

# Blood Amino Acid Compartmentation in Mice Bearing Lewis Lung Carcinoma<sup>1</sup>

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## ABSTRACT

Blood amino acid compartmentation between plasma and red blood cells was studied in mice bearing the Lewis lung carcinoma. The animals showed a change in compartmentation with an increase in the concentration of most amino acids in the plasmatic fraction with the exception of glycine, glutamine, aspartate, asparagine, and taurine. This work focuses on the importance of studying the distribution of amino acids in both fractions when performing studies concerning amino acid metabolism in tumor-bearing animals.

## INTRODUCTION

RBC have been recognized as having an important role in the interorganic transport of several metabolites (1, 2) including amino acids (3, 4). Although plasma amino acids are generally used as an index of the amino acid-transporting ability of blood, the erythrocyte content is not always dependent on the blood levels (5). On the other hand, the quantitative importance of RBC in amino acid transport has not thus far been completely established. In addition, blood amino acid compartmentation may depend on the physiological conditions of the animal and the measurement of plasma levels underestimate the actual blood capacity for amino acid transport.

The Lewis lung carcinoma has been described as an anaplastic epidermoid carcinoma with a marked hemorrhagic tendency (6). It is basically a well-known neoplasia that has a short infective cycle linked to a huge growth and a lung metastatic process which causes a fast death (7). The metabolic interactions between host and tumor are not yet well understood and they have received little attention compared with other aspects of tumor biochemistry (8).

The majority of studies on tumor action devote themselves to their physiological/biochemical differences, the degree of infectiousness together with the possible measures that can be used to eradicate it. However, the actual biochemical events that take place in the host have not been studied extensively. Malignant tumors compete with their host for certain amino acids and glucose to satisfy their needs; this results in a progressive hypoglycemia (9) and host hepatic glycogen depletion (10). It has also been speculated that the tumor also functions as a nitrogen trap (11) actively competing with their host for nitrogen compounds.

It was our aim to estimate blood amino acid content and distribution in the plasmatic and cellular compartment in mice bearing the Lewis lung carcinoma.

## MATERIALS AND METHODS

Consanguineous C57BL/6 mice were used. The animals were kept in a light (on from 9 a.m. to 9 p.m.), temperature (21–22°C), and humidity

(70–80%) controlled room. They remained housed in collective polypropylene-bottomed cages with wood shavings as absorbent material. They were fed *ad libitum* on AO3 type rat chow pellets (Panlab, Barcelona, Spain) and offered tap water. The tumor was inoculated via an i.m. (left thigh) injection containing  $5 \times 10^5$  tumor cells. The Lewis lung carcinoma had been maintained by successive implantation in our laboratory. The injection resulted in the appearance of lung metastases from day 7 onward and a growth of the primary tumor at the site of the injection that attained about one-half of the gross body weight on day 25. The mice died between days 27 and 29.

Blood samples were obtained by heart puncture, using heparinized syringes, on day 0, 15, and 25 min after the infection. Blood was collected into dry heparinized plastic beakers and also into heparinized capillary tubes for hematocrit determination. The hematocrit values obtained were  $43.7 \pm 2.1$  (SE) for controls and  $26.4 \pm 3.2$  and  $21.8 \pm 1.5$  for tumor-bearing mice (days 15 and 25, respectively). Plasma was separated by means of a refrigerated centrifuge. The blood pellet was resuspended in distilled water (1:4) in order to attain cellular lysis. Aliquots of plasma and resuspended cells were deproteinized with 10% (w/v) trichloroacetic acid and after centrifugation the clear supernatant was used for amino acid determination by means of a Chromaspek S-180 (Rank-Hilger) amino acid autoanalyzer equipped with a fluorimetric detector. Norleucine was used as an internal standard.

Blood amino acid compartmentation was calculated correcting plasma and blood levels by hematocrit values (5) as  $E = [WB - (1 - H)^*P]/H$ ; free amino acid concentrations in erythrocytes ( $E$ ); free amino acid concentrations in whole blood ( $WB$ ); free amino acid concentrations in plasma ( $P$ ); hematocrit ( $H$ ) values are expressed as a fraction. No differences in the amino acid compartmentation were observed in animals with different hematocrit. Statistical differences were calculated using Student's  $t$  test and analysis of variance computer programs.

## RESULTS

Table 1 shows the whole blood amino acid concentration found in C57BL/6 mice. It is interesting to point out that the highest concentration found is that of taurine, its value in the control animals being nearly double that of glutamine. The presence of the tumor increased the whole blood concentration of alanine, glutamine, aspartate, histidine, and taurine and decreased that of asparagine, isoleucine, valine, cysteine, tyrosine, and tryptophan.

The blood amino acid compartmentation is shown in Fig. 1. The majority of amino acids are more concentrated in the plasmatic fraction with the exception of glutamate, aspartate, asparagine, and arginine. The total concentrations of amino acids in each fraction were  $4013 \pm 380.1$  (controls),  $3818.2 \pm 252.4$  (tumor 15) and  $4158.8 \pm 308.6$  (tumor 25) for the cellular fraction, and  $2224 \pm 162.3$  (controls),  $2533.7 \pm 363.9$  (tumor 15), and  $2892.6 \pm 330.2$  (tumor 25) for the plasmatic fraction. The presence of the tumor causes significant variations in the plasmatic concentrations of all the amino acids considered with the exception of aspartate, arginine, and ornithine. On the other hand, glycine, aspartate, taurine, and proline increased significantly their cellular concentration in tumor-bearing mice. Conversely, glutamine, asparagine, leucine, isoleucine, valine, arginine, histidine, ornithine, citrulline, tyrosine, and tryptophan decreased their concentration values in the cellular fraction with the implantation of the carcinoma while, alanine,

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BLOOD AMINO ACID LEVELS IN MICE

Table 1 Whole blood amino acid concentration and plasma/cell ratios in tumor-bearing mice

Amino acid	Concentration ( $\mu\text{M}$ )						Analysis of variance <sup>d</sup>
	Control		Tumor, 15th day		Tumor, 25th day		
	Total	Ratio	Total	Ratio	Total	Ratio	
Alanine	476.4 ± 39.2 <sup>b</sup>	1.32 ± 0.11	637.2 ± 65.5	2.13 ± 0.22 <sup>c</sup>	913.6 ± 55.6 <sup>c, d</sup>	2.71 ± 0.16 <sup>c</sup>	c
Glycine	501.4 ± 54.3	1.34 ± 0.14	588.1 ± 54	0.66 ± 0.06 <sup>c</sup>	662.1 ± 59.4	0.75 ± 0.07 <sup>c</sup>	c
Glutamate	147.0 ± 23.1	0.39 ± 0.06	153.0 ± 25.4	0.37 ± 0.06	225.2 ± 26.7 <sup>c</sup>	0.64 ± 0.07 <sup>c, d</sup>	c
Glutamine	701.6 ± 70.0	1.19 ± 0.12	928.5 ± 79.0	3.54 ± 0.30 <sup>c</sup>	665.7 ± 72.5 <sup>d</sup>	1.63 ± 0.18 <sup>c, d</sup>	c
Aspartate	222.5 ± 32.8	0.21 ± 0.03	498.2 ± 71.4 <sup>c</sup>	0.09 ± 0.01 <sup>c</sup>	446.5 ± 71.6 <sup>c</sup>	0.12 ± 0.02 <sup>c</sup>	c
Asparagine	66.1 ± 8.4	0.78 ± 0.01	38.1 ± 6.7 <sup>c</sup>	1.80 ± 0.32 <sup>c</sup>	36.6 ± 1.8 <sup>c</sup>	0.15 ± 0.01 <sup>c, d</sup>	c
Threonine	196.3 ± 20.2	1.66 ± 0.17	179.8 ± 16.8	2.15 ± 0.20	217.4 ± 19.2	3.47 ± 0.31 <sup>c, d</sup>	NS <sup>e</sup>
Serine	161.8 ± 14.0	1.55 ± 0.13	108.1 ± 11.4 <sup>c</sup>	2.69 ± 0.28 <sup>c</sup>	183.4 ± 17.8 <sup>d</sup>	2.42 ± 0.23 <sup>c</sup>	c
Leucine	272.4 ± 16.7	2.34 ± 0.14	182.0 ± 11.1 <sup>c</sup>	2.40 ± 0.15	256.6 ± 18.8 <sup>d</sup>	3.53 ± 0.26 <sup>c, d</sup>	c
Isoleucine	161.0 ± 8.8	2.96 ± 0.16	106.1 ± 6.3 <sup>c</sup>	2.90 ± 0.17	143.4 ± 9.9 <sup>d</sup>	4.10 ± 0.28 <sup>c, d</sup>	c
Valine	352.0 ± 12.4	2.80 ± 0.10	239.8 ± 35.6 <sup>c</sup>	4.23 ± 0.63	334.8 ± 23.2 <sup>d</sup>	4.60 ± 0.32 <sup>c</sup>	c
Lysine	474.0 ± 23.6	1.02 ± 0.05	472.8 ± 35.6	1.11 ± 0.08	611.0 ± 33.0 <sup>d</sup>	2.19 ± 0.12 <sup>d</sup>	NS
Arginine	137.4 ± 14.1	0.50 ± 0.05	121.1 ± 13.3	0.66 ± 0.07	122.8 ± 17.9	0.73 ± 0.11	NS
Histidine	88.4 ± 7.3	3.42 ± 0.28	91.7 ± 5.4	5.19 ± 0.30 <sup>c</sup>	119.4 ± 6.8 <sup>c, d</sup>	5.32 ± 0.30 <sup>c</sup>	c
Ornithine	197.5 ± 26.2	10.1 ± 1.34	179.7 ± 25.3	10.90 ± 1.53	161.9 ± 11.8	14.42 ± 1.05	c
Citrulline	162.2 ± 12.2	2.80 ± 0.21	56.8 ± 8.0 <sup>c</sup>	3.58 ± 0.50	38.0 ± 13.7 <sup>c</sup>	3.31 ± 1.19	c
Methionine	61.0 ± 5.5		83.1 ± 23.8	1.00 ± 0.29	75.0 ± 12.3	4.55 ± 0.74 <sup>d</sup>	NS
Taurine	1285.2 ± 93.3	2.86 ± 0.20	1361.9 ± 93.2	1.70 ± 0.12 <sup>c</sup>	1326.0 ± 115.7	0.70 ± 0.06 <sup>c, d</sup>	NS
Tyrosine	140.5 ± 9.8	2.03 ± 0.14	95.1 ± 6.5 <sup>c</sup>	2.53 ± 0.17	108.9 ± 7.4	2.79 ± 0.19 <sup>c</sup>	c
Tryptophan	62.4 ± 6.5	9.06 ± 0.94	33.9 ± 2.5 <sup>c</sup>		21.0 ± 8.7 <sup>c</sup>		c
Phenylalanine	102.3 ± 6.7	5.82 ± 0.38	86.6 ± 5.0	5.80 ± 0.33	125.5 ± 14.1 <sup>d</sup>	6.69 ± 0.75	c
Proline	268.8 ± 42.7		151.7 ± 17.7 <sup>c</sup>	1.26 ± 0.15	256.5 ± 20.9 <sup>d</sup>	2.94 ± 0.24 <sup>d</sup>	

<sup>a</sup> Related to total amino acid concentrations.  
<sup>b</sup> Mean ± SE. The number of animals used was 5–8 per group.  
<sup>c</sup> Control versus tumor-bearing  $p < 0.05$ .  
<sup>d</sup> 15th versus 25th day tumor-bearing  $p < 0.05$ .  
<sup>e</sup> NS, not significant.

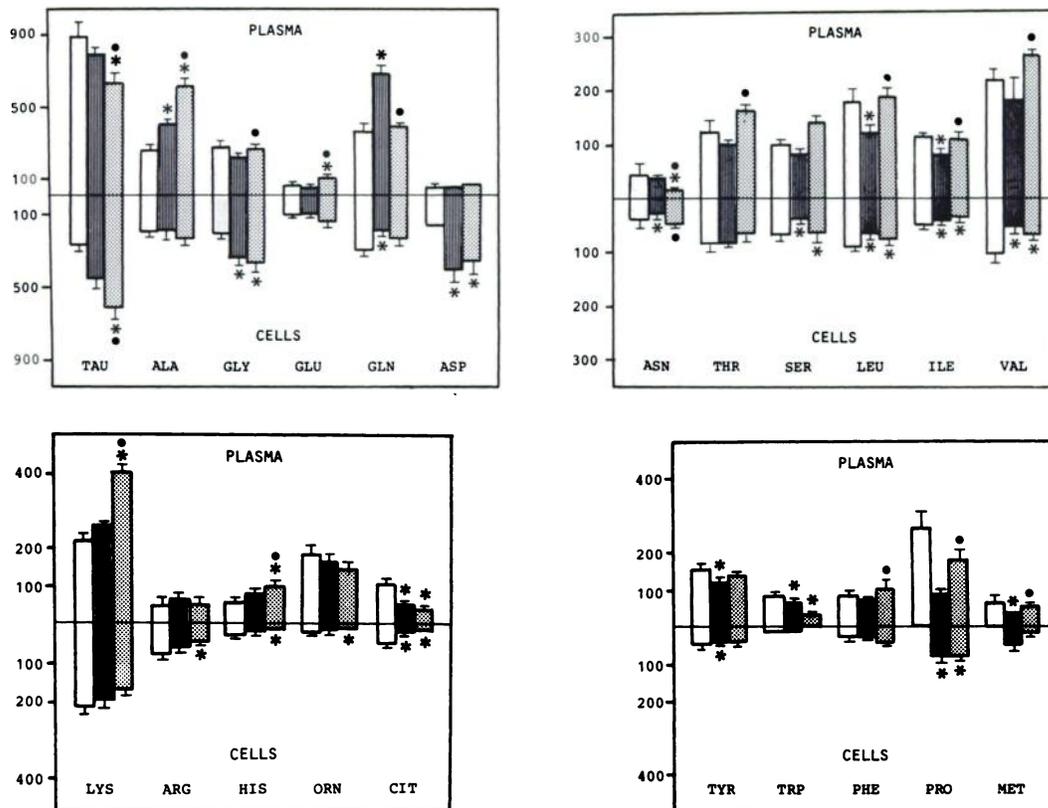


Fig. 1. Blood amino acid compartmentation in mice. Concentrations are micromolar. Open bars, controls; striped bars, day 15 after the implantation; dotted bars, day 25 after the implantation; \*, control versus tumor bearing,  $p < 0.05$ ; ●, 15 versus 25th day tumor bearing. ORN, ornithine; CIT, citrulline; TAU, taurine.

glutamate, lysine, methionine, and phenylalanine did not show significant variations.

Table 1 also shows the plasma/cell ratio for the different amino acids studied. Tumor-bearing mice show increased ratios for all the amino acids considered with the exceptions of glycine, glutamine, aspartate, asparagine, and taurine.

DISCUSSION

In recent years accumulated data have indicated that glucose, and therefore pyruvate, may not be a major respiratory source for cellular energy in some tumors (12, 13) and that amino acids may play an important role in tumor cell proliferation. In fact, a substantial body of experimental evidence indicates that

glutamine is a very important respiratory fuel for tumor cells. Glutamine has been shown to be an unusually good substrate for oxidation by tumor cell mitochondria (14), and intact Ehrlich cells oxidize glutamine to CO<sub>2</sub> at higher rates than any other amino acid present (15, 16). Indeed, the mitochondrial glutaminase activity of several rat hepatomas was linearly correlated with the growth rates and degree of malignancy (17). In addition to their role as energetic substrates amino acids can serve as amide or amino nitrogen in reactions leading to the biosynthesis of a number of important metabolites including the pyrimidine, purine, and pyridine nucleotides and amino sugars (18).

It can be stated that tumors function as nitrogen traps and they compete with their hosts for nitrogen compounds. This process produces in the host a negative nitrogen balance, a characteristic weight loss, and a reciprocal nitrogen increase in the tumor. The biochemical mechanisms underlying these phenomena still remain unclear (19). A hypothesis, based on the observation of the leucine requirements of tumor, advanced by Lazo (20) is that concentration gradients are established between the free amino acid pools, with a net flux of amino acids taking place toward the tumor cell. Similar results were reported by Fürst *et al.* (21) who studied the amino acid pools in several groups of cancer patients.

*In vitro* experiments have shown that the equilibration ratio between blood fractions is very slow (22) having been postulated that there is no rapid amino acid exchange. However, despite these low *in vitro* rates of interchange between both blood pools, considerable changes are found in blood amino acid compartmentation in mammals under different physiological concentrations. In addition, it was demonstrated, however, that *in vivo* the equilibration between blood fractions occurs very rapidly (23). At the same time, a direct, although not well defined, interrelationship for amino acid exchange between erythrocytes and tissue cells has also been suggested.

From our results it is interesting to point out the high levels of taurine found in blood. This observation has also been reported in other animals (24, 25). The precise function of this amino acid still remains controversial it having been suggested that taurine is involved in a wide range of metabolic responses that include a functional role in membranes, calcium flux, and possibly cyclic AMP activity (26). The dramatic influx of taurine into plasma cells that takes place as tumor development progresses is a very interesting observation that unfortunately we are not able, at present, to explain. The significant rise in whole blood alanine and glutamine concentrations in tumor-bearing mice could be related to an enhanced skeletal muscle protein depletion (10, 27).

In mice, blood amino acid compartmentation follows a different pattern than that of rat where blood amino acids are more concentrated in the cellular fraction (28). Although blood amino acid compartmentation could be influenced by the type and size of tumor, in this particular case the implantation of the tumor results in an increase in the majority of plasma/cell steady state ratios, which would seem to imply either an enhanced utilization of these compounds by the mouse erythrocytes or an increased release of amino acids from the host tissues into the plasmatic fraction. This observation agrees with the general pattern of tissue protein mobilization and amino acid release found in the majority of tumor studies (21).

Although erythrocyte amino acid levels as compared to

plasma levels rarely have been used in metabolic disorders in patients (29), the present study is the first one to consider blood amino acid compartmentation in tumoral situations, an interesting approach that may lead to future promising research in amino acid interchange between the tumor and the host.

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