GLUTAMINE-DEPENDENT CARBAMYL PHOSPHATE SYNTHETASE in PLACENTA AND FETAL STRUCTURES OF THE RAT

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Summary: Glutamine-dependent carbamyl phosphate synthetase activity has been demonstrated in fetal liver, gut, carcass, and brain and in the placenta of the 19-day pregnant rat. The widespread distribution of this enzyme within the conceptus indicates that even the first step in the de novo biosynthesis of pyrimidines can occur in situ. It also suggests that formation of nucleic acids by the conceptus during fasting may be supported by products of maternal protein catabolism.

Introduction:

Pregnant rats in late gestation display much greater rates of gluconeogenesis during fasting than age-matched non gravid animals (1). Formation of urea, on the other hand, is not increased commensurately (1,2). Our experiences with perfused rat livers (3,4) have reinforced the proposition that fasting in pregnancy may be attended by novel mechanisms for conserving the nitrogen of maternal amino acids despite an increased diversion of the carbon atoms for gluconeogenesis. We have observed that isolated livers from fasted 19-day pregnant rats when perfused with 17 mM L-alanine form much more glucose and ammonia and significantly less urea than livers from fasted male or nulliparous female animals (4). The present studies were initiated to assess whether the conceptus has enzymatic capabilities for incorporating such ammonia, or derivatives of ammonia such as glutamine, into nucleic acids. Placentas and tissues from the feti of 19-day pregnant rats were assayed for the recently described, soluble carbamyl phosphate synthetase, by which glutamine or ammonia may be utilized to form carbamyl phosphate for the de novo biosynthesis of pyrimidines (5-9). Although glutamine-dependent carbamyl phosphate synthetase (Synthetase II) has already been demonstrated in fetal

rat liver (6,9) other portions of the conceptus have not been examined for this enzyme previously.

Methods:

Primiparous rats were obtained from Charles River Laboratories and housed as described previously (1). On day 19 of gestation, following a 24-hour fast, rats were stunned and the concepti were delivered by hysterotomy. Placentas and fetal brain, gut, liver and carcass were dissected free and washed in 0.9 % saline. Appropriate tissues from all the feti (8-10) of an individual mother were pooled. Tissue homogenates (10% or 20%, w/v) were prepared in 20 mM Tris buffer (pH 7.4), containing 1 mM ATP; 0.5 mM $MgSO_4$; 5 mM mercaptoethanol; and 30% (v/v) glycerol. The homogenates were centrifuged 30 minutes at 110,000 x g and the soluble supernatants were assayed for Synthetase II activity by a modification of the assays of Hager and Jones (5,6) and Tatibana and Ito (7,8). Herein, carbamyl phosphate formed during incubation is trapped as ^{14}C-citrulline by excess ornithine transcarbamylase and ornithine. Reaction mixtures consisted of 1 ml and contained: 50 mM potassium phosphate buffer (pH 7.4); 25 mM ATP; 15 mM $MgSO_4$; 2.5 mM L-glutamine; 5 mM $NaH^{14}CO_3$ (1 µc); 1 mM dithiothreitol; 5 mM L-ornithine; 27 units ornithine transcarbamylase; and 0.5 ml of the 110,000 x g supernatants. Reaction blanks containing all components except L-ornithine and ornithine transcarbamylase were incubated concurrently. After incubation for 40 minutes at 37^{o}, reactions were terminated by the introduction of 0.5 ml of 10% trichloracetic acid. A portion of the acid soluble material was combined with carrier citrulline and applied to a 0.5 ml column of Dowex 50 W-X 8 (100-200 mesh) in the acid form. Radioactive citrulline was eluted with 10% pyridine, evaporated to dryness and assayed by liquid scintillation counting as described elsewhere (10). Two dimensional paper chromatography with the upper phase of n-butanol-acetic acid-water (5:1:4, v/v) followed by 90% phenol saturated with water (11) was employed to document that labeled citrulline accounted for more than 90% of the eluted radioactivity. Synthetase II

activity was expressed on the basis of the mμmoles of citrulline formed per minute at 37° under the above conditions.

Results and Discussion:

Glutamine-dependent carbamyl phosphate synthetase activity was demonstrable in all the 110,000 x g supernatants which we examined. Mean ± SEM values are summarized below.

TABLE 1

Glutamine-dependent Carbamyl Phosphate Synthetase Activity in the 19-day Rat Conceptus*

		mμmoles citrulline/min/g	
Fetal:	Liver	4.53 ± 0.39	(10)
	Gut	2.69 ± 0.24	(7)
	Carcass	1.06 ± 0.07	(9)
	Brain	1.04 ± 0.08	(8)
Placenta:		0.47 ± 0.04	(9)

* Activity in the 110,000 x g soluble supernatants has been expressed on the basis of the wet weights of the tissues prior to homogenization. Brackets denote the number of pools of the appropriate tissues which were assayed.

During assay with L-glutamine, as described above, none of the extracts displayed further augmentation of activity upon the addition of N-acetyl-L-glutamate (5 mM), the cofactor for the ammonia-dependent mitochondrial Synthetase I by which carbamyl phosphate is formed for urea synthesis (12). The ubiquitous distribution of soluble Synthetase II throughout the conceptus supports the contention (13) that preformed pyrimidines need not cross the placenta nor be transported from fetal liver or gut to the periphery for the synthesis of nucleic acids. Our findings indicate that even the first step in the de novo biosynthesis of pyrimidines, the generation of carbamyl phosphate, can occur in situ in the portions of the 19-day rat conceptus which we have examined.

The existence of such enzymatic mechanisms within the conceptus has certain additional implications with regard to the function of maternal gluconeogenesis. If less of the amino acid nitrogen were converted to urea, and more

escaped the maternal liver in a potentially reutilizable form in vivo, as in vitro (4), a unique cycle could result: Maternal amino acids could then provide the energy (i.e. glucose) for anabolism of the conceptus coincident with furnishing the nitrogenous building blocks (i.e. ammonia and/or glutamine). Thus, the qualitative disposition of maternal fuels during starvation would be parsimonious despite the quantitatively accelerated nature of their mobilization (1, 14). It remains to be seen whether these mechanisms within the mother for generating enzyme substrate can account for the observation of Yip and Knox (9) that there is more tissue growth per unit glutamine-dependent Synthetase II in fetal liver than in tumors.

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