Effect of Ethionine Alone or with Amino Acids on Tumor Growth, Carcass Weight, and Nonprotein Amino Acids in Rat Liver*

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Studies have been made previously on the effect of ethionine on the free amino acids in the livers of tumorous and nontumorous rats of the Long-Evans (6, 8), Sprague-Dawley (13), and Wistar (9) strains. Attempts at dual blocking, with pairs of antimetabolites, have also been reported (12). The observation that certain amino acids may function as antimetabolites in inhibiting tumor growth (3, 7) led to the present study of attempted dual blocking by multiple injections of ethionine alone and in combination with various amino acids.

MATERIALS AND METHODS

Male Wistar rats[1] (140–150 gm.) were maintained on Purina Laboratory Chow. A small cube (about 75 c. mm.) of Sarcoma R-1[2] (1, 2) was implanted subcutaneously by trocar at one lateral axillary site in each animal. On the 7th day following implantation, the rats were weighed, and the area of the tumors was estimated from caliper measurements. The takes averaged 96 per cent. On the 10th day after implantation the tumorous rats were divided into five groups of fifteen rats each, and each rat of a group was given intraperitoneal injections, on each of 7 consecutive days, of a control solution (0.85 per cent NaCl) or of an aqueous solution of ethionine alone or with an amino acid (see Tables 1–3). Tumor areas were estimated periodically, and the experiments were terminated 8–12 hours after the seventh injection (17th day after implantation). Prior to the last injection five animals of each group (with tumors of similar size) were fasted for 4 hours before, and 8 hours after, the last injection.

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[2] Obtained through the courtesy of Dr. J. B. Allison, Rutgers University, New Brunswick, N.J.

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The rats were anesthetized (ether) and weighed. The livers were excised rapidly, blotted free of excess blood, weighed, and pooled. Protein-free extracts were prepared by the method of Schurr *et al.* (11). The tumors were removed, sliced open, blotted free of excess fluid, and weighed. The remaining animals (not fasted) were sacrificed about 12 hours after the last injection, and their body and tumor weights were determined.

Nonprotein amino acids were determined before and after hydrolysis (24 hours' reflux in 6 N HCl) by the assay procedures cited previously (9). In addition to the seven amino acids studied previously, L-arginine was determined by the method of Dunn *et al.* (4) with *Lactobacillus casei* as the assay organism. By reducing the concentration of amino acids in the standard and unknown solutions to about half that employed previously, more analyses per liver sample could be made, and the drift encountered in the serine assay (6, 9, 10) was eliminated. The averages and ranges of the mean deviations from the mean at the different (usually five) levels for the ten pooled-liver samples were as follows: arginine, 3.6 (1.7–9.3); aspartic acid, 2.5 (0.49–14); glutamic acid, 5.0 (1.5–6.0); glycine, 4.3 (2.5–6.0); lysine, 5.1 (2.7–11); methionine, 5.8 (0.85–9.5); proline, 2.4 (0.31–6.2); and serine, 5.0 (0.85–19).

RESULTS AND DISCUSSION

The effect of ethionine alone and with arginine, glycine, or lysine on tumor and carcass weights is shown in Table 1. Progressive tumor areas were determined but are omitted to conserve space. Tumors did not regress, but their weight averaged 39 per cent less than that of the controls in the ethionine-treated animals and 28–33 per cent in the rats receiving ethionine plus an amino acid. These results indicate that the tumor-inhibiting effect of ethionine was not altered significantly by any amino acid investigated. The carcass weight of the experimental animals was decreased markedly by ethionine, but the added amino acids did not alleviate this effect.

TABLE 1

Effect of Multiple Injections of Ethionine Alone or with Amino Acids on Tumor and Carcass Weights of Male Wistar Rats

Treatment*	Rats No.	Rats Deaths	Body wt. Initial (gm.)	Body wt. Terminal (gm.)	Body wt. Change (gm.)	Tumor area Initial (sq. mm.)	Tumor area Terminal (sq. mm.)	Tumor wt. (gm.)	Carcass wt. change† (gm.)
Physiological saline solution	15	0	151 (126–179)‡	199 (161–237)	48 (28–88)	59 (25–100)	1077 (567–1,995)	18 (4–29)	30 (7–63)
50 mg. ethionine	15	1	149 (123–172)	164 (136–210)	15 (− 7–42)	55 (25– 90)	819 (238–1,147)	11 (2–19)	3 (−19–19)
50 mg. ethionine 50 mg. L-arginine§	15	3	144 (124–172)	157 (121–184)	13 (−19–24)	50 (25– 72)	817 (475–1,330)	13 (6–20)	0 (−26–11)
50 mg. ethionine 125 mg. glycine	15	3	148 (123–182)	163 (117–215)	15 (−15–41)	48 (9– 72)	839 (154–1,504)	12 (1–25)	3 (−17–20)
50 mg. ethionine 50 mg. L-lysine§	14#	0	150 (130–177)	170 (131–209)	19 (− 2–42)	63 (16–132)	940 (306–1,428)	13 (2–22)	7 (−12–25)

* Seven daily injections (each 2.5 ml. of solution).
† Body weight minus tumor weight.
‡ Range.
§ Administered as the equivalent amount of the hydrochloride.
# One rat with erratic tumor growth deleted.

TABLE 2

Effect of Multiple Injections of Ethionine Alone or with Amino Acids on the Nonprotein Amino Acids in the Livers of Tumorous Male Wistar Rats*

Treatment†	Arginine Free	Arginine Total‡	Aspartic acid Free	Aspartic acid Total	Glutamic acid Free	Glutamic acid Total	Glycine Free	Glycine Total	Lysine Free	Lysine Total	Methionine Free	Methionine Total	Proline Free	Proline Total	Serine Free	Serine Total
Physiological saline solution	21	16	78	143	172	512	397	403	47	100	15	16	27	40	331	158
50 mg. ethionine	21	21	96	183	378	1,410	516	587	63	133	28	28	38	54	750	349
50 mg. ethionine 50 mg. L-arginine	31	19	120	200	369	1,280	549	651	79	143	24	29	36	46	730	550
50 mg. ethionine 125 mg. glycine	55	31	93	175	383	1,270	867	908	82	146	27	27	36	50	1,270	865
50 mg. ethionine 50 mg. L-lysine	38	20	129	211	445	1,400	588	723	63	127	25	27	36	47	740	526

* Results expressed as micrograms/gm (wet wt.) five pooled livers.
† Seven daily injections (each 2.5 ml. of solution).
‡ In HCl hydrolysate.

TABLE 3

Effect of Single and Multiple Injections of Ethionine Alone or with Lysine on the Nonprotein Amino Acids in the Livers of Tumorous Male Wistar Rats*

Treatment	Arginine Free	Arginine Total†	Aspartic acid Free	Aspartic acid Total	Glutamic acid Free	Glutamic acid Total	Glycine Free	Glycine Total	Lysine Free	Lysine Total	Methionine Free	Methionine Total	Proline Free	Proline Total	Serine Free	Serine Total
50 mg. ethionine (single)‡	20	15	110	178	358	1,130	442	496	55	100	17	21	35	44	282	176
50 mg. ethionine (multiple)§	21	21	96	183	378	1,410	516	587	63	133	28	28	38	54	750	349
50 mg. ethionine 50 mg. L-lysine (single)	23	16	100	162	361	1,350	489	595	65	115	21	24	22	37	294	154
50 mg. ethionine 50 mg. L-lysine (multiple)	38	20	129	211	445	1,400	588	723	63	127	25	27	36	47	740	526

* Results expressed as micrograms/gm (wet wt.) five pooled livers.
† In HCl hydrolysate.
‡ 2.5 ml. solution administered at same time as seventh multiple injection.
§ Seven daily injections (each 2.5 ml. of solution).

The results of the amino acid assays are given in Table 2. It can be noted that the levels of the free amino acids (except arginine) were increased markedly over those of the controls. With single injections of larger doses (200 mg.) of ethionine it was observed previously (9) that free glycine and aspartic acid decreased. In the present experiments free and bound (nonprotein) amino acids in the livers of tumorous rats generally increased, percentage-wise, to about the same extent following injections of ethionine or of ethionine plus an amino acid. Exceptions are methionine, for which the concentrations of the free and bound (nonprotein) forms were about the same, and serine and arginine, for which there was an apparent decrease following hydrolysis.

It is of interest that ethionine was more effective at the same level (daily injections of 50 mg.) as a regressor of the Jensen sarcoma or the U.C.L.A. fibrosarcoma in Long-Evans rats (5) than of Sarcoma R-1 in Wistar rats. These results suggest that the Wistar rats are more tolerant to ethionine than the Long-Evans strain and/or that the Sarcoma R-1 is more resistant to ethionine than the two other tumors. That ethionine inhibited Adenocarcinoma 755 but not Sarcoma 180 in mice was reported by Skipper *et al.* (12). Experiments are planned with the use of larger dosages of ethionine, since the toxicities found by Levy *et al.* (8) with the Long-Evans rat may not be encountered with the Wistar strain. In this way some of the effects (e.g., increase of free serine following injection of ethionine and glycine, increase of free lysine after injection of ethionine and arginine or glycine, and no change in free lysine after injection of ethionine and lysine) may be further accentuated and others revealed.

In Table 3 are compared the effects of a single injection (50 mg.) and the seventh of the multiple injections of ethionine on free and combined (nonprotein) amino acids in the livers of tumorous male Wistar rats. Some tendency may be noted toward increase of these amino acid forms as induced by the multiple injections. From the data in Table 3 it may be seen that these effects appear to be enhanced somewhat by dual blocking with ethionine and lysine.

The close correlation between tumor weights and tumor sizes observed by Allison *et al.* (1) for Sarcoma R-1 in untreated male Wistar rats was not found in the present experiments, owing possibly to the variable amount of fluid, as has been observed with other treated tumors (11).

SUMMARY

Studies have been made on the effect of multiple injections of ethionine alone and with arginine, glycine, or lysine on the carcass weight, growth of Sarcoma R-1, and free and combined (nonprotein) amino acids in the livers of male Wistar rats. Carcass weights were decreased, and the tumor growth was inhibited to about the same degree by ethionine as by ethionine-amino acid combinations.

With the exception of methionine, serine, and arginine, the free and bound (nonprotein) amino acids in the livers of tumorous rats generally increased percentage-wise to about the same extent following injections of ethionine or of ethionine plus an amino acid. Higher concentrations of nonprotein amino acids were observed following multiple injections of ethionine or ethionine and lysine than were obtained with a single injection of these substances.

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