Effect of Ethionine on the Free Amino Acids in Liver of Tumorous and Nontumorous Rats*

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The ability of ethionine to inhibit the growth or to induce regression of a number of tumors in rats and mice has been reported (8, 12). The observation that these effects were accompanied by an increase in the concentration of free amino acids in the liver of the rat (8) led to studies of the amino acid concentration in livers of fed and fasted nontumorous rats, with and without injection of ethionine (6). Further studies are reported here on the changes occurring in the concentration of free amino acids in the liver of tumorous and nontumorous rats in fasting and force-feeding experiments with and without injections of DL-ethionine.

Since it has been observed (3, 4, 7) that, under some conditions, the presence of an excess of certain amino acids may cause metabolic disturbances or inhibitions of certain reactions (resulting in inhibition of tumor growth), it is believed that the judicious use of amino acid excesses in conjunction with certain amino acid antagonists may result in conditions favoring maximum damage to tumor tissue with minimum damage to the host. The objective of the present experiment, therefore, was to obtain information with which to orient future studies of augmented or dual blocking (12).

EXPERIMENTAL

Young male rats (120–180 gm.) of the Wistar strain (Pacific Animal Farms, Los Angeles, California) were maintained for 2 weeks on a diet of Rockland pellets with a weekly ration of fresh lettuce. A small cube (about 75 c. mm.) of R-1 sarcoma (1) was implanted subcutaneously by trocar at one lateral axillary site in each animal. Two weeks later the rats bearing large tumors (average area, 1,594 sq. mm.; range, 672–3,480 sq. mm.) and those in which tumor grafts failed to develop were divided into groups. The experimental conditions for the various groups are given in Table 1.

TABLE 1

EXPERIMENTAL DESIGN

Experiment no.	Type of rat	Group	No. animals	Material injected	Diet
1	Nontumorous	Control	16	Saline*	None
	"	Experimental	16	DL-Ethionine†	"
2	Tumorous	Control	20	Saline	None
	"	Experimental	20	DL-Ethionine	"
3	"	Control	20	Saline	Force-fed at 0 hours‡
	"	Experimental	20	DL-Ethionine	"
4	"	Control	20	Saline	Force-fed at 4 hours‡
	"	Experimental	20	DL-Ethionine	"

* Ten ml. of 0.85 per cent NaCl solution.

† Ten ml. of aqueous solution containing 200 mg. of DL-ethionine.

‡ Ten ml. of solution containing (per liter) 20 gm. of glucose, 5 gm. each of the amino acids, and 206 mg. of the vitamin-mineral mixture used in the methionine assay medium (5).

All the rats were fasted for 6 hours prior to the injection of either DL-ethionine or saline solution. Sets of four or five rats from each group were sacrificed at 6, 12, 18, and 24 hours after injection. The rats were anesthetized (ether), and the livers

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were rapidly excised, blotted free of excess blood, weighed, and pooled. Protein-free extracts were prepared according to the method of Schurr *et al.* (11), and the free amino acids (plus active peptides) were determined by the assay procedures

TABLE 2

CONCENTRATION OF FREE AMINO ACIDS IN THE LIVERS OF FASTED CONTROL AND TUMOR-BEARING RATS

Amino acid	Type of animal	Ethionine dosage (200 mg.)	Concentration of free amino acid* Hours under experimental conditions 6	12	18	24
Aspartic acid	n-t†	−	123	111	105	99
	t	−	128	135	143	114
	n-t	+	82	98	128	149
	t	+	85	86	71	92
Glutamic acid	n-t	−	264	218	293	218
	t	−	242	284	258	220
	n-t	+	204	400	374	374
	t	+	420	598	700	704
Glycine	n-t	−	249	288	251	294
	t	−	278	252	252	300
	n-t	+	268	253	244	273
	t	+	258	246	238	242
Lysine	n-t	−	82	70	63	59
	t	−	91	105	81	85
	n-t	+	131	144	79	89
	t	+	400	903	490	200
Methionine	n-t	−	17	16	17	16
	t	−	16	14	15	14
	n-t	+	45	50	31	28
	t	+	38	52	42	31
Proline	n-t	−	39	27	30	36
	t	−	38	37	37	32
	n-t	+	37	30	29	35
	t	+	81	73	80	64
Serine	n-t	−	233	186	189	202
	t	−	365	383	248	332
	n-t	+	260	199	156	214
	t	+	559	657	532	685

* Micrograms of amino acid/gm of wet liver. Each value is that for four or five (pooled) livers.

† n-t = nontumorous; t = tumorous.

given previously (6), with the exception that glutamic acid was determined with *Leuconostoc mesenteroides* P-60 rather than *Lactobacillus arabinosus* 17-5. The former organism gives results which are consistent at the different levels of unknown solution and which agree with those reported recently by Wu and Bollman (14). Because of the systematic drift at high levels in the serine assay (6, 10) the values obtained cannot be taken as true concentrations and, therefore, are presented as activities, calculated from the volume of sample required to give half-maximum growth response. The average and range of the mean deviation from the mean for each of 32 samples were as follows: aspartic acid, 2.8 per cent (0.42–10); glutamic acid, 5.0 per cent (0.86–13); glycine, 4.0 per cent (0.0–13); lysine, 3.6 per cent (0.9–10); and proline, 3.6 per cent (0.0–9.2). For 24 of the samples the average and range for methionine were 4.9 per cent (2.0–8.9); the remaining eight samples (force-fed treated tumorous rats) showed inhibition at the higher levels due to some unknown factor in the samples.

RESULTS

The changes induced by ethionine in the concentration of free amino acids in the liver of fasted tumorous and nontumorous rats are summarized in Table 2 and the changes in force-fed rats in Table 3.

The concentration of three amino acids (aspartic acid, lysine, and serine) was consistently higher in the untreated tumor-bearing rats fasted during the experiment than in similar nontumorous rats. The differences observed for the other amino acids studied were small or inconsistent.

In the fasted nontumorous rats the concentra-

TABLE 3

CONCENTRATION OF FREE AMINO ACIDS IN THE LIVERS OF FORCE-FED TUMOROUS RATS

Amino acid	Ethionine (200 mg.)	Time of force-feeding (hr.)	Concentration of free amino acid* Hours under experimental conditions 6	12	18	24
Aspartic acid	−	0	97	120	123	126
	−	4	114	124	106	117
	+	0	72	65	59	63
	+	4	82	63	68	103
Glutamic acid	−	0	375	278	256	278
	−	4	580	278	255	270
	+	0	463	587	654	559
	+	4	490	485	543	495
Glycine	−	0	358	372	388	346
	−	4	389	326	362	383
	+	0	420	380	376	398
	+	4	488	392	344	448
Lysine	−	0	66	86	82	56
	−	4	83	81	72	82
	+	0	298	471	910	239
	+	4	277	255	253	206
Methionine	−	0	15	14	14	13
	−	4	14	12	14	13
	+†	0	56	64	77	47
	+†	4	60	68	56	64
Proline	−	0	37	30	29	27
	−	4	49	27	27	25
	+	0	160	88	60	101
	+	4	218	80	68	79
Serine	−	0	535	319	336	212
	−	4	580	244	224	244
	+	0	435	415	589	507
	+	4	433	396	503	

* Micrograms of amino acid/gm of wet liver. Each value is that for five (pooled) livers.

† Each value is that calculated at the lowest level of tissue sample, since inhibition was pronounced at higher levels of tissue sample.

tion of three amino acids (methionine, lysine, and glutamic acid) increased significantly after ethionine injection, while that of two others (serine and proline) did not change. The concentration of glycine decreased slightly, while that of aspartic acid increased steadily from a value markedly below to a level considerably above that of the controls (no ethionine).

In the tumor-bearing fasted rats the injection of ethionine caused a significant increase in the concentration of five amino acids (serine, proline, methionine, lysine, and glutamic acid), but a decrease in the concentration of two amino acids (glycine and aspartic acid). The effect on aspartic acid persisted for 24 hours.

Increasing the supply of amino acids (except methionine) by force-feeding the untreated tumorous rats affected the amino acid levels as follows: (*a*) Proline, glutamic acid, and serine were markedly increased but fell to "normal" fasting levels by the 12th hour. (*b*) Glycine was markedly increased throughout the 24-hour experimental period. (*c*) Aspartic acid, lysine, and methionine changes were small or inconsistent.

Force-feeding the ethionine-treated tumor-bearing rats affected the amino acid levels as follows: (*a*) Proline, glycine, and aspartic acid changed in the same direction and to about the same relative degree as in the untreated rats. (*b*) Methionine was markedly and persistently higher than in the ethionine-treated fasted rats. (*c*) The increase in lysine and in glutamic acid observed in fasted, treated rats was delayed or reduced as a result of force-feeding. (*d*) Serine was initially reduced but began rising between the 12th and 18th hours.

DISCUSSION

The term "free amino acid" used herein refers to microbiologically available amino acids of peptides as well as free amino acids. Babson and Winnick (2) reported that the level of conjugated amino acids in the liver, muscle, and plasma of tumor-bearing rats was higher than in nontumorous controls. It has been observed, however, that apparently both the bound and free forms of some amino acids are altered by ethionine administration to about the same degree (6).

It is reasonable to assume that the rate of catabolism is greater in fasted tumor-bearing rats than in similar nontumorous animals, since it has been observed that a tumor maintains a high rate of growth at the expense of body weight in a fasted rat (8). That the increased level of total nonprotein amino acids in the liver of tumorous rats was associated with decreased levels in muscle has also been reported (2). The similarity in the concentration of some amino acids (glycine, methionine, proline, and glutamic acid) found here in the liver of fasted tumorous and nontumorous rats would seem to indicate that the rate of utilization of these amino acids keeps pace with the increased rate of supply in the tumor-bearing rat. The increased concentration of aspartic acid, lysine, and serine might indicate, however, that the rate of utilization of these particular amino acids lagged behind that of supply or, on the other hand, that the presence of the tumor induced a changed equilibrium level (or metabolic pattern) of some amino acids. Forcibly increasing the supply of amino acids (except methionine) caused changes which were consistent with the latter interpretation. Sassenrath and Greenberg (10) have concluded, however, that there is no pattern of change in the free amino acid levels of tissues of tumor-bearing animals which might be considered characteristic of the neoplastic process, ascribing the changes observed to a generalized condition of stress. White *et al.* (13), on the other hand, concluded that the presence of a neoplasm increased the level of free glutamic acid in the blood plasma of humans and rats and altered the glutamic acid metabolism of chick embryos bearing implanted tumors.

In the present experiments the presence of a tumor increased the degree (but did not alter the direction) of change in the concentration of some amino acids following the injection of ethionine into fasted rats. While there was no significant difference in the methionine or glycine response, the changes in lysine, glutamic acid, serine, and proline concentrations were much greater in tumorous than in nontumorous rats. The decrease in aspartic acid was greater in both degree and duration in tumorous rats. These differences may reflect both the increased rate of supply and the change in equilibrium levels or metabolic pattern discussed above. That the latter is an important factor is evidenced by the response obtained on forcibly increasing the supply of amino acids to treated tumorous rats. It is interesting to note that White *et al.* (13) found a significant increase in aspartic-glutamic transaminase in the livers of tumor-bearing rats.

The apparent diminution of the effect of injected ethionine after about 12 hours, reported previously (6), was again observed in these experiments. Wu and Bollman (14) have reported that, while only about one-third of the dose of ethionine was excreted by the 12th hour, the excretion of amino acids returned to approximately normal levels by the 10th–14th hour following injection of ethionine. Potter (9) pointed out that the accumulation

of a metabolite of a blocked reaction may make the enzyme involved resistant to the antagonist resulting in a "break-through."

The differences observed between the concentration levels given here and those reported earlier (6) for male Long-Evans rats might be attributed to strain differences. The possibility of such differences between strains of rats has been suggested by Sassenrath and Greenberg (10). This problem is being investigated.

SUMMARY

Changes in the free amino acids have been studied in the liver of fasted and force-fed tumorous and nontumorous rats with and without the injection of DL-ethionine.

In the presence of the tumor the level of aspartic acid, lysine, and serine increased in the fasted, untreated animals. The injection of ethionine caused an increase (methionine, lysine, and glutamic acid), no change (serine and proline), or a decrease (aspartic acid and glycine) of amino acids in the liver of nontumorous animals. In tumorous rats the injection of ethionine caused an increase of some amino acids (methionine, lysine, glutamic acid, serine, and proline) and a decrease of others (aspartic acid and glycine) in the liver. The changes in amino acids were greater in tumorous than in nontumorous animals.

It was concluded that the normal equilibrium levels of some amino acids of the liver were altered in animals bearing tumors. This conclusion was supported by the results from the force-feeding experiments.

REFERENCES

1. BABSON, A. L. Some Host-Tumor Relationships with Respect to Nitrogen. Cancer Research, **14**:89–93, 1954.
2. BABSON, A. L., and WINNICK, T. Protein Transfer in Tumor-bearing Rats. Cancer Research, **14**:606–11, 1954.
3. BEARD, H. H. Effect of Subcutaneous Injection of Individual Amino Acids upon the Appearance, Growth and Disappearance of the Emge Sarcoma in Rats. Exper. Med. & Surg., **1**:123–35, 1943.
4. CHRISTENSEN, H. N.; STREICHER, J. A.; and ELBINGER, R. L. Effects of Feeding Individual Amino Acids upon the Distribution of Other Amino Acids between Cells and Extracellular Fluid. J. Biol. Cem., **172**:515–24, 1948.
5. DUNN, M. S.; CAMIEN, M. N.; MALIN, R. B.; MURPHY, E. A.; and REINER, P. J. Percentages of Twelve Amino Acids in Blood, Carcass, Heart, Kidney, Liver, Muscle and Skin of Eight Animals. Univ. Calif. Publ. in Physiol., **8**:293–326, 1949.
6. LEVY, H. M.; MONTAÑEZ, G.; and DUNN, M. S. Effect of Ethionine and Fasting on the Free Amino Acids of Rat Liver. J. Biol. Chem., **212**:985–90, 1955.
7. LEVY, H. N.; MONTAÑEZ, G.; FEAVER, E. R.; MURPHY, E. A.; and DUNN, M. S. Effect of Arginine on Tumor Growth in Rats. Cancer Research, **14**:198–200, 1954.
8. LEVY, H. M.; MONTAÑEZ, G.; MURPHY, E. A.; and DUNN, M. S. Effect of Ethionine on Tumor Growth and Liver Amino Acids in Rats. Cancer Research, **13**:507–12, 1953.
9. POTTER, V. R. Sequential Blocking of Metabolic Pathways *in Vivo*. Proc. Soc. Exper. Biol. & Med., **76**:41–46, 1951.
10. SASSENRATH, E. N., and GREENBERG, D. M. Tumor Host Relationships. I. Effects on Free Amino Acid Concentrations of Certain Tissues. Cancer Research, **14**:563–69, 1954.
11. SCHURR, P. E.; THOMPSON, H. T.; HENDERSON, L. M.; and ELVEHJEM, C. A. A Method for the Determination of Free Amino Acids in Rat Organs and Tissues. J. Biol. Chem., **182**: 29–37, 1950.
12. SKIPPER, H. E.; THOMPSON, J. R.; and BELL, M. Attempts at Dual Blocking of Biochemical Events in Cancer Chemotherapy. Cancer Research, **14**:503–7, 1954.
13. WHITE, J. M.; OZAWA, G.; ROSS, G. A. L.; and MCHENRY, E. W. An Effect of Neoplasms on Glutamic Acid Metabolism in the Host. Cancer Research, **14**:508–12, 1954.
14. WU, C., and BOLLMAN, J. L. Effect of Ethionine on the Free Amino Acids in the Rat. J. Biol. Chem., **210**:673–80, 1954.