Alanine and lactate as gluconeogenic substrates during late gestation

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Rates of alanine incorporation into glucose by isolated liver cells of fed rats are 5-fold higher than those observed when lactate was used as substrate. The rates of gluconeogenesis from alanine and lactate in isolated liver cells of fed pregnant rats increase 50 and 200–400%, respectively, over virgins during the last 3 days of gestation. The results support the existence of an increase in the alanine-glucose cycle in the late pregnancy as an important homeostatic pathway in the supply of glucose to the growing fetus.

Gluconeogenesis *Gestation* *Hepatocyte* *Lactate* *Alanine*

1. INTRODUCTION

In the pregnant rat, the fetal weight increases 3–4-fold during late gestation. Maternal glucose is the primary fuel for the growing fetus [1–4] and its demands increase as gestation proceeds. It has been reported that blood glucose concentrations decrease continuously during gestation in several species, i.e. rabbit [5], guinea-pig [6], human [4,7] and rat [8–11], either in the postabsorptive state or after an overnight fast. Hence, it has been suggested that gluconeogenesis decreases in the fed pregnant animal during late gestation [5,12,13]. However, we have recently shown that the in vivo rates of gluconeogenesis from lactate increase 2-fold in fed pregnant rats at term gestation [14], which represents almost 50% of the glucose utilized by the conceptus [10,14]. Nevertheless, decreased operation of the glucose-alanine cycle has been reported to occur in late pregnancy [15].

The increased availabilities of gluconeogenic substrates, i.e. lactate and alanine [5,14,15] and the occurrence of lower blood glucose concentrations during late pregnancy have prompted us to study further the glucose production rates from these substrates in isolated liver cells of fed rats during late gestation. The aims of the present research were to establish if the rates of gluconeogenesis from alanine are impaired in the liver of late pregnant rats as suggested by others [15] and to evaluate the relative importance of lactate and alanine as gluconeogenic substrates during late pregnancy.

2. MATERIALS AND METHODS

2.1. *Materials*

L-[U-^{14}C]Lactate and L-[U-^{14}C]alanine were obtained from the Radiochemical Centre, Amersham, England. Analytical-grade anion-exchange resin Dowex AG1-X8 (Cl^- form; 200–400 mesh) and cation-exchange resin Dowex 50W (50x4-400) (hydrogen form, 200–400 mesh) were obtained from Bio-Rad, USA, and Sigma, USA, respectively. Bovine serum albumin (fraction V) was supplied by Armour Pharmaceutical, USA. Collagenase (lot no. 1074259), glucose-6-phosphate dehydrogenase, hexokinase, lactate dehydrogenase, β-nicotinamide adenine dinucleotide and adenosine 5-triphosphate were obtained from Boehringer (Mannheim). All other chemicals were of the highest purity grade available.

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2.2. *Animals*

Time-pregnant and virgin albino Wistar rats fed on standard laboratory chow and water ad libitum were used for the experiments between 09.00 and 10.00 hours. The presence of spermatozoa in the vagina had been assumed to indicate conception, and gestational age was confirmed by the fetal weight [14,16].

2.3. *Liver cell preparation*

Isolated liver cells were prepared essentially as in [17–19] with minor modifications. Briefly, the rats were anaesthetized with nembutal (40 mg/kg body wt) and the liver perfused with Ca^{2+}-free Krebs-Henseleit buffer containing 0.5 mM EGTA for 10 min. After this initial perfusion, the liver was perfused for another 20 min with normal Krebs-Henseleit buffer containing 0.05% (w/v) collagenase. The isolated cells were filtered and washed 3 times in normal Krebs-Henseleit buffer. When judged by the exclusion of 0.02% (w/v) trypan blue, the percentage of viable cells was always higher than 90–95% and 85–90% for virgin and pregnant rats, respectively.

2.4. *Liver cell incubation*

4–5 × 10^6 liver cells were incubated in 3 ml normal Krebs-Henseleit buffer containing 2.5% (w/v) bovine serum albumin, 5 mM glucose, 5 mM lactate and 1 mM alanine at 37°C with continuous shaking (100 strokes/min) in 25-ml Erlenmeyer flasks with a mixture of 95% O_2 and 5% CO_2 as gas phase. 0.25 μCi L-[U-^{14}C]lactate or L-[U-^{14}C]alanine were added to the incubations to measure rates of gluconeogenesis from both substrates. At 0, 10, 20, 30, 40 and 50 min, incubations were terminated by adding 1 ml of 10% (w/v) perchloric acid. After removing the precipitated protein, the supernatants were neutralized with 20% (w/v) KOH.

2.5. *Ion-exchange chromatography*

0.4 ml of the neutralized supernatants were chromatographed on anion-exchange or cation-exchange resin to separate glucose and lactate [16,20] or glucose and alanine, respectively. Glucose (95%) and alaine (95%) were recovered in eluate fractions 2 and 6, respectively. The radioactivity of [^{14}C]glucose, L-[U-^{14}C]lactate and L-[U-^{14}C]alanine in the chromatographic fractions was measured by liquid-scintillation counting and the concentration of each metabolilte assayed by standard enzymatic procedures as in [20,21]. The specific activities of the metabolites were expressed as dpm/μmol substrate. Statistical analysis was performed by Student's *t*-test. Statistical linear-regression correlation was applied to the linear plots. *P* values of 0.05 or less were taken as significant, and the results expressed as the means ± SE.

3. RESULTS AND DISCUSSION

The linear increase in specific activity of glucose/g dry wt liver cells from fed pregnant rats incubated in the presence of L-[U-^{14}C]lactate is presented in fig.1A. It has been reported that in freshly prepared isolated rat liver cells from 48 h starved rats, there is a 20 min lag in gluconeogenesis from lactate [18,22]. This effect has also been observed in isolated guinea-pig liver cells [19]. In our preparations, this effect was not observed; in fact, the rates of gluconeogenesis from L-lactate were linear during 40–50 min of incubation (fig.1A). The existence of the lag period was explained by Cornell et al. [22] by the precedence of the reoxidation of cytosolic NADH over gluconeogenesis, meaning that much of the oxaloacetate formed by the pyruvate carboxylase reaction has to be transferred 'twice' from the mitochondria to the cytosol by the aspartate shuttle before being converted into phospho*enol*pyruvate. The observed discrepancy could be explained on the basis of the previous hypothesis [22]: the cytosolic redox state of the liver of fed rats is more oxidized than that of the liver from starved rats [23], therefore, under a fed condition there would be no need of oxaloacetate diversion and the lag period would not be expected to occur.

Using the results of fig.1A and the specific activity of L-[U-^{14}C]lactate, the rates of gluconeogenesis were calculated and presented in fig.1B. The rates of gluconeogenesis from lactate in isolated liver cells of fed pregnant rats showed a 3–4-fold increase at days 20, 21 and 22 of gestation when compared to the rates found in fed virgin rats (fig.1B). It should be pointed out that the basal rates of gluconeogenesis observed in virgin rats are lower than those previously reported [18,19,22,24]. This difference is most likely due to the use in our experimental protocol of: (i) liver

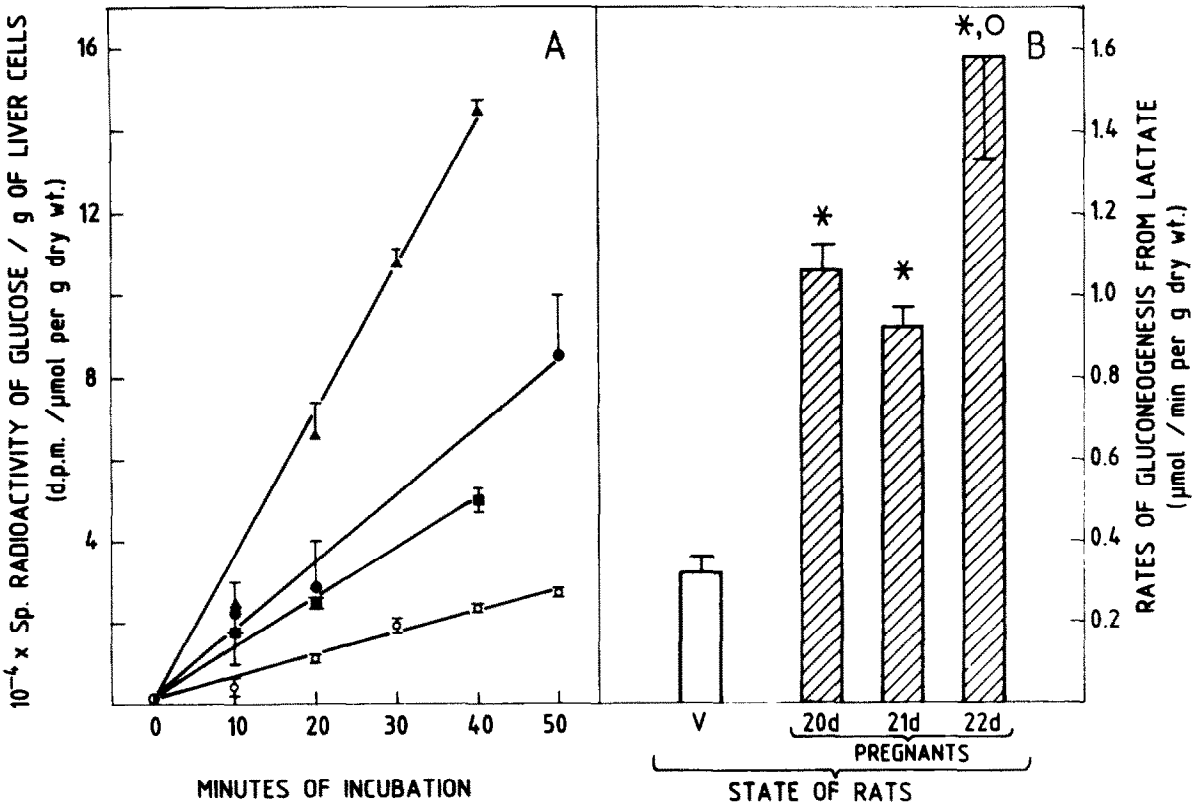

Fig.1. Rates of gluconeogenesis from lactate in isolated liver cells of fed virgin and fed pregnant rats during late gestation. Liver cells were isolated and incubated as described in section 2.2. Glucose was separated from lactate by anion-exchange chromatography. (A) Linear increase in the specific activity of [^{14}C]glucose formed from L-[U-^{14}C]lactate in one preparation of isolated liver cells of virgin (○) and 20 (●), 21 (■) and 22 (▲) days pregnant rats. Correlation coefficients (r) and significance (p) for the slopes: (○) $r = 0.984$, $p < 0.001$; (●) $r = 0.992$, $p < 0.01$; (■) $r = 0.989$, $p < 0.01$; (▲) $r = 0.993$, $p < 0.001$. (B) Rates of gluconeogenesis from lactate in isolated liver cells of virgin and pregnant rats. The results shown are means ± SE ($n = 3$–9). * $p < 0.0005$ for comparison of virgins and pregnant rats. ° $p < 0.0005$ for comparison of 21 and 22 day pregnant rats.

cells isolated from rats fed ad libitum, since starvation is well known to increase the gluconeogenic capacity of the liver [25,26]; and (ii) an incubation medium that included glucose and half the concentration of lactate used by others, but that was designed with the purpose of simulating the physiological concentrations of both metabolites to which liver cells are exposed in vivo [11,14].

The increase in specific activity of glucose/g dry wt was linear for 30–40 min of incubation in the 4 groups of liver cells studied when measured from L-[U-^{14}C]alanine (fig.2A). The rates of gluconeogenesis from alanine (fig.2B) showed a 50–60% increase in the isolated liver cells of 20, 21 and 22 day pregnant rats vs those found in virgins. Interestingly, in isolated liver cells of fed virgin rats 1 mM alanine (fig.2B) was almost 5-times more potent a gluconeogenic substrate than 5 mM lactate (fig.1B). This result is the first experimental evidence to support alanine as being a major liver gluconeogenic substrate in the postabsorptive mammal [27,28]. Although during late pregnancy the 5-fold difference mentioned above is significantly reduced, alanine is still a better gluconeogenic substrate than lactate for liver cells isolated from postabsorptive pregnant rats.

Our results support the existence of an increase in the alanine-glucose cycle during late gestation as recently reported for the Cori cycle [14]. Any interference in the maternal gluconeogenic capacity,

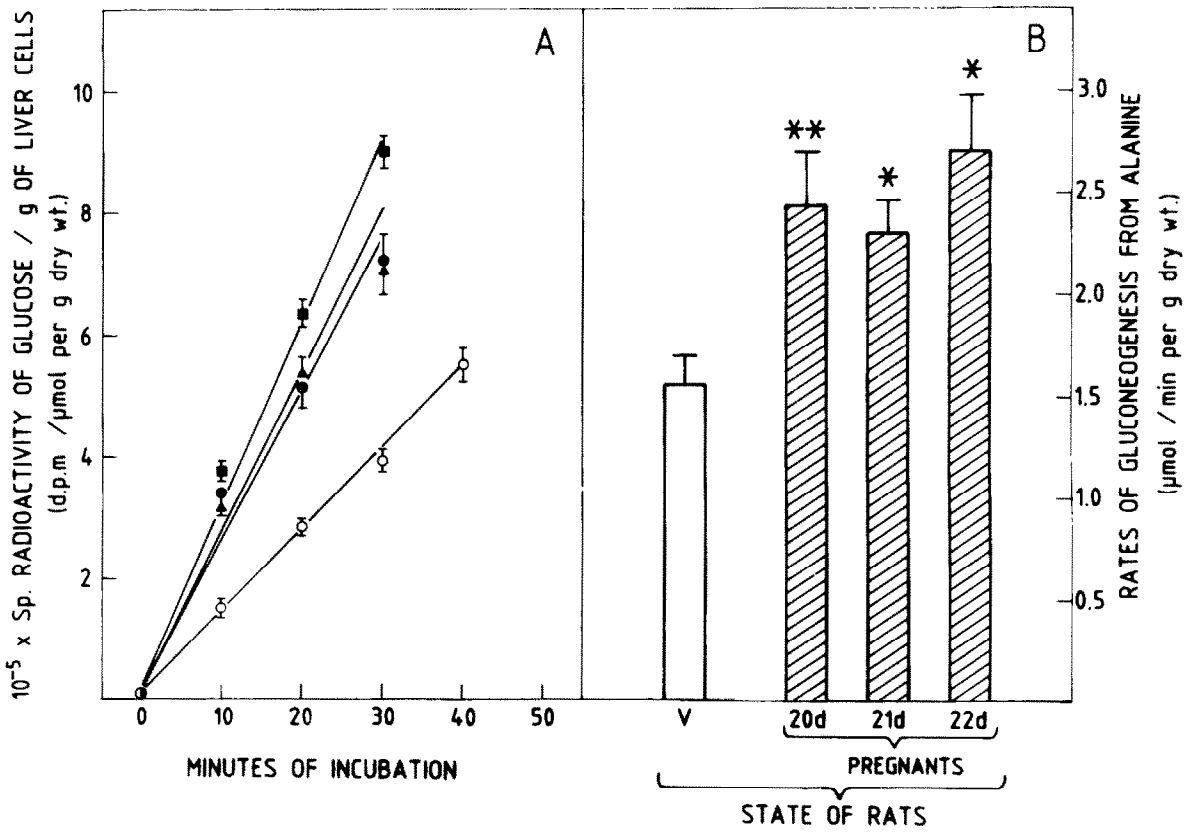

Fig.2. Rates of gluconeogenesis from alanine in isolated liver cells of fed virgin and fed pregnant rats during late gestation. Liver cells were isolated and incubated as described in section 2.2. Glucose was separated from alanine by cation-exchange chromatography. (A) Linear increase in specific activity of [^{14}C]glucose formed from L-[U-^{14}C]alanine in one preparation of isolated liver cells of virgin (○) and 20 (●), 21 (■) and 22 (▲) day pregnant rats. Correlation coefficients (r) and significance (p) for the slopes: (○) $r = 0.997$, $p < 0.001$; (●) $r = 0.986$, $p < 0.01$; (■) $r = 0.999$, $p < 0.01$; (▲) $r = 0.981$, $p < 0.02$. (B) Rates of gluconeogenesis from alanine in isolated liver cells of virgin and pregnant rats. Results shown are means ± SE ($n = 3$–6). * $p < 0.0025$ and ** $p < 0.0005$ for comparison of virgin and pregnant rats.

such as ethanol ingestion, administration of hypoglycemic drugs or homones during late gestation that could result in an impairment of these cycles may limit the glucose available to the fetus and hence handicap its future fetal and/or neonatal development.

In conclusion, this study demonstrates that gluconeogenesis from alanine and lactate is significantly increased in isolated liver cells from fed pregnant rats during late gestation, alanine being at least 2-times more potent substrate for gluconeogenesis than lactate.

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