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Amino acid metabolism in tumour-bearing mice

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Mice bearing the Lewis lung carcinoma showed a high tumour glutaminase activity and significantly higher concentrations of most amino acids than in both the liver and the skeletal muscle of the host. Tumour tissue slices showed a marked preference for glutamine, especially for oxidation of its skeleton to CO_2. It is proposed that the metabolism of this particular carcinoma is focused on amino acid degradation, glutamine being its preferred substrate.

INTRODUCTION

The metabolic interactions between host and tumour are not yet well understood, and they have received little attention compared with other aspects of tumour biochemistry (Schaur *et al.*, 1980). Malignant tumours compete with their host for glucose to satisfy their needs; this results in a progressive hypoglycaemia (Romeu *et al.*, 1986) and host hepatic glycogen depletion. It has also been proposed that the tumour functions as a nitrogen trap (Mider, 1951), actively competing with their host for nitrogen compounds (Landel *et al.*, 1985). The presence of a growing tumour has a pronounced effect on the nitrogen metabolism of the host, causing a general wastage of tissues which is particularly reflected in a loss of muscular protein (Clark & Goodlad, 1971).

A large amount of experimental evidence points towards amino acids, especially glutamine, as being the major respiratory fuels for cancer cells. Similarly, glutamine has been shown to be an unusually good substrate for oxidation by tumour cell mitochondria (Abou-Khalil *et al.*, 1983; Kovacevic & McGivan, 1983; Moreadith & Lehninger, 1985). Ehrlich ascites-tumour cells oxidize glutamine to CO_2 at higher rates than for any other amino acid present. Several studies have correlated the glutaminase activity of several rat hepatomas with the degree of malignancy (Linder-Horowitz *et al.*, 1969). These findings strengthen the possibility that tumours could impose a significant demand for glutamine in the host, and suggest that alterations in the supply of glutamine could provide a means of controlling tumour growth.

The Lewis lung carcinoma (3LL) originated in a spontaneous tumour of a C57 black mouse (Sugiura & Stock, 1955) and has been maintained by successive transplantation. The tumour has been described as an anaplastic epidermoid with a marked haemorrhagic tendency, which produces multiple lung metastasis regularly, spontaneously and consistently (Hellman & Burrage, 1969), and it is extremely refractory to most chemotherapeutic agents (Lippman *et al.*, 1975). It is basically a well-known neoplasia that has a short infective cycle linked to a huge growth and a lung metastatic process which soon causes death (Henry *et al.*, 1983).

It was our aim to study amino acid metabolism in tumour-bearing mice, and in the present work we report different enzyme activities and amino acid contents of both the tumour tissue and different host tissues.

MATERIALS AND METHODS

Materials

D-[U-^{14}C]Glucose (10.09×10^9 Bq/mmol), L-[U-^{14}C]-alanine (6.38×10^9 Bq/mmol), [U-^{14}C]glycine (4.14×10^9 Bq/mmol), L-[U-^{14}C]glutamate (10.6×10^9 Bq/mmol), L-[U-^{14}C]glutamine (10.57×10^9 Bq/mmol) and L-[U-^{14}C]-leucine (12.77×10^9 Bq/mmol) were purchased from Amersham International, Amersham, Bucks., U.K. The other chemicals used were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals and tumour inoculation

Consanguineous mice of the C57BL/6 strain were used. The animals were kept in a light- (on from 09:00 to 21:00 h), 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 A03 rat chow pellets (Panlab, Barcelona, Spain) and given tap water. As infective tumoural agent, the Lewis lung carcinoma 3LL was used. The initial inoculum was kindly given by the Unitat de Recerca Biomèdica from the Hospital de la Santa Creu i de Sant Pau of Barcelona, and was maintained alive by consecutive inoculation of mice of the same strain. The tumour was inoculated via an intramuscular (left thigh) injection containing 5×10^5 viable vegetative cells obtained from a mature similar tumour. The development of a nodule at the site of injection, growing in size up to almost half the animal weight, was considered as an index of effectiveness of the inoculation. Nearly 100 % of the injected animals developed hind-leg tumours, and all of them were affected by lung metastasis from day 7 onwards. The mice died between days 27 and 29.

Metabolite and enzyme assays

On days 0 (non-inoculated animals), 15 and 25 after the inoculation, groups of six to seven animals were

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Table 1. Characteristics of the animals affected with the Lewis lung carcinoma

Results are expressed as means ± S.E.M. for 10–15 different animals.

Parameter	Time after inoculation of the tumour (days)		
	0	15	25
Animal weight (g)	23.4±0.3	26.9±0.5	32.0±1.0
Primary tumour weight (g)		6.2±0.2	11.3±0.5
Lung weight (mg)	129±2.2	174±2.3	310±35.1
Metastasis weight (mg)		6.5±1.2	159±22.0
Pulmonar invasion (%)		3.73	51.3
Haematocrit value (%)	43.7±2.1	26.4±3.2	21.8±1.5

killed, and their tumour mass, metastasis weight and percentage of pulmonar invasion were assessed. Samples of blood were taken and centrifuged in a refrigerated centrifuge for 30 min at 3000 rev./min. Plasma samples were used for haematocrit estimation with capillary tubes, plasma glucose (Huggett & Nixon, 1957) and urea (Fawcett & Scott, 1960) determinations. Samples of liver, tumoural tissue and hind-leg striated muscle were dissected and frozen immediately in liquid N_2 and kept frozen at −70 °C. Then they were homogenized in 10 vol. of chilled 0.25 M-sucrose containing 2.5 mM-2-mercaptoethanol, with an all-glass motor-driven Tenbroeck homogenizer.

Crude homogenates were used for determinations of protein with the Folin phenol reagent (Lowry *et al.*, 1951), glycogen (Good *et al.*, 1933) and amino acids, as well as for the spectrophotometric assay of glutamate dehydrogenase (with NAD^+ as coenzyme) (Schmidt, 1974) and alanine transaminase (Bergmeyer & Bernt, 1974*b*) and aspartate transaminase (Bergmeyer & Bernt, 1974*a*). The activities of adenylate deaminase (Arola *et al.*, 1981*b*), glutamine synthetase (Arola *et al.*, 1981*a*) and glutaminase (Curthoys & Lowry, 1973) were also determined in the homogenates by determining the activity several times and calculating the corresponding initial velocities. All activities were expressed as microkatals (μkat) per g of tissue or, alternatively, per g of protein to render them more comparable. Total lipids in the different tissues were measured after extraction with chloroform/methanol (Folch *et al.*, 1957). Tissue water content was estimated after dehydration of the tissue for 24 h at 110 °C.

Experiments *in vitro*

Pieces (25–30 mg) of tumour tissue were incubated in specially designed flasks in a Krebs–Ringer (Umbreit *et al.*, 1964) bicarbonate buffer, pH 7.4, containing 1% fatty-acid-free bovine serum albumin. The medium also contained 100 μl of one of the different radioactive substrates, of specific radioactivity 1833.3 Bq/μmol for glucose and 4583.3 Bq/μmol for the different amino acids. Flasks were sealed with rubber stoppers equipped with wells hanging from the stoppers, gassed with O_2/CO_2 (19:1) and incubated for 60 min in a thermostatically controlled bath (37 °C) with a shaking device (80 cycles/min). Hyamine hydroxide (0.2 ml) was added to hanging wells, and the reactions were stopped by addition of 3 M-$HClO_4$ (0.5 ml) to the reaction medium. The wells were counted for radioactivity in order to assess the amount of the different substrates that was oxidized to CO_2 during the incubation time. The incorporation of the different tracers to the tissues was estimated after NCS solubilization of the tissue fragments for 2 h at 50 °C and decoloration with H_2O_2 (Peng, 1977). The solubilized tissue content was then mixed with liquid-scintillation cocktail and its radioactivity content measured in a Packard 460C apparatus.

RESULTS

The total weight of inoculated mice is increased as a result of tumour proliferation (Table 1). Tumour weight can account for 23 or 35% of the total body weight, 15 or 25 days after the inoculation respectively. The actual weight loss observed in tumour-bearing mice represents 10% of the body weight (excluding the tumour mass). The weight of the pulmonar metastasis increases tremendously during the last 10 days, the tumour-bearing animals having 51.3% of pulmonar invasion 25 days after inoculation of the tumour. The haematocrit value is decreased to half the value for control animals at day 25, showing values of only 22%.

Table 2 shows the different amino acid-metabolic enzyme activities measured in the different experimental groups. AMP deaminase activity in mice, 25 days after the inoculation of the carcinoma, shows values similar to those found in the liver of control mice, and the presence of the tumour causes decreases in this activity in both liver and muscle. The tumour itself decreased significantly AMP deaminase activity from day 15 to day 25.

The activity of glutamine synthetase detected in the tumour is of the same order as that found in the skeletal muscle of control animals. The presence of the tumour causes a significant decrease of this enzymic activity in liver, and an increase in muscle.

Glutamate dehydrogenase activity in tumour does not vary throughout the tumoural cycle, and shows values similar to those found in muscle of control mice. However, in this last tissue, there is a significant increase in this enzymic activity throughout the tumoural cycle. On the other hand, there are no changes in the activity of this enzyme in liver during the cycle.

Glutaminase activity is very high in tumour, in contrast with liver and muscle, where the activities found for this enzyme were very low.

Tumour alanine transaminase activity is very low.

Table 2. Amino acid-metabolism enzyme activities in mice bearing the Lewis lung carcinoma

Enzyme activities are expressed as (a) μkat/g of protein, or (b) μkat/g fresh tissue wt. Values are means ± S.E.M. for five to seven different animals. Statistical significance of the results: * non-tumour versus tumour, $P < 0.05$; †day 15 versus day 25, $P < 0.05$.

Enzyme	Tissue	Mice ...	Non-tumour-bearing	Tumour-bearing	
				Day 15	Day 25
AMP deaminase	Tumour	a	–	0.84±0.80	0.26±0.02†
		b	–	10.6±2.4	2.30±0.44†
	Liver	a	0.16±0.09	0.07±0.01*	0.05±0.00*
		b	2.69±0.10	1.16±0.06*	1.40±0.19*
	Muscle	a	47.0±3.24	50.6±9.42	18.9±3.8*
		b	789.6±60.6	461.7±67.0*	219.8±63.6*†
Glutamine synthetase	Tumour	a	–	0.10±0.01	0.17±0.01†
		b	–	1.43±0.27	2.24±0.11†
	Liver	a	10.8±0.69	4.55±1.04*	7.89±0.46*†
		b	191.5±14.1	94.1±22.4*	193±5.82†
	Muscle	a	0.12±0.02	0.21±0.01*	0.34±0.03*†
		b	2.16±0.32	2.02±0.21	3.23±0.43
Glutamate dehydrogenase	Tumour	a	–	0.43±0.06	0.39±0.03
		b	–	5.15±0.15	5.02±0.28
	Liver	a	5.39±0.15	6.14±0.82	5.41±0.41
		b	93.8±2.0	119.7±14.9	117.2±6.9*
	Muscle	a	0.30±0.02	0.63±0.06*	0.62±0.08*
		b	5.18±0.42	5.39±0.52	6.14±0.15
Glutaminase	Tumour	a	–	1.12±0.06	1.26±0.12
		b	–	13.30±0.70	15.97±1.54
	Liver	a	0.32±0.02	0.43±0.10*	0.45±0.04*
		b	5.92±0.39	8.97±0.23*	9.78±0.98*
	Muscle	a	0.49±0.01	0.01±0.00*	0.02±0.01*
		b	0.75±0.06	0.15±0.07*	0.24±0.15*
Alanine transaminase	Tumour	a	–	0.14±0.01	0.09±0.02
		b	–	1.77±0.53	1.12±0.16†
	Liver	a	1.87±0.13	3.16±0.27*	2.38±0.01*†
		b	33.74±1.95	62.52±3.20*	54.20±1.64*
	Muscle	a	0.34±0.01	0.71±0.07*	0.62±0.11*
		b	5.48±0.35	7.50±0.37	5.05±0.78
Aspartate transaminase	Tumour	a	–	1.47±0.12	1.02±0.13†
		b	–	18.7±0.77	12.55±1.18†
	Liver	a	7.59±0.15	6.67±0.72	6.73±0.31†
		b	132.6±1.85	129.1±10.25	151.6±9.47
	Muscle	a	4.79±0.52	9.01±0.27*	4.89±0.56†
		b	80.04±6.13	86.26±7.49	53.0±2.46*†

However, the presence of the tumour causes significant increases in the liver and muscle activities of this enzyme.

The aspartate transaminase activity is very low in the tumour tissue. Tumour-bearing mice show significantly higher activity of this enzyme 15 days after the inoculation, and significantly lower activity at the last phase of the tumoural cycle. On the other hand, liver aspartate transaminase does not change much after the inoculation of the tumour.

Table 3 shows the different amino acid concentrations in tumour, liver and skeletal muscle of mice bearing the Lewis lung carcinoma. The concentrations of alanine, glycine, asparagine, threonine, serine, leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine and proline in tumour tissue are higher than those found in muscle and liver. Only glutamine, histidine, ornithine, citrulline and methionine are at lower concentrations in tumour than in liver and muscle. The concentration of glutamate in tumour is higher than that found in liver, and similar to that found in muscle. Aspartate is at a higher concentration in tumour than in muscle, although less than in liver. With a few exceptions (aspartate, serine, valine, lysine, tyrosine, tryptophan, phenylalanine, proline and taurine) the different amino acid concentrations are similar in tumour-bearing animals throughout the proliferative cycle. During the cycle the concentrations of certain amino acids are significantly increased in both liver and muscle (leucine, isoleucine, histidine, phenylalanine and taurine), decreased in both (glycine, aspartate and ornithine), increased in muscle and decreased in liver (tyrosine) or decreased in muscle and increased in liver (glutamate and lysine). In other cases there are no changes in the muscle and liver concentrations (asparagine, threonine, tryptophan and alanine); however, there are increases (valine) or decreases (citrulline) in the muscle concentrations. Finally, in some cases there are no changes in the muscle concentrations, and

Table 3. Amino acid concentrations in tumour, liver and skeletal muscle in mice bearing the Lewis lung carcinoma

Abbreviations: T, tumour; L, liver; M, muscle. Results are expressed as means ± S.E.M. for five to seven different animals. Amino acid concentrations are in μmol/g of fresh tissue; N.D., not detectable. Statistical significance of the results: * non-tumour versus tumour, $P < 0.05$; † day 15 versus day 25, $P < 0.05$.

Amino acid	Tissue	Mice ... Non-tumour-bearing	Tumour-bearing	
			Day 15	Day 25
Alanine	L	5.43±0.22	6.71±0.28*	5.83±0.26†
	M	4.63±0.40	5.01±0.36	4.47±0.02
	T		8.04±0.77	7.05±0.01
Glycine	L	4.25±0.20	3.65±0.06*	3.15±0.15*†
	M	5.03±0.37	4.20±0.23	2.74±0.07*†
	T		6.42±0.19	5.88±0.34
Glutamate	L	2.33±0.23	2.75±0.16	3.73±0.26*†
	M	1.63±0.18	0.97±0.15*	0.89±0.13*
	T		6.91±0.56	6.18±0.48
Glutamine	L	8.34±1.40	7.56±0.25	4.85±0.61*†
	M	4.71±1.78	4.84±0.26	4.56±1.21
	T		0.68±0.22	0.51±0.08
Aspartate	L	3.89±0.44	3.83±0.33	2.54±0.21*†
	M	0.94±0.05	0.61±0.05*	0.47±0.08*
	T		1.70±0.14	1.20±0.12†
Asparagine	L	0.13±0.02	0.11±0.02	0.06±0.03
	M	0.11±0.06	0.16±0.02	0.11±0.05
	T		0.33±0.11	0.31±0.03
Threonine	L	0.33±0.04	0.32±0.02	0.32±0.02
	M	0.60±0.06	0.55±0.04	0.76±0.08†
	T		1.11±0.14	1.16±0.11
Serine	L	0.63±0.07	0.27±0.02*	0.35±0.04*
	M	0.78±0.14	0.67±0.04	0.77±0.10
	T		0.85±0.06	1.00±0.08
Leucine	L	0.37±0.02	0.56±0.02*	0.50±0.01*
	M	0.28±0.02	0.32±0.02	0.48±0.04*†
	T		1.10±0.03	0.85±0.01†
Isoleucine	L	0.23±0.01	0.32±0.02*	0.52±0.02
	M	0.18±0.01	0.20±0.01	0.28±0.03*
	T		0.59±0.02	0.47±0.01†
Valine	L	0.50±0.04	0.48±0.02	0.51±0.02
	M	0.48±0.03	0.46±0.03	0.66±0.04*†
	T		1.06±0.04	0.86±0.02†
Lysine	L	0.60±0.03	0.95±0.05*	1.20±0.08*†
	M	1.48±0.02	2.22±0.11*	1.86±0.19
	T		1.22±0.03	1.01±0.03†
Arginine	L	0.08±0.02	0.06±0.03	0.10±0.02
	M	0.75±0.06	0.95±0.05*	0.75±0.06†
	T		0.44±0.03	0.42±0.01
Histidine	L	0.88±0.10	1.04±0.02	1.22±0.03*†
	M	0.35±0.003	0.36±0.01	0.50±0.02*†
	T		0.48±0.02	0.43±0.03
Ornithine	L	0.63±0.05	0.60±0.04	0.53±0.05
	M	0.33±0.04	0.22±0.02*	N.D.
	T		0.28±0.02	0.22±0.01†
Citrulline	L	N.D.	N.D.	0.04±0.002
	M	0.46±0.08	0.34±0.04	0.24±0.07
	T		0.09±0.05	N.D.
Methionine	L	N.D.	N.D.	0.04±0.002
	M	0.15±0.01	0.17±0.02	0.21±0.02
	T		0.32±0.03	0.24±0.01†
Taurine	L	25.0±0.83	28.7±0.60*	33.8±1.02*†
	M	53.1±2.46	52.1±3.22	64.0±1.38*†
	T		22.9±0.61	24.2±1.04
Tyrosine	L	0.12±0.003	0.19±0.01*	0.17±0.01*†
	M	0.21±0.01	0.20±0.01	0.29±0.02*†
	T		0.51±0.04	0.33±0.01†
Tryptophan	L	0.03±0.002	0.05±0.003*	0.03±0.002†
	M	N.D.	N.D.	0.03±0.02
	T		0.09±0.01	0.07±0.003†
Phenylalanine	L	0.14±0.003	0.22±0.01*	0.23±0.01*
	M	0.16±0.01	0.20±0.01	0.30±0.01*†
	T		0.53±0.04	1.52±0.07†
Proline	L	0.61±0.03	0.76±0.06	0.52±0.01*†
	M	0.54±0.21	0.63±0.12	0.58±0.08
	T		2.04±0.04	1.52±0.07†

Table 4. Utilization of ^{14}C-labelled glucose and amino acids by tumour tissue slices

Results are expressed as means ± S.E.M. for five to seven different animals. Oxidation of the different substrates to CO_2 and incorporation into the tissue are expressed as μmol/h per g of tumour tissue. Statistical significance of the results: * day 15 versus day 25, $P < 0.05$.

	Oxidation		Incorporation		Oxidation/incorporation ratio	
Substrate	Day 15	Day 25	Day 15	Day 25	Day 15	Day 25
Glucose	1.45±0.11	1.08±0.13	1.62±0.13	1.61±0.23	0.90	0.67
Alanine	0.90±0.21	0.72±0.14	0.28±0.04	0.34±0.04	3.25	2.09
Glycine	0.22±0.02	0.08±0.07*	0.30±0.02	0.20±0.01	0.73	0.43
Glutamate	0.25±0.06	0.14±0.01	0.08±0.02	0.04±0.01	3.25	3.89
Glutamine	1.58±0.12	1.57±0.03	0.63±0.05	0.53±0.04	2.52	2.95
Leucine	0.15±0.03	0.25±0.02	0.37±0.05	0.33±0.05	0.40	0.75

Table 5. Tissue and circulating metabolites in tumour-bearing animals

Results are means ± S.E.M. for five to seven different animals. Statistical significance of the results: * non-tumour versus tumour, $P < 0.05$; † day 15 versus day 25, $P < 0.05$.

		Mice ... Non-tumour-bearing	Tumour-bearing	
Metabolite	Tissue		Day 15	Day 25
Glycogen (g/100 g of tissue)	Tumour		0.012±0.0012	0.00073±0.0006
	Liver	5.76±0.45	3.26±0.14*	2.54±0.15*†
	Muscle	0.30±0.02	0.18±0.024*	0.19±0.03*
Total lipids (% of fresh wt.)	Tumour		1.76±0.1	1.94±0.11
	Liver	2.84±0.19	2.77±0.14	2.45±0.04†
	Muscle	2.66±0.27	1.80±0.14*	1.50±0.99*
Protein (g/100g of tissue)	Tumour		11.8±0.63	12.6±0.38
	Liver	17.1±0.37	20.7±0.59*	21.4±1.19*
	Muscle	15.5±0.33	8.95±0.32*	10.2±1.04*
Water content (% of fresh wt.)	Tumour		84.2±0.50	84.0±0.59
	Liver	70.5±0.29	74.0±0.97*	73.2±0.32*
	Muscle	75.0±0.78	73.7±1.11	75.7±0.33
Glucose (mM)	Plasma	7.85±0.16	6.32±0.22*	6.99±0.25*
Urea (mM)	Plasma	9.01±0.84	6.56±0.50*	14.2±1.28*†

decreases in the liver concentrations (proline, serine and glutamine).

Table 4 shows the results of the experiments in which tumour slices were incubated with different ^{14}C-labelled compounds. The tumour oxidized glutamine and glucose preferably and, to a lesser extent, alanine, followed by glycine and glutamate. The different substrates were oxidized/incorporated to a similar extent at day 15 or 25 after the inoculation, except for glycine, which was significantly less oxidized at day 25.

Table 5 shows the different tissue and circulating metabolite concentrations in tumour-bearing animals. The glycogen content of the tumour tissue was very low, and decreased even more throughout the tumoral cycle. The same thing happened with liver and muscle glycogen, which were both much decreased in tumour-bearing animals.

The lipid content of tumour tissue was lower than that found in muscle and liver of control animals. Inoculation of the tumour significantly decreased the lipid content in both liver and muscle.

The protein content of the tumour tissue was high, but lower than that found in muscle and liver of control animals. In these last tissues there were significant increases as a result of the inoculation. The tumour is a very highly hydrated tissue, with 84% of water.

The plasma glucose concentration in tumour-bearing mice was significantly lower than that found in controls, and led to a mild hypoglycaemia. Circulating urea concentrations were also low in these tumour-bearing animals. However, the urea concentration rose significantly in the last phase of tumour growth.

DISCUSSION

In order to grow, the tumour requires energy substrates that must be supplied by the host. When the tumour

demands become greater than the host can supply, host weight loss occurs, as shown by our data after the inoculation of the tumour.

It has been previously reported that amino acids, especially glutamine, may play an important role in tumour cell proliferation (Linder-Horowitz *et al.*, 1969). The data obtained in the present work point towards this idea in the so-called Lewis lung carcinoma. Indeed, the glutamine content of the tumour is very low, because it is rapidly utilized and poorly synthesized (Carrascosa *et al.*, 1984; Sauer *et al.*, 1982). This observation, together with the high glutaminase activity found in the tumour, suggests that this carcinoma is highly glutamine-dependent. In fact, a number of relatively different tumours have been shown to be glutamine utilizers. In addition to their role as energy substrates, amino acids can serve as amide or amino-nitrogen donors in reactions leading to the synthesis of several important metabolites, including the pyrimidine, purine and nicotinamide nucleotides (Tate & Meister, 1973), which are absolutely vital for tumour cell proliferation.

Energy production resulting from the oxidation of glutamine or other amino acids is envisaged as occurring simultaneously with and in addition to that derived from glycolysis (Sauer *et al.*, 1982). Tumours function as nitrogen traps, and they compete with their host for nitrogen compounds. This process produces in the host a negative nitrogen balance and a characteristic weight loss and, in the tumour, a reciprocal nitrogen increase. This is also the case of the Lewis lung carcinoma, where the concentration of most amino acids is higher than that found in other host tissues such as skeletal muscle or liver.

Considering that the tumour might depend basically on the host for part of its amino acid requirements, it is noteworthy that there is a growing number of studies that support the idea that muscle protein synthesis is impaired in the tumour-bearing host. It has been demonstrated in both tumour-bearing patients (Lundholm *et al.*, 1976) and laboratory animals (Lundholm *et al.*, 1978) by means of [^{14}C]leucine-incorporation studies. It should also be pointed out that, although one can consider that the tumour's amino acid demand may be responsible for protein depletion in the host, the situation is probably not as clear-cut as this. Our data show a significant increase in the activity of skeletal-muscle glutamine synthetase after the inoculation of the tumour. This enhanced activity could be related to an increased skeletal-muscle proteolysis, since there is a proved correlation between these phenomena. Glutamine is mainly exported by muscle (it is indeed the main nitrogen compound released by muscle) as a means of transporting ammonia innocuously from peripheral tissues to the liver and possibly the tumour.

Aspartate transaminase is the most widespread transaminase, as it links glutamate metabolism with that of aspartate and oxaloacetate. Rather than being actually involved in aspartate utilization, the enzyme controls the key pathway for α-amino-group transfer from amino acids, through glutamate, to aspartate for utilization in the urea and purine nucleotide cycles, as well as to provide a means of ammonia transport between cytoplasmatic and mitochondrial spaces. Tumour shows rather a low activity of the enzyme. However, aspartate transaminase activity shows significant changes in skeletal muscle, particularly at day 15. This fact could be related to an enhanced transaminase activity linked to protein mobilization and amino acid release by muscle.

The significant decrease in glutamate dehydrogenase activity found in muscle of tumour-bearing mice, in relation to non-inoculated animals, can be related to a decreased tendency to export ammonia after the inoculation of the tumour and a less pressing demand for carbon skeletons, once a high muscular proteolysis has taken place. The contribution of AMP deaminase to ammonia export is also significantly decreased in all the host tissues studied.

The significantly enhanced muscle alanine transaminase activity found in tumour-bearing mice somehow indicates that alanine could be exported to the liver in a higher proportion for gluconeogenesis. The liver itself has also an increased alanine transminase activity in tumour-bearing animals, in order to cope with the alanine released by the muscle. In a previous study we have seen that the circulating alanine concentration is increased in mice bearing the Lewis lung carcinoma, this amino acid being transported preferably in the plasmatic rather than the cellular fraction (Rivera, 1986). There have been several reports indicating that hepatic gluconeogenesis is increased in several tumour-bearing situations (Levin & Gevers, 1981), partly in order to fulfil the tumor's demand for glucose. This demand is related to an increased glucose transport to the tumour cells, together with an accelerated glycolysis (Levin & Gevers, 1981). In addition, increased concentrations of gluconeogenic amino acids may be associated with preferential amino acid sequestration by the tumour, causing decreased muscle protein synthesis. It may be then postulated that the tumour somehow enhances protein catabolism of the host tissues and at the same time hinders protein synthesis in them. The dependence of the cancer cells on glucose is well described by Demetrakopoulos *et al.* (1978), who found that malignant transformed cells were unable to maintain ATP concentrations for more than 4 h in the absence of glucose, whereas normal cells maintained adequate ATP at least 24 h.

From our results on tissue amino acid concentrations, it is interesting to point out the high concentrations of taurine found in muscle and liver of tumour-bearing mice. After the inoculation, the concentration of this amino acid rises significantly in both tissues. A similar observation concerning the concentration of taurine in blood cells has also been made in the same tumour model (Rivera, 1986). The precise function of this amino acid still remains controversial; it has been suggested that it is involved in a wide range of metabolic responses, including a functional role in membranes, calcium flux and possibly cyclic AMP response (Hayes, 1986).

Liver and muscle glycogen concentrations sharply decrease after the implantation of the tumour. This represents a homoeostatic response for glycaemia maintenance (the concentration of glucose in blood is only slightly decreased in mice bearing the carcinoma), and can be accounted for by taking into consideration the tumour's increased glucose demand.

The data obtained in the experiments *in vitro* support the idea that both glutamine and glucose are used extensively by the tumour for both oxidation and incorporation into the tissue. Glucose, glycine and leucine are preferably incorporated into the tissue, whereas alanine, glutamate and glutamine were preferably oxi-

dized to CO_2. Although this observation has been made for the first time in the carcinoma concerned, several previous studies in other types of tumours confirm these results (Lazo, 1981).

The results presented here support the idea that glutamine utilization in the Lewis lung carcinoma plays an important role in the proliferation of the cells and that glutamine is probably supplied, in part, by liver and muscle of the host. However, the metabolism of its skeleton and its possible role in the bioenergetics of malignant cells remain to be investigated, and suggest a field for promising future research.

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