Hepatic uptake of amino acids in late-pregnant rats

Effect of food deprivation

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Hepatic availability, uptake and fractional extraction of amino acids were estimated in anaesthetized 21-day-pregnant and age-matched virgin rats, either fed or after 24 h starvation. Amino acid availability was unaltered in fed pregnant rats as compared with fed virgin controls. However, the hepatic uptake of these compounds was higher in the former than in the latter. These adaptations were mediated by an increase in the hepatic capability to take up amino acids in late-pregnant rats, as reflected by the changes found for the fractional extraction rates. The decrease in amino acid availability found after starvation was more pronounced in pregnant than in virgin rats. Nevertheless, the hepatic uptake was similar in both groups. These results indicate that amino acids are not limiting for ureagenesis during late pregnancy, strongly suggesting that the mechanism(s) which modulate urea synthesis may be intracellular in origin.

INTRODUCTION

High nitrogen retention during pregnancy (Bourdel & Jacquot, 1956) is mediated by several nitrogen-sparing mechanisms. Among them, a low ureagenic capacity seems to play a key role in preserving nitrogen compounds to support fetal growth (Freinkel *et al.*, 1972). What causes this decrease in urea synthesis during gestation is not yet known. Some intracellular events, such as depletion of mitochondrial *N*-acetyl glutamate (Rabier *et al.*, 1985) or ornithine (Hitier *et al.*, 1983, 1986), have been suggested as eventual causes of impaired ureagenesis. Furthermore, the orotic aciduria associated with gestation may be largely reversed by giving an arginine-supplemented diet (Milner & Visek, 1978) and strongly enhanced by feeding an arginine-free diet (Hassan & Milner, 1981). These findings may be explained from the point of view that arginine availability should modulate the reciprocal flows through the pyrimidine- and urea-synthesis pathways.

Because amino acids are necessary for fetoplacental growth, their availability has been often suggested to be limiting for ureagenesis, especially when food is withheld (Freinkel *et al.*, 1972; Kalhan *et al.*, 1982). However, there are no direct measurements of the hepatic availability and uptake of ureagenic substrates in late-pregnant rats. In the present work we have quantified amino acid availability, uptake and fractional extraction of livers from virgin and 21-day-pregnant rats, either fed or starved for 24 h.

MATERIALS AND METHODS

Animals

Virgin rats of the Wistar strain bred in our laboratory were used. They were mated at 65 days of age (200 g body wt.). Impregnation was assessed by the presence of spermatozoa in vaginal smears. That day was designated as day 0 of pregnancy. Animals were caged individually and maintained under controlled conditions of light (12 h on-off light cycle) and temperature (20 °C). They were fed *ad libitum* on a laboratory chow (Panlab, Spain)

of the following composition (w/w): protein 17.2%; lipid 3.5%; fibre 4.5%; free sugars 4.1%; starch 45%; minerals 4.5%.

Experimental design

Two sets of experiments were carried out on 21-day-pregnant rats and age-matched virgin controls, either 'fed' (150 min after the beginning of the light cycle) or 'starved' (24 h starved). The first set was aimed at the measurement of portal and hepatic blood flows. The second was performed to estimate the arteriovenous substrate differences across the liver.

Blood-flow procedure and substrate determinations

Portal and hepatic blood flows were measured, under sodium pentobarbital anaesthesia (60 mg/kg body wt., intraperitoneally), by the indicator-dilution method, with *p*-aminohippuric acid, as previously described (Casado *et al.*, 1987). The achievement of steady-state arterial concentrations of the indicator was assessed in a previous set of experiments on rats provided with indwelling arterial catheters and infused with *p*-aminohippuric acid in a mesenteric vein (results not shown).

Blood samples for substrate determinations were withdrawn from the hepatic and portal vein and aorta in less than 55 s. Under these conditions haemorrhage and hypovolaemic shock were avoided, and hematocrit values remained unchanged (Pastor-Anglada *et al.*, 1987). Blood samples were deproteinized by adding HClO₄ (final concn. 5%, w/v). Free amino acids were measured in the protein-free extracts with an amino acid analyser, as previously reported (Pastor-Anglada & Remesar, 1986).

Calculations

Substrate availability (*A*) was calculated as follows:

$$A = (F_p \times C_p) + (F_a \times C_a)$$

where *F_p* and *F_a* represent the portal and hepatic-artery blood flows, and *C_p* and *C_a* the substrate concentrations

Table 1. Arterial concentrations of amino acids

Statistical differences: starved versus fed, * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$; pregnant versus virgin, † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$).

No. of animals ...	Concn. (μM)			
	Virgin		Pregnant	
	Fed 8	Starved 7	Fed 6	Starved 6
Threonine	239 ± 24	220 ± 13	192 ± 14	189 ± 16
Methionine	18.1 ± 4.5	18.6 ± 5.2	17.7 ± 3.4	8.6 ± 2.6
Valine	138 ± 11	174 ± 14	104 ± 16	112 ± 13††
Isoleucine	61.8 ± 5.6	96.9 ± 10**	56.1 ± 12	71.2 ± 8.6
Leucine	101 ± 11	142 ± 15	88.4 ± 20	101 ± 15
Phenylalanine	43.4 ± 3.0	65.5 ± 3.9**	44.4 ± 7.0	58.5 ± 2.0
Tryptophan	24.1 ± 5.3	23.6 ± 2.9	18.0 ± 3.8	19.9 ± 4.2
Lysine	502 ± 24	561 ± 51	465 ± 31	598 ± 24**
Total essential	1123 ± 60	1356 ± 70*	910 ± 62†	1160 ± 69*
Taurine	304 ± 23	385 ± 26*	222 ± 27†	300 ± 27†
Serine	172 ± 13	159 ± 13	148 ± 12	136 ± 11
Aspartate	40.2 ± 5.1	35.4 ± 6.7	34.1 ± 7.7	26.9 ± 6.6
Asparagine	51.4 ± 4.3	43.8 ± 4.8	68.9 ± 4.0†	55.1 ± 3.8*
Glutamate	123 ± 5.9	174 ± 20*	126 ± 16	134 ± 18
Glutamine	614 ± 43	689 ± 64	869 ± 80†	814 ± 74
Glycine	200 ± 8.2	216 ± 9.2	115 ± 6.5†††	131 ± 4.4†††
Alanine	422 ± 50	278 ± 8.3*	557 ± 28	316 ± 34***
Citrulline	97.4 ± 4.3	108 ± 5.3	76.7 ± 1.9††	89.4 ± 4.8†
Tyrosine	32.2 ± 3.3	49.7 ± 2.1***	26.3 ± 3.9	45.5 ± 2.3**
Histidine	68.7 ± 4.2	72.3 ± 4.5	68.5 ± 7.6	56.5 ± 3.7†
Ornithine	67.3 ± 3.6	63.3 ± 3.7	58.9 ± 5.7	50.0 ± 3.0†
Arginine	227 ± 12	224 ± 11	199 ± 20	207 ± 13
Total amino acids	3477 ± 112	3732 ± 104	3607 ± 256	3605 ± 194

in porta and aorta respectively. Substrate uptake (U) by the liver was determined as:

$$U = A - (F_h \times C_h)$$

where $F_h = F_p + F_a$, and C_h is the substrate concentration in the hepatic vein. Substrate fractional extraction (FE) was calculated as follows:

$$\text{FE} = (U/A) \times 100.$$

Statistics

All results are expressed as means ± S.E.M. Significance of changes induced by either gestation or starvation was tested by the Student's t test.

RESULTS

Body and liver weights

Pregnancy induced a significant increase in body weight (345 ± 8 and 314 ± 8 g for fed and starved pregnant rats, against 209 ± 7 and 208 ± 6 g for fed and starved age-matched virgin controls respectively; $P < 0.001$), which correlated well with changes in liver weight. Starvation induced a net decrease in liver weight in both pregnant (13.8 ± 0.5 and 10.8 ± 0.4 g for fed and starved rats respectively; $P < 0.001$) and age-matched virgin controls (8.8 ± 0.2 and 6.6 ± 0.2 g for fed and starved rats respectively; $P < 0.001$).

Blood flows

Fed late-pregnant rats showed unaltered hepatic (3.1 ± 0.4 ml/min per g of liver) and portal (2.0 ± 0.2 ml/min per g of liver) blood flows compared with those of fed virgin rats (3.8 ± 0.4 and 2.1 ± 0.2 ml/min per g of liver for hepatic and portal flows respectively). Starvation induced a significant decrease in the portal blood flow in pregnant rats (1.2 ± 0.2 versus 2.0 ± 0.2 ml/min per g of liver; $P < 0.01$), but not in virgin rats (1.8 ± 0.3 versus 2.1 ± 0.2 ml/min per g of liver). However, starvation resulted in lower hepatic blood flows in both pregnant (1.6 ± 0.2 versus 3.1 ± 0.4 ml/min per g; $P < 0.01$) and age-matched virgin controls (2.4 ± 0.1 versus 3.8 ± 0.4 ml/min per g; $P < 0.01$), compared with the fed state.

Substrate arterial concentrations

Amino acid concentrations as μM in aorta are shown in Table 1.

Pregnancy in the fed state was associated with low concentrations of the whole essential amino acids, in spite of the differences found among the individual amino acid concentrations not being significant. As for the non-essential amino acids, pregnancy induced lower concentrations of taurine, glycine and citrulline and higher concentrations of asparagine and glutamine.

Starvation induced a significant increase of the whole essential amino acids in both pregnant and virgin rats. As for individual amino acids, significant increases

Table 2. Hepatic availability of amino acids

Availability is expressed as μmol reaching the liver/min per 100 g body wt. Statistical significances are the same as in Table 1.

No. of animals ...	Availability			
	Virgin		Pregnant	
	Fed 8	Starved 7	Fed 6	Starved 6
Threonine	3.39 ± 0.26	$1.74 \pm 0.05^{***}$	2.66 ± 0.27	$1.16 \pm 0.12^{***\dagger\dagger\dagger}$
Methionine	0.28 ± 0.06	0.17 ± 0.03	0.40 ± 0.07	$0.06 \pm 0.01^{***\dagger\dagger}$
Valine	2.05 ± 0.14	$1.37 \pm 0.12^{**}$	1.56 ± 0.25	$0.70 \pm 0.09^{***\dagger\dagger}$
Isoleucine	1.06 ± 0.11	0.79 ± 0.08	0.83 ± 0.19	$0.43 \pm 0.05^{\dagger\dagger}$
Leucine	1.56 ± 0.14	$1.15 \pm 0.11^*$	1.35 ± 0.32	$0.53 \pm 0.03^{***\dagger\dagger\dagger}$
Phenylalanine	0.75 ± 0.07	$0.54 \pm 0.03^*$	0.66 ± 0.10	$0.36 \pm 0.03^{***\dagger\dagger}$
Tryptophan	0.42 ± 0.06	$0.22 \pm 0.02^*$	0.29 ± 0.04	$0.12 \pm 0.02^{***\dagger\dagger}$
Lysine	7.93 ± 0.39	$4.68 \pm 0.27^{***}$	$6.42 \pm 0.60^{\dagger}$	$3.70 \pm 0.13^{***\dagger\dagger}$
Total essential	16.9 ± 0.75	$10.6 \pm 0.44^{***}$	$12.9 \pm 1.32^{\dagger}$	$7.14 \pm 0.46^{***\dagger\dagger\dagger}$
Taurine	5.54 ± 0.48	$3.55 \pm 0.20^{**}$	$3.25 \pm 0.44^{\dagger\dagger}$	$2.14 \pm 0.25^{\dagger\dagger\dagger}$
Serine	2.68 ± 0.24	$1.32 \pm 0.07^{***}$	2.34 ± 0.41	$0.84 \pm 0.06^{***\dagger\dagger\dagger}$
Aspartate	0.74 ± 0.09	$0.34 \pm 0.04^{**}$	$0.38 \pm 0.08^{\dagger}$	$0.16 \pm 0.03^{***\dagger\dagger}$
Asparagine	0.83 ± 0.06	$0.39 \pm 0.03^{***}$	$1.07 \pm 0.09^{\dagger}$	$0.33 \pm 0.03^{***}$
Glutamate	2.13 ± 0.11	$1.46 \pm 0.15^{**}$	$1.61 \pm 0.13^{\dagger\dagger}$	$0.88 \pm 0.06^{***\dagger\dagger}$
Glutamine	8.12 ± 0.59	$5.00 \pm 0.62^{**}$	10.2 ± 1.15	$4.19 \pm 0.29^{***}$
Glycine	3.69 ± 0.26	$1.94 \pm 0.10^{***}$	$2.25 \pm 0.18^{\dagger\dagger}$	$0.92 \pm 0.05^{***\dagger\dagger\dagger}$
Alanine	7.65 ± 0.85	$2.90 \pm 0.17^{***}$	9.52 ± 0.61	$2.72 \pm 0.48^{***}$
Citrulline	1.79 ± 0.13	$0.95 \pm 0.03^{***}$	$1.22 \pm 0.04^{\dagger\dagger}$	$0.59 \pm 0.04^{***\dagger\dagger\dagger}$
Tyrosine	0.55 ± 0.07	0.41 ± 0.02	0.39 ± 0.06	$0.27 \pm 0.02^{\dagger\dagger\dagger}$
Histidine	1.15 ± 0.12	$0.62 \pm 0.03^{**}$	0.97 ± 0.11	$0.35 \pm 0.03^{***\dagger\dagger\dagger}$
Ornithine	1.10 ± 0.06	$0.59 \pm 0.04^{***}$	$0.84 \pm 0.07^{\dagger}$	$0.34 \pm 0.02^{***\dagger\dagger\dagger}$
Arginine	3.53 ± 0.24	$2.00 \pm 0.12^{***}$	$2.61 \pm 0.28^{\dagger}$	$1.28 \pm 0.05^{***\dagger\dagger\dagger}$
Total amino acids	58.3 ± 5.02	$31.9 \pm 1.50^{***}$	51.1 ± 4.43	$22.4 \pm 1.32^{***\dagger\dagger\dagger}$

caused by starvation were only evident for isoleucine and phenylalanine in virgin rats, and for lysine in late pregnant rats. Starvation induced significant increases in some non-essential amino acid concentrations (taurine and tyrosine in both pregnant and virgin rats, and glutamate in controls only). On the other hand, alanine was depleted in starved pregnant and virgin animals when compared with the fed state.

Substrate availability

Amino acid availabilities, expressed as μmol reaching the liver/min per 100 g body wt., are shown in Table 2.

In the fed state, the availability of taurine, aspartate, glutamate, glycine, citrulline, ornithine, lysine, arginine and essential amino acids (when considered as a whole) was lower in late-pregnant rats than in age-matched virgin controls.

Starvation induced a general decrease in hepatic amino acid availability in pregnant and virgin rats. The only amino acids whose availability was not altered during starvation were methionine in controls and isoleucine and tyrosine in both pregnant and virgin rats.

Substrate uptake

Amino acid uptake by the liver, expressed as μmol taken up/min per 100 g body wt., is shown in Table 3.

Fed pregnant rats showed a net hepatic uptake of threonine, asparagine, glycine, alanine, valine, leucine, tyrosine, phenylalanine and histidine. When the total

content of either the whole amino acids, or only the essential ones, is considered, their uptake was significantly different from zero and from the values found in fed virgin controls. This fact was mainly accounted for by greater uptakes of threonine, glycine, alanine, valine, leucine and phenylalanine by the liver of pregnant animals compared with those from age-matched virgin controls. When pregnant rats were starved, a general decrease in the hepatic amino acid uptake was observed. This was mainly due to a decrease in the uptake of glycine, alanine and essential amino acids such as threonine, valine, leucine and methionine.

Fed virgin rats showed a net hepatic uptake of glycine, alanine, asparagine, tyrosine, phenylalanine and arginine, but when amino acids were considered as a whole, the balance was not different from zero. Starvation did not modify the uptake of the amino acids mentioned above, and induced a further net uptake of taurine, threonine, histidine and lysine. The liver of starved rats, conversely to what was found for the fed animals, showed a net uptake of essential and total amino acids.

Fractional extraction

Fed pregnant rats showed higher fractional extractions than did fed virgin rats, for the essential amino acids (0.3 ± 2.2 versus $16.4 \pm 2.6\%$; $P < 0.001$) (mainly accounted for by threonine, valine, leucine and phenylalanine), as well as for tyrosine and glycine (results not shown). The fractional extraction rates for the whole amino acids were $3.3 \pm 3.0\%$ for fed virgins and

Table 3. Hepatic uptake of amino acids

Uptake is expressed as $\mu\text{mol}/\text{min}$ per 100 g body wt. Statistical significances are the same as in Table 1.

No. of animals ...	Uptake			
	Virgin		Pregnant	
	Fed 8	Starved 7	Fed 6	Starved 6
Threonine	0.11 ± 0.09	0.29 ± 0.04	$0.57 \pm 0.08^{\dagger\dagger}$	$0.24 \pm 0.05^{**}$
Methionine	-0.24 ± 0.13	0.01 ± 0.03	0.08 ± 0.05	$-0.03 \pm 0.01^*$
Valine	-0.18 ± 0.13	0.01 ± 0.08	$0.50 \pm 0.07^{\dagger\dagger}$	$-0.03 \pm 0.01^*$
Isoleucine	0.00 ± 0.05	0.05 ± 0.03	0.08 ± 0.05	0.03 ± 0.04
Leucine	0.07 ± 0.05	0.16 ± 0.10	$0.39 \pm 0.11^{\dagger}$	$0.10 \pm 0.07^*$
Phenylalanine	0.06 ± 0.02	0.08 ± 0.02	$0.19 \pm 0.04^{\dagger\dagger}$	0.09 ± 0.02
Tryptophan	-0.06 ± 0.03	$0.02 \pm 0.01^*$	0.00 ± 0.02	$-0.04 \pm 0.01^{\dagger\dagger}$
Lysine	0.08 ± 0.30	0.30 ± 0.12	0.38 ± 0.34	0.01 ± 0.02
Total essential	-0.42 ± 0.54	0.76 ± 0.28	$2.14 \pm 0.50^{\dagger}$	$0.74 \pm 0.19^*$
Taurine	0.39 ± 0.25	0.50 ± 0.14	-0.13 ± 0.24	0.41 ± 0.20
Serine	0.14 ± 0.06	0.26 ± 0.11	0.30 ± 0.14	0.17 ± 0.04
Aspartate	0.07 ± 0.08	0.10 ± 0.04	0.07 ± 0.04	0.06 ± 0.02
Asparagine	0.24 ± 0.04	0.17 ± 0.02	0.28 ± 0.03	$0.11 \pm 0.03^{**}$
Glutamate	-0.07 ± 0.13	0.21 ± 0.11	0.00 ± 0.14	0.02 ± 0.07
Glutamine	-0.25 ± 0.37	0.10 ± 0.26	-0.85 ± 0.42	-0.27 ± 0.22
Glycine	0.50 ± 0.16	0.39 ± 0.09	$1.02 \pm 0.16^{\dagger}$	$0.36 \pm 0.02^{**}$
Alanine	1.62 ± 0.57	1.07 ± 0.18	$3.01 \pm 0.10^{\dagger}$	$1.22 \pm 0.22^{***}$
Citrulline	-0.13 ± 0.07	-0.05 ± 0.06	$0.12 \pm 0.07^{\dagger}$	0.04 ± 0.02
Tyrosine	0.10 ± 0.04	0.09 ± 0.02	0.16 ± 0.03	0.11 ± 0.02
Histidine	0.07 ± 0.04	0.08 ± 0.01	0.13 ± 0.04	0.05 ± 0.02
Ornithine	-0.10 ± 0.08	0.03 ± 0.02	0.09 ± 0.04	0.01 ± 0.02
Arginine	0.29 ± 0.10	0.23 ± 0.07	0.16 ± 0.16	0.21 ± 0.05
Total amino acids	0.13 ± 1.11	3.03 ± 1.02	$7.07 \pm 2.09^{\dagger\dagger}$	3.36 ± 0.71

$12.8 \pm 3.5\%$ for fed late-pregnant rats. Starvation induced an increase in the fractional extraction of the overall essential amino acids in virgin ($0.3 \pm 2.2\%$ versus $8.8 \pm 1.9\%$; $P < 0.05$), but not in pregnant rats (16.4 ± 2.6 versus $10.0 \pm 2.8\%$). The fractional extraction rates for the whole amino acids after food deprivation were $11.3 \pm 2.2\%$ and $14.1 \pm 2.8\%$ for virgin and pregnant rats respectively.

DISCUSSION

Hepatic blood flow

Hepatic blood flow in the rat has been usually estimated by using two different methods, the injection of radiolabelled microspheres (Tuma *et al.*, 1986) or the indicator-dilution technique. The latter was first utilized in large mammals, and involved multiple catheterization (Katz & Bergman, 1969). It has been adapted to small animals such as the rat by using *p*-aminohippuric acid as an indicator. Our results for age-matched virgin control rats are similar to those previously reported with the *p*-aminohippuric acid dilution method (Rémésy *et al.*, 1983; Casado *et al.*, 1987).

There are only a few reports describing hepatic blood flows during pregnancy in the rat (Buelke-Sam *et al.*, 1982; Ahokas *et al.*, 1984; Rémésy & Demigné, 1986). Ahokas *et al.* (1984) found, as we have shown here, unaltered portal and low arterial flows in late-pregnant rats (when corrected for liver weight). Rémésy & Demigné (1986) more recently described a decrease in hepatic blood flow when animals were starved, with lower values

in pregnant than in virgin rats. The effect of starvation was, however, less acute than that found in the present work.

Substrate arterial concentrations

The effect of starvation on arterial amino acid concentrations of virgin and pregnant rats is less acute than that reported by others when amino acids were measured in plasma from mixed blood (Metzger *et al.*, 1971; Zorzano *et al.*, 1986). This may be explained by the changes reported to occur in blood amino acid compartmentalization during pregnancy (López-Tejero *et al.*, 1986) or starvation (Soley *et al.*, 1982). In fact, plasma amino acid determinations usually mask whole-blood changes.

An accelerated metabolic response to starvation is a characteristic feature of pregnant mammals (Freinkel *et al.*, 1972); the depletion of alanine was more accelerated in pregnant than in virgin animals (43% and 34% of the fed values respectively). However, it must be noted that our fed rats were in a near-post-absorptive state, because food intake of either pregnant or virgin rats, during the first 3 h after the beginning of the light cycle, is only just over 1 g (Hitier *et al.*, 1982).

Substrate availability

A decrease in substrate availability has been suggested as the eventual limiting step for ureagenesis in late pregnancy, mainly in post-absorptive conditions or during short periods of starvation, both situations closely similar to our present experimental groups. Low avail-

ability of amino acids during late pregnancy had been inferred by interpreting the concentrations found either in mixed blood plasma from starved rats (Metzger *et al.*, 1971; Zorzano *et al.*, 1986) or in plasma from an antecubital vein of humans in post-absorptive conditions (Felig *et al.*, 1972; Kalhan *et al.*, 1982).

However, 'availability' measures have never been done at the actual site of hepatic substrate uptake, i.e. the portal vein. This vessel accounts for about 70% of the hepatic blood flow, and its substrate concentrations are higher than or equal to the arterial values, as found in the present experiments (results not shown).

Amino acid availability at the hepatic level was more decreased in starved pregnant than in starved virgin rats; this fact was mainly due to the lower hepatic blood flow of the former, rather than to a decrease in substrate blood concentrations. This finding may be also interpreted as additional evidence for an 'accelerated starvation' response. Nevertheless, our results make it clear that, at least in the fed (near-post-absorptive state) rat the low ureagenic flux may not be due to a low amino acid availability, although in any case the eventual limiting role of these compounds on ureagenesis will be determined by their actual net uptake.

Hepatic uptake and fractional extraction of substrates

Different amino acid transport systems have been identified at the hepatocyte plasma membrane. Some of them may be modulated by different stimuli (Kilberg, 1982), thus implying that the hepatic availability of amino acids will not be the only factor controlling their uptake.

Our results suggest that the hepatic uptake of amino acids during late pregnancy is not a mere function of their availability, because, firstly, it is clear that, for an unaltered availability of substrates, the livers of fed pregnant rats were taking up much more amino acids than those of virgin controls, and, secondly, for a lower availability of substrates, the livers of starved pregnant rats were taking up the same amounts of amino acids as did those of virgin controls.

Starvation induced a depletion in the total amounts of amino acids reaching the liver in both pregnant and virgin rats, but simultaneously induced an increase in the hepatic uptake of amino acids in starved virgin rats (in comparison with fed virgin rats) and a decrease in starved pregnant rats (in comparison with fed pregnant rats). This event was especially evident for the whole essential amino acids and should be understood as an eventual mechanism to divert these compounds towards the fetoplacental unit. In fact, it has been shown that short periods of starvation induce slighter variations in the amino acid plasma composition of the fetus than in that of the mother (Girard *et al.*, 1977; Arola *et al.*, 1982).

The mechanism which allows the liver to adapt its uptake independently of substrate availabilities is reflected by the fractional extraction rates. Nowadays, there is some evidence showing that alterations in this parameter are usually accompanied with changes in the transport system kinetics at the hepatocyte plasma-membrane level. In the rat, this occurrence has been shown for starvation (Fafournoux *et al.*, 1983), feeding high-protein diets (Fafournoux *et al.*, 1983) or mid-pregnancy (Pastor-Anglada *et al.*, 1987). Thus the fractional extraction rate may give an approximate estimation of the hepatic transport capacity. The liver of

fed late-pregnant rats showed a high capacity to take up amino acids, which was not altered when food was withdrawn.

Regarding individual amino acids, alanine and glycine accounted for a major proportion of amino acids taken up by the liver, especially in fed pregnant rats. This finding is in accordance with the higher rates of conversion of alanine into glucose found in hepatocytes isolated from fed late-pregnant rats compared with those from fed virgin controls (Cuezva *et al.*, 1986).

The present results strongly suggest that the low ureagenic capacity of late-pregnant rats is due to intracellular events only. Some of them have been recently proposed by others and summarized in the Introduction.

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