Effect of Ethionine on Tumor Growth, Carcass Weight, and Nonprotein Amino Acids in the Liver of Male and Female Wistar Rats*

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It has been shown in the authors' laboratory that a low level of DL-ethionine, alone or in combination with certain amino acids, decreased carcass weight and inhibited the growth of Sarcoma R-1 in male Wistar rats (3). The degree of tumor inhibition, however, was not as great as that observed with comparable levels of ethionine administered to Long-Evans rats bearing the Jensen sarcoma or the U.C.L.A. fibrosarcoma (5). It was suggested (3) that Wistar rats may be more tolerant to ethionine than the Long-Evans rats and/or that the Sarcoma R-1 may be more resistant to ethionine treatment than the two other tumors. Skipper *et al.* (12) have reported that ethionine was effective in inhibiting the growth of Adenocarcinoma 755 but failed to inhibit Sarcoma 180 in mice. It was of interest, therefore, to determine the effect of ethionine at relatively high levels on the growth of Sarcoma R-1 and Walker carcinoma 256 and to determine the concomitant concentration of apparent free amino acids in the liver of Wistar rats.

Mixtures containing vitamin B_{12} and/or choline, as well as ethionine, were injected in an attempt to reduce the toxicity anticipated at the high levels of ethionine (5). Levy (4) found that the addition of choline to a choline-free diet reduced the toxicity of ethionine ingested at a level of 0.5 per cent of the diet and that the addition of vitamin B_{12} to an otherwise adequate diet "had a beneficial effect" in reducing the toxicity of ethionine administered to Long-Evans strain rats.

MATERIALS AND METHODS

Two groups of ten male Wistar rats received daily intraperitoneal injections (10 ml. of 0.85 per cent saline containing 100 or 200 mg. of DL-ethionine) from the 5th through the 12th day following subcutaneous implantation of Sarcoma R-1. Similar groups of rats were injected with 100 or 200 mg. of ethionine plus (*a*) 25 mμg. of vitamin B_{12}, (*b*) 25 mμg. of B_{12} and 10 mg. of choline chloride, or (*c*) 10 mg. of choline chloride. In all of the experiments the rats were maintained on Purina Laboratory Chow.

The animals were fasted 6 hours before and 8 hours after the last (seventh) injection and were then sacrificed. The tumors were removed (ether anesthesia), sliced open, blotted free of excess fluid, and weighed. The livers were excised quickly, blotted free of excess blood, weighed, and pooled. Protein-free extracts of the livers were prepared by the method of Schurr *et al.* (9, 10). The concentration in the liver of apparent free arginine, aspartic acid, glutamic acid, glycine, lysine, methionine, proline, and serine was determined by the microbiological procedures described previously (3, 6).

In a second experiment two groups of male Wistar rats (five control, twelve experimental) and two groups of similar female rats (ten each, control and experimental) received daily intraperitoneal injections of 0.85 per cent saline, alone or containing ethionine, from the 8th through the 15th and from the 5th through the 12th day, respectively, following subcutaneous implantation of Walker carcinoma 256. The dosage of ethionine (105 mg/100 gm body weight) was equivalent to that (200 mg/rat/day) used previously (5). Two additional groups of female rats (eight control, fourteen experimental) were treated in exactly the same manner as the above females except that they were implanted with Sarcoma R-1. The surviving rats were sacrificed and tumor and carcass weights were determined in the manner already described.

In the third experiment twenty male Wistar rats were inoculated with Sarcoma R-1. Twenty similar rats were used as nontumorous controls. Eleven days after inoculation the rats, in groups of five, were fasted for 4 hours before and 6 hours after a single intraperitoneal injection of 10 ml. of 0.85 per cent saline, alone or containing 50, 100, or 200 mg. of ethionine. The animals were then anesthetized with ether, the livers were excised quickly, and samples for amino acid analysis were prepared as described above.

RESULTS AND DISCUSSION

The carcass weight of tumorous (Sarcoma R-1) male Wistar rats receiving a series of seven (daily) injections of ethionine decreased progressively with increase of ethionine dosage. The amount of ethionine injected and the average carcass weight changes, respectively, ([3] and this paper) were as follows: 0 mg., +30 gm.; 50 mg., +3 gm. (3); 100 mg., −33 gm.; 200 mg., −49 gm. (Table 1).

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Similar data for the heavier rats bearing Walker carcinoma 256 were: 0 mg., +10 gm.; and 200 mg., −27 gm. (Table 2). The change in carcass weights of tumorous male Wistar rats receiving 200 mg. of ethionine per day was comparable to that obtained with as little as 20 mg/day administered to tumorous male Long-Evans rats (4). The low mortality (only four of 75) of the male rats and the observations that 43 per cent of the female rats survived seven consecutive injections of ethionine (Table 2) whereas only two equivalent injections killed 50 per cent of female Long-Evans rats (5) would seem to indicate a relatively high tolerance of Wistar males and females to ethionine.

TABLE 1

EFFECT OF MULTIPLE* INJECTIONS OF ETHIONINE ALONE OR WITH VITAMIN B_{12} AND/OR CHOLINE ON TUMOR AND CARCASS WEIGHTS IN MALE WISTAR RATS

Material injected	No. tumorous rats	Initial weight† (gm.)	Final weight‡ (gm.)	Carcass weight§ (gm.)	Change in carcass wt. (gm.)	Tumor weight# (gm.)
Ethionine (100 mg.)	6	179	146	142	−37	3.6‖ (2.6–6.9)**
" +vitamin B_{12} (25 mμg.)	7	194	168	161	−33	6.1 (1.7–12)
" + " +choline (10 mg.)	8	204	179	172	−32	6.6 (3.7–8.8)
" +choline (10 mg.)	7	191	166	160	−31	5.7 (2.6–10)
Ethionine (200 mg.)	5	191	153	149	−42	4.2 (2.7–5.8)
" +vitamin B_{12} (25 mμg.)	8	190	142	137	−53	4.7 (2.3–6.6)
" + " +choline (10 mg.)	8	185	135	130	−55	4.6 (1.3–8.4)
" +choline (10 mg.)	6	191	145	142	−49	2.5 (0.72–3.6)

* Seven daily injections.

† Total weight at time of first injection.

‡ Total weight at time of sacrifice, approximately 8 days after first injection.

§ Total weight − tumor weight.

Average weights of all tumors were: (100 mg. ethionine etc.), 5.5 gm.; (200 mg. ethionine etc.), 4.0 gm. The average weight of comparable control tumors was 6.4 gm.

‖ Average weight.

** Range of weights.

TABLE 2

EFFECT OF MULTIPLE* INJECTIONS OF ETHIONINE ON TUMOR GROWTH AND CARCASS WEIGHT OF MALE AND FEMALE WISTAR RATS

Group	No. rats	Tumor	Initial weight (gm.)	Final weight (gm.)	Carcass weight† (gm.)	Change in carcass weight (gm.)	No. of survivors	Tumor weight (gm.)	No. tumors	Tumor growth inhibition (per cent)
Control (male)	5	Walker carcinoma	268	273	258	10	5	8.3‡ (7.1–32.)§	9	0
Experimental (male)	10	Walker carcinoma	277	260	250	−27	10	6.6 (2.0–23.)	17	20
Control (female)	7	Walker carcinoma	146	183	165	19	7	9.4 (2.7–16.)	13	0
Experimental (female)	9	Walker carcinoma	148	119	124	−24	4	1.5 (0.90–2.0)	8	84
Control (female)	8	Sarcoma R-1	223	239	221	− 2	8	8.9 (4.8–12.)	16	0
Experimental (female)	14	Sarcoma R-1	213	163	160	−53	6	2.4 (0.50–4.0)	12	73

* Seven daily injections (105 mg ethionine/100 gm body weight).

† Total weight − tumor weight.

‡ Average weight.

§ Range of weights.

It may be observed (Table 1, footnote #, and Table 2) that seven consecutive daily injections of 100 mg. of ethionine decreased the growth of Sarcoma R-1 14 per cent, and similar injections of 200 mg. decreased the growth of Sarcoma R-1 and Walker carcinoma 22 per cent and 20 per cent, respectively, in male Wistar rats. The administration of comparable doses of ethionine to female Wistar rats bearing the Sarcoma R-1 or Walker carcinoma decreased tumor growth by 73 per cent and 84 per cent in those rats which survived (six of fourteen rats and four of nine rats, respectively). It is apparent, therefore, that Sarcoma R-1 and Walker carcinoma 256 have similar susceptibilities to ethionine treatment but that there is a marked difference in this respect between male and female animals.

rats. The addition of choline alone to the ethionine lowered the concentration of arginine and glycine at the 100-mg., but not the 200-mg., ethionine level. It should be noted that the same ratio of choline to ethionine was not used at both levels of ethionine. The amount of choline used (10 mg.) was the estimated daily requirement per rat (2).

It may be observed in Table 4 that the concentration of apparent free arginine, lysine, and methionine increased (nonlinearly) in both tumorous and nontumorous rats with increase in ethionine dosage. There were no significant changes in aspartic acid, glycine, proline, or serine.

It is not yet clear whether the effect of ethionine on tumor growth is due to inhibition of protein synthesis through direct competitive inhibition of methionine incorporation into proteins (5) or an

TABLE 3

APPARENT FREE AMINO ACIDS IN LIVERS OF TUMOROUS MALE WISTAR RATS RECEIVING MULTIPLE INJECTIONS OF VARYING LEVELS OF ETHIONINE, WITH AND WITHOUT VITAMIN B_{12} AND/OR CHOLINE

Material injected	Amino acids (μg/gm liver)							
	Arginine	Aspartic acid	Glutamic acid	Glycine	Lysine	Methionine*	Proline	Serine
Ethionine (100 mg.)	28±3.0†	96±5.6	160±5.5	462±2.7	60±7.0	25	27±3.7	146±10.0
" +vitamin B_{12} (25 mμg.)	26±1.8	122±5.4	103±10.0	413±7.6	44±4.4	25	35±3.9	146±6.7
" + " +choline (10 mg.)	18±2.0	113±3.6	169±7.1	347±4.0	55±3.4	29	29±1.0	257±3.3
" +choline (10 mg.)	17±1.0	105±3.6	156±1.6	382±2.3	48±5.4	25	30±4.8	177±0.6
Ethionine (200 mg.)	29±1.5	79±6.3	148±5.9	430±3.2	67±4.1	36	29±4.0	276±0.6
" +vitamin B_{12} (25 mμg.)	42±2.0	88±11.0	121±5.8	458±1.7	52±3.2	38	29±1.0	137±8.2
" + " +choline (10 mg.)	31±1.6	84±3.7	110±2.1	450±2.3	80±3.2	39	31±1.0	400±8.9
" +choline (10 mg.)	30±3.6	80±5.7	116±5.6	412±2.7	53±5.3	40	37±9.3	144±3.8

* Used only the lowest of five levels of sample concentration owing to marked inhibition of growth of assay organism with increased sample concentration.
† Mean deviation from the mean, in per cent, over a fivefold range of concentrations of assay samples.

The addition of vitamin B_{12} and/or choline had no significant effect on the tumor or carcass weights of the ethionine-treated male rats (Table 1); however, the physical appearance of the rats receiving B_{12} (with or without choline) was markedly superior to that of the rats injected with ethionine alone (lack of yellow matted hair in the pubic region typical of ethionine-treated rats).

It is evident from the data in Table 3 that consecutive daily injections of ethionine at the 200-mg. level induced higher concentrations of methionine in the liver than did similar injections of 100 mg. of ethionine. The markedly higher level of serine found previously (3) is probably due to the larger tumors present in those animals, since it has been reported that the concentration of apparent free serine in the liver was greater in ethionine-treated rats bearing large tumors than in similar animals with small tumors (5).

While the presence of vitamin B_{12} had no apparent effect on the amino acid levels, the combination of B_{12} and choline markedly increased the liver serine in ethionine-treated tumorous male

indirect result of imbalances induced in other amino acids (6). The effect of ethionine on the concentration of apparent free amino acids in the liver observed in the present study can be explained, in large part, by inhibition of the transfer of methyl groups from methionine (1, 14) and inhibition of the oxidation of choline and sarcosine (7, 15) (Chart 1). It has been observed that injection of ethionine increased the concentration of apparent free arginine. This result would be expected from the inhibition by ethionine of the methionine-specific methylation of glycocyamine to creatine. Evidence for (14) and against (12) such an inhibition has been reported. The addition of choline to the injected ethionine reduced the concentration of arginine (and glycine) possibly through alleviation of competition for the methionine-CH_3 by the production of methyl groups via formate (11) and/or the reduction of use of methyl groups to convert ethanolamine to choline (1).

The slight (and variable) decrease in glycine following ethionine administration might reflect the reduced production of glycine as well as the in-

creased use of formate (from glycine) as a methyl-group source. These results are consonant with the inhibition by ethionine of the production of glycine and formate from sarcosine by sarcosine oxidase (15). The production of sarcosine, itself, from choline (via betaine) by choline oxidase is inhibited by ethionine (1, 15).

The increase in serine following the injection of ethionine plus B_{12} and choline may be a composite result of increased production of formate (1, 11, 14) and a reduction in the formation of choline from serine via ethanolamine (1).

carcass weight, tumor growth, and/or the concentration of apparent free amino acids in the liver of tumorous and nontumorous rats of the Wistar strain.

There was a progressive decrease in carcass weight with increase in ethionine dosage in tumorous male rats.

The growth of Sarcoma R-1 was inhibited by 22 per cent and that of Walker carcinoma 256 by 20 per cent, in male rats receiving multiple injections of high levels of ethionine, while comparable treatment inhibited the growth of these tumors by 73 per cent and 84 per cent, respectively, in female rats. It was concluded that these tumors were similar in their response to ethionine treatment but that there was a marked difference in the response observed in male or female animals.

The concentration of apparent free arginine, lysine, and methionine in the liver increased with increased ethionine dosage following a single injection into either tumorous or nontumorous male rats. Consecutive daily injections of ethionine into tumorous male rats induced higher concentrations of apparent free methionine in the liver with increase in ethionine dosage.

The addition of vitamin B_{12} and/or choline to the ethionine injected had no significant effect on the carcass or tumor weight in male rats, although the presence of B_{12} improved the physical appearance of the rats. While vitamin B_{12} had no apparent effect on the liver amino acid levels, the addition of B_{12} plus choline to the injected ethionine markedly increased the liver serine concentration.

It was concluded that tumorous Wistar rats have a higher tolerance to ethionine than tumorous Long-Evans strain rats.

TABLE 4

EFFECT OF A SINGLE INJECTION OF DL-ETHIONINE AT VARYING LEVELS ON APPARENT FREE AMINO ACIDS IN THE LIVERS OF TUMOROUS AND NON-TUMOROUS MALE WISTAR RATS

Ethionine level	Nontumorous Amino acids (μg/gm liver) Arginine	Aspartic acid	Glycine	Lysine	Methionine	Proline	Serine
None*							
50 mg.	13±2.5†	84±2.6	344±2.9	64±2.7	22±9.1	20±2.2	132±1.9
100 "	14±4.5	99±2.4	333±5.6	72±7.1	24±8.4	20±3.1	117±8.2
200 "	19±1.5	122±1.0		97±6.8	52±8.0	30±3.0	145±3.5
				Tumorous‡			
None	16±2.6	116±1.4	376±8.0	73±3.3	16±6.4	24±3.1	178±1.5
50 mg.	17±1.4	122±4.3		77±1.5	26±8.6	25±2.0	207±9.7
100 "	18±3.1	99±1.5	328±1.9	80±4.2	28±5.6	23±2.1	115±3.9
200 "	25±3.1	100±7.0	346±4.8	94±13.0	52±12.0	22±6.0	127±13.0

* Sample lost.

† Mean deviation from the mean, in per cent, over a fivefold range of concentrations of assay sample.

‡ Average tumor weight, 3.4 gm. (1.0–6.3 gm.).

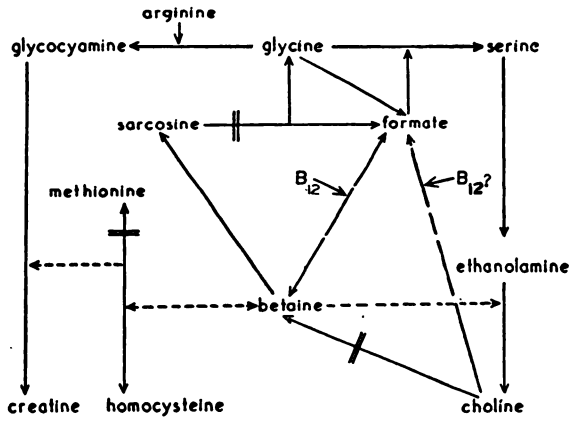

CHART 1.—Pathways of amino acid metabolism in rat liver (1, 11).

Conversion ——→
Methyl-transfer - - - - - - - - - →
Methyl-conversion —— —— →

SUMMARY

Studies have been made of the effect of single or multiple injections of ethionine, alone or in combination with vitamin B_{12} and/or choline, on

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