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Galactose insulin
glucose liver
glycogen newborn

Glycogen Regulation in Isolated Perfused Near Term Monkey Liver

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Extract

Glycogen metabolism was studied in the isolated perfused liver of the monkey conceptus at 90% of gestation using an *in situ* recirculating perfusion system. Net uptake of glucose and galactose and the activities of the enzymes, glycogen synthetase and phosphorylase, were studied in response to varied perfusate composition. Synthetase activity was expressed as %I, the percentage of total synthetase activity in the active form. Perfusate glucose concentrations as high as 700 mg/100 ml did not lead to net glucose uptake or to an increase in the baseline %I synthetase (4 ± 1, mean ± SEM). In the presence of 300 mg/100 ml glucose, insulin at 10^{-7} M increased %I to 8 ± 2, and galactose > 75 mg/100 ml increased %I to 8 ± 1. The combination of galactose, glucose, and insulin increased %I to 40 ± 5. With this latter combination, synthetase activity was proportional to perfusate glucose concentration above 100 mg/100 ml. Phosphorylase activity was diminished by either galactose or insulin, and phosphorylase activity was lowest in the group receiving galactose, glucose, and insulin. Galactose was taken up by all livers, but net glucose uptake was not observed under any condition; net hexose uptake was observed in perfusions with galactose. Glycogen levels did not vary significantly with varied perfusate composition during the 30-min perfusion periods.

Speculation

We speculate that galactose may be uniquely important for neonatal liver glycogen synthesis, and that its action is mediated through reciprocal changes in the activities of the enzymes, glycogen synthetase and phosphorylase. If liver glycogen is important for acute neonatal glucose homeostasis, then galactose may also be essential for maintaining circulating glucose concentration by ensuring glycogen synthesis during feeding.

The infant has a continuing obligatory requirement for glucose (7, 23). During feeding, this requirement is met by exogenous glucose, the excess carbohydrate being stored principally as glycogen. During fasting, the liver derives glucose both from gluconeogenesis and from mobilization of stored carbohydrate, principally liver glycogen (1, 23). Since gluconeongenesis is limited in the infant (1, 15), liver glycogen synthesis and mobilization may be particularly important for maintaining circulating glucose levels.

Glycogen metabolism in liver is regulated by reciprocal changes in the activities of the rate-limiting enzymes of glycogen synthesis and degradation, glycogen synthetase and glycogen phosphorylase, respectively. The isolated adult rat liver regulates the activities of these two enzymes directly in response to circulating glucose concentration as well as in response to hormones (4, 12, 13, 20). The relative physiologic contributions of glucose and hormonal regulation of glycogen metabolism *in vivo* are unknown.

In a previous study with isolated, perfused newborn monkey liver (11), we provided evidence that glucose mobilization occurred in response to hypoglycemia or glucagon. However, net glucose uptake and activation of glycogen synthetase did not occur in response to hyperglycemia. This lack of response to hyperglycemia suggested to us that the infant primate liver may be limited in its ability to store glucose as glycogen in the presence of hyperglycemia, such as might occur in the portal vein during feeding. We also considered the possibility that other dietary constituents might regulate glycogen synthesis directly.

In an companion study (28), we have provided evidence that galactose as well as glucose regulates glycogen cycle enzyme activities and promotes glycogen synthesis in the isolated perfused liver of the adult rat. Galactose and glucose form lactose, the principal dietary carbohydrate in milk (6). In human milk, lactose provides 40% of the total calories (19). Galactose-metabolizing enzymes are well developed at birth (6). Because of the abundance of galactose in the newborn period, and the known enzymatic pathways linking galactose, glucose, and glycogen metabolism, we questioned whether galactose might play a major role in glycogen metabolism of the newborn primate liver.

In the present study we provide evidence that the isolated perfused liver of the near term monkey regulates glycogen enzyme activities in response to a combination of galactose, glucose, and insulin. We speculate that galactose may play a unique role in glycogen synthesis during the newborn period.

MATERIALS AND METHODS

ANIMALS

Thirteen pregnant rhesus monkeys (*Macaca mulatta*) with timed gestation ± 1 day were obtained from the primate colony at the National Institutes of Health. The gestational age ranged from 144–152 days (term = 164 days). After a 16-hr fast, animals were tranquilized with ketamine-HCl before anesthesia with halothane-nitrous oxide administered via an endotracheal tube. Immediately after delivery by hysterotomy, the livers of the newborn monkeys were perfused as described below.

The pregnant animals were used for *in utero* studies before liver perfusion. Six fetuses received intravenously 20 μg luteinizing hormone-releasing factor 1.5 hr before delivery. Six other fetuses received 20 μg thyroid hormone-releasing factor, 10 mg L-dihydroxyphenylalanine, or both, 1.5 hr before delivery. In all animals, fetal blood pH, pO_2, pCO_2, and glucose were in the normal range before delivery. In all animals receiving thyroid hormone-releasing factor or L-dihydroxyphenylalanine, levels of insulin and glucagon were in the normal range before delivery. Results of these intrauterine studies will be presented elsewhere (5). The observed normal baseline values at delivery and the similarity of responses within groups regardless of prior experience suggest no effect of *in utero* testing on perfusion results. The responses obtained in this study were independent of gestational age.

PERFUSION

The present study used an *in situ* recirculating perfusion system used previously for studies of liver glycogen metabolism in adult rats and near term monkeys (4, 11–13, 20). Perfusate, operative technique, and perfusion conditions are shown in Figure 1. This system provides perfusion *via* the umbilical vein and outflow *via* the inferior vena cava above the diaphragm. A closed system is established by clamping the hepatic artery, the portal vein, and the inferior vena cava above the renal vein. Perfusate oxygen tensions of 400–450 mm Hg and carbon dioxide tensions of 35–45 mm Hg are maintained; perfusate pH is between 7.39 and 7.42. Perfusate (3 ml/g liver) is recirculated to the livers at a rate of 1.5–2.0 ml/g liver/min, allowing slight expansion of the liver during the filling phase.

A standard protocol was used for the experiments presented in the figures and tables, except where noted. According to this protocol, each liver was perfused for three consecutive 30-min experimental periods. In any given experiment, insulin was either present or absent throughout the entire 90-min experiment, except as noted in text. The initial period began with glucose concentrations between 100 and 125 mg/100 ml, without added galactose. In the second and third periods, glucose and galactose concentrations were as indicated in the text. After the second period, the perfusate from the first flask was stopped and perfusion from a second flask was started without interruption of blood flow. A washout with approximately 20 ml perfusate preceded establishment of recirculating flow with the second flask.

Galactose was added to the perfusate in either the second or third experimental period. Livers removed galactose from the perfusate equally well when added in either the second or third periods. Galactose added in the second period was effectively removed from the perfusate by the washout between the second and third periods. The observed changes in perfusate glucose and galactose concentrations and in liver enzyme activities were similar when galactose was added in either period, and the results in the text are grouped according to perfusate composition (glucose, galactose, and insulin) but without regard to the order of testing.

In two perfusions as noted in the text, galactose was added during the first perfusion period in the absence of insulin, and insulin was added in the subsequent two periods.

The experimental design permitted comparison in the same liver of responses to different perfusate composition. Each liver thus served as its own control in order to cancel any variation in responses because of variation in perfusion or prior *in utero* testing.

SAMPLES

Serum samples at the experimental times indicated were taken from the pooled perfusate in the oxygenator. A liver biopsy was taken at the end of each experimental period. Hemostasis of the liver was accomplished with a ligature. Liver tissue samples were frozen immediately between aluminum blocks at the temperature of liquid nitrogen, and liver samples were stored in liquid nitrogen until assayed for enzyme activity.

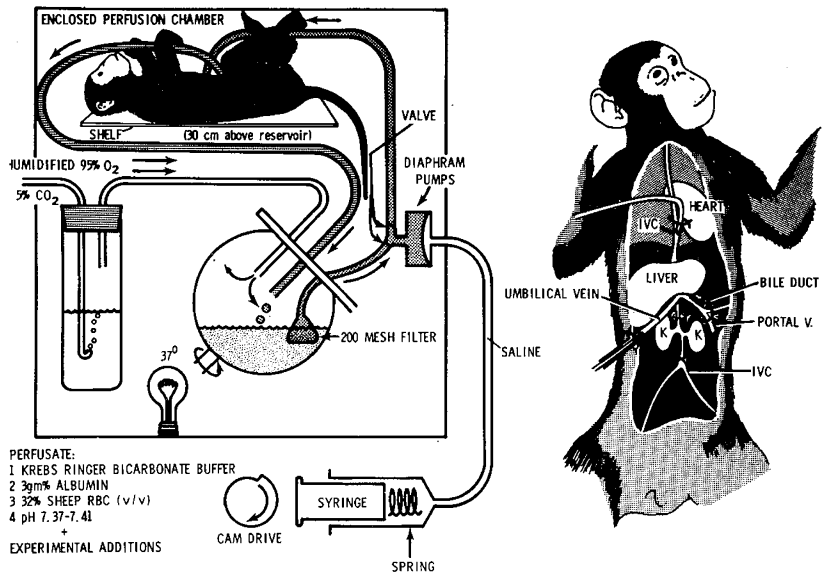

Fig. 1. Liver perfusion system. Perfusate is oxygenated with humidified 5% CO_2–95% O_2 in a rotating 500-ml film oxygenator and is pumped to the liver by a cam-driven diaphragm pump. Effluent perfusate returns from liver directly to the oxygenator. Operative procedure involves placing an inflow catheter in the umbilical vein and starting perfusate flow. The inferior vena cava and aorta are then transected below the left renal vein. The return catheter is placed through the right atrium into the inferior vena cava above the diaphragm and tied in place. The hepatic artery, portal vein, and inferior vena cava (*IVC*) above the renal veins are clamped, establishing a closed recirculating system. The bile duct is transected to prevent biliary stasis. *RBC*: red blood cells.

REAGENTS

Glucagon-free insulin, donated by Lilly Research Laboratories, was dissolved (1 mg/ml) and stored in 1 mM HCl. Before use, this stock solution was diluted with 0.9% NaCl containing 0.5% albumin. In experiments using insulin, insulin was present at an initial concentration of 10^{-7} M.

Galactose, obtained from Sigma Chemical Corporation, contained no detectable glucose by the glucose oxidase method used. Fraction V bovine serum albumin was purchased from Armour Pharmaceutical Company. Heparinized sheep erythrocytes were obtained from the National Institutes of Health animal facility and were washed three times in 0.9% NaCl before resuspension at 32% (v/v) in Krebs-Ringer bicarbonate buffer containing 3% albumin.

ASSAYS

Galactose and glucose were measured in plasma using Galactostat and Glucostat reagents from Worthington Biochemical Corporation. Glycogen levels, glycogen synthetase activity, and glycogen phosphorylase activity were assayed in liver tissue as described previously for fetal rat and monkey liver (10, 11, 26). Synthetase activity was assayed in the presence and the absence of 10 mM glucose 6-phosphate (10). The percentage of synthetase in the physiologically active, glucose 6-phosphate-independent form (I-form) was calculated (7) as: %I = activity without glucose 6-phosphate divided by activity with glucose 6-phosphate times 100. Total synthetase activity was 18.7 ± 0.9 units/g protein. Phosphorylase activity was assayed in the presence of 50 mM glucose 1-phosphate without added 5′-AMP, ATP, or Mg^{++} (26). Synthetase and phosphorylase were determined at a final tissue dilution of 1:200. Activity was proportional to time and to the dilution of tissue between 50- and 300-fold.

Radiochemicals were purchased from New England Nuclear Corporation. A synthetase unit is defined as the amount of enzyme which incorporates 1 μmol [^{14}C]glucose into glycogen from UDP[^{14}C]glucose per min at 30°. A phosphorylase unit is the amount of enzyme which incorporates 1 μmol [^{14}C]glucose into glycogen from [^{14}C]glucose 1-phosphate per min at 37°.

Glucose or galactose uptake or release from liver were estimated from observed changes in glucose or galactose concentrations in a known recirculating perfusate volume. Calculations involving liver weight were based on an estimation of liver weight as 5% of total body weight. Galactose elimination capacity was calculated as milligrams of galactose removed from the perfusate during each 30-min period per g liver.

RESULTS

GLYCOGEN SYNTHETASE

Liver samples were frozen rapidly at the end of each 30-min perfusion period and were assayed for glycogen synthetase activity in the presence and the absence of glucose 6-phosphate. The percentage of synthetase in the active form (%I) was calculated (Figs. 2, 3).

After 30 min of perfusion with glucose alone at concentrations as high as 700 mg/100 ml, the perfused monkey livers had consistently low %I synthetase activity (4% ± 1). When either insulin or galactose was added at the beginning of the perfusion period to achieve perfusate concentrations of 10^{-7} M or 250 mg/100 ml, respectively, %I glycogen synthetase at the end of 30 min increased, respectively, to 8% ± 2 ($P < 0.1$) and 8 ± 1% ($P < 0.05$).

When the combination of insulin and galactose was added to perfusate containing glucose, glycogen synthetase activity increased in proportion to perfusate glucose concentration between 200 and 400 mg/100 ml glucose. Glycogen synthetase activity observed with glucose concentrations between 300 and 400 mg/100 ml (40 ± 5%) was significantly higher than that which occurred in any other experimental group ($P < 0.01$). In the group containing glucose, galactose, and insulin, we observed no correlation between synthetase activity and galactose concentration when galactose concentration was varied between 100 and 300 mg/100 ml.

PHOSPHORYLASE

Figure 2 also shows liver phosphorylase activities after 30 min of perfusion. All values shown are low when compared with the values of 150–200 units/g protein which occur after exposure of the livers to 45 sec of hypoxemia.

Liver phosphorylase activity was highest in the group perfused with the lower glucose concentration in the absence of galactose or insulin. Activity in the higher glucose group was significantly reduced compared with the lower glucose group ($P < 0.05$). Addition of either galactose or insulin also reduced mean phosphorylase activity compared with the low glucose group ($P < 0.05$). Mean phosphorylase activity was lowest in the group which had high glucose, galactose, and insulin added to perfusate.

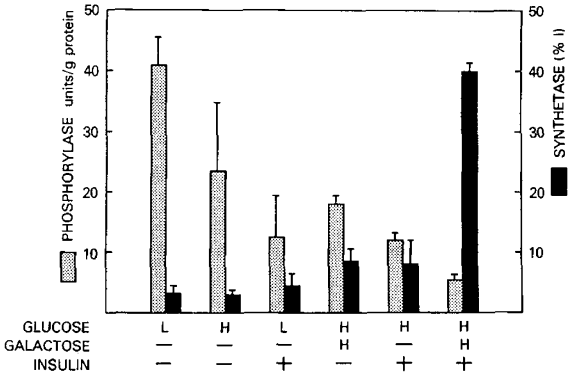

Fig. 2. Glycogen synthetase and phosphorylase activities with various combinations of perfusate glucose, galactose, and insulin. Values shown are means ± SEM of synthetase and phosphorylase activity after 30 min of perfusion under conditions shown. Numbers in each group are given in Table 1. For comparison, animals are grouped by presence (+) or absence (−) of insulin, at 10^{-7} M, and by glucose and galactose concentration. High concentrations (*H*) are between 275 and 400 mg/100 ml; lower concentrations (*L*) are between 100 and 250 mg/100 ml.

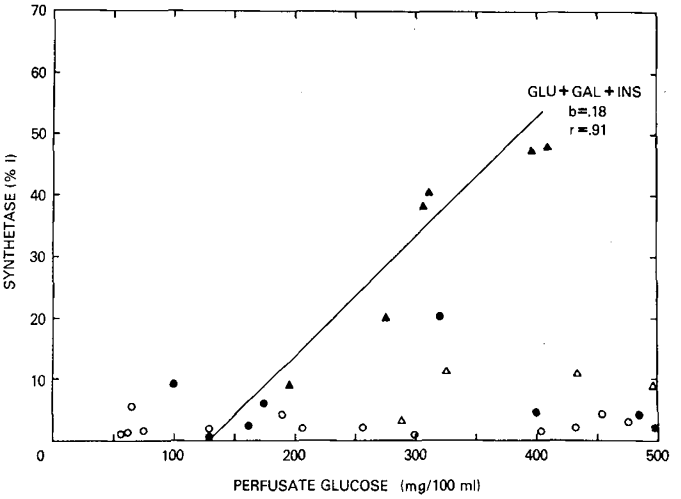

Fig. 3. Synthetase activities as a function of perfusate glucose concentration. Each point is the activity observed after 30 min of perfusion with glucose as shown. Circles represent perfusions with glucose added to perfusate with (●) and without (○) added insulin. Triangles represent perfusions with both glucose (*GLU*) and galactose (*GAL*) added to perfusate with (▲) and without (△) added insulin (*INS*). Regression line for combination of glucose, galactose, and insulin added to perfusate was calculated by the least-squares method.

GLYCOGEN

The mean glycogen level for all tissue samples assayed was 60.6 mg/g liver; standard deviation of the mean was 20.3 mg/g liver. Differences in the mean glycogen levels between any of the groups were not significantly different from zero.

Table 1 shows the net changes in liver glycogen concentration over the 30-min perfusion periods for the experimental group where initial and final samples were available. Mean increases in glycogen content were observed only in livers perfused with galactose, but the amount of net synthesis calculated was not significantly greater than zero.

HEXOSE UPTAKE AND RELEASE

Net uptake or release of hexose was estimated from the product of the fixed perfusate volume and the observed change in perfusate hexose concentration during the experimental period.

Each experimental group showed a net release of glucose into the perfusate. Addition of galactose or insulin to the perfusate decreased mean glucose release, but these decreases were not statistically significant. Galactose was taken up by all livers. The amount of galactose uptake exceeded the amount of glucose released, and significant total hexose uptake was observed in both groups of livers perfused with galactose.

Figure 4 details the time course of liver hexose uptake and release in the group given glucose, galactose, and insulin. It demonstrates the continuous uptake of galactose at a rate greater than the rate of glucose release. Figure 5 depicts the uptake of galactose in a single perfused liver before and after addition of insulin. Insulin did not appreciably alter galactose uptake. The rate of galactose uptake was independent of initial galactose concentration, supporting the possibility that uptake was maximal in the concentration range tested.

DISCUSSION

In a previous study with the isolated perfused liver of the near term rhesus monkey conceptus (11), we demonstrated that glucose release from liver was stimulated by glucagon, cyclic AMP, or hypoglycemia. The effects of these stimuli in monkey are thus similar to their effects in isolated, perfused liver of the adult rat (11, 20). However, the effects of hyperglycemia in the near term monkey were significantly different from its effects in adult rat liver (4, 13, 28). In the near term monkey (10), hyperglycemia did not stimulate glycogen synthesis, glucose uptake, or activation of glycogen synthetase. Insulin activated synthetase to a small degree, and this activation was not increased by increasing glucose concentration.

The present study confirms the lack of responsiveness of the near term monkey liver to increasing glucose concentrations. With increasing perfusate glucose concentration, no increase occurred in synthetase activity, glycogen levels, or glucose uptake. Glycogen phosphorylase activity was not reported in the previous study, and we now note additionally that phosphorylase activity is decreased to very low levels when perfusate glucose is increased above 100 mg/100 ml. We also note decreased phosphorylase activity in response to insulin.

In the previous study we speculated that the failure of newborn monkey liver to take up glucose, activate synthetase, and synthesize glycogen rapidly in response to hyperglycemia would not permit rapid synthesis of glycogen during feeding. An analagous situation in the human could contribute to glycogen depletion and

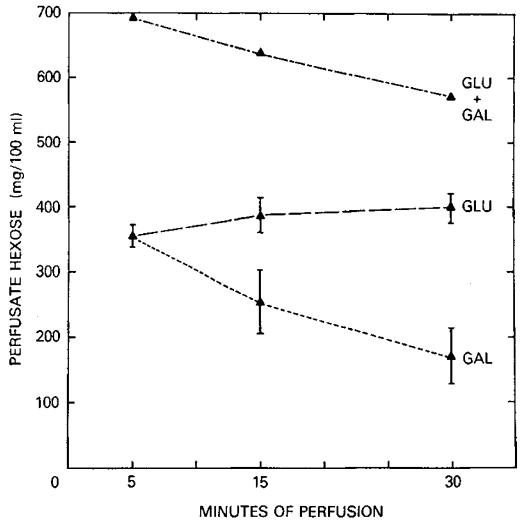

Fig. 4. Uptake and release of galactose (▲----▲) and glucose (▲- - -▲) in the presence of 10^{-7} M insulin as a function of time of perfusion. The top curve (▲----▲) represents arithmetic sum of the individual curves.

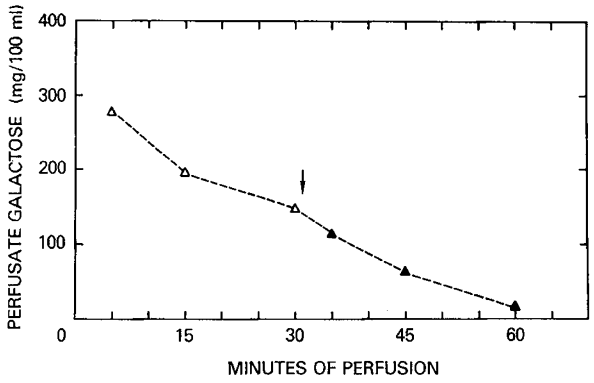

Fig. 5. Effect of insulin on galactose uptake. An individual liver was perfused for 30 min without insulin (△). Insulin was then added at 30 min (↓), and the liver was perfused an additional 30 min (▲). Galactose was added only at $t = 0$ and galactose concentrations were observed as shown.

Table 1. *Relationship between starting conditions, hexose uptake, and glycogen synthesis during 30-min perfusion periods*[1]

Initial			Net uptake (mg/g liver)			ΔGlycogen mg/g liver	*n*
Glucose	Galactose	Insulin	ΔGlucose	ΔGalactose	ΔHexose		
H	H	–	−0.45 ± 0.81	+2.73 ± 0.91	+2.28 ± 1.68	+5 ± 7	4
H	H	+	−0.99 ± 0.28	+4.1 ± 0.99	+3.1 ± 1.0	+4 ± 3.5	6
H	0	–	−1.35 ± 0.60	0	−1.35 ± 0.60	0 ± 9	4
H	0	+	−0.69 ± 1.68	0	−0.69 ± 1.68	0 ± 7	4
L	0	–	−2.67 ± 1.05	0	−2.67 ± 1.05		4
L	0	+	−2.37 ± 0.60	0	−2.37 ± 0.60		4

[1] H: High concentration; L: low concentration. For the initial conditions, H = 275–400 mg/100 ml, L = 125–250 mg/100 ml. Hexose uptake is expressed in milligrams hexose per 30-min period per g liver. Positive values reflect uptake; negative values reflect release. Values are mean ± SEM.

hypoglycemia during fasting or when peripheral glucose utilization is increased. However, hypoglycemia is not frequent in otherwise normal human newborns (24), and we considered that other dietary components might regulate or provide substrate for liver glycogen synthesis.

Glycerol and fatty acids have been observed in the rat to provide substrate for glycogen synthesis only after the newborn period (17), and gluconeogenesis from protein in many species, including the human, occurs only at a slow rate in newborns (1, 15). In contrast, galactose infusion is accompanied by rapid elevation of blood glucose in the human neonate (2, 16, 21), and the metabolic pathway of galactose metabolism (Fig. 6) involves its conversion to the substrate for glycogen synthesis, UDP-glucose (6). Although it is generally assumed (1, 23) that galactose is metabolized to glycogen in infants, both increased and decreased liver glycogen levels have been observed after large galactose feedings in adults (3, 14). In a recent study using the isolated perfused adult rat liver, we demonstrated increased liver glycogen after perfusion with galactose (28). We also noted that galactose regulated the activities of glycogen synthetase and phosphorylase. We therefore questioned whether galactose regulation of glycogen synthesis, observed in the adult rat liver, is developed in the perfused newborn monkey liver.

The observed data in the present series of isolated monkey liver perfusions are consistent with a role for galactose in newborn monkey liver glycogen regulation. When the livers were perfused with glucose and galactose, we observed net hexose uptake and significant activation of glycogen synthetase, both with and without added insulin. Phosphorylase activity was diminished by galactose. Significant net hexose uptake was observed only with galactose present. When the combination of glucose, galactose, and insulin was added to the perfusate, synthetase was not only activated 10-fold, but also the degree of activation was proportional to perfusate glucose concentration. This is qualitatively similar to the glucose concentration dependence observed in the adult rat liver perfused under similar conditions (28), and this concentration dependence of synthetase activation may be important for the rapid synthesis of glycogen during feeding.

The lack of glucose uptake by the near term monkey liver in perfusion is a consistent finding under all conditions tested. It is possible that the lack of glucose uptake reflects some acute operative consequence of the perfusion procedure. However, galactose was taken up rapidly in the perfused near term monkey liver. As galactose uptake is used in humans and experimental animals in both adults (3, 18) and infants (2, 16) as an index of liver function, this same index provides no evidence of significant impairment of carbohydrate metabolism in our system. Additionally, the perfused newborn monkey liver regulates pH, pCO_2, and pO_2 within an appropriate range, and we have previously shown maintainance of potassium with the normal range (11). Finally, using the same perfusion system, the adult rat takes up glucose during hyperglycemia (28). For these reasons, we conclude that the lack of net glucose uptake by the perfused near term monkey liver is not artifactual, and it is possible that this may be characteristic of newborn primate liver. While newborn human and nonhuman primates fed glucose demonstrate net glucose uptake, the contribution of liver to this glucose uptake in the whole organism is difficult to assess.

The levels of glucose and galactose used in the present study were chosen to be comparable with the portal vein hexose concentrations after a lactose feeding. A newborn receiving from milk 100 cal/kg/24 hr will ingest approximately 10 g lactose /kg and therefore 5 g each of glucose and galactose/kg/24 hr. If fed five to eight times/24 hr, ingested glucose and galactose may each be on the order of 600–1000 mg/kg/feeding. Portal vein glucose concentrations after ingestion of large amounts of glucose in adult dogs with chronic portal vein catheters may reach 400 mg/100 ml (27); similar data in newborns or with galactose are not available.

The role of insulin in the perfused newborn monkey liver is not clear. In the perfused adult rat liver, insulin does not activate synthetase directly (28), although it antagonizes the inactivation of synthetase in response to glucagon, cyclic AMP, or hypoglycemia (12, 20). However, insulin has been reported to activate synthetase in cultured fetal rat liver explants (9, 10), and we also observed activation in the perfused monkey liver (11). In the absence of net carbohydrate uptake or net glycogen synthesis, it is difficult to interpret this enzymatic change. In further studies, we are attempting to define the role of insulin.

We conclude that the perfused near term monkey liver, unlike the perfused adult rat liver, does not regulate glycogen metabolism directly in response to glucose concentration alone and that galactose is necessary in this system for substrate regulation of glycogen synthesis.

We speculate that galactose is uniquely necessary in the regulation of liver glycogen metabolism in the newborn primate.

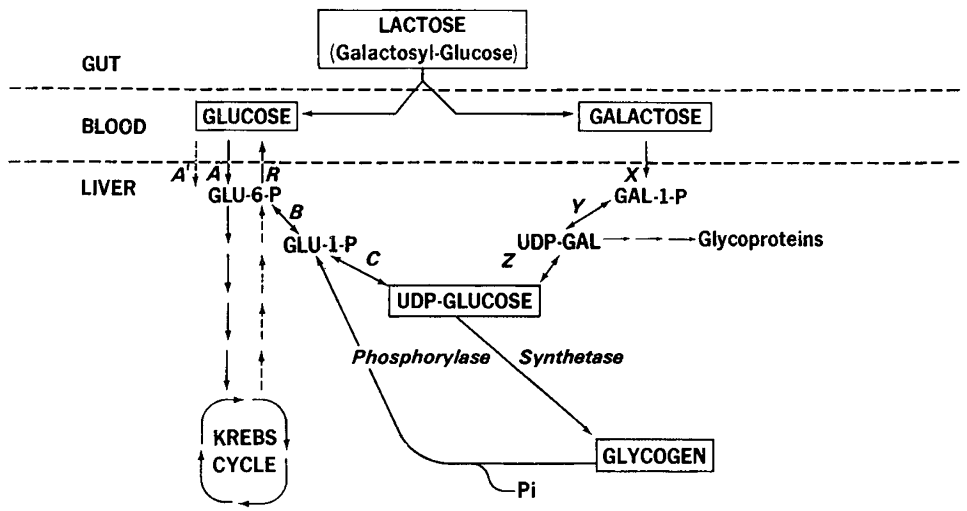

Fig. 6. Metabolism of lactose. Ingested dietary lactose is hydrolyzed in the gastrointestinal tract into the component sugars galactose and glucose. These sugars are transported to the liver *via* the portal vein. Glucose is phosphorylated to glucose 6-phosphate by hexokinase, enzyme A (*GLU-6-P*) (*A*), in adults and newborns, and by glucokinase (*A'*) in adults but not newborns. Glucose 6-phosphate can enter Emden-Myerhof or other metabolic pathways, or be converted to glucose 1-phosphate (*GLU-1-P*) by phosphoglucomutase (*B*), and proceed to UDP-glucose by action of enzyme (*C*) UDP-glucose pyrophosphorylase. Galactose is phosphorylated in liver by galactokinase (*X*) to galactose 1-phosphate (GAL-1-P), which is metabolized to UDP-galactose (*UDP-GAL*) by galactose 1-phosphate uridyltransferase (*Y*) and epimerized to UDP-glucose by epimerase (*Z*). Glycogen is synthesized from UDP-glucose by glycogen synthetase and degraded by glycogen phosphorylase to glucose-1-phosphate. (Adapted from Reference 6.)

Galactose provides approximately 20% of the total calories in mature human milk, and a similar proportion is found in human colostrum and transitional milk (19). Galactose is thus abundant throughout infancy and, in particular, during the immediate postnatal period (25) when glycogen levels and blood glucose concentrations are lowest. If liver glycogen plays an important role in acute glucose homeostasis in the newborn (25), then it follows from our speculation that galactose may be secondarily important by ensuring glycogen stores (Fig. 6) adequate to maintain normoglycemia.

In spite of its abundance, the significance of galactose in normal milk is not known. It seems unlikely that its presence is accidental (6). It is possible that the regulation of glycogen metabolism by galactose observed in the present study may be important to the newborn. Galactose has also been found to augment glucose utilization in isolated adult adipose cells (22), and galactose could alter other organ systems in an analagous manner. Clearly, further studies are warranted to determine the physiologic role of galactose in the normal infant diet.

SUMMARY

The isolated, perfused liver of the near term newborn monkey does not regulate glycogen synthesis in response to glucose concentration. Addition of either insulin or galactose to the perfusate elevates glycogen synthetase activity and decreases phosphorylase activity without an accompanying net glucose uptake. When a combination of galactose insulin and glucose are added to the perfusate, the perfused newborn liver regulates synthetase activity in response to perfusate glucose concentration. With this combination, net hexose uptake is observed and phosphorylase activity is very low.

REFERENCES AND NOTES

1. Ballard, F. J.: Carbohydrates. In: U. Stave: Physiology of the Perinatal Period, Vol. 1, Chap. 13, pp. 417-440 (Appleton-Century-Crofts, New York 1970).
2. Beyreiss, K.: Quantitative Untersuchungen über den Umsatz von Galaktose Neugeborener, Saüglinge und Erwachsener bei intravenöser Galaktose-infusion. Acta Biol. Med. Germ., *27:* 125 (1971).
3. Bollman, J. L., Mann, F. C., and Power, M. H.: The utilization of galactose following complete removal of the liver. Amer. J. Physiol., *111:* 483 (1935).
4. 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. Nat. Acad. Sci. U. S. A., *65:* 383 (1970).
5. Chez, R. A.: Personal communication.
6. Cohn, R. M. and Segal, S.: Galactose metabolism and its regulation, Metabolism, *22:* 627 (1973).
7. Cornblath, M., Wybregt, S. H., and Baens, G. S.: Studies of carbohydrate metabolism in the newborn infant. Pediatrics, *32:* 1007 (1963).
8. Curnow, R. T., Rayfield, E. J., George, D. T., Zenser, T. V., and DeRubertis, F.: Control of hepatic glycogen metabolism in the rhesus monkey: The effect of glucose, insulin and glucagon administration. Amer. J. Physiol., *228:* 80 (1974).
9. Eisen, H. J., Glinsmann, W. H., and Sherline, P.: Effect of insulin on glycogen synthesis in fetal rat liver in organ culture. Endocrinology, *92:* 584 (1973).
10. Eisen, H. J., Goldfine, I. D., and Glinsmann, W. H.: Regulation of hepatic glycogen synthesis during fetal development: Roles of hydrocortisone, insulin and insulin receptors. Proc. Nat. Acad. Sci. U. S. A., *70:* 3454 (1973).
11. Glinsmann, W. H., Eisen, H. J., Lynch, A., and Chez, R. A.: Glucose regulation by isolated near term monkey liver. Pediat. Res., *9:* 600 (1975).
12. Glinsmann, W. H., and Mortimore, G. E.: Influence of glucagon and 3',5' AMP on insulin responsiveness of the perfused rat liver. Amer. J. Physiol., *215:* 553 (1968).
13. Glinsmann, W. H., Pauk, G., and Hern, E.: Control of rat liver glycogen synthetase and phosphorylase activities by glucose. Biochem. Biophys. Res. Commun., *39:* 774 (1970).
14. Handler, P.: The biochemical defect underlying the nutritional failure of young rats on diets containing excessive quantities of lactose or galactose. J. Nutr., *33:* 221 (1947).
15. Haymond, M. W., Karl, I. E., and Pagliura, A. S.: Increased gluconeogenic substrates in the small-for-gestational age infant. N. Engl. J. Med., *291:* 322 (1974).
16. Hjelm, M., and Sjölin, S.: Changes in the elimination rate from blood of intravenously injected galactose during the neonatal period. J. Clin. Invest., *18*(suppl. 92): 126 (1966).
17. Johnson, J. D., Hurwitz, R., and Kretchmer, N.: Utilization of fat and glycerol for glycogenesis by the neonatal rat. J. Nutr., *101:* 299 (1971).
18. Keiding, S., Andreansen, P. B., and Fauerholdt, L.: Effect of phenobarbital induction on galactose elimination capacity in the rat. Biochem. Pharmacol., *22:* 2293 (1973).
19. Macy, I. G., Kelly, H. J., and Sloan, R. E.: The Composition of Milks (National Academy of Sciences—National Research Council Publication no. 254, Washington, D. C., 1953).
20. Mortimore, G. E., King, E., Jr., Mondon, C. E., and Glinsmann, W. H.: Effects of insulin on net carbohydrate alterations in perfused rat liver. Amer. J. Physiol., *212:* 179 (1967).
21. Mulligan, P. B., and Schwartz, R.: Hepatic carbohydrate metabolism in the genesis of neonatal hypoglycemia. Pediatrics, *30:* 125 (1962).
22. Naito, C., and Okada, K.: Stimulation of glucose metabolism by galactose in isolated adipocytes from rats. J. Biol. Chem., *249:* 1657 (1974).
23. Pagliara, A. S., Karl, I. E., Haymond, M., and Kipnis, D. M.: Hypoglycemia in infancy and childhood. J. Pediatr., *82:* 365, 558 (1973).
24. Pildes, R. Forbes, A. E., O'Connor, S. M., and Cornblath, M.: The incidence of neonatal hypoglycemia—A completed survey. J. Pediat., *70:* 76 (1967).
25. Shelley, H. J., and Neligan, G. A.: Neonatal hypoglycemia. Brit. Med. Bull., *22:* 34 (1966).
26. Sherline, P., Eisen, H., and Glinsmann, W.: Acute hormonal regulation of cyclic AMP content and glycogen phosphorylase in fetal liver in organ culture. Endocrinology, *94:* 935 (1974).
27. Shoemaker, W. C., Yanof, H. M., Turk, L. N., and Wilson, T. H., Glucose and frutose absorption in the unanesthetized dog. Gastroenterology, *44:* 654 (1963).
28. Sparks, J. W., Lynch, A., and Glinsmann, W. H.: Regulation of rat liver glycogen synthesis and activities of glycogen cycle enzymes by glucose and galactose. Metabolism (In press).
29. Widdows, S. T., Lowenfeld, M. F., Bond, M., Shiskin, C., and Taylor, E. I.: A study of the antenatal secretion of the human mammary gland and a comparison between this and the secretion obtained directly after birth. Biochem. J., *29:* 1145 (1935).
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