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Effect of Ethionine on Amino Acid Composition of Rat Tumors.* (25546)

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We have shown that ethionine inhibited growth of 4 different tumors in rats but its effectiveness varied with strain and sex of host animals(4,12,14,18). Inhibition by ethionine of growth of tumors in mice has been reported from other laboratories(6,23, 26). Although many studies have been made (7,8,11,16,19,24,25,28-32), it is not clear whether ethionine inhibits incorporation of methionine *per se,* or of other amino acids, into proteins of the tumor or causes decreased growth of tumors by affecting adversely protein synthesis or other metabolic processes elsewhere in the body. It was reported(4,5, 12-15,18) that concentration of some "free" amino acids is decreased, and some increased, in liver of rats treated with ethionine. Our object was to determine whether composition of proteins, or concentration of "free" amino acids, in tumors of rats is affected by administration of ethionine.

Materials and methods. Young male and female rats (average weight 174 and 162 g, respectively) of Wistar strain, maintained on *ad lib.* diet of Purina Chow supplemented with weekly ration of fresh carrots, were divided into groups and treated as shown in Table I. Eighteen to 24 hours after last injection, the tumors were removed under ether anesthesia, freed as much as possible from connective tissue, rinsed with distilled water, blotted and weighed. The method of sample preparation was based on that of Schurr *et al.*(22). The viable tissue of each tumor was separated quickly from the necrotic portion, rinsed, and heated for 3 minutes in boiling water. The boiled viable tissues of each group were blotted free of water, separated from small amount of necrotic tissue remaining, cut into small pieces and pooled. The pooled samples were homogenized in Waring Blender and concentrated *in vacuo* to an appropriate volume depending on size of group sample. These suspensions were analyzed for dry weight, total nitrogen(9,27), total amino acids, and apparent free amino acids. Ash and mineral data obtained on these samples will be reported elsewhere. *Total Amino Acids*: Aliquots of tissue suspensions were refluxed in 8 N HCl for 24 hours and amino acids determined by microbiological assay methods previously described(2,3,14). *Apparent Free Amino Acids*: Aliquots of tissue suspensions were deproteinized with tungstic acid(22) and the amino acids determined by microbiological assay procedures described previously(2, 4,14).

Results. Data on number and sex of animals, tumors inoculated and material injected are given in Table I. Dosage of ethionine in females was reduced to ½ that used for males, to obtain a comparable degree of tumor inhibition in both sexes, since Dunn *et al.*(5,18) and Simpson *et al.*(25) reported that ethionine was more toxic, a more potent tumor-growth inhibitor, and affected liver composition to greater degree in female than in male rats.

Seven injections of ethionine inhibited growth of Sarcoma R-1 55% and 40%, and Walker carcinosarcoma 256 74% and 39% in males and females, (data omitted to conserve space). Degree of tumor inhibition ob-

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TABLE I. Experimental Design.

Animals* No./group	Sex	Tumor inoculated†	Material inj. Type	Material inj. Daily amt‡
15	♂	Sarcoma R-1	Saline§	10 ml
20		*Idem*	Ethionine‖	100 mg/100 g body wt
23		Walker carcinosarcoma 256	Saline	10 ml
30		*Idem*	Ethionine	100 mg/100 g body wt
20	♀	Sarcoma R-1	Saline	5 ml
39		*Idem*	Ethionine	50 mg/100 g body wt
24		Walker carcinosarcoma 256	Saline	5 ml
35		*Idem*	Ethionine	50 mg/100 g body wt

* Range of avg weights of each group was as follows: males, 173-174 g; females, 158-164 g.
† A cube, approximately 85 mm^3, was implanted subcut. by trocar technic into 2 axiliary sites of each animal.
‡ From 5th to 8th, and 11th to 13th day, inclusive, following tumor implantation.
§ 0.85% aqueous solution of sodium chloride.
‖ 2% solution of DL-ethionine in 0.85% aqueous solution of sodium chloride.

served in present experiments was about the same for females, but was greater for males, compared to results obtained previously(18). Growth of both tumors in male rats was less than expected, the decreased tumor growth and poor condition of treated males necessitating a 2-day interruption of injections (Table I). There is no simple explanation for this slower tumor growth but apparently it was not due to decreased viability of tumors *per se*, since normal growth was observed only a month before and subsequent to present experiment. As animals were purchased from commercial source, it is possible that some variation in host animals was responsible for this result.

Total Amino Acids. Percentages (dry-weight basis) of total amino acids in viable tissue of the 2 tumors are given in Table II. There was no significant difference in amino acid composition between the 2 tumors grown in male or female, treated or untreated rats whether values were calculated on dry-weight basis or on nitrogen content (data omitted to conserve space) of samples. Close agreement (average difference, 3.5%) between duplicate values was observed for aspartic acid, glutamic acid, histidine, isoleucine, leucine, lysine, proline, serine and threonine. Only single assay values were obtained for arginine, glycine, methionine, valine and the indicated samples of the other amino acids.

There were no significant differences between percentages of 13 amino acids found for the 2 rat tumors studied, the UCLA rat fibrosarcoma(3) and the 4 rat tumors investigated by Sauberlich and Baumann(21). It is of interest, therefore, to note the marked differences in aspartic acid, lysine and serine for these rat tumors compared to mouse tumor (Sarcoma 180) reported by Mickelson and Barvick(17). The problem of possible species difference in this regard is under investigation.

Apparent Free Amino Acids. μg/100 mg (dry-weight basis) of apparent free amino acids in viable tissue of the 2 tumors are given in Table III. The average difference between analyses made in duplicate was relatively high (11%) owing to difficulties encountered in handling and assaying the small quantities of available material. Levels of threonine in tumors were consistently higher in female than in male rats (Table III). Injection of ethionine had no effect on level of methionine in the tumors; the one inconsistent value (Sarcoma-males) is believed not to be significant.

Levels of arginine and histidine in viable tissue of the Walker tumor and of lysine in both tumors were lower in experimental animals of both sexes. It is of interest that "free" arginine and lysine (histidine was not determined) were elevated in liver after treatment with ethionine(4,5) while generally they were decreased in tumors in present experiments. It is of further interest that Busch and Davis(1) reported that specific activity of histones of Walker and Jensen tumors as measured by uptake of L-lysine-U-C^{14} or of glycine-1-C^{14}, was greater than those of pro-

TABLE II. Total Amino Acid Concentration of Viable* Tissue of Sarcoma R-1 and Walker Carcinosarcoma 256 Tumors Grown in Control and Ethionine-Treated Male and Female Rats of Wistar Strain. Values expressed in % of dry wt of original tissue.

	Sarcoma R-1				Walker carcinosarcoma 256			
	♂		♀		♂		♀	
Amino acids	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.
Arginine	5.0 (.71)†	5.4 (1.1)	5.0 (.89)	5.1 (1.7)	5.1 (2.6)	5.0 (2.4)	5.3 (2.0)	5.7 (1.6)
Aspartic acid	6.8 (.25)	6.9‡ (3.4)	7.0‡ (2.0)§	6.6 (2.8)	7.2‡ (2.0)	7.3‡ (4.5)	7.1‡ (.82)	7.2‡ (4.6)
Glutamic acid	9.6 (8.9)	9.6‡ (9.5)	10.0‡ (10)§	10.2‡ (8.3)	9.6 (7.3)	9.5‡ (4.0)	10.0 (8.8)	9.8 (5.9)
Glycine	4.3 (1.6)	4.5 (1.2)	4.3 (2.5)	4.6 (.34)	4.7 (.68)	4.5 (2.3)	4.3 (.14)	4.7 (3.0)
Histidine	1.8 (3.4)	1.9 (4.8)	1.8‖	1.9‡ (3.4)	2.0‡ (3.6)	1.9 (2.6)	2.0‡ (2.4)	2.1‡ (2.4)
Isoleucine	3.5 (1.8)	3.7 (.33)	3.4‡ (1.5)§	3.3‡ (4.2)	3.6‡ (.53)§	3.5‡ (1.0)	3.6‡ (.87)§	3.8‡ (2.4)
Leucine	6.3 (.66)	6.6 (2.8)	6.1 (2.5)	6.0 (.87)	6.4‡ (3.2)	6.4‡ (1.6)	6.4‡ (.88)	6.5‡ (1.4)
Lysine	7.0‡ (3.6)	6.6 (2.9)	7.1‡ (1.7)§	7.2‡ (4.2)	7.2‡ (4.1)	7.0‡ (1.8)	7.1‡ (2.0)	7.8‡ (4.2)
Methionine	1.6 (3.7)	2.0 (7.1)	1.7 (1.8)	1.6 (4.7)	1.8 (3.5)	1.8‖	1.7 (4.2)	1.8 (4.3)
Proline	3.6 (3.3)	3.7‡ (3.5)	3.7 (1.8)	3.7 (2.2)	3.6 (3.2)	3.7 (1.5)	3.7 (2.1)	3.6 (4.0)
Serine	3.8‡ (4.2)	3.8‡ (6.1)	3.7 (3.1)	3.6 (3.8)	3.8 (5.5)	4.2‡ (6.5)	4.0‡ (4.9)	4.1 (3.2)
Threonine	3.7‡ (.59)§	3.6‡ (2.4)	3.6‖	3.6‡ (2.7)§	3.4 (1.7)	3.6‡ (1.6)§	3.4‡ (2.8)	3.6‡ (1.4)
Valine	4.3 (.67)	4.6 (1.1)	4.2‖	4.4 (1.2)	4.6 (.67)	4.3 (.36)	4.4 (.13)	4.6 (1.4)

* Comprised approximately 85% of Sarcoma R-1 and 55% of Walker carcinosarcoma 256 on dry wt basis.
† Mean deviation from mean, MDM, in %, over a 3- to 5-fold range of concentration of arginine and all other assay samples.
‡ Avg of duplicates, all others single values. Mean of deviations among duplicates, 3.48%.
§ MDM of second determination; in original assay only 2 levels fell on standard curve.
‖ MDM not calculated since only 2 values for levels of samples fell within standard curve; assay not repeated owing to insufficient amount of sample.

TABLE III. Apparent Free Amino Acid Concentration of Viable Tissue of Sarcoma R-1 and Walker Carcinosarcoma 256 Grown in Control and Ethionine-Treated Male and Female Wistar Rats. Values expressed as μg/100 mg dry wt of tissue.

	Sarcoma R-1				Walker carcinosarcoma 256			
	♂		♀		♂		♀	
Amino acids	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.
Arginine	12 (6.5)*	9 (3.4)	11 (4.7)	11 (3.5)	21 (4.1)	13 (1.2)	19 (4.1)	13 (2.6)
Aspartic acid	20†	26†	40§ (4.4)‡	37§ (4.2)‡	35 (2.2)‡		27 (3.7)	27 (4.7)
Glutamic acid	113 (35)‡	138 (23)	197§ (4.5)‡	178§ (11)‡	123§(4.5)‡	107 (6.2)‡	105 (3.2)‡	132§(4.0)‡
Glycine	79 (1.6)	93 (1.7)	97 (2.4)	108 (2.7)	97 (3.0)	103 (2.5)	105 (2.1)	109 (3.2)
Histidine	4†		4 (8.4)‡	6 (4.5)‡	7 (4.5)	3 (5.3)‡	8 (2.7)‡	5 (1.4)
Isoleucine	10 (27)‡	14 (4.9)	16 (2.8)	14 (1.0)	16 (.46)	7 (7.7)	13§(5.2)	12 (8.8)
Leucine	20†	26†	28 (4.9)	25 (2.2)	32 (4.7)	17 (10)	20 (3.7)	18 (2.3)
Lysine	31 (5.6)	25 (3.6)	40 (.78)	34 (.50)	52 (4.8)	19 (5.2)	37 (2.2)	29 (5.3)
Methionine	16 (11)‖	25 (5.5)‖	14 (4.4)‖	13 (7.4)‖	15 (2.2)‖	15 (11)‖	11 (5.5)‖	11 (7.9)‖
Proline	43 (19)‡	45 (8.5)‡	73 (4.5)‡	52§ (5.2)‡	33 (7.5)‡	34 (6.4)‡	36 (6.6)‡	37 (.96)
Serine	35 (3.3)‖	45 (6.3)‖	31 (13)‖	53§ (7.5)‖	47§(6.1)‖	45 (7.0)‖	47§(7.8)‖	56 (6.9)‖
Threonine	38 (9.8)‡	36 (8.0)‡	56 (5.9)‡	60 (3.3)‡	36 (2.6)	37 (1.5)	40 (3.1)	72 (.90)
Valine	17 (23)‡	19 (21)‡	36 (6.5)‡	22§ (6.9)‡	27 (3.6)‡	13 (11)‡	18 (2.5)‡	17 (3.9)‡

* Mean deviation from mean, in %, over a 3- to 5-fold range of concentration of arginine and all other assay samples (MDM).
† Fifth level of sample concentration only.
‡ Drift (values *increased* from 1st toward 5th level of sample concentration).
§ Analysis in duplicate.
‖ Drift (values *decreased* from 1st toward 5th level of sample concentration).

teins of any other cellular fraction of the tumor, and that specific activity of histones and other nuclear proteins of these tumors was greater than that of 11 other tissues. It is possible that interference with metabolism of one or more of the basic amino acids in liver following ethionine injection could contribute to "starvation" of tumor cells.

Lack of consistency in differences observable in isoleucine, leucine, proline, and valine is believed to render these differences insignificant.

No analogous microbiological assay data are available but the present values for aspartic acid, glutamic acid, glycine, and valine are within, while that for histidine is below, the range of values reported by Kit and Awapara(10) and Sassenrath *et al.*(20) who used chromatographic technics. It is possible that some free amino acids originally present in viable tissue were removed by boiling. Since relative (not absolute) values are being sought here this should not be a major objection to the method.

Summary. 1. Percentages of total and "free" amino acids in viable tissues of Sarcoma R-1 and Walker carcinosarcoma 256 grown in control and ethionine-treated male and female rats of the Wistar strain have been determined by microbiological assay methods. 2. Ethionine treatment induced no change in total amino acids but caused an increase or decrease in concentration of some "free" amino acids in viable tissue but was without marked effect on methionine. 3. It was concluded that inhibition of tumor growth by ethionine probably results largely from indirect effects on utilization of amino acids other than methionine.

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