Regulation of glucose-6-phosphate dehydrogenase synthesis and mRNA abundance in cultured rat hepatocytes

Patricia MANOS,* Roderick NAKAYAMA and Darold HOLTEN†

Department of Biochemistry, University of California at Riverside, Riverside, CA 92521, U.S.A.

Conditions were identified which, for the first time, demonstrate that primary hepatocytes can express the same range of glucose-6-phosphate dehydrogenase (G6PD) synthesis and mRNA as in live rats. Primary hepatocytes were cultured without prior exposure to serum, hormones or carbohydrates. Five modulators implicated in G6PD induction *in vivo* were examined: insulin, dexamethasone, tri-iodothyronine (T_3), glucose and fructose. T_3 did not affect G6PD activity, and did not interact with carbohydrate to affect the activity of G6PD. Neither glucose nor fructose alone affected G6PD activity, and they did not interact with insulin to increase G6PD activity. Hepatocytes isolated from fasted rats and cultured in serum-free media with amino acids as the only energy source show a 12-fold increase in G6PD synthesis and mRNA (measured by a solution-hybridization assay). This induction does not require added hormones or carbohydrate. The addition of insulin alone caused another increase in G6PD synthesis and mRNA. There are at least three distinct phases to G6PD induction under these conditions. The largest increase in G6PD synthesis (12-fold) occurs in the absence of any hormones and with amino acids as the only energy source. This phase is due to increased G6PD mRNA. Insulin causes an additional 2–3-fold increase in G6PD synthesis and mRNA. However, dexamethasone and insulin are both required before G6PD synthesis is equal to that in rats which are fasted and refed on a high-carbohydrate diet.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) is generally considered to be the rate-determining enzyme of the oxidative portion of the pentose phosphate pathway, which has been estimated to contribute 50–75% of the NADPH used in hepatic lipid synthesis [1]. It and a number of other key enzymes in fatty acid synthesis are regulated in co-ordinate fashion by the dietary and hormonal state of the animal. G6PD levels decrease during fasting [2–4], feeding on a high-fat diet [5–7], in alloxan-induced diabetes [2,8], with glucagon injections [9] and after thyroidectomy [10]. G6PD activity shows a strong positive correlation with the fraction of carbohydrate in the diet [11,12], and increases with exogenously administered hormones such as insulin [12,13] and thyroid hormones [2,14]. In addition, G6PD activity undergoes very large changes (60–70-fold) when fasted rats are refed on a high-carbohydrate fat-free diet [4].

Although there is general agreement that the synthesis of G6PD *de novo* can account for the changes in G6PD activity [3,15,16], the mechanism by which individual hormones or dietary components regulate the rate of G6PD synthesis is poorly understood. There has been considerable controversy concerning the molecular events predominantly responsible for the enhanced rate of G6PD synthesis, with both transcriptional and post-transcriptional mechanisms accruing experimental support [15–18].

The regulation of G6PD synthesis *in vivo* by diet and hormones could occur by a direct effect of dietary components on the liver, or indirectly, by those components eliciting a specific endocrine response. We have previously reported the characterization of a cell-culture system for hepatocytes where the cells could survive for at least 6 days in culture without exposure to serum, hormones or carbohydrate [19]. This culture system is the only one which reproduces the full induction of G6PD that we observe *in vivo* [4]. It offered the possibility of unambiguously studying the signals that regulate G6PD induction, and the mechanism by which they

act. In the present report, we examine in more detail the role of insulin, dexamethasone (Dex) and carbohydrate in G6PD rates of synthesis and cytoplasmic mRNA accumulation, as well as the effects of other potential modulators of lipogenesis on G6PD activity.

EXPERIMENTAL

Materials

Sprague–Dawley rats were purchased from Bantin and Kingman; collagenase (120–150 units/mg) was from Cooper Biomedical, and Nitex nylon mesh from Tetko. L-[4,5- 3H]Leucine (130–180 Ci/mmol) and [α - ^{32}P]dGTP (3000 Ci/mmol) were from Amersham Corp. All other materials were of reagent grade.

Hepatocyte isolation and culture conditions

Hepatocytes were isolated from 48 h-fasted adult male Sprague–Dawley rats by a modification of the collagenase-perfusion method of Berry & Friend [20,21], as described in detail previously [19]. The cells were cultured on a hydrated collagen-gel substratum which was reinforced with a nylon mesh [19]. The medium was made from the components listed for Leibovitz's L-15, with the following modifications: galactose and pyruvate were omitted, arginine was replaced with ornithine to prevent non-parenchymal-cell overgrowth [22,23], the amino acid composition was as presented in Oliver *et al.* [24], and Hepes (25 mM), $NaHCO_3$ (1.19 g/l), BSA (2 mg/ml) and penicillin/streptomycin (50 μ g/ml each) were added. This serum-, hormone-, carbohydrate- and lipid-free medium is referred to as basal medium. The medium was changed 4 h after the initial plating, and the collagen-gel substratum was dislodged. The medium was changed every 24 h thereafter.

G6PD activity measurements

Cultured hepatocytes were harvested, a post-mitochondrial supernatant was prepared and G6PD activity was measured

Abbreviations used: Dex, dexamethasone; G6PD, glucose-6-phosphate dehydrogenase; T_3 , 3,3',5-tri-iodothyronine.

* Present address: Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024, U.S.A.

† To whom all correspondence should be addressed.

spectrophotometrically at 340 nm as the production of NADPH, as described previously [12,25], with modifications [19]. One unit of activity is defined as that amount of enzyme capable of producing 1 μ mol of NADPH/min at 30 °C. The amount of DNA was determined [19], and the specific activity of G6PD was expressed as m-units of enzyme activity/ μ g of DNA.

G6PD synthetic rates

Hepatocytes (approx. 5×10^6 cells in 5 ml of medium) cultured in 100 mm-diam. plates were labelled for 90 min in medium prepared without leucine, but supplemented with 200 μ Ci of [3 H]leucine (sp. radioactivity 128–184 Ci/mmol). For immunoprecipitation of G6PD, 1 ml of post-mitochondrial supernatant (0.05–0.35 unit of G6PD) was used, as previously described [3,26].

The amount of [3 H]leucine incorporated into immunoprecipitated G6PD protein was quantified by resolving the immunoprecipitate by SDS/PAGE and solubilizing the gel slices as described [7]. The radioactivity in total trichloroacetic acid-precipitable proteins was determined [27], and the relative rate of synthesis of G6PD was expressed as the ratio of the radioactivity (d.p.m.) in immunoprecipitated G6PD protein to that in the total soluble protein.

RNA isolation and G6PD mRNA quantification

Ten 100 mm culture plates (1.02–1.38 g of cells) were pooled for each condition, and total RNA was isolated by a modification of the method of Chirgwin *et al.* [28]. All solutions were prepared with diethylpyrocarbonate-treated water. Cells were harvested along with the substratum into 5 ml of lysis buffer (5 M-guanidinium thiocyanate, 50 mM-Tris/HCl, 5 mM-EDTA, pH 7.5) and stored at -70 °C. The samples were thawed in the presence of an additional 3 ml of lysis buffer, and 0.8 ml of β -mercaptoethanol was added. After homogenizing the samples for 1–2 min at setting 8.5 with a Polytron homogenizer, 2.5 ml of 12 M-LiCl and 4.0 ml of water were added, and the mixture was stored on ice at 4 °C. The RNA precipitate was collected by sedimentation (5500 rev./min at 4 °C for 75 min in a JA-20 rotor) and extracted at 50 °C for 2×15 min with 4.8 ml of water, 0.04 ml of 250 mM-EDTA, pH 7.5, 0.10 ml of 10% SDS and 0.05 ml of 2 M-Tris/HCl, pH 7.5. The supernatants were pooled and extracted with an equal volume of water-saturated phenol. The aqueous layer was then extracted with an equal volume of chloroform/3-methylbutan-1-ol (24:1, v/v), and RNA was precipitated from the aqueous phase by addition of 0.1 vol. of 3 M-sodium acetate and 2.5 vol. of ethanol.

G6PD mRNA was quantified by an S1-nuclease solution-hybridization assay recently characterized by Louie *et al.* [29]. A single-strand cDNA probe specific for G6PD mRNA [16] was synthesized as described by Williams *et al.* [30] from a 203-base G6PD cDNA subcloned into bacteriophage M13MP18. After purification of the single-stranded (SS) cDNA probe by electrophoresis and chromatography on hydroxyapatite, the background S1-nuclease-resistant radioactivity was decreased to 0.5% or less. The SS-cDNA probe corresponds to bases 1924–2126 of the sequence published by Ho *et al.* [31].

Total RNA from cultured hepatocytes (10–200 μ g) was hybridized to 3000 c.p.m. of SS cDNA in 0.03 M-Tris/HCl (pH 7.0)/0.3 M-NaCl/0.02 M-EDTA, containing 5 μ g of yeast tRNA and 100 μ g of denatured salmon sperm DNA/ml in a final volume of 100 μ l. Each sample was overlaid with 50 μ l of paraffin oil and incubated for 72 h at 69 °C. Digestion by 100 units of S1 nuclease was stopped after 30 min by adding 100 μ g of carrier DNA and 200 μ l of 50% (v/v) trichloroacetic acid. After 10 min on ice, the precipitated nucleic acid was filtered through a

Whatman GF/C glass-fibre filter and washed sequentially with 7.5% trichloroacetic acid and 95% ethanol before scintillation counting.

By comparing the amount of SS-cDNA probe hybridized to G6PD mRNA with that hybridized to a standard curve constructed by using several concentrations of the M13 mRNA template used for SS-cDNA probe synthesis, one can calculate the pg of G6PD mRNA/ μ g of total RNA isolated from hepatocytes cultured under various conditions. Each value was calculated from the slope of a line by using two different concentrations of total RNA run in duplicate [29].

RESULTS

Regulation of G6PD specific activity by hormones and carbohydrates

Elucidation of hepatocyte culture conditions which replicate the 70-fold induction of G6PD observed in fasted-refed rats provides an opportunity to identify unambiguously metabolites or hormones which regulate G6PD synthesis. Here, we examine the effect of carbohydrates and hormones which have been shown to stimulate the synthesis of lipogenic enzymes. Primary adult-rat hepatocytes were cultured on a floating collagen-gel substratum in a chemically defined Leibovitz L-15 medium lacking serum, hormones, lipids or carbohydrates. G6PD activity in hepatocytes isolated from 2-day-fasted rats and cultured in this basal L-15 medium (which contained only amino acids as an energy source), increased 5–10-fold over the activity measured at the time the cells were isolated ([19], and Fig. 1). Therefore, the effects of hormones and carbohydrates added to the medium are in addition to the effect of the basal medium alone. Fig. 1(a) illustrates the effect of insulin on G6PD activity during 6 days in culture. G6PD activity increased in a dose-dependent manner in response to insulin; the maximum increase, of about 4-fold, was generally observed after 4 days in culture (results not shown), and was maintained to day 6 with 0.1 μ M-insulin. In contrast with the effect of insulin alone, Dex alone did not affect G6PD activity, even at micro-molar concentrations (Fig. 1b). The third hormone examined was tri-iodothyronine (T_3) (Fig. 1c). T_3 alone had no effect on G6PD activity throughout a concentration range known to saturate the liver T_3 nuclear receptors (1 nM–0.1 μ M; [32,33]). Further, T_3 did not interact with glucose when it was at a physiological concentration of 10 mM [34], or at a higher concentration (30 mM). Under these culture conditions, T_3 has no effect on G6PD activity and does not interact with glucose to affect G6PD activity at day 4 (Fig. 1c) or day 6 (results not shown). T_3 also did not interact with fructose to alter the activity of G6PD (results not shown).

Glucose is thought to be poorly utilized by the liver unless it is at high concentrations and insulin is present [35]. We examined the effect of glucose alone and with insulin on G6PD activity. Glucose alone, at a physiological or supraphysiological concentration (30 mM), had no effect on G6PD activity. Further, there did not appear to be any interaction between glucose and insulin, although insulin, in the absence of glucose, increased G6PD activity (results not shown).

We have previously reported that under the culture conditions used here G6PD activity was profoundly increased by the interaction among insulin, Dex and fructose. After 6 days in culture, this combination produced an increase in G6PD activity comparable with that measured *in vivo* in fasted-refed rats [19]. These studies have been extended by an analysis of the role of fructose and Dex in the induction of G6PD *in vitro* in the presence of insulin. In contrast with glucose, fructose is utilized rapidly by the liver. It is catabolized by a set of liver-specific

Fig. 1. Effects of insulin, Dex and T_3 on the activity of G6PD in primary cultures of hepatocytes

Hepatocytes isolated from 2-day-fasted rats were cultured as described in the Experimental section. (a) The basal medium was supplemented with insulin at the concentrations indicated. (b) Serial dilutions were made of stock $1 \mu\text{M}$ -Dex in 11% ethanol for concentrations less than $1 \mu\text{M}$, and were added to the medium before the start of the experiment. An 11%-ethanol solution had no effect on G6PD activity (1.35 ± 0.29 m-units/ μg of DNA). (c) T_3 was added alone at the concentrations indicated (●) or in the presence of 10 mM- (○) or 30 mM- (△) glucose. The filled bars indicate enzyme activity at the time the cells were isolated. The cells were harvested after 6 days (a,b) or 4 days (c) in culture. The values are means $\pm$ S.D. of three plates and are representative of three to four experiments.

enzymes, including fructokinase and aldolase type B, which enables fructose to by-pass glucokinase and phosphofructokinase. It is thus more rapidly glycolysed, and it is thought to be a good substrate for lipogenesis [36]. However, fructose alone had no effect throughout a physiological range, and did not interact with insulin or Dex, in any significant manner, to increase the activity of G6PD, although insulin again increased G6PD activity about 3-fold (Fig. 2). The combination of insulin and Dex, however, increased G6PD activity relative to insulin alone, and about 25-fold over the activity measured when the cells were isolated. The addition of 5 mM-fructose to the medium containing insulin and Dex produced an additional 1.3-fold increase in G6PD activity. However, increasing fructose to 10 mM decreased G6PD activity. As previously reported [37], this may be due to the depletion of ATP stores as a result of the rapid catabolism of fructose. The energy status of the cells may have been compromised.

Fig. 3 illustrates the effect of different Dex concentrations on G6PD in hepatocytes cultured for 6 days in a medium containing insulin and fructose. Although an increase can be seen at 10 nM-Dex, the largest increase occurs at $1 \mu\text{M}$. The large increase in G6PD observed when cells are cultured with insulin, Dex and a

Fig. 2. Effect of interactions between carbohydrate, insulin and Dex on G6PD activity

Hepatocytes were isolated from 2-day-fasted rats. The cells were cultured for 6 days in the presence of insulin ($0.1 \mu\text{M}$), Dex ($1 \mu\text{M}$), or variable concentrations of fructose as indicated. The black bar indicates the activity of G6PD present at the time the cells were isolated. The values are means $\pm$ S.D. of five plates. ●, No additions; ○, Dex; △, insulin; ▲, insulin + Dex.

Fig. 3. Dex concentration required for maximal induction by insulin and carbohydrate

Cells from 2-day-fasted rats were cultured for 6 days in basal medium (B) or in the presence of $0.1 \mu\text{M}$ -insulin (I), 5 mM-fructose or variable concentrations of Dex as indicated. IDG indicates cells cultured with $0.1 \mu\text{M}$ -insulin, $1 \mu\text{M}$ -Dex and 5 mM-glucose. The filled bar indicates the G6PD activity in freshly isolated hepatocytes. All values are means $\pm$ S.D. of five plates.

carbohydrate appears to be specific for the type of carbohydrate. G6PD activity in cells cultured with $1 \mu\text{M}$ -Dex and insulin was 2-fold greater with 5 mM-fructose as the carbohydrate than with 5 mM-glucose (IDG bar, Fig. 3).

Of the three major hormones implicated in the induction of

Table 1. Effects of insulin, Dex and fructose on G6PD activity and synthesis

Hepatocytes were cultured in the presence of insulin (0.1 μ M), Dex (1 μ M) or fructose (5 mM) as indicated. The cells were labelled with [3 H]leucine for 90 min before harvest. G6PD was immunoprecipitated, and resolved by SDS/PAGE. The radioactivity (c.p.m.) in total soluble protein was used to normalize the radioactivity in the immunoprecipitated G6PD. The values are means $\pm$ s.d. of three plates from three experiments. Values in parentheses are fold increases.

	G6PD (m-units/ μ g of DNA)	Relative rate of G6PD synthesis [100 $\times$ G6PD (d.p.m.)/ total protein (d.p.m.)]
Liver*		
Fasted	0.2 $\pm$ 0.04	0.0015 $\pm$ 0.0002
Induced	12.5 $\pm$ 2.4	0.11 $\pm$ 0.02
Hepatocytes (day 4)		
Basal media	1.2 $\pm$ 0.45 (1)	0.018 $\pm$ 0.005 (1)
Dex	1.4 $\pm$ 0.38	0.017 $\pm$ 0.007
Insulin	4.5 $\pm$ 0.34 (3.8)	0.068 $\pm$ 0.015 (3.8)
Insulin, fructose	4.2 $\pm$ 0.10 (3.5)	0.056 $\pm$ 0.006 (3.1)
Insulin, Dex	5.8 $\pm$ 1.8 (4.8)	0.12 $\pm$ 0.05 (6.7)
Insulin, Dex, fructose	6.8 $\pm$ 0.23 (5.7)	0.11 $\pm$ 0.018 (6.1)
Hepatocytes (day 6)		
Insulin	4.5 $\pm$ 0.69 (3.8)	0.035 $\pm$ 0.008 (2.0)
Insulin, Dex	5.6 $\pm$ 1.0 (4.7)	0.063 $\pm$ 0.028 (3.5)
Insulin, Dex, fructose	10.6 $\pm$ 2.6 (9.0)	0.11 $\pm$ 0.012 (6.1)

* From Morikawa *et al.* [26]. Hepatocyte suspensions prepared from 2-day-fasted and fasted/carbohydrate-refed (induced) rats.

G6PD, only insulin directly increased G6PD activity in cultured hepatocytes. The other two hormones examined individually, Dex and T_3 , did not have a stimulatory effect on G6PD. However, Dex seems to enhance the induction of G6PD when insulin is present.

Regulation of G6PD synthesis by insulin, Dex and fructose

The changes in G6PD activity in response to hormones or carbohydrates could arise from alterations in the rate of synthesis or degradation, or from activation of pre-existing enzyme. To discriminate between these possibilities, the rate of synthesis of G6PD *de novo* was examined by immunoprecipitating the radiolabelled enzyme from cultured hepatocytes. A comparison of the activity and the corresponding relative rates of synthesis for G6PD are presented in Table 1. Data obtained from freshly isolated hepatocytes [26] are also presented for comparison.

Hepatocytes cultured for 4 days in the basal medium show increased G6PD activity and synthesis (6-fold and 12-fold respectively) relative to measurements made in hepatocytes freshly isolated from fasted rats. The effects of hormones and fructose are expressed relative to the basal medium (numbers in parentheses). At day 4, hepatocytes cultured with insulin increased G6PD activity 3–4-fold, and this was accompanied by commensurate changes in the rate of synthesis. Fructose added with insulin had no additional effect on G6PD activity or synthesis. Dex alone had no effect on G6PD synthesis, whereas the combination of insulin and Dex increased G6PD synthesis 6–7-fold relative to the basal medium. The addition of fructose to a medium containing insulin and Dex had little effect on G6PD synthesis at day 4.

These experiments show that the combination of the basal medium, with amino acids as its only energy source, and insulin and Dex, produces a rate of G6PD synthesis equal to that in rats which are maximally induced by fasting and refeeding on a high-carbohydrate fat-free diet. To our knowledge, this is the first demonstration of a complete induction of G6PD synthesis in cultured hepatocytes like that *in vivo*. Although carbohydrate is not required to attain a high rate of G6PD synthesis, it seems to

play a role in the maintenance of these high rates. By day 6, unless fructose is present as an alternative energy source, the cells cannot maintain the maximal rate of G6PD synthesis.

Changes in G6PD mRNA in cultured hepatocytes

The abundance of G6PD mRNA was quantified to gain insight as to whether a pre-translational or a translational mechanism was involved in the increased rate of G6PD synthesis. This was

Fig. 4. Summary of the effects of insulin and Dex on the induction of G6PD activity and mRNA in cultured hepatocytes

G6PD activity (▨) and mRNA abundance (■) are expressed relative to those in basal medium, which was normalized to unity. Cells were cultured for 4 days in the presence of insulin (INS; 0.1 μ M) or insulin and 1 μ M-Dex before measurement of G6PD activity and mRNA as described in the Experimental section.

achieved by using a sensitive and accurate solution-hybridization assay which allows one to calculate molar amounts of G6PD mRNA [29]. Total RNA was isolated from hepatocytes cultured for 4 days in basal medium alone, with added insulin or with insulin and Dex.

Freshly isolated hepatocytes from a fasted rat contain about 0.02 pg of G6PD mRNA/ μ g of total RNA. After 4 days in the basal medium, this increased about 13-fold, in excellent agreement with the 12-fold increase in G6PD synthesis (Table 1).

Fig. 4 summarizes results typical of three experiments on the effects of insulin and Dex on G6PD activity and mRNA. The results are expressed relative to the basal condition, which was set at unity. Although the increase caused by insulin was somewhat less than usual (possibly because of seasonal variation in the extent of G6PD induction [2]), the most obvious result is that insulin alone increased G6PD mRNA sufficiently to account for the increase in G6PD activity. Dex in the presence of insulin did not cause an additional increase in G6PD mRNA. The amount of G6PD mRNA in hepatocytes cultured with insulin (0.72 pg/ μ g of total RNA) was 36 times that in the hepatocytes isolated from a fasted rat.

DISCUSSION

The ability to use cultured primary hepatocytes and observe the full induction of G6PD seen in live rats [19] allows us to draw some unique conclusions from the results reported here. The most important of these is that a very simple medium with amino acids as the only energy source, and with insulin and a synthetic glucocorticoid as the only hormones, is all that is required to produce this large increase in G6PD synthesis. The culture conditions allow identification of at least two levels for the regulation of G6PD synthesis. One is the approx. 12-fold increase in G6PD synthesis and mRNA which occurs in the basal media lacking serum, hormones, carbohydrate or fat. Thus, added carbohydrate is not required for the induction of G6PD, and the addition of glucose or fructose does not cause an increase in G6PD synthesis under these culture conditions (Table 1).

There have been several studies suggesting that an intermediate in carbohydrate metabolism may be responsible for regulating the induction of lipogenic enzymes [32,38–41]. Since the hepatocytes used in the work reported here were from fasted rats and presumably were using gluconeogenesis from amino acids to form intermediates in carbohydrate metabolism, our results do not exclude the possibility that some intermediate in carbohydrate metabolism is involved in G6PD induction. However, the concentration of this putative intermediate would need to be changed in the same way in fasted rats refed on a high-carbohydrate diet [3,16] or a high-protein carbohydrate-free diet [15] as in hepatocytes cultured under our conditions, since all of these cause a substantial induction of G6PD and increase its mRNA.

There are conflicting reports on the effect of carbohydrate on G6PD activity, synthesis and mRNA levels in cultured hepatocytes. Several groups find no effect of carbohydrate ([42,43], and Fig. 2), whereas others find that G6PD activity or synthesis is increased by carbohydrate [44,45] or ethanol [44,46]. Kletzein's group has proposed that carbohydrate and T_3 increased G6PD synthesis at the translational level in hypothyroid rats [47]. However, we see no effect of T_3 on hepatocytes from euthyroid rats (Fig. 1).

It is unclear why only some culture conditions allow induction by carbohydrate. When hepatocytes from fasted rats (which are gluconeogenic) are placed in media containing amino acids as the only energy source, they are primed to metabolize those amino acids. However, when hepatocytes from an induced rat are

cultured in media with amino acids as the only energy source, they are not primed for gluconeogenesis and may not be able to use those amino acids in a way which is consistent with the induction of G6PD. This may explain why G6PD activity decreases rapidly when hepatocytes from induced rats are cultured in basal media, even in the presence of insulin, Dex and fructose, which support a high rate of G6PD synthesis in hepatocytes isolated from fasted rats (Table 1). Kelly *et al.* [48] have recently reported a rapid decline in G6PD, fatty acid synthase and acetyl-CoA carboxylase activities when hepatocytes from induced rats are cultured under conditions similar to those used here, and we have confirmed these results for G6PD [49]. One possibility which we think is consistent with these data is that hepatocytes from a fasted rat, upon removal from the animal, are no longer exposed to hormones or intermediates which normally repress G6PD mRNA levels and synthesis in the intact animal. In the absence of these hormones or intermediates, G6PD induction can occur as long as the energy needs of the cell are met and the metabolic state of the cell allows appropriate use of that energy source.

A second mechanism regulating G6PD synthesis is the insulin-mediated increase in G6PD mRNA. This does not require the addition of carbohydrate or any other hormone (Fig. 4). In three separate experiments, culturing hepatocytes from a fasted rat in medium containing amino acids as the only energy source and insulin as the only hormone caused a 19–36-fold increase in G6PD mRNA (from 0.02 to 0.72 pg/ μ g of total RNA). This is equivalent to the 33-fold increase in G6PD mRNA that we observed in live rats which were fasted and refed on a high-carbohydrate diet [50]. Thus these simple culture conditions allow full expression of the changes in G6PD mRNA observed in live rats.

However, the induction of G6PD synthesis to the full extent observed in live rats requires the addition of insulin and Dex to cells cultured for 4 days, or insulin, Dex and fructose to cells cultured for 6 days (Table 1). Fructose added for the first 4 days has no effect on G6PD synthesis, but, unless fructose is present, that rate of synthesis decreases by day 6 (Table 1). Fructose may simply be acting as an additional energy source, which helps maintain the general health of these primary hepatocytes beyond day 4, under these stringent culture conditions. In several experiments, Dex alone usually causes a small or no increase in G6PD mRNA and no increase in G6PD activity. As shown in Fig. 4, Dex does not increase G6PD mRNA in the presence of insulin. Thus our results are consistent with the report by Fritz *et al.* [51] that, although Dex alone does not increase G6PD, the combination of insulin and Dex causes a greater induction of G6PD than does insulin alone. Additional experiments will be required to elucidate the mechanism of this effect.

It is noteworthy that the amount of G6PD mRNA in whole livers is higher than in cultured hepatocytes (G6PD mRNA is 0.07 and 2.3 pg/ μ g of total RNA in whole livers from fasted and induced rats respectively [50]). Although hepatocytes are the predominant cell type, Kupffer and endothelial cells represent about 25% of the cells in liver [52], and they both have much higher levels of G6PD than do hepatocytes [53]. We believe the high amounts of G6PD mRNA in Kupffer and endothelial cells may account for the greater amount of G6PD mRNA in total liver cells than in isolated hepatocytes.

The very simple culture conditions used here, which still allow a full induction of G6PD like that *in vivo*, are unique, since most primary hepatocyte culture systems do not permit the full range of regulation shown in live animals [51,54]. It should be investigated whether the culture conditions described here might be a superior model for the regulation of other classes of liver proteins.

This work was supported in part by a grant from the American Diabetes Association (California Affiliate). We thank Guy K. Bryan for insightful suggestions during the preparation of this manuscript.

REFERENCES

- Rognstad, R. & Katz, J. (1979) *J. Biol. Chem.* **254**, 11969–11972
- Glock, G. E. & McLean P. (1955) *Biochem. J.* **61**, 390–396
- Winberry, L. & Holten, D. (1977) *J. Biol. Chem.* **252**, 7796–7801
- Manos, P., Taylor, N., Rudack-Garcia, D., Morikawa, N., Nakayama, R. & Holten, D. (1986) in *Glucose-6-Phosphate Dehydrogenase* (Yoshida, A. & Beutler, E., eds.), pp. 324–359, Academic Press, Orlando, FL
- Nace, C. S. & Szepesi, B. (1975) *Nutr. Rep. Int.* **12**, 305–310
- Wolfe, R. G. & Holten, D. (1978) *J. Nutr.* **108**, 1708–1717
- Tomlinson, J. E., Nakayama, R. & Holten, D. (1988) *J. Nutr.* **118**, 408–415
- Berdanier, C. D. & Shubeck, D. (1979) *J. Nutr.* **109**, 1766–1771
- Rudack, D., Davie, B. & Holten, D. (1971) *J. Biol. Chem.* **246**, 7823–7824
- Novello, F., Gumaa, J. A. & McLean, P. (1969) *Biochem. J.* **111**, 713–725
- Michaelis, O. E. & Szepesi, B. (1973) *J. Nutr.* **103**, 697–705
- Rudack, D., Chisholm, E. M. & Holten, D. (1971) *J. Biol. Chem.* **246**, 1249–1254
- Weber, G. & Convery, H. J. H. (1966) *Life Sci.* **5**, 1139–1146
- Diamant, S., Gorin, E. & Shafrir, E. (1972) *Eur. J. Biochem.* **26**, 553–559
- Katsurada, A., Iritani, N., Fukuda, H., Matsumura, Y., Noguchi, T. & Tanaka, T. (1989) *Biochim. Biophys. Acta* **1006**, 104–110
- Kletzien, R. F., Prostko, C. R., Stumpo, D. J., McClung, J. K. & Dreher, K. L. (1985) *J. Biol. Chem.* **260**, 5621–5624
- Miksicek, R. J. & Towle, H. C. (1982) *J. Biol. Chem.* **257**, 11829–11835
- Sun, J. D. & Holten, D. (1978) *J. Biol. Chem.* **253**, 6832–6836
- Manos, P. & Holten, D. (1987) *In Vitro Cell Dev. Biol.* **23**, 367–373
- Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520
- Bonney, R. J., Walker, P. R. & Potter, V. R. (1973) *Biochem. J.* **136**, 947–954
- Acosta, D., Anuforo, D. C. & Smith, R. V. (1978) *In Vitro* **14**, 428–436
- Leffert, H. L. & Paul, D. (1972) *J. Cell Biol.* **52**, 559–568
- Oliver, I. T., Edwards, A. M. & Pitot, H. C. (1978) *Eur. J. Biochem.* **87**, 221–227
- Glock, G. E. & McLean, P. (1953) *Biochem. J.* **55**, 400–408
- Morikawa, N., Nakayama, R. & Holten, D. (1984) *Biochem. Biophys. Res. Commun.* **120**, 1022–1029
- Winberry, L., Nakayama, R., Wolfe, R. & Holten, D. (1980) *Biochem. Biophys. Res. Commun.* **96**, 748–755
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
- Louie, P., Nakayama, R. & Holten, D. (1990) *Biochim. Biophys. Acta* **1087**, 25–30
- Williams, D. L., Newman, T. C., Shelness, G. S. & Gordon, D. A. (1986) *Methods Enzymol.* **128**, 671–689
- Ho, Y. S., Howard, A. J. & Crapo, J. D. (1986) *Nucleic Acids Res.* **16**, 7746
- Oppenheimer, J. H. (1979) *Science* **203**, 971–979
- Oppenheimer, J. H. & Schwartz, H. L. (1980) *Endocrinology* (Baltimore) **107**, 1460–1467
- Newgard, C. B., Hirsch, L. J., Foster, D. W. & McGarry, J. D. (1983) *J. Biol. Chem.* **258**, 8046–8052
- Smith, E. L., Hill, R. L., Lehman, I. R., Lefkowitz, R. J., Handler, P. & White, A. (1983) *Principles of Biochemistry*, pp. 389–392, McGraw-Hill, New York
- Van Den Berghe, G. (1978) *Curr. Top. Cell Regul.* **13**, 97–135
- Burch, H. B., Max, P., Chyu, K. & Lowry, O. H. (1969) *Biochem. Biophys. Res. Commun.* **34**, 619–625
- Mariash, C. N., Kaiser, F. E., Schwartz, H. L., Towle, H. C. & Oppenheimer, J. H. (1980) *J. Clin. Invest.* **65**, 1126–1134
- Noguchi, T., Inoue, H. & Tanaka, T. (1982) *Eur. J. Biochem.* **128**, 583–588
- Hamblin, P. S., Ozawa, Y., Jefferds, A. & Mariash, C. N. (1989) *J. Biol. Chem.* **264**, 21646–21651
- Mariash, C. N. (1989) *Endocrinology* (Baltimore) **124**, 212–217
- Kurtz, J. W. & Wells, W. W. (1981) *J. Biol. Chem.* **256**, 10870–10875
- Spence, J. T. & Pitot, H. C. (1982) *Eur. J. Biochem.* **128**, 15–20
- Kelly, D. S. & Kletzien, R. F. (1984) *Biochem. J.* **217**, 543–549
- Salati, L. M., Adkins-Finke, B. & Clarke, S. D. (1988) *Lipids* **23**, 36–41
- Stumpo, D. J. & Kletzien, R. F. (1985) *Biochem. J.* **226**, 123–130
- Fritz, R. S. & Kletzien, R. F. (1987) *Mol. Cell. Endocrinol.* **51**, 13–17
- Kelly, D. S., Nelson, G. J. & Hunt, J. E. (1986) *Biochem. J.* **235**, 89–90
- Manos, P. (1987) Ph.D. Dissertation, University of California, Riverside
- Kim, M.-H., Nakayama, R. & Holten, D. (1990) *Biochim. Biophys. Acta* **1049**, 177–181
- Fritz, R. S., Stumpo, D. J. & Kletzien, R. F. (1986) *Biochem. J.* **237**, 617–619
- Smedsrod, B., Pertoft, H., Gustafson, S. & Laurent, T. C. (1990) *Biochem. J.* **266**, 313–327
- Battistuzzi, G., D'Urso, M., Toniolo, D., Persico, G. M. & Luzzatto, L. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1465–1469
- Decaux, J.-F., Antoine, B. & Kahn, A. (1989) *J. Biol. Chem.* **264**, 11584–11590

Received 16 August 1990/18 December 1990; accepted 14 January 1991