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STUDY ON TYROSINE: 2-OXOGLUTARATE AMINOTRANSFERASE ACTIVITY IN THE LIVER OF TUMOR-BEARING AND INJURED MICE

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ABSTRACT

Elevation of the liver tyrosine: 2-oxoglutarate aminotransferase (EC 2. 6. 1. 5.) activity was observed in Ehrlich ascites tumor bearing mice and also in mice with nonspecific stimuli, such as talcum injection and high protein diet feeding.

Regarding the intracellular distribution of the enzyme activity, a higher percentage was demonstrated in the supernatant fraction of mice with acute nonspecific stimuli than in that of the control mice but not in that of mice with tumor. In case of chronic nonspecific stimuli such as repeated talcum injections or feeding on high protein diet for a long term, however the intracellular distribution of enzyme activity resembled that of the control mice. These changes in enzyme activity and its intracellular distribution were found to be closely related to the adrenal steroid hormone.

INTRODUCTION

Since Girkin[1] first reported in 1960 on the elevation of liver tyrosine: 2-oxoglutarate aminotransferase in tumor bearing rats, numerous studies on the elevation of this enzyme activity in the liver of tumor bearing animals have been reported[2)-5)]. In these studies, however, the authors discussed mainly the relationship between protein catabolism and adrenal hyperfunction in the later stage of tumor bearers. Furthermore, liver tyrosine: 2-oxoglutarate aminotransferase (LTA) activity has been proved to rise not only in tumor bearers but also in animals receiving Celite injection[6] or laparotomy[7] both of which are nonspecific stimuli.

The present study was designed to investigate the mechanism of elevation of this enzyme activity in the liver of Ehrlich ascites tumor bearing mice by comparison with animals receiving so-called nonspecific stimuli.

MATERIALS AND METHODS

Animals: SMA male mice weighing 20 to 30 g, were used. They were fed on mouse food (MF) manufactured by Oriental Yeast Co., Japan, and fresh

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water ad libitum. In some experiments, synthetic food was given (Table 1).

TABLE 1. Composition of Experimental Diet

	High protein carbohydrate free diet
Casein	81.5
Corn starch	0
Sucrose	0
Oil	10
Salt mixture	5
Vitamin mixture	0.85
Choline chloride	0.15
CMC	2.5

Tumor: Ehrlich ascites tumor was used throughout this study, prepared by intraperitoneal inoculation of 5×10^6 cells into each mouse. Mean survival time was about eighteen days.

Tissue injury: A suspension for injection was prepared by mixing 0.5 g of autoclaved talcum powder in 1 ml of sterile physiological saline, which was administered subcutaneously into the back of each mouse. Control animals were injected with equal volumes of sterile physiological saline.

Adrenal cortical hormone: Prednisolone hemisuccinate was used.

Adrenalectomy: Adrenals were extirpated by bilateral subcostal incision under Nembtal anesthesia. Adrenalectomy was performed on the 2nd day after intraperitoneal inoculation of tumor cells or on the 4th day after talcum injection. After surgical operation, mice were given mouse food and physiological saline instead of fresh water.

Preparation of liver homogenate: The animals were killed by decapitation. The livers were removed, weighed immediately, and homogenized in ice-cold isotonic 0.15 M KCl solution. A 5% homogenate was used for assay of the enzyme.

Preparation of subcellular fraction: The livers pooled from four mice for each group were homogenized in ice-cold 0.25 M sucrose. Differential centrifugation of 20% homogenate in 0.25 M sucrose was carried out according to the method of Kenney[8]. Nuclear, mitochondrial, lysosomal and supernatant fractions were isolated from the homogenates by centrifugation at $600 \times g$ for 5 minutes, $5000 \times g$ for 10 minutes and $20000 \times g$ for 15 minutes, respectively. Each fraction was treated with desoxycholate, in a final concentration of 0.5%, before enzyme assay according to the method of Palade and Siekevitz[9]. All procedures were carried out at 0–5°C.

Enzyme assay: L-tyrosine: 2-oxoglutarate aminotransferase activity was determined by the method of Kenney[10]. Reaction was initiated by the addition of 30 μmoles of 2-oxoglutarate after 5 minutes preincubation. After incubation for 10 minutes at 37°C, the reaction was stopped by adding 0.5 ml of 30% trichloroacetic acid. After centrifugation, the *p*-hydroxyphenylpyruvate in the supernate was measured by the modification[11] of Briggs reaction[12].

Tyrosine: 2-oxoglutarate aminotransferase activity was expressed as μmoles of *p*-hydroxyphenylpyruvate produced per hour per 100 g of carcass weight.

RESULTS

1. LTA activity in Ehrlich ascites tumor bearing mice: LTA activities were determined on the 2nd, 4th, 6th, 9th, 11th, 13th and 16th days after intraperitoneal inoculation of Ehrlich ascites tumor cells. As shown in Fig. 1, LTA activity was markedly elevated in the later stage, to as high as 2412 μmoles on the 13th and 2599 μmoles on the 16th day, while in the early stage this activity was not much higher than in normal mice.

2. LTA activity in injured mice: LTA activities were determined on the 1st, 2nd, 3rd, 5th, 7th, 9th and 11th days after talcum injection. As shown in Fig. 2, LTA activity was markedly elevated in the early stage to 2475 μmoles which remained so for about seven days and then fell rapidly to the level in the control animals.

3. LTA activity in mice after injection of glucocorticoid: Four hours after intraperitoneal injection of prednisolone hemisuccinate, LTA activity showed

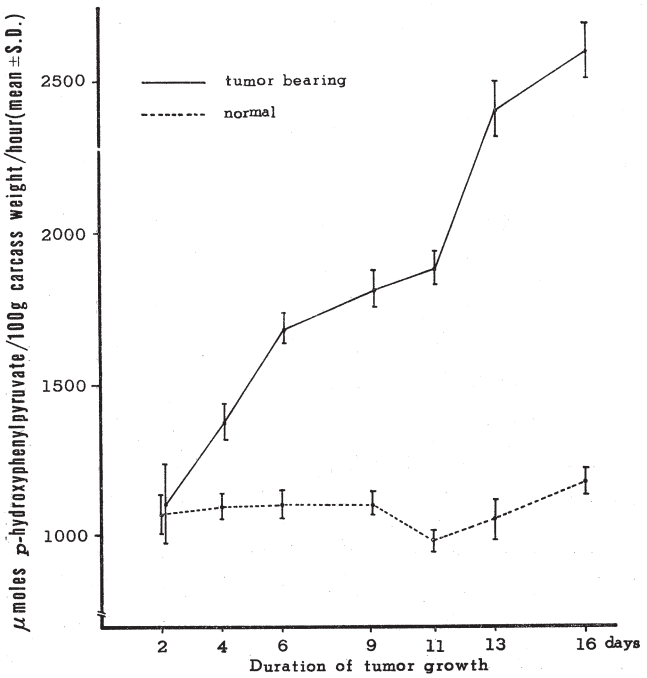

FIG. 1. LTA activity in tumor bearing and normal mice. Each group consisted of 5 mice.

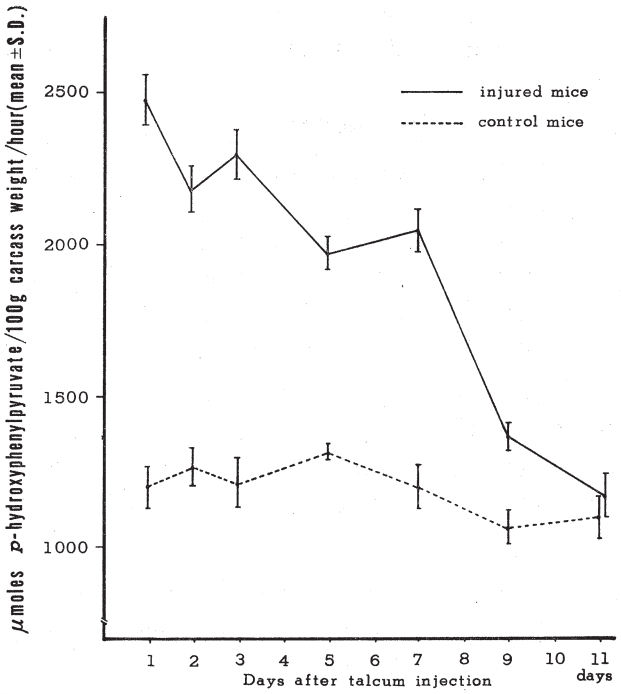

FIG. 2. LTA activity in injured and control mice. Each group consisted of 5 mice.

TABLE 2. LTA Activity in Mice after Injection of Glucocorticoid

Group	No. of mice	LTA activity (mean±S.D.)
control	5	1040.3±48.0
glucocorticoid*	5	3220.8±86.4

* 4 hours after intraperitoneal injection of 1 mg of prednisolone hemisuccinate into each mouse.

a marked elevation, as indicated in Table 2, to 3220.8 μmoles in the glucocorticoid injected group as compared with 1040.3 μmoles in the untreated group.

4. Effect of adrenalectomy on LTA activity in tumor bearing mice: After the inoculation of Ehrlich ascites tumor cells, adrenalectomy was performed on the 2nd day, and LTA activity was measured on the eighth day. As shown in Table 3, LTA activity in adrenalectomized tumor bearing group was 1014.4 μmoles in contrast to 1671.3 μmoles in sham-operated group, and 576.2 μmoles, 1232.7 μmoles in adrenalectomized and sham-operated tumor free groups,

TABLE 3. Effect of Adrenalectomy on LTA Activity in Tumor Bearing and Normal Mice

Group		No. of mice	LTA activity (mean±S.D.)
control	sham-operated	5	1232.7±40.8
	adrenalectomized	5	576.2±23.0
tumor-bearing*	sham-operated	5	1671.3±53.2
	adrenalectomized**	4	1014.4±39.2

* 8 days after intraperitoneal inoculation of Ehrlich ascites tumor.

** Adrenalectomy was performed on the 2nd day after the inoculation.

TABLE 4. Effect of Adrenalectomy on LTA Activity in Injured and Control Mice

Group		No. of mice	LTA activity (mean±S.D.)
control	sham-operated	5	1105.5±49.9
	adrenalectomized	4	584.9±24.6
injured*	sham-operated	5	1899.6±87.9
	adrenalectomized**	5	1006.5±29.6

* 5 days after talcum injection.

** Adrenalectomy was performed on the 4th day after the injection.

respectively. Thus, LTA activity was markedly lowered by adrenalectomy in both tumor bearing and tumor free mice.

5. Effect of adrenalectomy on LTA activity in injured mice: On the 4th day after the talcum injection, adrenalectomy was performed and 24 hours later LTA activity was determined. As shown in Table 4, LTA activity was 1006.5 μmoles in the adrenalectomized injured group and 1899.6 μmoles in the sham-operated group. In control mice, LTA activity was 584.9 μmoles and 1105.5 μmoles in adrenalectomized and sham-operated groups, respectively. Thus it is clear that LTA activity was markedly lowered by adrenalectomy in both injured and control mice.

6. Intracellular distribution of LTA activity in Ehrlich ascites tumor bearing mice: Eleven days after the tumor transplantation, LTA activity was determined in each subcellular fraction of the liver homogenates. As shown in Table 5, LTA activity in tumor bearing mice was elevated about two-fold as much as in control mice. Distribution of this enzyme activity in each of the subcellular

TABLE 5. Intracellular Distribution of LTA Activity in Ehrlich Ascites Tumor Bearing and Control Mice

Fractions	Control		Tumor bearing▲		
	LTA activity	Distribution (%)	LTA activity	Distribution (%)	Rate of elevation*
nuclear	196.6	18.4	454.4	21.2	231.1
mitochondrial	45.9	4.3	111.2	5.2	242.5
lysosomal	42.1	4.0	131.0	6.1	311.2
supernatant	785.3	73.3	1482.1	67.5	188.7
total activity	1069.9	100	2178.7	100	203.8

▲ 11 days after intraperitoneal inoculation of Ehrlich ascites tumor.

* Each value was expressed as $\frac{\text{LTA activity in tumor-bearers}}{\text{LTA activity in control}} \times 100$.

fracitons was 21.2%, 5.2%, 6.1% and 67.5% in nuclear, mitochondrial, lysosomal and supernatant fractions, while in control mice the respective distributions were 18.4%, 4.3%, 4.0% and 73.3%. These results indicate that in tumor bearing mice the distribution of this enzyme in the subcellular fraction did not differ much from that in control mice.

7. Intracellular distribution of LTA activity in injured mice: One group of four mice was given talcum injection and sacrificed 24 hours later, while another group received two injections of talcum at a week interval and killed 4 days after the second injection. As shown in Table 6, the LTA activity in

TABLE 6. Intracellular Distribution of LTA Activity in Injured and Control Mice

Fractions	Control		Injured					
			Single injection▲			Two injections△		
	LTA activity	Distribution (%)	LTA activity	Distribution (%)	Rate of elevation*	LTA activity	Distribution (%)	Rate of elevation*
nuclear	215.3	20.2	251.1	10.3	116.6	488.5	19.6	226.9
mitochondrial	45.8	4.3	39.6	1.6	86.5	111.5	4.5	234.4
lysosomal	45.3	4.3	73.2	3.0	161.5	157.5	6.3	347.6
supernatant	763.5	71.2	2065.9	85.1	270.6	1729.6	69.6	226.5
total activity	1069.9	100	2427.8	100	227.9	2487.1	100	233.6

▲ Each mouse of this group was given a talcum injection and sacrificed 24 hours later.

△ Each mouse of this group was given two injections of talcum at a week interval and killed 4 days after the second injection.

* Each value was expressed as $\frac{\text{LTA activity in injured}}{\text{LTA activity in control}} \times 100$.

injured mice with a single injection was 2.3 times of that in control mice and the distribution of enzyme activity in nuclear, mitochondrial, lysosomal and supernatant fraction was 10.3%, 1.6%, 3.0% and 85.1%, respectively. In the injured mice with two injections, although the LTA activity was elevated as much as in mice given a single injection, distribution of enzyme activity in each subcellular fraction was 19.6%, 4.5%, 6.3% and 69.6%, while in control mice the distribution was 20.2%, 4.3%, 4.3% and 71.2%, respectively. Thus the elevation of LTA activity in injured mice with a single injection was mainly due to the elevation in the supernatant fraction. On the other hand, the elevation of LTA activity in injured mice with two injections was due to elevation of activity in all subcellular fractions and the distribution of LTA activity was, therefore, almost the same as in the control group.

8. Intracellular distribution of LTA activity in mice fed on high protein diet: Mice were fed on high protein diet and sacrificed on the third and eleventh days after the start of the feeding. As shown in Table 7, on the third day after feeding on high protein diet, LTA activity in mice was 2.0 times that in control mice and the distribution of the enzyme activity in nuclear, mitochondrial, lysosomal and supernatant fractions, was 10.6%, 2.0%, 4.5%, and 82.9%, respectively. On the eleventh day after feeding on the high protein diet, although the LTA activity became about 2.2 times of that in control mice, distribution of enzyme activity in each subcellular fraction was 22.7%, 5.0%, 6.6% and 65.7%, while in the control group the distribution was 19.2%, 4.3%, 4.4% and 72.1%, respectively. From the above results, the activity of this enzyme increased remarkably only in the supernatant fraction in mice fed on high protein diet for three days but in mice fed on the same diet for eleven days, the activity of the enzyme increased not only in the supernatant

TABLE 7. Intracellular Distribution of LTA Activity in Mice Fed on High Protein Carbohydrate Free Diet

Fractions	Control		High protein carbohydrate free diet					
			For 3 days			For 11 days		
	LTA activity	Distribution (%)	LTA activity	Distribution (%)	Rate of elevation*	LTA activity	Distribution (%)	Rate of elevation*
nuclear	190.2	19.2	215.1	10.6	113.1	489.1	22.7	257.2
mitochondrial	42.2	4.3	40.4	2.0	95.7	106.8	5.0	253.1
lysosomal	43.2	4.4	90.4	4.5	209.3	143.1	6.6	331.3
supernatant	717.4	72.1	1681.1	82.9	234.3	1417.2	65.7	197.5
total activity	993.0	100	2027.0	100	204.1	2156.2	100	217.1

* Each value was expressed as $\frac{\text{LTA activity in high protein diet}}{\text{LTA activity in control}} \times 100$,

TABLE 8. Intracellular Distribution of LTA Activity in Mice with Glucocorticoid Injection

Fractions	Control		Glucocorticoid					
			Single injection▲			Repeated injection△		
	LTA activity	Distribution (%)	LTA activity	Distribution (%)	Rate of elevation*	LTA activity	Distribution (%)	Rate of elevation*
nuclear	181.7	18.6	271.4	7.3	149.4	356.7	14.2	196.3
mitochondrial	44.7	4.6	43.4	1.2	97.1	108.6	4.3	242.9
lysosomal	48.7	5.0	97.4	2.6	200.0	97.6	3.9	200.4
supernatant	702.8	71.8	3291.1	88.9	468.3	1957.4	77.6	278.5
total activity	977.9	100	3703.3	100	378.7	2520.3	100	257.4

▲ Each mouse of this group was given 1 mg of prednisolone and sacrificed 4 hours later.
△ Each mouse of this group was given 1 mg of prednisolone per day for 11 days and was killed 4 hours after the last injection.
* Each value was expressed as $\frac{\text{LTA activity in glucocorticoid}}{\text{LTA activity in control}} \times 100$.

fraction but also in the particle fractions, and the distribution of enzyme activity was, therefore, almost the same as in the control group.

9. Intracellular distribution of LTA activity in mice with glucocorticoid injection: One group of four mice was given 1 mg of prednisolone per mouse by intraperitoneal injection and sacrificed four hours later. Another group of mice was given the same amount of prednisolone intraperitoneally per day for eleven days and was killed four hours after the last injection. As shown in Table 8, the LTA activity in mice with a single injection of prednisolone was about 3.8 times of the control and the distribution of the enzyme activity in nuclear, mitochondrial, lysosomal and supernatant fractions, was 7.3%, 1.2%, 2.6% and 88.9%, respectively. In the group of mice with eleven injections of prednisolone LTA activity was about 2.6 times of that of the control group and its distribution in each subcellular fraction was 14.2%, 4.3%, 3.9% and 77.6%, respectively. In the control group, distribution of the enzyme activity was 18.6%, 4.6%, 5.0% and 71.8%, respectively. Thus, the elevation of enzyme activity in the supernatant fraction was marked in the group treated with a single injection of prednisolone but less so in the group with repeated injection.

DISCUSSION

Tyrosine aminotransferase acts on the first step of tyrosine oxidizing system and its activity in the liver is elevated by the administration of adrenal cortical hormone[13)-18)], ACTH[19)] and tyrosine[20)21)]. An elevation of this enzyme activity has also been recognized in the liver of animals fed on high protein

diet[2)19)] or received nonspecific stimuli, such as laparotomy[7)] and Celite injection[6)]. It is known that these elevatons in enzyme activity above mentioned are inhibited by adrenalectomy. The rise in liver tyrosine aminotransferase (LTA) activity in tumor bearing animal was first observed by Girkin *et al.*[1)], and later many authors reported that the elevation of this enzyme activity is lowered by adrenalectomy[2)-5)22)].

In the present report, LTA activity showed marked elevation in Ehrlich ascites tumor bearing, injured and high protein diet fed mice, and this elevation was also lowered by adrenalectomy. However, the time changes in enzyme activity showed different patterns in tumor bearing mice and nonspecifically stimulated mice, namely, LTA activity showed a marked elevation in the later stage in the former group and in the early stage in the latter.

To study this difference, the intracellular distribution of LTA activity was investigated. There have been only a few studies made on the intracellular distribution of LTA activity. Lin *et al.*[23)] first described the existence of tyrosine aminotransferase activity in the supernatant fraction obtained from rat liver homogenate by centrifugation at 13000-14000 × g. This was confirmed by other authors[24)-27)] and the greater part of LTA activity has been shown to be in the supernatant fraction and not in the other fractions. However, enzyme activity has also been demonstrated in the nuclear, mitochondrial, and lysosomal fractions by Litwack *et el.*[25)], Rowsell *et al.*[26)] and Kenney[8)], respectively, although very low in each fraction. Litwack *et al.*[25)] and Fellmann *et al.*[27)] reported that in the liver of rat with steroid hormone induction, the elevation of this enzyme activity was significant in the supernatant fraction but not in the particle fractions.

In the present report, similar results to the above were obtained in mice with tissue injury and fed on high protein diet as well as in mice with steroid hormone injection. Kenney *et al.*[6)] and Nakata *et al.*[2)3)] suggested that the elevation of LTA activity in the rat with tissue injury and fed on high protein diet was due to adrenal hyperfunction. The existence of adrenal hyperfunction in tumor bearing animals has been confirmed by Savard[28)], Ball *et al.*[29)], Peric-Golia *et al.*[30)] and others[31)32)]. From these findings, it was considered that in the liver of mice with nonspecific stimulus, the increased activity of enzyme in the supernatant fraction might be due to adrenal steroid hormone, and also that in the tumor bearing mice the intracellular distribution of the enzyme activity would show a similar pattern as that in mice with nonspecific stimulus. However, in the present study, the intracellular distribution of the enzyme activity in the liver of tumor bearing mice differed clearly from that in mice with nonspecific stimulus. In the tumor bearing mice the enzyme activity was elevated almost equally in all subcellular fractions and hence, the intracellular distribution of enzyme activity showed a similar pattern as in the control mice.

It can be said that a tumor acts as a chronic never ending stimulus, but talcum injection and high protein diet feeding for only a few days can be considered to be short term irritations. It was important, therefore, to compare the distribution of the enzyme in tumor bearing mice with that in mice with nonspecific stimulus maintained for a relatively long duration. In this study, chronically stimulated states were induced by repeated talcum injections, prolonged high protein diet feeding and repeated steroid hormone injections, and the intracellular distribution of the enzyme in these mice with chronic stimuli was examined. It was then found that in these mice with chronic nonspecific stimuli the percentage of enzyme activity in the supernatant fraction which was high in short term irritation, tended to fall to the level of the controls so that the intracellular distribution returned to the pattern of the controls.

It is proper, therefore, to consider that the distribution of the enzyme in the later stage of tumor bearing host is similar to that in mice with chronic nonspecific stimuli, and the elevated LTA activity and its location are hence not specific findings in the tumor bearing host. A growing tumor acts as a chronic and continuous stress stimulating the adreneal function, and this plays an important role in the elevation and changes in the distribution of this enzyme activity.

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