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Original Paper

Tumour Growth and Fetal Uptake of Amino Acids in the Pregnant Rat

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The aim of the present investigation was to determine the effects of maternal tumour burden on fetal growth and to relate them to amino acid availability to the fetus. A fast-growing tumour, the Yoshida AH-130 ascites hepatoma, was inoculated into rats during pregnancy. Late pregnant rats bearing a rapidly-growing tumour presented a normal conceptus mass while the tumour cell content was unaffected by gestation. In addition, no changes were found in fetal uptake of amino acids as measured by the fetal accumulation of [^{14}C]aminoisobutyrate and [^{14}C] cycloleucine. However, increased alanine and leucine concentrations in the fetal circulation of the tumour-bearing rats suggest an enhanced fetal amino acid availability which does not seem to be the result of changes in placental or fetal relative blood flow, as indicated by the tissue accumulation of [^{14}C]DDT, which were actually lower in the tumour-bearing rats. It may be suggested that tumour burden induces changes in placental amino acid transport systems.

Key words: pregnancy, amino acids, tumour, fetal growth
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INTRODUCTION

PREGNANCY IS a physiological state characterised by increased food intake and important metabolic changes involving carbohydrate, lipid and protein metabolism. These adaptations are essential to sustain exponential fetal growth at the end of gestation. Especially relevant are the changes associated with increased protein degradation in skeletal muscle [1] and decreased ureogenic capacity of the pregnant rat [2], both allowing for a nitrogen-saving mechanism which is essential to sustain the high nitrogen demand of the growing fetus. Indeed, growth involves the accretion of a net quantity of protein, emphasising the importance of amino acids as nutrients in fetal life. In this way, the umbilical uptake of amino acids is the primary nutritional supply line for these compounds during fetal life. However, the role of amino acids in fetal metabolism is not restricted to the sole process of tissue protein accretion, since the quantity of amino acids supplied to the fetus exceeds by far the rate of accretion of tissue protein [3, 4]. The majority of amino acids (except glutamate and aspartate) are transferred to the fetus in large amounts where they are oxidised to CO_2 [5]. This is supported by the high rates of urea synthesis present in the fetus of most species [6]. Very few studies have related pathological states with alterations in amino acid transport to the fetus. A number of studies have attempted to demonstrate an effect of maternal ethanol ingestion upon fetal uptake of amino acids [7]. In spite of the methodological problems involved in this kind of study, the data suggest an alteration in the placental uptake of amino acids from the maternal circulation during ethanol ingestion. Chronic fetal hyperglycaemia does not seem to have any effects upon alanine transport to the fetus [8]. In the rat, the implantation of a rapidly growing tumour, the Walker 256 carcinosarcoma, to the mother during gestation results in impaired early neonatal growth [9], but there are no data relating this phenomenon with a decreased fetal amino acid availability caused by tumour burden.

To some extent, the growth and metabolic characteristics of the fetus share important similarities with those of a fast-growing tumour. Both consume appreciable amounts of glucose and amino acids for oxidation (conversion of glucose to lactate) and protein synthesis involved in growth. The control of glucose utilisation by the ovine fetus is closely related to its insulin levels [8], whereas in the case of most tumours, glucose utilisation appears to be unaffected by insulin and is solely dependent on glucose availability [10]. A similar situation

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may exist for the utilisation of amino acids. Consequently, competition may occur between the fetus and the tumour and the interactions may affect their growth. We have, therefore, compared the effect of a rapidly growing tumour, the AH-130 Yoshida ascites hepatoma, implanted during gestation so that the tumour is growing exponentially concomitantly, with the exponentially growing fetus. In particular, we have assessed the fetal amino acid uptake of maternally ingested amino acids as measured by the tissue accumulation of α-amino [1-^{14}C]isobutyrate and [1-^{14}C]cycloleucine in order to relate possible changes on fetal growth with altered amino acid uptake brought about by tumour burden.

MATERIALS AND METHODS

Animals

All animals (female Wistar rats) were fed *ad libitum* on a chow diet consisting (by weight) of 54% carbohydrate, 17% protein and 5% fat (the residue was non-digestable material) with free access to drinking water, and were maintained at an ambient temperature of 22 ± 2°C with a 12 h-light/12 h-dark cycle (lights on from 08:00 h). They were mated at 60 days of age (180 g body weight approximately). Day 0 of pregnancy was considered when the presence of spermatozoa in vaginal smears was detected. Twenty-day pregnant rats were used in all the experiments.

Biochemicals and radioactive compounds

All materials were of reagent grade and obtained either from Boehringer Mannheim S.A. (Barcelona, Spain) or from Sigma Chemical Co. (St Louis, Missouri, U.S.A.). α-Amino[1-^{14}C]isobutyrate (specific activity 55 mCi/mmol), [^{14}C]-1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane ([^{14}C]DDT; specific activity 104 mCi/mmol) and 1-aminocyclopentane-[1-^{14}C]carboxylic acid [(1-^{14}C]cycloleucine; specific activity 55 mCi/mmol) were purchased from Amersham International (Amersham, U.K.).

Tumour implantation

A Yoshida AH-130 ascites hepatoma cell suspension (approximately 120×10^6 cells/2 ml) was injected intraperitoneally, the control rats being injected with 2 ml of 0.9% (w/v) NaCl solution. The Yoshida AH-130 is a rapidly-growing tumour with a volume doubling time of 1 day. Total cell content was estimated using Trypan Blue staining. All the experiments took place either 4 or 7 days after tumour transplantation, when the cells grow exponentially or reach the stationary phase, respectively [11, 12]. In order to carry out the experiments with 20-day pregnant rats, they were previously inoculated with the tumour (at day 13 or 16 of gestation depending on the experimental groups).

Flow cytometry

Flow cytometry analysis was carried out using an *Epics Elite* flow cytometer (Coulter Electronics Corporation, Hialeah, Florida, U.S.A.). Excitation was carried out using a standard 488 nm air-cooled argon-ion laser at 15 mW power. The instrument was set up with the standard configuration. Forward scatter (FSC), side scatter (SSC) and propidium iodide red fluorescence (675 nm bandpass filter) were used. Optical alignment was performed on an optimised signal from 10 nm fluorescent beads (DNA-check, Coulter Electronics Corporation). Propidium iodide was applied simultaneously with RNase (DNase free) after methanol fixation. Chicken erythrocytes and peripheral rat blood leucocytes were used as a calibration standard for DNA diploidy. Time was used as a control of the stability of the instrument in a fluorescence-versus-time dot plot. The cell cycle analysis was carried out using *Multicycle Software* (Phoenix Flow Systems, San Diego, California, U.S.A.). The variation coefficient of the diploid peaks varied from 4.5 to 6.6, the number of nuclei analysed being usually 20 000.

Measurement of AIB and CLEU absorption and tissue uptake

The animals received 2 μCi (about 40 μmol) of either α-amino[1-^{14}C]isobutyrate (AIB) or [1-^{14}C]cycloleucine (CLEU) dissolved in 0.5 ml of distilled water enterally by gastric intubation, without anaesthetic but with minimal stress to the animal. The animals were killed 90 min after either the AIB or CLEU administration under pentobarbital anaesthesia. Arterial blood was taken by means of an heparinised syringe, and samples of liver, heart, white and brown adipose tissues, placenta, fetus and skeletal muscle were rapidly excised and freeze-clamped. The tissues were powdered in liquid N_2 and deproteinised in four volumes of cold 6% (w/v) $HClO_4$. After homogenisation and centrifugation at 15 000 rpm for 15 min, the acid supernatants were neutralised with 30% (w/v) KOH. After centrifugation at 3000 rpm for 10 min, the clear supernatants were used for measurement of ^{14}C radioactivity with a liquid scintillation spectrometer. The gastro-intestinal tract (plus contents) was homogenised in 150 ml of 3% (w/v) $HClO_4$. [^{14}C]AIB or [^{14}C]CLEU absorption was calculated by subtracting total gastro-intestinal radioactivity from that administered.

Relative blood-flow measurements by the accumulation of [^{14}C]*DDT*

Relative tissue blood-flows were measured by the method of Herd and colleagues [13], which exploits the high lipid/aqueous partition coefficient (approximately 4000:1) of the insecticide DDT. After cannulation of the jugular vein, rats were rapidly administered (2–3 sec) with 0.3 ml of [^{14}C]DDT in saline containing 2.5% (v/v) Tween 20 (DDT 8 μCi/kg body weight, stock solution 80 μCi/ml in ethanol). A blood sample from the tip of the tail was removed at 1 and 21 min after administration of DDT. Following pentobarbital anaesthesia, the animals were sacrificed and samples of tissues (1 g) were saponified and the lipid was extracted [14]. The extracted fatty acids were dissolved in 10 ml of scintillation fluid (NEF-989G, DuPont, Boston, Massachusetts, U.S.A.) for measurement of radioactivity and hence determination of [^{14}C]DDT accumulation.

Plasma insulin and blood metabolites

Whole-blood glucose was determined by the method of Slein [15] and lactate by the method of Hohorst [16]. Plasma insulin was determined by radioimmunoassay with a rat insulin standard [17].

Amino acid analysis

Blood samples were sonicated for 5 min and then diluted 1:1 with distilled water. The mixture was deproteinised using 10% trifluoroacetic acid. Following centrifugation at 12 000*g* for 30 min, the clear supernatants were filtered with Millipore filter (10,000 NMWL) and then submitted to vacuum evaporation to eliminate the acid. After resuspension of the deproteinised samples in distilled water, the amino acid analysis was carried out by means of an Alpha Plus (Pharmacia LKB

Table 1. Carcass, tissue weight and energetic efficiency in 20-day pregnant rats bearing the AH-130 Yoshida ascites hepatoma

	Experimental groups		
	Control	Tumour day 4	Tumour day 7
Carcass weight	278 ± 5 (5)	281 ± 9 (4)	282 ± 3 (5)
Food intake (a)	218 ± 3 (4)	210 ± 4 (5)	168 ± 4 (4)*††
(b)	126 ± 2 (4)	110 ± 4 (5)**	95 ± 8 (5)**
Energetic efficiency			
Fetus (a)	2.23 ± 0.8 (4)	2.42 ± 0.03 (4)	2.75 ± 0.02 (4)*†
(b)	3.86 ± 0.13 (4)	4.63 ± 0.03 (4)*	4.86 ± 0.01 (4)*†
Carcass (a)	13 ± 8 (4)	6 ± 1 (4)*	−2 ± 1 (4)*†
(b)	12 ± 8 (4)	−9 ± 2 (4)*	−38 ± 9 (4)*†
Tissue weights			
Conceptus	58.3 ± 3.0 (5)	61.1 ± 1.3 (4)	55.4 ± 1.1 (5)†
Placenta	0.484 ± 0.022 (5)	0.422 ± 0.008 (4)*	0.392 ± 0.007 (5)**
Fetus	4.86 ± 0.25 (5)	5.09 ± 0.11 (4)	4.62 ± 0.09 (5)
Liver	13.28 ± 0.31 (5)	14.74 ± 0.53 (5)*	14.85 ± 0.24 (5)**
Heart	0.941 ± 0.023 (8)	0.836 ± 0.015 (4)*	0.830 ± 0.012 (5)**

For full details see Materials and Methods. Food intake and weights are expressed in grams. Carcass weight was calculated by subtracting both the conceptus and tumour weights from the final body weight. Conceptus weight is referred to twelve fetuses. Energetic efficiency (Δ carcass or fetus weight/food intake × 100) was calculated between the intervals: (a) 13–20; and (b) 16–20 days of gestation. In the case of the fetus, the placenta and the amniotic fluid were included. The results are mean ± S.E.M. for the number of animals indicated in parentheses. Statistical significance of the results (Wilcoxon two-sample rank sum test): tumour versus control: * $P < 0.05$; ** $P < 0.01$; tumour day 4 versus tumour day 7: † $P < 0.05$; †† $P < 0.01$.

Biotechnology) amino acid analyser using ninhydrine as amino group reagent.

RESULTS

Effects of tumour growth on food intake, carcass and tissue weights

No differences in the carcass weights were found in the tumour-bearing rats as a consequence of tumour implantation (Table 1). The food intake was significantly lower in the tumour-bearing rats at day 7 with a 23% decrease. This decrease in food intake was lower than that observed during the growth of this tumour on non-pregnant animals [12]. Associated with this decrease in food intake, was an increase in fetal energetic efficiency, in spite of the fact that the energetic efficiency of the carcass was much lower in the tumour-bearing rats, being, in fact, negative (Table 1).

There were significant increases in liver mass, as has been observed in non-pregnant animals after the implantation of this tumour type [11] or other fast-growing ones [18, 19]. The presence of the tumour also resulted in a decrease in heart size both at days 4 and 7 following tumour inoculation. Although no changes were observed in the fetus, the placentas of the tumour-bearing pregnant rats were considerably smaller (19% decrease at day 7) (Table 1).

Tumour volume, mass and flow DNA analysis

Although the tumour volume (Table 2) represented nearly 10% of body weight at day 7, the actual tumour mass was relatively small. Non-pregnant animals have shown a similar cell content and mass, although the density was considerably lower [12]. The flow DNA analysis was carried out at day 7 and shows that most cells were in the G_0–G_1 phases (Table 2).

Table 2. Tumour volume, cell content and flow DNA analyses of the cell cycle distribution of Yoshida AH-130 cells

	Experimental groups	
	Tumour day 4	Tumour day 7
Volume (ml)	9.1 ± 1.4 (5)	30.2 ± 1.5 (5)
Cell content (× 10^6)	1706 ± 118 (5)	4813 ± 301 (5)
Percentage of cells in phase:		
G_0–G_1		63.54 ± 3.09 (5)
S		29.32 ± 1.77 (5)
G_2–M		7.10 ± 1.52 (5)

For full details see Materials and Methods. Data are expressed as percentage (%) of cells. The results are mean ± S.E.M. for the number of animals indicated in parentheses.

[^{14}C]*AIB accumulation*

The tissue uptake of a trace amount of AIB was used as a measure of the net influx into tissues [20]. This compound is a non-metabolised analogue of alanine, the principal amino acid released by muscle in catabolic states and the major gluconeogenic amino acid in the liver. The net influx of AIB is the result of a carrier-mediated uphill influx and a passive AIB efflux out of the tissue [21]. The tissue AIB uptake represents an estimate of the transmembrane passage of neutral amino acids transported by system A such as glycine, alanine, serine, proline and other amino acids.

In our experiments, the trace amount of AIB was enterally administered because our aim was to study the effects of the tumour on the delivery of dietary amino acid to the fetus. The results presented in Table 3 show that although there was a tendency for increased absorption in the pregnant rats bearing the tumour for 7 days, that difference did not reach statistical significance. The AIB uptake has been expressed in two ways: (a) as a percentage of the absorbed dose per g of tissue or per

Table 3. Effects of tumour growth on tissue α-aminoisobutyrate uptake in 20-day pregnant rats

Tissue AIB uptake	Experimental group: Control % of ABD	Control T/P	Tumour day 4 % of ABD	Tumour day 4 T/P	Tumour day 7 % of ABD	Tumour day 7 T/P
Heart	(a) 0.377 ± 0.033 (4)	2.11 ± 0.35 (4)	0.307 ± 0.037 (4)	1.37 ± 0.08 (4)	0.326 ± 0.040 (4)	1.77 ± 0.22 (5)
Skeletal muscle	(a) 0.138 ± 0.009 (4)	0.76 ± 0.09 (4)	0.150 ± 0.027 (4)	0.66 ± 0.07 (4)	0.130 ± 0.012 (5)	0.70 ± 0.06 (5)
WAT	(a) 0.049 ± 0.005 (4)	0.30 ± 0.07 (4)	0.089 ± 0.020 (4)	0.39 ± 0.06 (4)	0.123 ± 0.021 (5)**	0.67 ± 0.12 (5)*
BAT	(a) 0.145 ± 0.015 (4)	0.80 ± 0.11 (4)	0.254 ± 0.015 (4)*	1.19 ± 0.095 (4)*	0.187 ± 0.015 (5)	1.01 ± 0.08 (5)
Liver	(a) 0.837 ± 0.072 (4)	4.58 ± 0.46 (4)	1.003 ± 0.079 (4)	4.51 ± 0.16 (4)	1.264 ± 0.028 (4)*	6.58 ± 0.05 (4)*
	(b) 12.69 ± 1.31 (4)		14.77 ± 1.31 (4)		18.49 ± 0.29 (4)*	
Fetus	(a) 0.389 ± 0.020 (4)	2.17 ± 0.28 (4)	0.441 ± 0.041 (4)	2.00 ± 0.17 (4)	0.355 ± 0.023 (5)	1.93 ± 0.15 (5)
	(b) 23.18 ± 1.34 (4)		27.05 ± 3.60 (4)		19.82 ± 1.27 (5)	
Fetal liver	(a) 0.387 ± 0.025 (4)	2.20 ± 0.22 (4)	0.361 ± 0.019 (5)	1.60 ± 0.12 (5)*	0.302 ± 0.021 (5)*	1.63 ± 0.10 (5)
Placenta	(a) 0.558 ± 0.028 (4)	3.05 ± 0.20 (4)	0.701 ± 0.044 (5)*	3.11 ± 0.20 (5)	0.758 ± 0.071 (5)**	4.12 ± 0.42 (5)*
Maternal plasma	(a) 0.183 ± 0.007 (4)	1.00	0.223 ± 0.018 (4)	1.00	0.185 ± 0.003 (5)	1.00
Fetal plasma	(a) 0.144 ± 0.026 (4)	0.91 ± 0.06 (4)	0.267 ± 0.012 (4)*	1.38 ± 0.06 (4)*	0.202 ± 0.015 (5)	1.09 ± 0.08 (5)
Intestinal absorption of AIB (%)	71.6 ± 6.2 (4)		78.7 ± 4.7 (4)		82.9 ± 1.9 (5)	

For full details see Materials and Methods. Tissue AIB uptake is expressed both as a percentage of the absorbed dose (ABD) and also as the tissue/maternal plasma ratio (T/P). (a) % of ABD/g; (b) % of ABD/total tissue. In the case of the fetus, (b) is referred to twelve fetuses. The fetal liver and fetal plasma T/P are calculated in relation to maternal plasma. In the case of the fetus, the placenta and the amniotic fluid were included. The results are mean ± S.E.M. for the number of animals indicated in parentheses. Statistical significance of the results (Wilcoxon two-sample rank sum test): tumour versus control: * $P < 0.05$, ** $P < 0.01$. WAT, white adipose tissue; BAT, brown adipose tissue.

Table 4. Effects of tumour growth on tissue cycloleucine uptake in 20-day pregnant rats

Tissue CLEU uptake		Experimental group: Control % of ABD	Control T/P	Tumour day 7 % of ABD	Tumour day 7 T/P
Heart	(a)	0.316 ± 0.015 (5)	1.50 ± 0.07 (5)	0.262 ± 0.021 (4)	1.00 ± 0.14 (4)**
Skeletal muscle	(a)	0.236 ± 0.006 (5)	1.11 ± 0.03 (5)	0.212 ± 0.008 (4)*	0.80 ± 0.07 (4)
WAT	(a)	0.040 ± 0.001 (5)	0.18 ± 0.01 (5)	0.131 ± 0.019 (4)**	0.49 ± 0.07 (4)**
BAT	(a)	0.146 ± 0.081 (5)	0.68 ± 0.03 (5)	0.198 ± 0.014 (4)*	0.74 ± 0.02 (4)
Liver	(a)	0.240 ± 0.010 (5)	1.14 ± 0.06 (5)	0.251 ± 0.018 (4)	0.95 ± 0.12 (4)
	(b)	2.914 ± 0.240 (5)		3.937 ± 0.151 (4)*	
Fetus	(a)	0.992 ± 0.091 (5)	4.71 ± 0.47 (5)	0.746 ± 0.035 (4)	2.83 ± 0.31 (4)**
	(b)	58.55 ± 6.21 (5)		55.29 ± 5.22 (4)	
Fetal liver	(a)	0.890 ± 0.034 (5)	4.20 ± 0.23 (5)	0.863 ± 0.084 (4)	3.28 ± 0.48 (4)
Placenta	(a)	0.676 ± 0.049 (5)	3.20 ± 0.29 (5)	0.599 ± 0.038 (4)**	2.23 ± 0.11 (4)*
Maternal plasma	(a)	0.214 ± 0.008 (5)	1.00	0.271 ± 0.022 (4)*	1.00
Fetal plasma	(a)	0.988 ± 0.064 (5)	4.69 ± 0.40 (5)	0.824 ± 0.058 (4)	3.09 ± 0.23 (4)*
Intestinal absorption of CLEU (%)		75.0 ± 4.04 (5)		81.3 ± 1.57 (4)	

For full details see Materials and Methods. Tissue CLEU uptake is expressed both as a percentage of the absorbed dose (ABD) and also as the tissue/maternal plasma ratio (T/P). (a) % of ABD/g; (b) % of ABD/total tissue. In the case of the fetus, (b) is referred to twelve fetuses. The fetal liver and fetal plasma T/P are calculated in relation to maternal plasma. In the case of the fetus, the placenta and the amniotic fluid were included. The results are mean ± S.E.M. for the number of animals indicated in parentheses. Statistical significance of the results (Wilcoxon two-sample rank sum test): * $P < 0.05$, ** $P < 0.01$. WAT, white adipose tissue; BAT, brown adipose tissue.

ml of plasma and (b) as the tissue/plasma ratio. Total tissue accumulation of AIB can thus be easily calculated from the former whereas the latter gives an indication of the ability of the tissue to concentrate AIB [22]. While no significant changes were found in AIB uptake in either heart or skeletal muscle of the tumour-bearing rats, the presence of the Yoshida AH-130 tumour caused a large increase in liver AIB accumulation at day 7 (46% increase in total tissue) and also in the liver to plasma ratio at day 7 (44%). Similar observations have previously been made in virgin rats [19]. There was also a significant increase in the accumulation of the tracer in white adipose tissue, 7 days after tumour inoculation, and in brown adipose tissue at day 4 (Table 3).

The feto-placental unit accumulated a similar amount of

[^{14}C]AIB in the tumour-bearing rats both at days 4 and 7. In these animals, there was a decreased accumulation (22% at day 7) and a decreased tissue to plasma ratio (27% and 26% at days 4 and 7, respectively) in fetal liver (Table 3). Conversely, there was an increase in AIB accumulation in the placenta as a result of tumour growth (26% increase at day 4, 36% increase at day 7) (Table 3).

[^{14}C]CLEU accumulation

With the aim of examining whether the effects of the tumour on placental amino acid transfer could also involve other amino acids, the tissue uptake of [^{14}C]cycloleucine (CLEU), an analogue of leucine, mainly transported by system L, was examined using a similar experimental procedure.

The tissue uptake of [^{14}C]CLEU reached a steady state from 60 min after the administration of the tracer in all the tissues studied both in virgin and pregnant rats (data not shown).

The uptake 90 min after the intragastric administration was measured for comparative purposes with the [^{14}C]AIB experiments. The results presented in Table 4 show that the presence of the tumour did not significantly influence the intestinal absorption of the tracer. In contrast, tumour growth (day 7) significantly increased the tissue uptake of CLEU (expressed as % of the absorbed dose) in white (3.3-fold) and brown (1.3-fold) adipose tissues while it decreased that of skeletal muscle (Table 4). When expressed as tissue to plasma ratio, at day 7, there was decreased uptake in heart, but increased uptake in white adipose tissue. No changes in CLEU uptake were found in the feto–placental unit or fetal liver as a result of tumour burden (Table 4). In contrast, when the CLEU uptake was expressed in terms of tissue to plasma ratio, tumour burden resulted in decreases in both the placenta and the fetus (Table 4).

Circulating metabolites

In contrast with the results observed with the *in vivo* administration of the amino acid analogues, the blood concentrations of both alanine (2.7-fold at day 4 and 2.6-fold at day 7) and leucine (1.5-fold at day 4) were significantly elevated in the fetuses of the tumour-bearing rats compared to control fetuses (Table 5). Total fetal blood amino acids were also significantly elevated (1.9- and 1.8-fold at days 4 and 7, respectively). In contrast, there were no significant differences in maternal alanine concentrations, but for leucine there was a significant decrease (32% at day 4) (Table 5). Total maternal amino acids also decreased 7 days after tumour inoculation.

Tumour growth also resulted in important increases in the concentration of both lactate (1.9-fold at day 4, 2-fold at day 7) and glucose (1.4-fold at day 4, 1.7-fold at day 7) compared to controls. These increases were associated with a significantly lower insulin concentration at day 7 (24%). In non-pregnant rats bearing this kind of tumour, normoglycaemia is maintained while lactate concentrations are also increased by tumour burden [23]. The increase in glycaemia could either be due to an enhanced hepatic glucose production [24] and/or an increased glucose sparing by the tumour [25].

[^{14}C]DDT accumulation

In spite of the fact that tumour growth did not affect the transplacental passage of the amino acid analogues, the fetal circulating amino acid concentration was significantly increased as a result of tumour burden. In order to determine if this was a consequence of an altered blood flow to the feto–placental unit, the relative tissue blood flow was estimated using [^{14}C]DDT. The insecticide DDT (1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane) has a high affinity for neutral fat with a lipid/aqueous partition coefficient of approximately 4000:1. In addition, equilibrium between the lipid and aqueous phase is not reached for many hours, and thus provides an average measure of blood flows over longer periods of time than is obtainable with other indirect methods. As can be seen from the data presented in Table 6, tumour growth decreased the amount of tracer per g of tissue in liver (26%) and fetus (24%), while it did not significantly affect the uptake of tracer in the rest of the tissues studied with the exception of skeletal muscle where the tumour induced a significant increase. In addition, the circulating levels of [^{14}C]DDT in the fetal plasma were much lower than those found in the mother, but higher in tumour-bearing animals in relation to the control group (Table 6).

DISCUSSION

To some extent, the fetus can be compared with a fast-growing tumour since their growth rates are exponential, both

Table 5. Plasma insulin, blood leucine, alanine, total amino acids and other metabolites after tumour growth in late pregnant rats

	Experimental groups					
	Control		Tumour day 4		Tumour day 7	
	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal
Alanine	506 ± 64 (5)	577 ± 66 (5)	521 ± 31 (5)	1567 ± 128 (5)**	535 ± 18 (7)	1481 ± 99 (7)***
Leucine	185 ± 11 (5)	211 ± 17 (5)	125 ± 7 (5)**	310 ± 11 (5)**	140 ± 13 (7)*	241 ± 16 (7)
Total amino acids	4053 ± 289 (5)	4339 ± 377 (5)	3830 ± 117 (5)	8369 ± 477 (5)**	3853 ± 57 (7)*	7865 ± 510 (7)***
Glucose	4.29 ± 0.26 (5)		6.09 ± 0.35 (5)**		7.43 ± 0.40 (5)**	
Lactate	1.80 ± 0.23 (4)		3.38 ± 0.31 (4)**		3.63 ± 0.47 (7)*	
Insulin	2.11 ± 0.15 (5)		1.62 ± 0.18 (5)		1.61 ± 0.12 (7)*	

For full details see Materials and Methods. The results are mean ± S.E.M. for the number of animals indicated in parentheses. Amino acid concentrations are in μM and glucose and lactate concentrations are in mM. Insulin is expressed as mU/ml. Statistical significance of the results (Wilcoxon two-sample rank sum test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ comparing data between groups.

Table 6. Effects of tumour growth on tissue [^{14}C]DDT uptake in 20-day pregnant rats

Tissue		Experimental groups Control	Tumour day 7
Liver		0.820 ± 0.080 (5)	0.610 ± 0.046 (5)*
Heart		0.237 ± 0.024 (4)	0.226 ± 0.009 (5)
Skeletal muscle		0.095 ± 0.007 (4)	0.123 ± 0.005 (5)*
WAT		0.143 ± 0.028 (5)	0.168 ± 0.008 (5)
BAT		0.869 ± 0.162 (3)	0.871 ± 0.134 (5)
Placenta		0.088 ± 0.013 (5)	0.071 ± 0.005 (5)
Fetus		0.033 ± 0.001 (5)	0.025 ± 0.001 (5)**
Fetal liver		0.069 ± 0.005 (5)	0.065 ± 0.004 (5)
Maternal plasma	*0 min.*	2.043 ± 0.370 (5)	1.902 ± 0.179 (5)
	20 min.	0.428 ± 0.072 (5)	0.216 ± 0.014 (5)*
Fetal plasma	*20 min.*	0.058 ± 0.005 (5)	0.118 ± 0.004 (5)**

For full details see Materials and Methods. Relative [^{14}C]DDT uptake was assessed as previously described [13]. In the case of the fetus, the placenta and the amniotic fluid were included. The results are mean ± S.E.M. for the number of animals indicated in parentheses and are expressed as % of administered dose per gram or ml. Statistical significance of the results (Wilcoxon two-sample rank sum test): * $P < 0.05$, ** $P < 0.01$. WAT, white adipose tissue; BAT, brown adipose tissue.

being highly dependent on glucose and amino acids. In the present study, our intention was to analyse the results of competition for growth between the fetus and a fast-growing implanted tumour. Previous results derived from the implantation of the Walker 256 carcinosarcoma to late pregnant rats have suggested that tumour burden impairs early neonatal development [9]. In contrast with these observations, we have seen that the implantation of the fast-growing Yoshida AH-130 ascites hepatoma results in normal fetal growth coexisting with a normal tumour growth. This is not related to changes in the fetal uptake of amino acids as measured by the accumulation of [^{14}C]AIB and [^{14}C]CLEU. The unexpectedly unaltered transplacental passage of amino acid analogues in the tumour-bearing animals does not seem to be a consequence of changes in blood flow to the fetus since this parameter was actually significantly decreased as a result of the presence of the tumour. The fact that [^{14}C]AIB and [^{14}C]CLEU uptake was not altered by tumour growth, in spite of the commented decreased fetal blood flow, suggests that placental amino acid transport is highly altered as a result of tumour burden. In addition, the effects of tumour growth increasing the fetal circulating amino acid concentration cannot be accounted for by an increase in the fetal blood flow. Conversely, this was actually lower in the tumour-bearing animals, suggesting that changes in the kinetics of the amino acid transporting systems must have taken place after tumour burden. Current studies using reconstituted placental membrane vesicles are being undertaken to characterise the kinetics of both alanine and leucine transport.

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