Effect of Ethionine on Tumor Growth and Liver Amino Acids in Rats*

HARVEY M. LEVY, GRACE MONTAÑEZ, EDWARD A. MURPHY, AND MAX S. DUNN

(Chemical Laboratory, University of California, Los Angeles, Calif.)

It was anticipated from earlier studies on growth retardation of rats (8) that ethionine might be somewhat specific for rapidly growing tumors. Since ethionine interferes with protein synthesis, possibly through its role as a competitive antagonist of methionine, it was assumed that its effect would be most marked in tissues with a low concentration of endogenous methionine. It has been reported in previous studies from this laboratory (2) that the methionine content of the U.C.L.A. fibrosarcoma was lower than that of normal rat connective tissue. The experiments described here were concerned with the growth of tumors and the levels of certain total and "free" amino acids in the livers of normal and tumor-bearing rats with and without the administration of ethionine.

EXPERIMENTAL

DL-Ethionine (U.S. Industrial Chemical Co.) was either incorporated into the basal ration (see Table 1) or injected subcutaneously into the rats (Long-Evans strain) as a physiological saline solution containing 20 mg of DL-ethionine/ml. Each animal in a given experiment received subcutaneous inoculations of either the U.C.L.A. fibrosarcoma or the Jensen sarcoma at one inguinal ventral site by trocar implantation of a cube of tissue approximately 75 c. mm. in volume taken from the same tumor. After the tumors became palpable, their areas were estimated from caliper measurements. The animals were divided into groups of individuals of approximately the same body weight and with tumors of approximately the same area. Possible systemic effects of DL-ethionine on nontumor-bearing rats were investigated in some experiments. The pertinent data are summarized in Table 2. In all experiments tumor growth was followed by means of caliper measurements, body weights were determined periodically, and food consumption was recorded (but omitted to conserve space) in the indicated experiments. In Experiment VII (see Table 2) the control rats were housed in individual cages and were restricted to the average food intake of the experimental animals.

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At the termination of Experiments V, VI, and VII, the tumors and livers were rapidly excised, weighed, frozen with solid carbon dioxide, and stored at about −20° C. The livers of animals from Experiment V, pooled in groups as shown in Table 3, were homogenized in a Waring Blendor. Aliquots were taken for analysis of total nitrogen by Kjeldahl, total fat by the method of Jensen *et al.*, (3) and arginine, glycine, methionine, and threonine by the microbiological assay procedures of Dunn *et al.* (1). For the determination of nonprotein liver amino acids, the protein was precipitated with tungstic acid by the method of Schurr *et al.* (7), the filtrate was reduced to a syrup by evaporation *in vacuo* at 60°C., and the syrup was refluxed for 24 hours with 6 N HCl to hydrolyze any peptides present.

TABLE 1

COMPOSITION OF BASAL RATION

Constituent	Per cent
Casein, vitamin-free (Nutritional Biochemicals)	22
Sucrose	67
Salt mixture (Sure's No. 1)	4.5
Corn oil (Mazola)	6.0
Cystine	0.3
	mg/kg
Thiamine chloride	5
Pyridoxine	5
Riboflavin	10
Niacin	100
Calcium DL-pantothenate	100
p-Aminobenzoic acid	150
Inositol	400
Choline hydrochloride	1,000
Biotin	0.2
Folic acid	0.2
Vitamin B_{12}	0.15
Vitamin E	50
Vitamin K	1
	units/kg
Vitamin A	20,000
Vitamin D	2,000

RESULTS AND DISCUSSION

The effect of DL-ethionine on the body weight of nontumorous rats is shown in Table 2. When fed at a level of 0.5 per cent, there was an early marked loss in weight followed by stabilization at a lower weight. At the lower injected dosages there was a tendency to regain weight after the initial loss. At very high dosages the weight loss

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TABLE 2

DATA ON EXPERIMENTAL ANIMALS AND TUMORS*

Exp. No.	Tumor	No. rats	Sex	Ethionine	Initial tumor Age (days)	Initial tumor Size (mm^2.)	Final tumor Age (days)	Final tumor Size (mm^2.)	Final tumor Weight (gm.)	Final body wt.	Wt. change	Liver wt. (wet)
	fibrosarc.	9	F.	none	8	132	43	none		205	+44	
	"	6	"	none		186	43	2,100		171	+19	
	"	1	"	none		56	55	2,232		230	+40	
I	"	17	"	0.5 per cent		139	43	none		168	+ 6	
	"	1	"	0.5 per cent		144	43	1,833		130	−10	
	none	9	"	none						209	+39	
	none	9	"	0.5 per cent						156	−15	
	fibrosarc.	10	"	none	4	58	34	none				
II	"	2	"	none		50		1,634; 240				
	"	7	"	1.0 per cent		60		none				
	"	7	"	1.0 per cent		80		1,396				
	fibrosarc.	6	"	none	12	143	35	none				
III†	"	2	"	none		183		933				
	"	4	"	0.5 per cent		150		none				
	"	4	"	0.5 per cent		171		1,155				
IV	Jensen	21	"	none	5	150	19	2,050		241	+ 1	
	"	24	"	1.0 per cent		150		1,530		194	−52	
	Jensen (small)	7	M.	none	5	83	26	282	2.1	336	+ 1	12.4
	Jensen (large)	17	"	none		150		2,400	46.9	313	−16	13.9
	Jensen (small)	16	"	0.25 per cent		125		145	1.3	308	−15	13.9
	Jensen (large)	10	"	0.25 per cent		125		1,680	28.2	300	−33	13.3
V	Jensen (small)	18	"	0.50 per cent		125		210	1.4	276	−46	13.0
	Jensen (large)	4	"	0.50 per cent		135		1,750	31.6	300	−31	15.2
	none	15	"	none						330	+ 9	13.6
	none	15	"	0.25 per cent						333	− 9	15.7
	none	14	"	0.50 per cent						320	−18	15.4
VI	Jensen	22	"	none	4	150	19	1,200	20.6	238	−68	10.1
	Jensen	22	"	20 mg.‡		155		975	20.9	222	−42	8.8
	Jensen	25	F.	none	7	300	16	1,400	26.9	229	−12	11.4
VII	Jensen	43	"	§		300		750	7.2	191	−51	7.9
	none	10	"	none						236	− 8	8.8
	none	15	"	§						198	−53	7.7

* The tumors which are included in each subgroup all showed the same type of growth curve.

† Not included are thirteen animals whose tumors regressed before the 18th day and were not treated.

‡ 20 mg/rat/day injected subcutaneously.

§ 200 mg. ethionine injected subcutaneously on the 7th and 8th days; 100 mg. ethionine injected daily from the 12th through the 15th day.

TABLE 3

TOTAL AMINO ACIDS IN LIVERS OF CONTROL AND TUMOR-BEARING RATS

Experiment V Tumor	No. Rats	Ethionine	Amino acid (per cent in protein calc. to 16 per cent N) Argi-nine	Gly-cine	Serine*	Methio-nine	Threo-nine
Jensen (small)	7	none	5.43	4.97	6.28	2.15†	4.40
						2.48	
Jensen (large)	17	none	5.44	4.88	6.48	2.09	4.31
						2.38	
Jensen (small)	16	0.25 per cent	5.08	4.77	6.24	2.07	4.52
						2.27	
Jensen (large)	10	0.25 per cent	5.48	4.74	6.37	2.13	4.39
						2.48	
Jensen (small)	18	0.50 per cent	5.25	4.74	6.37	2.11	4.54
						2.40	
Jensen (large)	4	0.50 per cent	5.43	4.80	6.54	2.07	4.54
						2.51	
None	15	none	5.34	4.64	6.65	2.18	4.69
						2.58	
None	15	0.25 per cent	5.28	4.88	6.41	2.12	4.70
						2.40	
None	14	0.50 per cent	5.42	4.99	6.60	2.14	4.83
						2.54	

* Serine was determined by Camien and Dunn's improved unpublished method.

† This value and the one listed first in each group was obtained with *Leuconostoc mesenteroides* P-60 which responds only to L-methionine (unpublished data from the authors' laboratory). The value listed second in each case was obtained with *Lactobacillus fermenti* (1) which responds to both L- and D-methionine. The higher values found with *L. fermenti* may indicate slight racemisation during hydrolysis of the livers.

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was continuous. The development of resistance may be explained by a detoxification mechanism or, possibly, by competitive antagonism of 'free' methionine which increased (see Table 4) in the livers of ethionine-treated rats. As would be expected on this basis, longer times were required to overcome the toxic effects of larger doses of ethionine. In nontumorous groups deaths occurred only in rats receiving two injections of 200-mg. each of DL-ethionine on consecutive days. Treated animals, bearing large tumors which did not regress, tended to die sooner than the controls. The weight changes were similar for nontumor-bearing animals and those with tumors which regressed or grew slowly.

The administration of ethionine tended to reduce food intake as observed in Experiment V where the average daily intake was 8.8 gm/day for control animals as compared to 7.4 gm/day for tumor-bearing animals fed 0.25 per cent ethionine and 6.6 gm/day for tumor-bearing animals fed 0.50 per cent ethionine. On the other hand in Experiment I there was no difference in food intake of the control and experimental groups, while the effect of ethionine was similar to that noted in Experiment V. In Experiment VII, in which the control rats were limited to the average food intake of the treated animals, the tumors grew very rapidly. The report of Sugiura and Stock (9) that there was little or no inhibition of tumors in mice maintained for 9–14 days on one-third to one-fourth of the normal dietary intake has been confirmed by the authors' experiments in which it was shown that the growth of established Jensen sarcoma (7-day implants, area 300 mm.²) was not altered significantly during 2 weeks of starvation. It appears, therefore, that neither the weight losses nor the effect on tumor growth induced by ethionine depended primarily on differences in food intake.

The administration of ethionine under the conditions of Experiment I resulted in an early retardation of growth and an increase in the number of regressions of the U.C.L.A. fibrosarcoma (Chart 1). The tumors of nine of the sixteen control rats and seventeen of the eighteen experimental animals regressed completely. In one control animal it was observed that, although there was no appreciable growth of tumor until the 32d day after implantation, the tumor grew rapidly thereafter and eventually caused the death of the animal. There is, therefore, a fine balance between factors operating to influence establishment and regression of some tumors. It is of interest that the U.C.L.A. fibrosarcoma had gradually decreased in viability during the previous year from 90–100 per cent takes in carrier animals.

TABLE 4

'FREE' AMINO ACIDS IN LIVERS OF CONTROL AND TUMOR-BEARING RATS

Experiment V			Amino acid (μg/mg of protein)			
Tumor	No. rats	Ethionine	Glycine	Methionine	Serine*	Threonine
Jensen (small)	7	none	53.5	4.78	56.9	18.4
Jensen (large)	17	none	62.3	8.30	83.0	33.2
Jensen (small)	16	0.25 per cent	60.8	5.78	62.9	25.0
Jensen (large)	10	0.25 per cent	65.9	8.35	82.7	37.0
Jensen (small)	18	0.50 per cent†	57.8	5.80	57.8	23.4
Jensen (large)	4	0.50 per cent†	67.0	9.51	99.8	31.9
None	15	none	45.6	5.24	55.9	16.5
None	15	0.25 per cent	86.0	11.8	113.0	44.2
None	14	0.50 per cent†	54.1	6.00	54.8	21.2

* Serine was determined by Camien and Dunn's improved unpublished method.
† 20 mg of ethionine/rat/day from the 14th to the 26th day.

At the time of Experiments II and III relatively few U.C.L.A. fibrosarcoma tumors normally failed to regress in carrier animals (Chart 2). In these experiments the administration of ethionine apparently interfered with the defense mechanism which was operating so efficiently in the control rats. This striking reversal of effect indicates that a metabolic antagonist, such as ethionine, may interfere with tumor resistance mechanisms under one set of conditions and with tumor growth under another. The marked increase in tumor necrosis, observed in Experiments I–III following the administration of ethionine, has been noted in greater or lesser degree in all experiments of this kind performed either with the U.C.L.A. fibrosarcoma or the Jensen sarcoma.

From studies with female rats in Experiment IV it was found that ethionine induced definite, but slight, retardation of tumor growth. The tumors of the treated animals were extremely necrotic with an orange discoloration, were soft and filled with a pink nonviscous fluid, and were commonly hemorrhagic and ulcerated. Most of the

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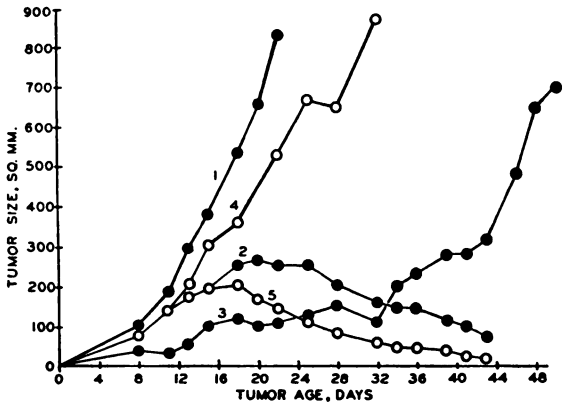

CHART 1 (Experiment I).—Curves showing the effect of ethionine on the growth of the U.C.L.A. fibrosarcoma in female rats. The notations are as follows:

● = Controls: No ethionine.
Curve 1: represents six rats; average tumor size at 36 days was 17,650 mm².
Curve 2: represents nine rats.
Curve 3: represents one rat.

○ = Experimental group; 0.5 per cent ethionine in diet from day 8 to day 20, 1.0 per cent ethionine in diet thereafter.
Curve 4: represents one rat; average tumor size at 48 days was 1,475 mm².
Curve 5: represents seventeen rats.

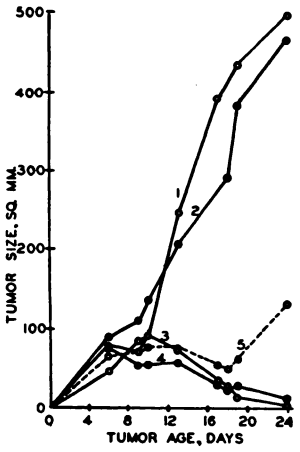

CHART 2 (Experiment II).—Curves showing the effect of ethionine on the growth of the U.C.L.A. fibrosarcoma in female rats. The notations are as follows:

● = Experimental diet contained 1.0 per cent ethionine.
○ = Control diet, no ethionine.
Curve 1: one animal.
Curve 2: seven animals.
Curve 3: ten animals.
Curve 4: seven animals.
Curve 5: one animal.

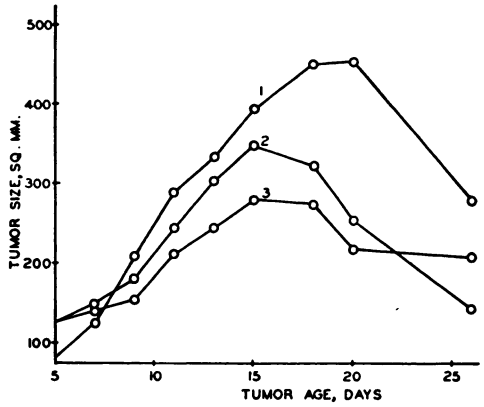

CHART 3 (Experiment V).—Curves showing the growth of Jensen sarcoma in male rats whose tumors regressed. The notations are as follows:

Curve 1: Control animals. Data represent 29 per cent of the total control animals.
Curve 2: Experimental animals; ethionine level was 0.25 per cent of the diet.* Data represent 62 per cent of the total animals.
Curve 3: Experimental animals; ethionine level was 0.50 per cent of the diet.* Data represent 82 per cent of the animals.

* Experimental animals were changed to control diet plus daily injections of 20 mg. of ethionine from 14th to 26th day.

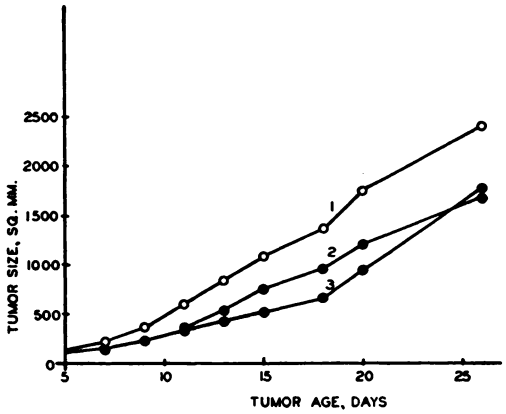

CHART 4 (Experiment V).—Curves showing the growth of Jensen sarcoma in male rats with nonregressing tumors. The notations are as follows:

Curve 1: Control animals. Data represent 71 per cent of the animals.
Curve 2: Experimental animals; ethionine level was 0.25 per cent of the diet.* The data represent 38 per cent of the animals.
Curve 3: Experimental animals; ethionine level was 0.50 per cent of the diet.* Data represent 18 per cent of the animals.

* Experimental animals were changed to control diet plus daily injections of 20 mg. of ethionine from the 14th to 26th day.

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rats had died by the 26th day after implantation of the tumor, possibly from the effects of toxic products of necrosis.

In Experiment V the tumors regressed in 29 per cent of the control animals (Charts 3 and 4). Tumor growth was retarded by ethionine and the percentage of regressions increased with increase in ethionine administered. Tumors regressed in 62 per cent of the animals receiving 0.25 per cent ethionine and in 82 per cent of the animals receiving 0.50 per cent ethionine. Retardation only in the early stages of tumor growth resulted from the injection of 20 mg. of ethionine per day.

In Experiment VII the growth of well established Jensen sarcoma tumors (7 days old, area 300 mm.^{2}) was markedly retarded by high toxic dosages of ethionine (Chart 5). It was observed, however, that the growth rate of the tumor became nearly normal following cessation of ethionine administration. In many instances the tumors of ethionine-treated animals were reduced almost completely to a black, pulpy, fluid-filled sac. On the other hand, more than 50 per cent of the animals died because of the toxicity of ethionine at the high levels employed.

There was no significant difference between the percentages of total amino acids in the livers of nontumor control, untreated tumor and ethionine-treated tumor rats (see Table 3).[1] On the other hand (see Table 4), the "free" amino acids (calculated in terms of μg of amino acid/mg of liver protein) invariably were present in larger (from about 15 to 65 per cent) amounts in livers of animals with large, as contrasted to small, tumors. It may be noted further that the "free" amino acids were markedly elevated in livers of nontumorous rats receiving 0.25 per cent ethionine in the diet until the end of the experiment, while the "free" amino acid levels in the livers of nontumor-bearing rats receiving injections of only 20 mg. of ethionine per day during the latter half of the experiment were essentially the same as in the livers of the nontumorous controls. Apparently, in nontumorous rats ethionine causes an accumulation of "free" amino acids in liver by interfering with protein synthesis. In animals bearing large tumors, "free" amino acids (presumably from the more expendable muscle tissue) accumulate in the liver in response to the increased demand for blood proteins and other essential metabolites consumed in inordinate amounts by the fast-growing tumor.

As shown in Table 3, the liver weight of animals with large tumors is maintained proportional to body weight (carcass plus tumor), even though the weight of the carcass per se is considerably reduced. In harmony with this observation is the report of Yeakel and Tobias (10) that the total nitrogen content of rats bearing an adenocarcinoma is proportional to the total body weight.

A tumor regresses whenever the tumor tissue is no longer able to overcome the growth controlling or retarding influences of its environment. Whether or not regression occurs depends upon the size, age, and viability of the tumor as well as upon the potency of the natural or artificial inhibitors. It is the view of Mefferd *et al.* (4, 5) that early tumor regression is a function of 'intrinsic host resistance,' since they have shown that, within a certain size range, tumor regression and the size of the tumor implant are inversely related. The inhibition of tumor growth observed in the present experiments may be explained on this basis, although ethionine is an effective regressing agent only when it is acting in conjunction with strong natural inhibitors. It appears that there is a critical tumor size beyond which ethionine induces necrosis but is too toxic to be used as a regressing agent. This may be explained, possibly, by the lethal toxicity of the products of necrosis.

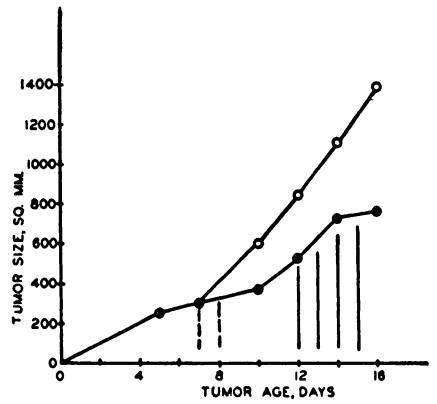

CHART 5 (Experiment VII).—Curves showing the effect of large doses of ethionine on the growth of Jensen sarcoma in female rats. The notations are as follows:

○ = Controls.

● = Experimental; dashed-bars indicate injections of 200 mg. of ethionine, solid-bars, 100 mg. of ethionine: the LD_{50} was apparently a cumulative dose of 400 mg. of ethionine.

SUMMARY

Growth of the U.C.L.A. fibrosarcoma and the Jensen sarcoma in male and female rats of the Long-Evans strain has been investigated with and without the influence of DL-ethionine. Total and

[1] The fat content (data omitted to conserve space) of the livers of the ethionine-treated rats was nearly the same for animals with small or large tumors and was only about 25 per cent greater than that of the controls.

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"free" amino acids (serine, threonine, methionine, arginine, glycine) have been determined in the livers of the rats by microbiological assay methods.

The administration of DL-ethionine retarded the growth of tumors, increased the tendency of tumors to regress, produced necrosis in all tumors examined, and, in nontumor-bearing animals, caused a marked increase in the "free" amino acids of the liver. There was also noted an increase in "free" amino acid content of livers of rats bearing large tumors. The weight of the liver was maintained proportional to the body weight (carcass plus tumor).

It was concluded that, when acting in conjunction with strong natural inhibitors, DL-ethionine is an effective regressing agent under the described experimental conditions.

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