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BRIEF NOTE

AMINO ACID LEVELS IN SARCOMA 37 ASCITES TUMOR CELLS COMPARED WITH ASCITIC FLUID AND NORMAL MURINE TISSUES[1]

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The functional characteristics of 2 amino acid transport systems known as Systems A and L have been studied as they occur in Sarcoma 37, (S37), murine ascites tumor cells (Matthews *et al* 1970, Matthews 1972, Matthews *et al* 1975, Matthews *et al* 1977, Matthews and Zand 1977). In order to examine the physiological significance of these active transport systems, we have measured levels of individual endogenous amino acids in S37 cells *in vivo* and in the surrounding cell-free ascitic fluid. For comparative purposes, we also measured levels of individual amino acids in blood plasma and in several normal murine tissues.

Analyses were performed on pooled samples from 5 S37 tumor-bearing mice. Solid tissues were freed of fat and connective tissues, and at least 1 g of solid tissue was used in each case. Freshly drawn blood, or ascites fluid (10 ml), was used for analysis. Picric acid was used for deproteinization and was subsequently removed using Dowex 2–X8 resin. Amino acids were eluted from the resin using 0.02 N HCl. The eluate was concentrated by lyophilization and samples were neutralized with NaOH. After 4 hours at 25 °C to convert cysteine to cystine, samples were adjusted to pH 2, transferred to a volumetric flask, and diluted to 5 ml using pH 2.2 acetate buffer. Amino acid analyses were carried out using a Beckman model 120–C amino acid analyzer. Cystine and valine, glutamine and asparagine, as well as the basic amino acids, were not well resolved by the methods employed and are not included. The results suggest that most of the amino acids of S37 cells have been accounted for in that the total amino acids measured for the S37 cell in the present study (32.3 mM) exceeded slightly the level of the total intracellular amino acid pool measured in another preparation of S37 cells by a non-discriminating method.

Earlier measurements showed amino acid levels were high in rapidly-growing tissues and in cancers in particular (Holden 1962, Ryan and Lorincz 1964). In the present study, total and neutral amino acids were higher for S37 cells than for any normal tissues assayed (tables 1 and 2). Acidic amino acids were higher in brain than in S37 cells due to the glutamic acid, which has a central metabolic role in brain (Neame 1968). High total amino acid and neutral amino acid levels were maintained in S37 cells despite the fact that the ascites fluid possesses lower total and neutral amino acid levels than does blood plasma. This finding that ascites fluid is poorer in amino acids than blood plasma is in accordance with the study by Drewes and McKee (1967) using Ehrlich tumors, and it suggests that diffusion of amino acids from plasma to ascites fluid is followed by active transport into tumor cells. We attribute the moderately high level of glutamic acid in S37 cells to metabolism rather than transport since S37 cells transport acidic amino acids relatively ineffectively. Glutamic acid is formed in metabolism by transamination of the amino group from other amino acids to α-ketoglutarate derived from the Krebs tricarboxylic acid cycle.

If we sum essential amino acids and

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TABLE 1
*Amino Acid Levels in Murine Tissues and Fluids.**

Amino Acid	Ascites Fluid	Blood Plasma	S37 Cells	Liver	Muscle	Brain
Phe	0.13	0.34	0.41	0.40	0.29	trace
Tyr	0.05	0.25	0.12	0.32	0.20	trace
Leu	0.34	0.90	0.81	1.16	0.76	0.46
Ile	0.14	0.58	0.44	0.62	0.46	0.18
Met	0.06	0.21	0.32	0.61	0.29	0.16
Ala	1.01	2.33	9.00	4.47	3.73	1.96
Gly	0.63	1.86	5.82	2.51	3.40	2.63
Pro	0.37	trace	3.64	trace	1.03	none
Ser	0.09	0.49	0.65	0.47	0.75	0.54
Thr	0.39	0.74	3.44	0.62	0.80	3.58
Glu	0.28	1.26	7.30	1.84	0.79	11.00
Asp	0.23	0.19	0.35	0.50	0.35	0.21

*Units are μmoles/g for solid tissues and μmoles/ml for fluids.

nonessential amino acids and compute the ratio (E/N) we note that it is marginally lower in ascites fluid than in plasma and markedly lower in S37 cells than in normal tissues (table 2). The low E/N ratio in S37 cells may reflect 2 aspects of cancer cell metabolism: first, a continuing protein synthesis and minimal protein breakdown associated with growth. The tendency of tumor cells to grow to the limits of essential amino acids available was suggested by tissue culture experiments in which it was shown that normal cells arrest in culture due to limitations in peptide growth factors, whereas transformed cells arrest due to limitations in glucose and some essential amino acids (Moses *et al* 1978). Second, stimulated growth has been associated with increased activity of transport system A, which favors several nonessential amino acids as substrates (Tramacere, Borghetti and Guidotti 1977). This stimulation of system A is reflected in the present study in the concentrating of several typical A system substrates (ala, gly, pro, ser and thr) by the S37 cell (table 1). Thus, the 2 factors of use of essential amino acids in ongoing protein synthesis and a high activity of a transport system favoring many nonessential amino acids leads to the observed low E/N ratio in the S37 cell.

Elevated amino acid levels may contribute to the unregulated growth characteristic of tumor cells in ways apart from supplying material for protein synthesis. Increased levels of amino acids appear to stimulate protein synthesis (Clemens and Korner 1971). Further-

TABLE 2
*Totals of Various Amino Acid Groups.**

Group	Ascites Fluid	Blood Plasma	S37 Cells	Liver	Muscle	Brain
Neutral	3.21	7.70	24.65	11.18	11.71	9.51
Acidic	0.51	1.45	7.65	2.34	1.14	11.21
Total	3.72	9.15	32.30	13.52	12.85	20.72
Essential	1.06	2.77	5.42	3.41	2.60	4.38
Nonessential	2.66	6.38	26.88	10.11	10.25	16.34
E/N Ratio**	0.40	0.43	0.20	0.34	0.25	0.27

*Units are μmoles/g for solid tissues and μmoles/ml for fluids.
**Essential to nonessential amino acid ratio.

more, glycine and glutamic acid are components of glutathione, which is synthesized and consumed in the γ-glutamyl cycle in a manner that may support amino acid transport. Some evidence for the operation of the γ-glutamyl cycle in support of transport systems L and A has been presented (Matthews and Sardovia 1975). γ-Glutamyl-cysteine synthetase has a K_m value of 1.6 mM for glutamic acid (Orlowski and Meister 1971), and glutathione synthetase has a K_m of 2 mM for glycine (Mooz and Meister 1967). The levels of glycine and glutamic acid in the S37 cell (table 1) far exceed these K_m values, so that these reactions would be catalyzed at maximum velocity in the S37 cell. This raises the possiblity of a positive feedback mechanism contributing to metabolic instability, since the γ-glutamyl cycle's operation at maximal velocity could work to increase amino acid transport, and increased amino acid transport could be contributing to the operation of the γ-glutamyl cycle at maximal velocity.

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