Glycogen synthase (%I)	Phosphorylase *a* (% of initial)	Glucose 6-phosphate (nmol/mg of cell protein)
None	38	100	0.27 ± 0.05
15 mM-Glucose	38 ± 3	61 ± 3	0.27 ± 0.02
15 mM-Fructose	40 ± 3	81 ± 8	0.31 ± 0.03
10 mM-Glucose + 5 mM-fructose	38 ± 2	60 ± 4	0.27 ± 0.02

phosphorylase with respect to P_i [22,23]. In the present work, we have confirmed fructose 1-phosphate to be a potent inhibitor of phosphorylase *a*, with a K_i of 1.1 ± 0.1 mM at physiological concentrations of P_i (4–5 mM).

When the glycogen in cultured hepatocytes was labelled by preincubation with 10 mM-[U-^{14}C]glucose in the absence or the presence of 5 mM unlabelled fructose, and then the incubation was continued in the presence of identical concentrations of unlabelled substrate, the loss of radioactivity in glycogen in cells preincubated with glucose alone was much greater than that noted in cells incubated with glucose in the presence of fructose (Fig. 4*a*). Parallel incubations indicated that the loss of radioactivity in prelabelled glycogen (in the absence of fructose) occurred concomitantly with the continued incorporation of carbon from glucose into glycogen (Fig. 4*b*).

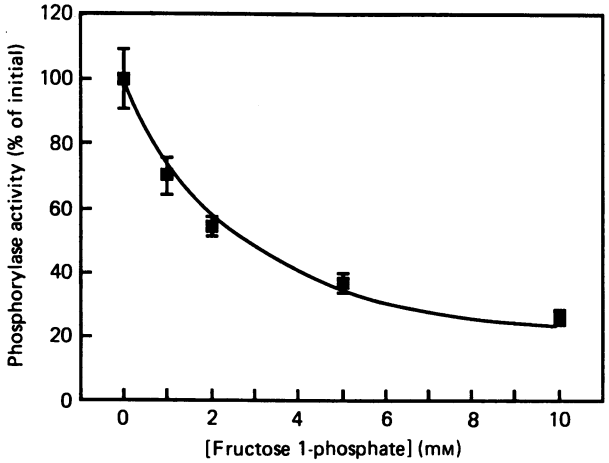

Fig. 3. Effect of fructose 1-phosphate on the activity of phosphorylase *a* in crude extracts prepared from isolated hepatocytes

Hepatocytes in primary culture were washed and scraped into 0.25 M-sucrose / 0.1 M-KF / 5 mM-EDTA, pH 7.0, rapidly frozen on solid CO_2, and thawed. After centrifugation at 12000 *g* for 15 min, the supernatants were used for assay. Phosphorylase *a* was measured in the direction of glycogen breakdown [18] in the presence of 4 mM-P_i. The activity of phosphorylase *a* was 30.9 ± 2.7 nmol/min per mg in the absence of fructose 1-phosphate. The continuous line is calculated for inhibition competitive with respect to P_i, with a K_i of 1.1 mM. Results are means ± S.D. ($n = 3$).

DISCUSSION

Glucose alone has been considered to be a rather poor precursor for glycogen synthesis in perfused liver [24] or in isolated hepatocytes [5,6,25]. In contrast, substantial glycogen deposition is noted in these systems when glucose and gluconeogenic substrates such as fructose are administered simultaneously. Indeed, it has been proposed that gluconeogenic precursors, and not glucose, serve as the major carbon source for glycogen repletion in fasted animals on re-feeding [7–9]. It has therefore become apparent that hepatic glycogen synthesis can proceed by two pathways, a direct mechanism which does not require the initial catabolism of glucose and an indirect pathway which uses gluconeogenic precursors. Since it has been found that isolated hepatocytes use glucose as a substrate for both of the glycogenic pathways [10,11], we decided to study the effect of a substrate which is able to traverse only one of the glycogenic paths on the use of glucose for glycogen synthesis in hepatocytes in primary culture.

Fructose is an excellent substrate for hepatic carbohydrate metabolism: it is a better gluconeogenic precursor than lactate [25] and is considered to be a more efficient carbon source for glycogen synthesis than

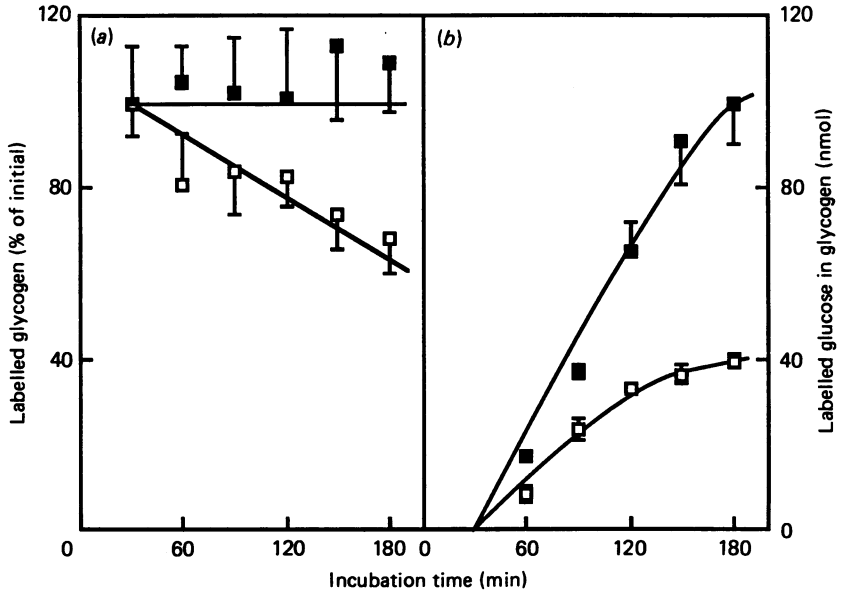

Fig. 4. Turnover of glycogen in primary cultures of rat hepatocytes incubated with 10 mM-glucose in the absence (□) or the presence (■) of 5 mM-fructose

(*a*) Cell glycogen was labelled with 10 mM-[U^{14}C]glucose in the absence or the presence of 5 mM unlabelled fructose for 30 min. Incubations were then continued in the presence of identical concentrations of unlabelled substrates. (*b*) Cells were incubated with 10 mM unlabelled glucose in the absence or the presence of 5 mM unlabelled fructose for 30 min. Incubations were then continued with 10 mM-[U-^{14}C]glucose in the absence or the presence of 5 mM unlabelled fructose. Each point is the mean ± S.D. for five dishes of cells. All values were normalized for mg of cell protein.

glucose [6]. Since fructose enters the glycolytic/gluconeogenic pathways at the level of triose phosphate [26], glycogen synthesis from this ketohexose must arise entirely via the indirect mechanism. With rat hepatocytes in primary culture, we found that fructose was a more effective glycogenic substrate than glucose at low concentrations of the hexoses (< 10 mM). When glucose (5 mM) and fructose (5 mM) were administered simultaneously to cultured hepatocytes, glycogen deposition was 3–5 times that with equivalent concentrations of either substrate alone. Under these conditions the glycogen was formed from approximately equal amounts of carbon from fructose and from glucose (Table 1); this result implies (i) a synergism in glycogen synthesis in the presence of the two substrates and (ii) that both glucose and fructose are equally good sources of glycogenic carbon during active glycogen deposition. Although fructose is incorporated into glycogen only by the indirect pathway, glucose can traverse either or both the indirect and the direct glycogenic pathways in cultured hepatocytes [10,11]. The concomitant presence of fructose enhances equally well the use of glucose for glycogen synthesis by both pathways, as indicated by the unchanged $^3H/^{14}C$ relative ratios in glycogen deposited in cells exposed to [3-^{3}H],[U-^{14}C]glucose in the absence or in the presence of fructose.

The ability of fructose to enhance glycogen synthesis via the direct route is particularly evident from our experiments with 2-deoxyglucose (Table 4). This glucose analogue can be taken up by a number of tissues and phosphorylated to 2-deoxyglucose-6-phosphate. This latter compound is unable to serve as a substrate for either glucose-6-phosphate dehydrogenase or phosphoglucose isomerase, and therefore cannot be further metabolized by the pentose phosphate route or by the glycolytic pathway. However, 2-deoxyglucose can be incorporated into hepatic glycogen [10,27]. Since it cannot be metabolized to C_3 intermediates via glycolysis, any glycogenic use of 2-deoxyglucose must be by the direct mechanism. The presence of fructose resulted in 5-fold increase in the incorporation of 2-deoxyglucose into glycogen compared with that seen with 2-deoxyglucose alone. It is also of particular interest that 2-deoxyglucose promoted a consistent 50% increase in the incorporation of fructose carbon into glycogen.

Enhanced glycogen synthesis in the presence of glucose and gluconeogenic substrates as compared with that in the presence of glucose alone has been described for perfused liver [6,13,24], isolated hepatocytes [5,6,25] and in primary cultures of hepatocytes (the present work). However, in previous studies the source of glycogenic carbon was not determined. We have demonstrated a synergism of glucose and the gluconeogenic substrate fructose in glycogen synthesis; the presence of fructose greatly stimulates the incorporation of carbon from glucose into glycogen, and the presence of glucose enhances the glycogenic utilization of fructose carbon, as compared with that seen in the presence of either glucose or fructose alone. Wood *et al.* [12] estimated that about 30% of the glucosyl units used for glycogen synthesis could arise from fructose in isolated hepatocytes exposed to 30 mM-glucose/2 mM-fructose. Although we have employed rather different experimental conditions, we find that fructose provides a strikingly similar value of 36–40% of the glycosyl units in newly deposited glycogen in cultured hepatocytes exposed to equimolar glucose and fructose (Table 1). It has been recently suggested, on the basis of kinetic modelling studies [13], that the

increased hepatic glycogen deposition in the presence of glucose and gluconeogenic substrates may be the result of decreased degradation of the polysaccharide. (After submission of the present manuscript, Youn *et al.* [28] presented evidence that fructose acts to inhibit phosphorylase and thereby promote net glycogen deposition in perfused liver.) Such a possibility is consistent with the known inhibition of phosphorylase by intermediates such as fructose 1-phosphate [22,23]. In the present work we provide experimental evidence for this possibility. In hepatocytes in primary culture, glycogen is in a dynamic state; degradation of newly laid-down glycogen occurs concomitantly with continued active incorporation of carbon into glycogen. The extent of glycogen deposition is therefore a function of the relative rates of synthesis and degradation of the polysaccharide. With glucose alone as glycogenic substrate, this degradation was such that approx. 50% of the carbon incorporated into glycogen during the labelling period was removed after an additional 2.5 h incubation. In the presence of fructose and glucose, however, no loss of prelabelled glycogen was noted. Since the activity of glycogen synthase in cells exposed to glucose and fructose was similar to that in cells exposed to glucose alone, it thus appears that glycogen degradation is substantially diminished in the former situation. The activity of phosphorylase *a* was the same in both incubation conditions; indeed the activity of this enzyme was lower in cells exposed to glucose (with or without fructose) than in cells exposed to fructose alone. The diminished degradation of glycogen in hepatocytes exposed to glucose/fructose is thus unlikely to be due to covalent modification of phosphorylase. Administration of fructose results in a rapid accumulation of fructose 1-phosphate in liver, up to 10 μmol/g [26]. Our studies have indicated that fructose 1-phosphate (at 10 mM-concn.) has no effect on the activity of glycogen synthase *in vitro*. However, under similar conditions, phosphorylase *a* is inhibited to a significant extent (up to 80% inhibition of phosphorylase by fructose 1-phosphate, (ii) covalent inactivation of phosphorylase induced by the binding of glucose and subsequent action of phosphorylase phosphatase [29,30], and (iii) activation of present simultaneously. On the basis of the information in the present report, we consider that this enhanced glycogenesis results from the combination of (i) allosteric inhibition of phosphorylase by fructose 1-phosphate, (ii) covalent inactivation of phosphorylase induced by the binding of glucose and subsequent action of phosphorylase phosphatase [29,30], and (iii) activation of glycogen synthase by a synthase phosphatase, owing to a decrease in the amount of its inhibitor, phosphorylase *a* [29,30]. Either substrate alone can induce only part of the total effect described. The observed synergism of glucose and fructose for glycogenesis results from a combination of the above three phenomena: fructose enhances the incorporation of glucose into glycogen by an inhibition of phosphorylase; glucose enhances the use of fructose for glycogenesis by inducing a covalent modulation of phosphorylase activity with a subsequent activation of glycogen synthase. Since the last two phenomena also occur in the presence of glucose alone, but not in the presence of fructose alone [26], and since fructose is a more efficient glycogenic substrate than is glucose, we must conclude that the inhibition of phosphorylase *a* by fructose 1-phosphate plays a major role in the increased hepatic glycogen deposition noted in the concomitant presence of glucose and fructose.

The repeated observation that glucose appears to be a rather poor precursor for glycogen synthesis in isolated liver systems [5,6,24,25] has been ascribed to an insufficient capacity of the hepatocyte to phosphorylate glucose [9,31]. This contention is controversial; a number of groups have demonstrated that the activity of hepatic glucokinase is more than sufficient to support the rates of glycogen synthesis observed *in vivo* [13,32,33]. We consider that the low net glycogen synthesis from glucose in these systems is due, in large part, to an incomplete inhibition of glycogen degradation under these conditions. We therefore conclude that glucose must be considered a major, perhaps the major, substrate for hepatic glycogen synthesis, whether by the direct path or by the indirect mechanism after the initial intrahepatocyte metabolism of glucose to at least the triose level.

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REFERENCES

1. Abumrad, N. H., Cherrington, A. D., Williams, P. E., Lacy, W. W. & Robin, D. (1982) Am. J. Physiol. **242**, E398–E406
2. Bergman, R. N., Bier, J. R. & Hourigan, P. M. (1982) Diabetes **31**, 27–35
3. DeFronzo, R. A., Ferrannini, E., Hendlor, R., Wahren, J. & Felig, P. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 5173–5177
4. Ishida, T., Chap, Z., Chou, J., Lewis, R., Hartley, C., Entman, M. & Field, J. B. (1983) J. Clin. Invest. **72**, 590–601
5. Katz, J., Golden, S. & Wals, P. A. (1979) Biochem. J. **180**, 389–402
6. Boyd, M. E., Albright, E. B., Foster, D. W. & McGarry, J. D. (1981) J. Clin. Invest. **68**, 142–152
7. Newgard, C. B., Hirsch, L. J., Foster, D. W. & McGarry, J. D. (1983) J. Biol. Chem. **258**, 8046–8052
8. Newgard, C. B., Moore, S. V., Foster, D. W. & McGarry, J. D. (1984) J. Biol. Chem. **259**, 6958–6963
9. Katz, J., Kuwajima, M., Foster, D. W. & McGarry, J. D. (1986) Trends Biochem. Sci. **11**, 136–140
10. Parniak, M. & Kalant, N. (1985) Can. J. Biochem. Cell Biol. **63**, 333–340
11. Spence, J. T. & Koudelka, A. P. (1985) J. Biol. Chem. **260**, 1521–1526
12. Wood, C. L., Babcock, C. J. & Blum, J. J. (1981) Arch. Biochem. Biophys. **212**, 43–53
13. Youn, J. H., Youn, M. S. & Bergman, R. N. (1986) J. Biol. Chem. **261**, 15960–15969
14. Leibovitz, A. (1963) Am. J. Hyg. **78**, 173–180
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
16. Lowry, O. H. & Passoneau, J. V. (1972) A Flexible System of Enzymatic Analysis, pp. 174–182, Academic Press, New York
17. Thomas, J. A., Schlender, K. K. & Larner, J. (1968) Anal. Biochem. **25**, 486–499
18. Lowry, O. H., Shulz, D. W. & Passonneau, J. V. (1967) J. Biol. Chem. **242**, 271–280

19. Gilboe, D. P., Larson, K. L. & Nuttall, F. Q. (1972) Anal. Biochem. **47**, 20–27
20. Katz, J. & Rognstad, R. (1976) Curr. Top. Cell. Regul. **10**, 237–289
21. Niewoehner, C. B., Nuttall, B. Q. & Nuttall, F. Q. (1987). Metab. Clin. Exp. **36**, 338–344
22. Van den Berghe, G., Hue, L. & Hers, H. G. (1973) Biochem. J. **134**, 637–645
23. Thurston, J. H., Jones, E. M. & Hauhart, R. E. (1974) Diabetes **23**, 597–604
24. Hems, D. A., Whitton, P. D. & Taylor, E. A. (1972) Biochem. J. **129**, 529–538
25. Seglen, P. O. (1974) Biochim. Biophys. Acta **338**, 317–336
26. Van den Berghe, G. (1986) Prog. Biochem. Pharmacol. **21**, 1–32
27. Pentreath, V. W., Seal, L. H. & Kai-Kai, M. A. (1982) Neuroscience **7**, 759–767
28. Youn, J. H., Kaslow, H. R. & Bergman, R. N. (1987) J. Biol. Chem. **262**, 11470–11477
29. Stalmans, W. (1976) Curr. Top. Cell. Regul. **11**, 51–97
30. Hers, H. G. (1976) Annu. Rev. Biochem. **45**, 167–189
31. Kuwajima, M., Newgard, C. B., Foster, D. W. & McGarry, J. D. (1986) J. Biol. Chem. **261**, 8849–8853
32. Stanley, J. C., Dohm, G. L., McManus, B. S. & Newsholme, E. A. (1984) Biochem. J. **224**, 667–671
33. Davidson, A. L. & Arion, W. J. (1987) Arch. Biochem. Biophys. **253**, 156–167

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