Effects of Acute Variation of Fetal Glycemia on Glycogen Storage and on Glycogen Synthase and Phosphorylase Activities in the Liver of the Rat Fetus

M. GILBERT AND J. BOURBON

SUMMARY

The effects of variations of glycemia from 1.7 to 35 mM on the activity of glycogen synthase and phosphorylase, on glycogen content, and on U-^{14}C-glucose incorporation into glycogen in the liver of the near-term rat fetus were investigated. Hypoglycemia did not affect the activities of phosphorylase and synthase; total glycogen content was not modified, but incorporation of labeled glucose was markedly decreased. This is consistent with a decreased glycogen synthesis. A slight hyperglycemia (about 5.5 mM) sharply decreased phosphorylase a (active) activity but increased slightly glycogen synthase a activity; liver glycogen content and labeled glucose incorporation were both enhanced. Higher levels of glycemia induced a decrease of phosphorylase a activity of the same order, but by contrast, glycogen synthase a activity increased progressively with increasing glycemia. Sequential study showed that hyperglycemia first induced the decrease of phosphorylase activity, then increased synthase activity. Marked hyperglycemia strongly enhanced liver glycogen content and labeled glucose incorporation. The fetal liver appears very responsive to acute variations of glycemia. The mechanisms seem to be oriented toward maximal glycogen accumulation. DIABETES 29:266–271, April 1980.

The fetal liver accumulates glycogen at the end of gestation. This accumulation is under hormonal control.[1] However, little is known about the influence of nutritional factors on glycogen accumulation. Severe starvation in the pregnant rat is associated both with decreased fetal glycemia and decreased fetal liver glycogen.[2–5] Glucose infusion to the pregnant starved rat increases fetal liver glycogen.[2]

From the Laboratoire de Physiologie du Développement du Collège de France, Paris.

Address reprint requests to M. Gilbert, Laboratoire de Physiologie du Développement, Collège de France, II, place Marcelin Berthelot, 75231 Paris Cédex 05, France.

Received for publication 13 September 1979 and in revised form 15 November 1979.

The present work is an attempt to determine to what extent variations of maternal glycemia affect glycogen accumulation in the fetal liver. We studied the effects of acute variations of plasma glucose (hypoglycemia, moderate hyperglycemia, and marked hyperglycemia) on glycogen storage and on synthase and phosphorylase activities in the liver of the fetuses of normal fed rats. These experimental conditions were obtained through maternal venous infusion of saline, insulin, or glucose solutions.

MATERIALS AND METHODS

The study was made on female rats of the Wistar strain weighing approximately 280–320 g and fed ad libitum. Gestation was verified 14 days after mating, by abdominal palpation, and the fetal age determined according to Jost and Picon.[1]

UDP-U-^{14}C-glucose (312 mCi/mmol) and D-U-^{14}C-glucose (3.05 mCi/mmol) came from the Radiochemical Centre (Amersham, Bucks, G.B.). UDP-glucose, glucose 6-phosphate, glucose 1-phosphate, and oyster glycogen were obtained from Calbiochem (San Diego, California). Amylo, α-1,4,α-1,6-glucosidase and glucose oxidase (Perid method) were provided by Boehringer (Mannheim, Germany). Other chemicals were obtained from Prolabo (Paris, France).

Pregnant rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg body wt). A primed steady state infusion technique was used. The rats were infused through the saphenous vein. For control fetuses, mothers were infused with saline solution (NaCl 0.9%); for hypoglycemic fetuses, infusion of insulin solution was used (5 iU/ml at a rate of 6 iU/h); and for hyperglycemic fetuses, infusion of glucose solution 30% w/v through the saphenous vein was given. A priming dose of 1 ml was rapidly infused (2 min), followed by a constant infusion. Fetal blood glucose levels in the range of from 5.5 to 32 mM were obtained by altering the infusion rate from 1.2 to 4.5 ml/h. In some experiments U-^{14}C-glucose was added in trace amount to the glucose, insulin, or saline infusions.

Fetal blood was collected from exteriorized fetuses, (whose placenta was left in situ) with a heparinized pipette

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/29/4/266/350828/29-4-266.pdf by guest on 25 March 2023

at the level of the armpit vessels. The livers were immediately placed in liquid N_2 and stored at −20°C for enzymatic assays and glycogen determinations.

Glycogen synthase (EC 2.4.1.11) activity was measured by incorporation of U-^{14}C-glucose from UDP-U-^{14}C-glucose into glycogen. The incorporated radioactivity was measured after adsorption of an aliquot of incubation mixture on filter paper. Active synthase a (active) was measured by the method of De Wulf and Hers.[6] The liver was homogenized with 3 vol of ice-cold solution containing 150 mM sucrose and 100 mM glycylglycine buffer (pH 7.4); 0.02 ml of homogenate was incubated for 10 min at 20°C in a final volume of 0.2 ml containing 0.2 μmol of UDP-glucose + UDP-U-^{14}C-glucose (100 c.p.m./nmol), 1 mg of glycogen, 6 μmol of glycylglycine buffer (pH 7.4), 1 μmol of Na_2SO_4, and 0.5 μmol of EDTA. Then 0.1 ml of the mixture was placed on a piece (2 × 2 cm) of Whatman 31 ET chromatography paper (according to Thomas et al.[7]) and the reaction stopped by placing the paper in cold ethanol. The papers were washed three times (3 × 10 min) with 66% ethanol. Papers rinsed with acetone and dried were placed in vials containing 10 ml of scintillation liquid (P.C.S. Amersham, Searle). The radioactivity was measured in a Nuclear Chicago (Mark 1) liquid scintillation spectrometer. Activity was expressed in μmoles of glucose incorporated into glycogen per min/g wet liver (i.e., units/g wet liver). In a few cases, total (a + b) glycogen synthase activity was measured according to Devos and Hers.[8]

Phosphorylase (EC 2.4.1.1) was assayed by measuring Pi released during incorporation of glucose 1-phosphate into glycogen. Only the active form of phosphorylase a was determined according to Hue et al.[9] Pieces of liver were homogenized in 10 vol of ice-cold solution containing 100 mM NaF, 20 mM EDTA, 0.5% (w/v) glycogen, and 50 mM glycylglycine (pH 7.4). One-tenth milliliter of the homogenate was incubated for 30 min at 20°C, with an equal volume of a mixture containing 100 mM glucose 1-phosphate, 2% (w/v) glycogen, 0.3 mM NaF, and 1 mM caffeine (pH 6.1). The reaction was stopped by adding 0.5 ml of 10% (w/v) trichloroacetic acid. The mixture was centrifuged (16,000 × *g*, 2 min) and Pi assayed in the supernatant as described by Fiske and Subbarow.[10] The activity was expressed as μmoles Pi released/min/g wet liver (i.e., units/g wet liver).

The technique for glycogen determination in the fetal liver was that described by Roehrig and Allred.[11] When U-^{14}C-glucose was added to the 30% glucose or saline infusion, the glycogen content of the liver and radioactivity incorporated into glycogen were simultaneously determined according to the method of Chan and Exton.[12] Frozen liver was weighed and homogenized (2.5%) in twice distilled water with a glass teflon homogenizer. One-tenth milliliter of the homogenate was spotted on a piece (2 × 3 cm) of filter paper (Whatman 3 MM chromatography paper). The papers were dropped into a beaker containing 66% ethanol and washed three times (3 × 10 min). They were then briefly rinsed with acetone, dried, and placed in plastic tubes containing 0.4 ml acetate buffer (0.05 M, pH 4.5), 1.6 ml H_2O, and 0.02 ml of amylo,α-1,4,α-1,6-glucosidase (3 U). The tubes were placed at 55°C for 30 min to allow hydrolysis of glycogen. Glucose was determined in 0.1 ml of hydrolysate with the glucose oxidase method. Hydrolyzed oyster glycogen was used as the standard. One milliliter of the hydrolysate was placed in a vial containing 10 ml of scintillation liquid (P.C.S. Amersham, Searle, G.B.), and radioactivity was measured in the liquid scintillation spectrometer.

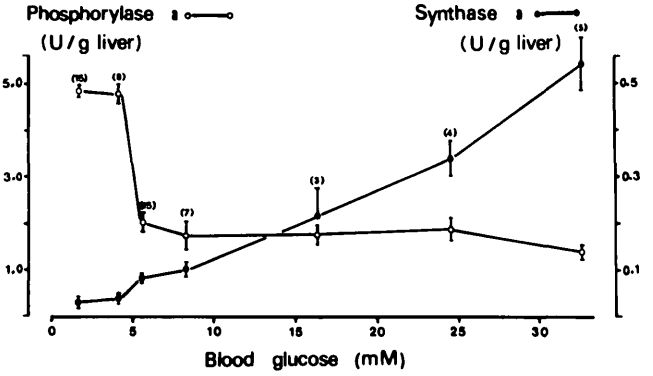


FIGURE 1. Effect of blood glucose concentration on glycogen synthase and phosphorylase activities in the fetal liver. On day 21.5 of pregnancy, maternal glucose or insulin infusion was sustained during 60 min. At the end of this time period the activity of phosphorylase (○——○) and synthase (●——●) was measured on the same liver. The activities have been measured at different fetal blood glucose concentrations (between 1.7 and 32 mM). Values are means ± SEM with the number of experiments in parentheses.

Means of the experimental series were compared by Student's *t* test.

RESULTS

Relation between fetal glycemia and enzyme activities. The activity of phosphorylase a and glycogen synthase a was measured at different blood glucose concentrations in 21.5-day-old fetuses. The maternal infusions of saline, insulin, or glucose were continued for 60 min in different experiments. Saline infusion did not modify fetal blood glucose level (Figure 3). Hypoglycemia between 3.5 and 1.7 mM did not affect the activity of phosphorylase and synthase. Hyperglycemia between 3.5 and 5.5 mM increased glycogen synthase and decreased phosphorylase activity. Higher levels of glycemia induced a decrease of the phosphorylase activity of the same order (Figure 1). By contrast, glycogen synthase activity increased progressively with increasing glycemia.

The activity of synthase was plotted versus the corresponding value of phosphorylase for each liver in Figure 2. There is an inverse correlation between the activities of these enzymes.

Sequential study of glycogen synthase and phosphorylase activities during glucose infusion (marked hyperglycemia). Glycogen synthase a and glycogen phosphorylase a activities were determined 15, 30, 60, and 150 min after the beginning of maternal infusion with 30% glucose solution (3 ml/h) on day 21.5 of gestation. Controls were obtained by saline infusion at the same rate. Saline infusion did not significantly modify fetal glycemia nor the enzymatic activities at any time.

Ten minutes after beginning glucose infusion, the fetal glycemia increased to 15 mM and afterward remained stable (Figure 3). Activity of glycogen synthase a was not modified for 15 min. A sharp increase occurred between 30 and 60 min, while between 60 and 150 min there was a decrease of enzymatic activity (Figure 3). A similar pattern was

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/29/4/266/350828/29-4-266.pdf by guest on 25 March 2023

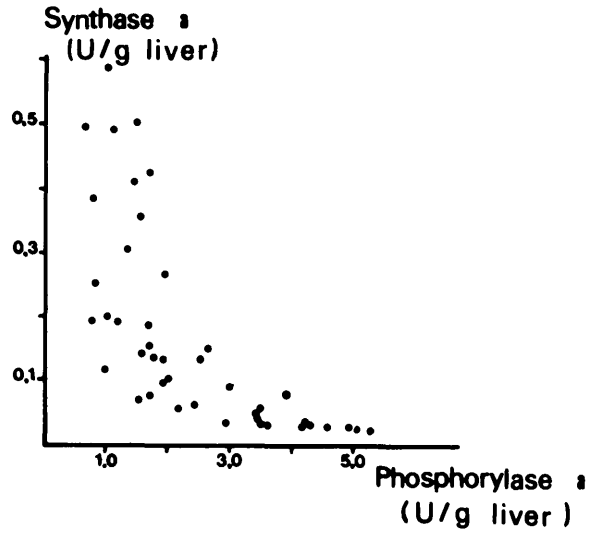


FIGURE 2. Correlation between the activity of synthase and phosphorylase in fetal liver. Values of glycogen synthase shown in Figure 1 have been plotted versus the corresponding values of glycogen phosphorylase.

observed in 18.5-, 19.5-, and 20.5-day-old fetuses (data not shown).

The increase of synthase a activity resulted from an activation of the enzyme, not from synthesis, since the total activity (a + b) was not enhanced (initial value = 1.18 ± 0.45 U/g liver; final value = 1.21 ± 0.40 U/g liver).

The activity of phosphorylase a decreased during the first 30 min and afterward remained practically unchanged (Figure 3). The same was observed on days 19.5 and 20.5. At 18.5 days, the decrease occurred only after 30 min and was transitory (data not shown).

Relation between fetal glycemia, liver glycogen content, and incorporation of labeled glucose into liver glycogen. On day 21.5 of gestation, maternal infusions of tracer amounts of U-^{14}C-glucose were made during 150 min under the following four experimental conditions: normoglycemia with saline infusion (2 μCi/ml, 2.2 ml/h); hypoglycemia with insulin infusion, 5 iU/ml (1.2 μCi/ml, 2.2 ml/h); moderate hyperglycemia with 30% glucose solution (3 μCi/ml, 1.2 ml/h); and marked hyperglycemia with 30% glucose solution (11 μCi/ml, 3 ml/h). Under these conditions specific radioactivities of blood glucose were approximately the same.

Fetal hypoglycemia did not modify the liver glycogen content compared with fetuses of normoglycemic mothers, but labeled glucose incorporation into glycogen was strongly decreased (Figure 4). Moderate hyperglycemia (about 5.5 mM) increased slightly but significantly the fetal liver glycogen content ($P < 0.01$) and glucose incorporation ($P < 0.001$) compared with fetuses of saline-infused mothers (Figure 4). Marked hyperglycemia (about 15 mM) strongly increased both fetal liver glycogen content and labeled glucose incorporation (Figure 4).

Effect of marked hyperglycemia on liver glycogen content as a function of fetal age. Fetal glycemia and liver glycogen were determined in 17.5–21.5-day-old fetuses under three experimental conditions: (1) immediately after anesthesia of the mother; (2) after a 150-min saline infusion to the mother; and (3) after a 150-min 30% glucose infusion to the mother. Fetal glycemia is reported in Table 1. After

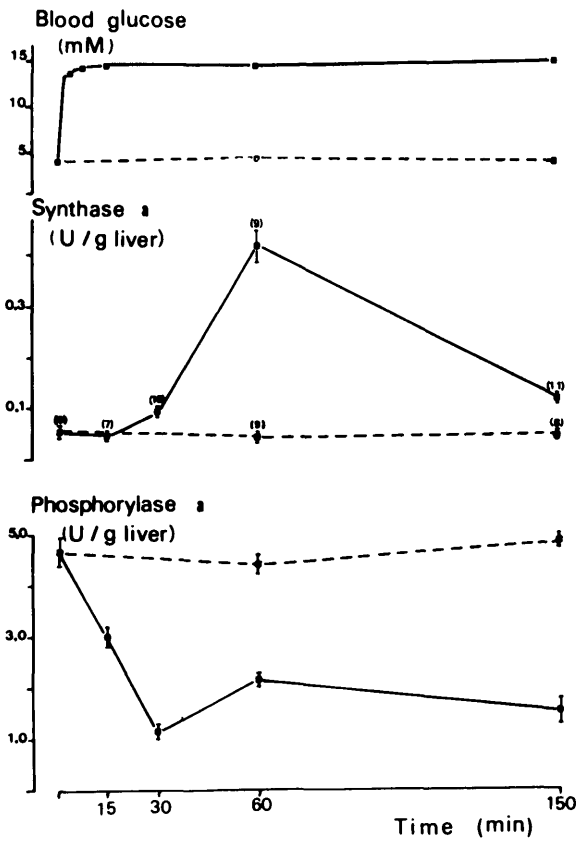


FIGURE 3. Sequential activation of glycogen synthase and inactivation of glycogen phosphorylase in the fetal liver on day 21.5 of gestation. The pregnant rat was infused with either saline (---) or glucose solution (——). At different time intervals after the beginning of maternal infusion, synthase and phosphorylase activities were measured in the same liver. Fetal hyperglycemia occurred only at 5, 15, 60, and 150 min. Values are means ± SEM of the number of determinations shown in parentheses.

glucose infusion, it was about 8–10 times higher than that of control fetuses (saline infusion) from day 17.5 to day 21.5. At all stages, there was no difference between the glycemia of fetuses taken at time zero and that of fetuses taken after saline infusion.

Liver glycogen is reported in Figure 5. Saline infusion did not affect glycogen content at any stage. In 17.5- or 18.5-day-old fetuses, liver glycogen concentration after glucose infusion was not modified compared with fetuses of saline-infused dams. In the liver of 19.5-, 20.5-, and 21.5-day-old fetuses, glycogen concentration was increased by about 30% after glucose infusion.

DISCUSSION

The results reported in Figure 1 indicate that a slight fetal hyperglycemia (5.5 mM) induced a decrease of glycogen phosphorylase activity as striking as that seen with very high glycemia (32 mM); the changes observed indicate that a slight increase of fetal blood glucose may be sufficient to control fetal liver glycogen metabolism.

By contrast, glycogen synthase activity progressively increased until glycemia reached 32 mM. Similar observations were obtained with glucose levels extending from 5 to 55 mM in the perfused adult rat liver,[13] in the isolated rat liver cells,[9] and in the liver of rats and mice in vivo.[14] The results plotted in Figure 2 indicates that the glycogen synthase activity is enhanced only in livers in which the level of

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/29/4/266/350828/29-4-266.pdf by guest on 25 March 2023

TABLE 1
Fetal glycemia (mM) immediately after anesthesia of the mother (controls) and after a 150-min maternal saline or glucose infusion at different stages of pregnancy

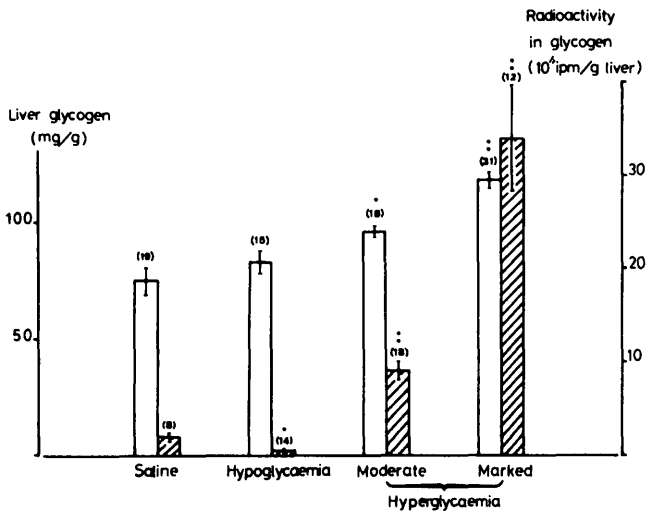
	Days of gestation				
	17.5	18.5	19.5	20.5	21.5
Controls		2.7 ± 0.3 (12)	2.4 ± 0.2 (25)	2.6 ± 0.2 (13)	3.6 ± 0.2 (32)
Saline infusion	1.1 ± 0.2 (14)	1.3 ± 0.1 (18)	1.8 ± 0.2 (12)	2.2 ± 0.3 (23)	3.3 ± 0.2 (18)
Glucose infusion	16.7 ± 0.2 (14)	14.3 ± 0.2 (20)	15.2 ± 0.1 (13)	13.1 ± 0.1 (22)	15.0 ± 0.1 (31)

Values are means ± SEM of the number of determinations shown in parentheses.

phosphorylase is lower than a value of 2 U. Since an increase of fetal blood glucose produced an inactivation of glycogen phosphorylase and an activation of glycogen synthase, this sequence of events might be the biochemical expression of the concept of hepatic threshold to glucose initially proposed by Soskin.[15]

Sustained fetal hyperglycemia (Figure 4) induced firstly an inactivation of the major part of the glycogen phosphorylase followed by an activation of the glycogen synthase. The same sequence has been observed in adult rats[14] and monkeys[16] as well as in the perfused liver[13,17] and in isolated hepatocytes.[9] The apparent existence of a threshold of phosphorylase activity for synthase activation suggests that the response of fetal liver to glucose obeys the pattern proposed by Hers and co-workers.[14] One can assume that, as shown in the adult rat liver, the primary effect of glucose is to bind to phosphorylase a[18] and to favor the conversion of this enzyme into phosphorylase b (inactive form) by phosphorylase phosphatase.[19] Since phosphorylase a is a strong inhibitor of glycogen synthase phosphatase,[20] its disappearance would be a prerequisite for the activation of glycogen synthase, which occurs in a second step. Thus, the difference in the response of the two enzymes may permit a better stimulation of glycogen synthesis when the degradation is diminished or stopped in the first step.

FIGURE 4. Relation between fetal glycemia, liver glycogen content, and incorporation of labeled glucose into liver glycogen. On day 21.5 of gestation, maternal infusions of U-^{14}C-glucose in trace amount were made during 150 min under four experimental conditions. For experimental details see text. Open bars, liver glycogen content; hatched bars, radioactivity in glycogen. *, significant statistical difference ($P < 0.01$); **, highly significant statistical difference ($P < 0.001$).



However, some findings which do not agree with this model have been reported in the adult rat. Nuttall et al.[21] in the rat liver in vivo and Gilboe and Nuttall[22] in acellular preparations of rat liver observed a significant glucose-induced synthase activation before a decrease of phosphorylase activity was detectable. In the fasted rat liver in vivo, glucose induced a striking activation of glycogen synthase without concomitant inactivation of phosphorylase, while phosphorylase inactivation occurred in the liver of the fed rat.[23] In a rat hepatocyte preparation, Ciudad et al.[24] reported a simultaneous increase of synthase and phosphorylase activity in the presence of fructose and postulated that inactivation of phosphorylase is not a prerequisite for synthase activation.

Roach and Larner[25] have proposed an alternative explanation for stimulation of glycogen synthesis: glycogen synthase activity depends on the phosphorylation state of the enzyme, and hormones modulate this phosphorylation state. However, metabolic activators such as glucose are able to induce an increase of activity at the same phosphorylation state without modifying it. In this model, inactivation of phosphorylase a, which inhibits synthase phosphatase, is not required, since activation can occur without dephosphorylation of synthase b by synthase phosphatase.

It is likely that in our fetal model (as in the adult rat liver) both mechanisms can occur: glucose might induce simultaneously a direct activation of synthase activity and an activation of synthase phosphatase through phosphorylase inactivation.

Recently Katz et al.[26] established the existence of two distinct systems of glycogen synthesis by isolated adult rat hepatocytes. In the first, glycogen is derived from gluconeogenic precursors, essentially lactate, pyruvate, and dihydroxyacetone;[27] glucose is produced concurrently with glycogen synthesis, and a high glucose concentration is not required to obtain glycogen accumulation. In the second system, synthesis of glycogen from glucose as sole substrate requires a high glucose concentration for a significant rate. In this second system only, glycogen accumulation is accompanied by a decrease of phosphorylase activity.

Since in the rat fetus the gluconeogenic pathway from lactate, pyruvate, and amino acids is not functional until birth,[28,29] one can assume that the first system cannot apply to our fetal model. Glucose of maternal origin is probably the main substrate for glycogen synthesis in the fetal rat liver. Consequently, glycogen synthesis in the liver of the rat fetus might be explained by Her's model. Any increase of maternal blood glucose would be of physiologic significance for glycogen storage in the fetal liver.

The possibility that the observed effect of glucose might

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/29/4/266/350828/29-4-266.pdf by guest on 25 March 2023

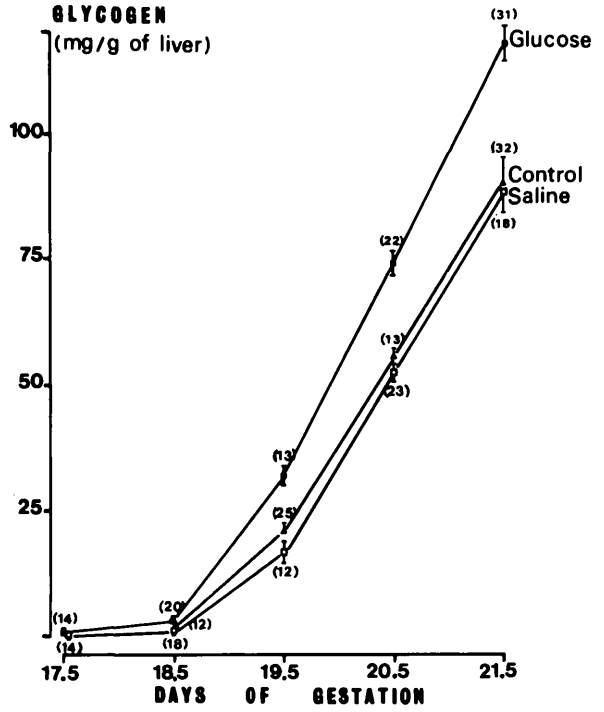


FIGURE 5. Effect of maternal glucose infusion on fetal liver glycogen concentration. Control fetuses (▲——▲) of nonperfused dams were studied immediately after maternal anesthesia. In fetuses of glucose-(■——■) and saline-perfused females (□——□), the liver glycogen was measured 150 min after the beginning of maternal infusion. Values are means ± SEM of the number of determinations shown in parentheses.

be mediated by insulin cannot be ruled out, since in the term rat fetus, an acute hyperglycemia stimulates insulin secretion.[30] However, in the perfused liver of the adult rat[13] and in isolated hepatocytes[9] as well as in adult rat liver in vivo,[14] the activation of glycogen synthase by high glucose concentrations does not depend on the presence of insulin. In preliminary experiments we injected insulin intravenously (40 mU) into the 21.5-day-old rat fetus. This rather high dose of insulin induced effects on glycogen synthesis qualitatively similar to those of glucose, but quantitatively lesser than those induced by a moderate increase of blood glucose from 3.8 to 5.5 mM. Therefore, it is unlikely that the glucose-induced increase of insulinemia is totally responsible for glycogen synthesis stimulation.

As expected from the effects on enzyme activities, fetal hyperglycemia increased glycogen content of the liver over a period of 150 min, i.e., it enhanced net glycogen synthesis. Since a moderate hyperglycemia (5.5 mM) was able to significantly enhance glycogen synthesis in the liver of the rat fetus, one can assume that physiologic increments of maternal glycemia as a result of nutritional state would affect fetal liver glycogen synthesis.

In contrast, acute fetal hypoglycemia was without effect on fetal liver glycogen content. No degradation of glycogen occurred, but, as shown by incorporation of labeled glucose, synthesis was markedly reduced. Girard et al.,[31] in similar experiments, observed reduced fetal insulinemia (half of the control value) after acute maternal hypoglycemia. In spite of the decrease, this level of fetal blood insulin might be high enough to protect glycogen against degradation.

Thus, the liver of the rat fetus seems to be able to resist an acute fall in maternal blood glucose, while it responds to slight maternal blood glucose increase by enhancement of glycogen synthesis. The mechanisms seem to be oriented toward maximal glycogen accumulation. Why this should be true is not known since, teleologically, glycogen breakdown would be expected during hypoglycemia.

As far as young gestational ages are concerned, the present study confirms previous results obtained in the rabbit fetus:[32] fetal hyperglycemia greatly enhances the rate of glucose incorporation into fetal liver glycogen only on and after a definite stage of gestation (19.5 days in the rat and 26 days in the rabbit). In the rat, the liver synthesizes glycogen from 18.5 days up to birth. Nevertheless, hyperglycemia is not sufficient to obtain an increase of glycogen storage before day 19.5. On days 17.5 and 18.5 other factors, such as hormone(s), are probably limiting for development of the physiologic maturation of hepatocytes necessary for glycogen accumulation.[1]

ACKNOWLEDGMENT

We thank Dr. F. Battaglia for helpful discussion and criticism.

Note added in proof. Hyperglycemic fetuses from 30% glucose-infused mothers were given an amount of anti-insulin serum from guinea pigs (through the vitellin vein) which was able to complex 2.5 mU of insulin; controls received normal guinea pig serum. This treatment did not affect either the increase of liver glycogen content or ^{14}C-glucose incorporation. Therefore, insulin suppression did not impair the increase of glycogen accumulation due to the rise of fetal blood glucose.

REFERENCES

[1] Jost, A., and Picon, L.: Hormonal control of fetal development and metabolism. Adv. Metab. Disord. *4*:123–84, 1970.

[2] Goodner, C. J., and Thompson, D. J.: Glucose metabolism in the fetus in utero. The effect of maternal fasting and glucose loading in the rat. Pediatr. Res. *1*:443–51, 1967.

[3] Bossi, E., and Greenberg, R. E.: Sources of blood glucose in the rat fetus. Pediatr. Res. *6*:765–72, 1972.

[4] Vinicor, F., Kohalmi, D., and Clark, C. M.: Characterization of carbohydrate metabolism in the isolated fetal rat heart. Effect of fasting and alloxan diabetes. Diabetes *23*:662–68, 1974.

[5] Girard, J. R., Ferre, P., Gilbert, M., Kervran, A., Assan, R., and Marliss, E. B.: Fetal metabolic response to maternal fasting in the rat. Am. J. Physiol. *1*:E456–63, 1977.

[6] De Wulf, H., and Hers, H. G.: The interconversion of liver glycogen synthetase a and b in vitro. Eur. J. Biochem. *6*:552–57, 1978.

[7] Thomas, J. A., Schlender, K. K., and Larner, J.: A rapid filter paper assay for UDP glucose glycogen glucosyl transferase including an improved biosynthesis of UDP-^{14}C-glucose. Anal. Biochem. *25*:486–99, 1968.

[8] Devos, P., and Hers, H. G.: Glycogen metabolism in the liver of the foetal rat. Biochem. J. *140*:331–40, 1974.

[9] Hue, L., Bontemps, F., and Hers, H. G.: The effect of glucose and of potassium ions on the interconversion of the two forms of glycogen phosphorylase and of glycogen synthetase in isolated rat liver preparation. Biochem. J. *152*:105–14, 1975.

[10] Fiske, C. H., and Subbarow, Y.: The colorimetric determination of phosphorus. J. Biol. Chem. *66*:375–80, 1925.

[11] Roehrig, K. L., and Allred, J. B.: Direct enzymatic procedure for the determination of liver glycogen. Anal. Biochem. *58*:414–21, 1974.

[12] Chan, T. M., and Exton, J. M.: A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. Anal. Biochem. *71*:96–105, 1976.

[13] Glinsmann, W., Pauk, G., and Hern, E.: Control of rat liver glycogen synthetase and phosphorylase activities by glucose. Biochem. Biophys. Res. Commun. *39*:774–82, 1970.

[14] Stalmans, W., De Wulf, H., Hue, L., and Hers, H. G.: The sequential inactivation of glycogen phosphorylase and activation of glycogen synthe-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/29/4/266/350828/29-4-266.pdf by guest on 25 March 2023

tase in liver after the administration of glucose to mice and rats. The mechanism of the hepatic threshold to glucose. Eur. J. Biochem. *41*:127–34, 1974.

[15] Soskin, S.: The liver and carbohydrate metabolism. Endocrinology *26*:297–308, 1940.

[16] Curnow, R. T., Rayfield, E. J., George, D., Zenser, T. V., and De Rubertis, F.: Control of hepatic glycogen metabolism in the rhesus monkey: effect of glucose, insulin and glucagon administration. Am. J. Physiol. *228*:80–87, 1975.

[17] Buschiazzo, H., Exton, J. H., and Park, C. R.: Effects of glucose on glycogen synthetase phosphorylase and glycogen deposition in the perfused rat liver. Proc. Natl. Acad. Sci. USA *65*:383–87, 1970.

[18] Stalmans, W., Laloux, M., and Hers, H. G.: The interaction of liver phosphorylase a with glucose and AMP. Eur. J. Biochem. *49*:415–27, 1974.

[19] Stalmans, W., De Wulf, H., Lederer, B., and Hers, H. G.: The effect of glucose and of a treatment by glucocorticoids on the inactivation in vitro of liver glycogen phosphorylase. Eur. J. Biochem. *15*:9–12, 1970.

[20] Stalmans, W., De Wulf, H., and Hers, H. G.: The control of liver glycogen synthetase phosphatase by phosphorylase. Eur. J. Biochem. *18*:582–87, 1971.

[21] Nuttall, F. Q., Gannon, M. C., and Larner, J.: Oral glucose effect on glycogen synthetase and phosphorylase in heart, muscle and liver. Physiol. Chem. Phys. *4*:497–515, 1972.

[22] Gilboe, D. P., and Nuttall, F. Q.: The regulation of liver glycogen synthetase D phosphatase by ATP and glucose. Biochem. Biophys. Res. Communun. *53*:164–71, 1973.

[23] Goldstein, D. E., and Curnow, R. T.: Effect of starvation on hepatic glycogen metabolism and glucose homeostasis. Metabolism *27*:315–23, 1978.

[24] Ciudad, C. J., Massague, J., and Guinovart, J. J.: The inactivation of glycogen phosphorylase is not a prerequisite for the activation of liver glycogen synthase. FEBS Lett. *99*:321–24, 1979.

[25] Roach, P. J., and Larner, J.: Regulation of glycogen synthase: a relation of enzymic properties with biological function. T.I.B.S. *1*:110–12, 1976.

[26] Katz, J., Golden, S., and Wals, P. A.: Glycogen synthesis by rat hepatocytes. Biochem. J. *180*:389–402, 1979.

[27] Katz, J., Golden, S., and Wals, P. A.: Stimulation of hepatic glycogen synthesis by amino acids. Proc. Natl. Acad. Sci. USA *73*:3433–37, 1976.

[28] Yeung, D., and Oliver, I. T.: Gluconeogenesis from amino-acids in neonatal rat liver. Biochem. J. *103*:744–48, 1967.

[29] Yeung, D., and Oliver, I. T.: Development of gluconeogenesis in neonatal rat liver. Effect of premature delivery. Biochem. J. *105*:1229–33, 1967.

[30] Kervran, A., and Girard, J.: Glucose-induced increase of plasma insulin in the rat foetus in utero. J. Endocrinol. *62*:545–51, 1974.

[31] Girard, J. R., Kervran, A., Soufflet, E., and Assan, R.: Factors affecting the secretion of insulin and glucagon by the rat fetus. Diabetes *23*:310–16, 1974.

[32] Gilbert, M., and Jost, A.: Hyperglycemia and glycogen storage in the rabbit fetal liver. Role of age and hormonal status. Biol. Neonate *32*:125–31, 1977.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/29/4/266/350828/29-4-266.pdf by guest on 25 March 2023