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COMPARATIVE UTILIZATION OF GLYCEROL AND ALANINE AS LIVER GLUCONEOGENIC SUBSTRATES IN THE FED LATE PREGNANT RAT

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Abstract—1. The appearance of plasma [^{14}C]glucose in the inferior cava vein after a pulse of 0.2 mmol of [U-^{14}C]L-alanine or [U-^{14}C]glycerol/200 g body wt given through the portal vein was studied in fed 21 day pregnant rats and virgin controls under pentobarbital anesthesia. In both groups values were much higher when [U-^{14}C]glycerol was the administered tracer than when [U-^{14}C]L-alanine, and they were augmented in pregnant versus virgin animals at 1 min when receiving [U-^{14}C]glycerol and at 2 min when receiving [U-^{14}C]L-alanine.

2. 20 min after the tracers rats receiving [U-^{14}C]glycerol showed much higher liver [^{14}C]glycogen and [^{14}C]glyceride glycerol than those receiving [U-^{14}C]L-alanine. Radioactivity present in liver as [^{14}C]glyceride glycerol was greater in pregnant than in virgin rats receiving [U-^{14}C]glycerol whereas radioactivity corresponding to [^{14}C]fatty acids was lower in the former group receiving either tracer.

3. At 20 min after maternal treatments fetuses showed lower plasma [^{14}C]glycerol than [^{14}C]alanine values but plasma [^{14}C]glucose and liver [^{14}C]glycogen values were much greater in fetuses from mothers receiving [U-^{14}C]glycerol than [U-^{14}C]L-alanine.

4. Besides showing the higher gluconeogenic efficiency in pregnant than in virgin rats, results indicate that at late gestation glycerol is used as a preferential substrate for both glucose and glyceride glycerol synthesis in liver.

INTRODUCTION

The continuous and intense extraction of maternal glucose by the fetoplacental structures in the late fed pregnant rat seems to be the primary cause for hypoglycemia (Herrera *et al.*, 1969; Battaglia and Meschia, 1978) since glucose utilization by maternal tissues is reduced (Leturque *et al.*, 1981) and gluconeogenesis was found either unchanged (Herrera *et al.*, 1969; Zorzano and Herrera, 1984a) or enhanced (Gilbert and Ricquier, 1977; Chaves and Herrera, 1980), depending on the nature of the substrate used. Following a comparative study with the same concentration of differently administered substrates we recently reported in the starved rat that glycerol was more efficiently converted into glucose than L-alanine or pyruvate. This process was enhanced in pregnant as compared with nonpregnant control animals (Zorzano *et al.*, 1986). Circulating levels of glycerol are enhanced at late gestation in both the fed and starved rat (Chaves and Herrera, 1980; Zorzano *et al.*, 1986) as a consequence of augmented adipose tissue lipolysis (Knopp *et al.*, 1970; Chaves and Herrera, 1978), raising the possibility that also in the fed state glycerol could constitute a preferential gluconeogenic substrate for the mother. We recently reported that in contrast to how it occurs in the starved animals, pentobarbital anesthesia does not modify the rate of gluconeogenesis in the late pregnant rat (Zorzano and Herrera, 1984a). In the present work the liver production of glucose after a pulse of [U-^{14}C]L-alanine or [U-^{14}C]glycerol in the portal vein to pentobarbital anesthesized fed 21 day pregnant rats and virgin controls was studied. This technique has been previously used to study liver gluconeogenesis in other experimental conditions (Soley *et al.*, 1983; Soley *et al.*, 1985a; Soley *et al.*, 1985b) allowing a rapid sample collection in order to avoid differences in peripheral glucose utilization which could vary between pregnant and nonpregnant rats. The investigation was extended to determine the appearance of radioactivity in liver glycogen and lipidic fractions as well as in fetal circulation.

MATERIALS AND METHODS

Sprague–Dawley female rats from our own colony were mated when weighing 160 g and gestation was timed from the appearance of spermatozoids in vaginal smears. Sex and age-matched virgin rats were studied in parallel. Animals were housed in collective cages in a light cycle and temperature controlled room (12 hr on–off; 23 ± 1°C) and fed *ad libitum* with Purina chow pellets. To determine the steady-state concentration of metabolites, animals were decapitated without anesthesia and blood samples collected from the neck wound. Protein precipitation was carried out in plasma aliquots with $ZnSO_4$ and $Ba(OH)_2$ (Somogyi, 1945) for the analysis of glucose (Huggett and Nixon, 1957),

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whereas it was done with 5% $HClO_4$ for the analysis of glycerol (Garland and Randle, 1962) and with 10% sulfosalycilic acid in 0.1 N HCl for the analysis of alanine by using a Beckman 121 MB autoanalyzer (Martin del Rio and Latorre Caballero, 1980). Immediately after killing the animals, an aliquot of liver was placed in liquid nitrogen. This was digested with 40% KOH in boiling water bath for 20 min and used for glycogen purification (Good *et al.*, 1933) and glucose determination (Huggett and Nixon, 1957) after acid hydrolysis. For gluconeogenetic studies, rats were anesthesized by the i.v. injection of 33 mg/kg body wt pentobarbital and were subjected to the surgical procedure previously described (Soley *et al.*, 1983). Briefly, it consisted in introducing a catheter (PE-10 Intramedic, Clay Adams, NJ, U.S.A.) through the ileocolic vein up to the level of the portal vein. At this step special attention was paid in handling the conceptus to avoid any alteration in blood flow. After suturing the abdominal wall, another cannula (PE-50 Intramedic, Clay Adams, NJ, U.S.A.) was introduced through the right jugular vein passing by the superior cava vein down to the level of the suprahepatic veins. Once surgery was finished, a saline–albumin solution (0.5 g bovine albumin/l of 0.9% NaCl) was infused through the cannula in the portal vein, at a rate of 5 ml/hr for 10 min, after which a pulse of 10 μCi and 0.2 mmol per 200 g body wt of either [U-^{14}C]L-alanine or [U-^{14}C]glycerol (The Radiochemical Centre, Amersham, U.K.) was administered. This was followed by an infusion with saline–albumin until the end of the experiment. Blood aliquots were collected from the catheter placed in the suprahepatic veins at different times after the tracer. At 20 min an aliquot of liver was placed into liquid nitrogen and fetuses were rapidly excised and decapitated for blood and liver collection to be processed as were the mother's. When [U-^{14}C]L-alanine was administered, plasma or blood aliquots were deproteinized with 5% $HClO_4$ and neutralized supernatants used for glucose and alanine determinations, as indicated above and for the purification of [^{14}C]alanine, [^{14}C]glucose and [^{14}C]lactate by ion-exchange chromatography (Zorzano and Herrera, 1984b), being 97, 88 and 90% their respective recoveries when [^{14}C]labelled standards were added to plasma samples. When [U-^{14}C]glycerol was used, plasma samples were deproteinized with acetone, and [^{14}C]glucose, [^{14}C]lactate and [^{14}C]glycerol were separated by ascending paper chromatography (Chaves and Herrera, 1980). With this technique, recuperation of [^{14}C]labelled standards of [^{14}C]glucose, [^{14}C]lactate and [^{14}C]glycerol was 92, 90 and 86% respectively. Aliquots of frozen livers were purified by ethanol precipitation (Good *et al.*, 1933) from alkali digests and hydrolyzed with 5 N H_2SO_4 for 2 hr at 100°C, for radioactivity counting and glucose determination (Huggett and Nixon, 1957). This procedure was previously validated for [^{14}C]glycogen estimations (Herrera *et al.*, 1969). Another aliquot of each frozen liver was used for lipid purification (Folch *et al.*, 1957) and fractionation (Carmaniu and Herrera, 1980). Radioactive measurements were performed in a PPO/POPOP based scintillation cocktail dissolved in xylene and Triton X-100. All radioactive values were adjusted to an initial value of 1×10^6 dpm/200 g body wt as the administered tracer. Statistical analysis of the data was done with the Student's *t*-test.

Table 1. Corporal and metabolic parameters in 21-day pregnant rat

	Virgin	Pregnant
Body wt (g)	231 ± 9	409 ± 9***
Liver wt (g)	7.1 ± 0.3	13.0 ± 0.6***
Blood glucose (mM)	5.4 ± 0.3	4.3 ± 0.1**
Hepatic glycogen (%)	3.9 ± 0.6	4.1 ± 0.3
Plasma alanine (μM)	579 ± 52	828 ± 42**
Plasma glycerol (μM)	81 ± 16	152 ± 21*

Rats were decapitated and blood collected from the neck wound into heparinized receptacles while liver aliquots were placed into liquid nitrogen. Results are means ± SE of 6–10 rats per group. Statistical comparisons between values from virgin and pregnant rats are indicated by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

RESULTS

At day 21 of gestation the pregnant rats showed augmented body and liver weights as compared to virgin controls (Table 1). Liver glycogen concentration was unchanged but blood glucose was reduced and plasma alanine and glycerol concentrations were enhanced in pregnant versus virgin rats (Table 1).

The appearance of plasma [^{14}C]glucose in the inferior cava vein after portal administration of a pulse of 0.2 mmol of [U-^{14}C]L-alanine or [U-^{14}C]glycerol per 200 g body wt, was used as an index of liver glucose production.

Results after [U-^{14}C]L-alanine administration are summarized in Table 2. Appearance of [^{14}C]glucose in plasma increased progressively with time reaching a plateau phase between 15 and 20 min. At 2 min after [U-^{14}C]L-alanine, plasma [^{14}C]glucose was higher in pregnant than in virgin animals but values were similar thereafter in both groups. Plasma [^{14}C]alanine was highest in both groups at 2 min and decreased

Table 2. Plasma [^{14}C]glucose and [^{14}C]alanine values and alanine concentration after portal injection of [U-^{14}C]alanine in 21-day pregnant rats

Min. after the tracer	Formation of [^{14}C]glucose (dpm/ml)	[^{14}C]alanine (dpm/ml)	Plasma alanine (μM)
	Virgin		
2	188 ± 27	9519 ± 812	
5	444 ± 52	3489 ± 93	2311 ± 180
10	626 ± 45	1435 ± 54	
15	703 ± 94		
20	885 ± 115	939 ± 26	851 ± 41
	Pregnant		
2	371 ± 60*	15,113 ± 1921	
5	538 ± 52	8412 ± 298***	3556 ± 84**
10	736 ± 37	2884 ± 336*	
15	830 ± 137		
20	800 ± 151	1502 ± 233	2217 ± 452*

Rats were injected through the portal vein with 10 μCi/0.2 mmol of [U-^{14}C]alanine per 200 g body wt and blood was collected at different times from the suprahepatic vein. Results are means ± SE of 5–6 rats per group. Statistical comparisons between values from virgin and pregnant rats are indicated by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3. Plasma [^{14}C]glucose and [^{14}C]glycerol values and glycerol concentration after portal injection of [U-^{14}C]glycerol in 21-day pregnant rats

Min. after the tracer	Formation of [^{14}C]glucose (dpm/ml)	[^{14}C]glycerol (dpm/ml)	Plasma glycerol (μM)
	Virgin		
1	782 ± 62	20,457 ± 3160	4000 ± 87
2	1735 ± 207	14,946 ± 1204	2987 ± 696
5	4620 ± 540	3713 ± 785	520 ± 135
20	5206 ± 428	836 ± 288	91 ± 40
	Pregnant		
1	1110 ± 169*	27,227 ± 3366	5260 ± 806
2	2182 ± 230	20,374 ± 2758	2608 ± 432
5	3900 ± 304	5749 ± 1062	795 ± 249
20	4055 ± 325	879 ± 210	137 ± 46

Rats were injected through the portal vein with 10 μCi/0.2 mmol of [U-^{14}C]glycerol per 200 g body wt and blood was collected at different times from the suprahepatic vein. Results are means ± SE of 5–6 observations per group. Statistical comparisons between values from virgin and pregnant rats are indicated by asterisks: *$P < 0.05$.

thereafter. This parameter always tended to be higher in pregnant than in virgin rats, the difference attaining statistical significance at 5 and 15 min. Plasma alanine concentrations were measured at 5 and 20 min after the tracer, and at both times values appeared higher in pregnant than in virgin rats. When [U-^{14}C]glycerol was the administered tracer, more rapid and much higher amounts of [^{14}C]glucose appeared in the inferior cava vein plasma (Table 3) than with [U-^{14}C]L-alanine (Table 2), the plateau level being reached at 5 min (Table 3). A slight but significant increase in plasma [^{14}C]glucose appearance was found 1 min after [U-^{14}C]glycerol in pregnant vs virgin rats but the difference disappeared thereafter. Plasma [^{14}C]glycerol values and glycerol concentration progressively decreased after the administration of the labelled substrate and no differences for either parameter were found between pregnant and virgin rats.

As shown in Table 4, at 20 min after either tracer liver [^{14}C]glycogen did not differ between pregnant and virgin rats but values were much higher when [U-^{14}C]glycerol was the administered tracer than when [U-^{14}C]L-alanine. Appearance of the label in liver fatty acids was similar from either tracer and it was lower in pregnant than in virgin rats whereas [^{14}C]glyceride glycerol was much greater from [U-^{14}C]glycerol than from [U-^{14}C]L-alanine, and it was significantly enhanced in pregnant rats with the former tracer (Table 4).

In Table 5 ^{14}C-values in fetuses at 20 min after maternal treatments with the labelled substrates are summarized. Plasma [^{14}C]glycerol was significantly lower than [^{14}C]alanine and fetal/maternal ratio of

Table 4. Incorporation of [^{14}C]alanine and [^{14}C]glycerol into hepatic glycogen and lipids in 21-day pregnant rats

	[^{14}C]glycogen (dpm/g)	^{14}C-fatty acids (dpm/g)	[^{14}C]glyceride glycerol (dpm/g)
	[U-^{14}C]alanine		
Virgin	127 ± 23	586 ± 156	287 ± 47
Pregnant	83 ± 25	149 ± 25*	112 ± 50
	[U-^{14}C]glycerol		
Virgin	1666 ± 393	359 ± 150	1931 ± 138
Pregnant	1087 ± 540	136 ± 24*	2357 ± 91*

Rats were injected through the portal vein with 10 μCi/0.2 mmol of [U-^{14}C]alanine or [U-^{14}C]glycerol per 200 g body wt. After 20 min an aliquot of liver was collected for purification of [^{14}C]glycogen and different fractions of [^{14}C]lipids. Results are means ± SE of 5–6 per group. Statistical comparisons between values from virgin and pregnant rats are indicated by asterisks: *$P < 0.05$.

Table 5. Metabolite concentration, radioactivity in [^{14}C]plasma metabolites and in hepatic [^{14}C]glycogen in fetuses from 21-day pregnant rats after maternal administration of [U-^{14}C]alanine or [U-^{14}C]glycerol

	[U-^{14}C]alanine	[U-^{14}C]glycerol
Fetal [^{14}C]substrate (dmp/ml)	882 ± 88	259 ± 104***
Fetal/maternal [^{14}C]substrate ratio	0.57 ± 0.08	0.26 ± 0.08*
Fetal [^{14}C]glucose (dpm/ml)	374 ± 66	2187 ± 270***
Fetal/maternal [^{14}C]glucose ratio	0.49 ± 0.04	0.51 ± 0.04
Fetal hepatic [^{14}C]glycogen	58 ± 6	188 ± 25***

Metabolite concentration and radioactivity in plasma and in liver were studied in fetuses after 20 min of the maternal administration of [U-^{14}C]alanine or [U-^{14}C]glycerol. Results are means ± SE of 5–6 observations per group. Statistical comparisons between fetal values after maternal administration of [^{14}C]alanine and [^{14}C]glycerol are indicated by asterisks: *$P < 0.05$; ***$P < 0.001$.

these parameters were lower for [^{14}C]glycerol than for [^{14}C]alanine. Conversely, fetal plasma [^{14}C]glucose was much higher when the mother received [U-^{14}C]glycerol than [U-^{14}C]alanine. This difference was similar to that found in mother's plasma, producing a fetal/maternal plasma [^{14}C]glucose ratio which did not differ for [U-^{14}C]glycerol or [U-^{14}C]L-alanine. Similarly to plasma [^{14}C]glucose values liver [^{14}C]glycogen appeared significantly higher in fetuses from mothers receiving [U-^{14}C]glycerol than those receiving [U-^{14}C]L-alanine (Table 5).

DISCUSSION

Present results show that early after a pulse of either labelled L-alanine or glycerol, liver glucose production in the late pregnant rat is slightly enhanced as compared to virgin animals shortly after the tracer whereas the difference disappeared at a longer period. These findings agree with reported unchanged or augmented *in vivo* gluconeogenesis from the same (Zorzano and Herrera, 1984a; Gilbert and Ricquier, 1977; Chaves and Herrera, 1980) or other substrates (Herrera *et al.*, 1969) and the unchanged activity of key gluconeogenic liver enzymes (Smith, 1975; Diamant and Shafrir, 1972) in fed pregnant rats. Altogether they indicate that maternal hypoglycemia is a consequence of enhanced glucose disappearance rather than impaired glucose synthesis. This effect must be the result of the intense use of glucose by the fetoplacental structures as glucose utilization by maternal tissues is reduced at late gestation, as already reported (Leturque *et al.*, 1981). Basal concentrations of plasma alanine and glycerol were found augmented in the pregnant rat, but the tracers were administered diluted with cold substrates to minimize endogenous differences between the groups. As the rate of hepatic gluconeogenesis is known to be limited in normal conditions by the availability of substrates (Exton and Park, 1967; Mallette *et al.*, 1969; Aikawa *et al.*, 1972), greater differences in glucose synthesis would have been found between pregnant and virgin animals if not such substrate dilution had been done. This manoeuver however allows to compare the gluconeogenic efficiency of the two substrates studied, and the observed results substantiate a much more efficient conversion of glycerol than alanine to glucose. It could be argued that administered alanine is diluted with an unlabelled pool of oxaloacetate as proposed in other conditions (Vinay *et al.*, 1978; Hetenyi, 1982), whereas glycerol enters gluconeogenesis at the dihydroxyacetone phosphate level. Nonetheless the low specific activity of the administered tracers and the enormous difference in the rate of glucose formation found between alanine and glycerol allow us to conclude that in equimolecular amounts, glycerol is a more efficient gluconeogenic substrate than alanine. This view acquires major importance at late gestation where maternal adipose tissue mass is augmented (Moore and Brasel, 1984), lipolysis accelerated (Knopp *et al.*, 1970; Chaves and Herrera, 1978) and materno-fetal glycerol transfer is lower than other metabolites, including alanine (Palacin *et al.*, 1983). These changes lead to a permanent and augmented availability of glycerol in maternal circulation to be preferentially used as gluconeogenic substrate. Present findings on the appearance of lower amounts of [^{14}C]glycerol than [^{14}C]alanine in fetal circulation after their respective administration to the mother, and the appearance of much greater amounts of [^{14}C]glucose when [^{14}C]glycerol was the administered tracer support this hypothesis. Observed differences in fetal/maternal ratios of plasma ^{14}C-substrates after their administration to the mother are also consistent with the lower placental transfer of glycerol than alanine. Unmodified fetal/maternal plasma [^{14}C]glucose ratio values in spite of great differences in absolute values between the two substrates clearly indicate the direct dependence of the fetus on mother's glucose. This finding supports the notion that late rat fetus does not make gluconeogenesis from either glycerol or alanine which is in agreement with most of the previous reports (Philippidis and Ballard, 1969; Provost *et al.*, 1980).

The injected gluconeogenic substrates were also partially converted into mother's liver lipids. Decreased appearance of liver ^{14}C-fatty acids after either [^{14}C]alanine or glycerol administration in pregnant rats, agrees with the reported decreased rate of lipogenesis from ^3H_2O (Lorenzo *et al.*, 1981). However this contrast with the unchanged (Fain and Scow, 1966) or enhanced (Dannenburg *et al.*, 1964; Smith, 1973) lipogenesis reported by others. The differences among these reports may reside in the gestational time studied, as it is known that at late gestation in the rat striking changes in the lipogenic rate occur, being enhanced at day 20, unchanged at day 21 and reduced at day 22 (Lorenzo *et al.*, 1981). It is then proposed that minor differences in the mating day (or even hour) may produce a completely different result in lipogenic activity. In addition, it is proposed that at late gestation common substrates for gluconeogenesis and lipogenesis are preferentially driven to the first pathway. The mechanism responsible for this shift is not known but the load of fatty acids arriving at the mother's liver in late gestation from enhanced adipose tissue lipolysis (Knopp *et al.*, 1970; Chaves and Herrera, 1978) may be one of the controlling factors. This may also be the explanation for the enhanced appearance of labelled glyceride glycerol after [U-^{14}C]glycerol in the liver of the pregnant rat, indicating an enhanced rate of fatty acids esterification which would agree with studies done in perfused liver showing an augmented fatty acid incorporation into perfusate triglycerides (Wasfi *et al.*, 1980). Present findings allow us to emphasize the role of glycerol as preferential substrate for both glucose and glyceride glycerol synthesis in the late pregnant rat.

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