Nagoya J. med. Sci. 33: 315-328, 1971

ACTIVITIES OF UREA CYCLE ENZYMES IN TUMOR-BEARING MICE

MINEO SANO

1st Department of Internal Medicine, Nagoya University School of Medicine
(Director: Assist. Prof. Itsuro Sobue)

ABSTRACT

The hepatic arginase activity was found to be elevated significantly in tumor-bearing mice at the terminal stage. Furthermore, other enzymes in the urea cycle, ornithine transcarbamylase, arginine synthetase (over all reaction) and argininosuccinate cleavage enzyme, also showed elevated activity with a concomitant increase in urinary excretion of urea.

The present experiments seem to show that the enhanced activities of these enzymes and the increased urea excretion both are related to protein catabolism under adrenal hyperfunction in tumor-bearing mice at the terminal stage. Possible explanations for the development of cachexia in tumor-bearing hosts were made.

INTRODUCTION

Since the concept of urea cycle was advocated by Krebs *et al.*[1] in 1932, the role of this cycle in protein metabolism has become, with the progress of biochemistry, increasingly clear in recent years[2)-10)]. On the other hand, although there have been many reports on metabolic abnormalities in a tumor-bearing host[11)-17)], the dynamics of urea cycle enzymes has been analyzed in only a few reports, where the authors have not been in full agreement.

The purpose of the present study is to analyze the dynamics of urea cycle enzymes in tumor-bearing animals and to investigate possible factors affecting the activity of arginase in the cycle.

MATERIALS AND METHODS

Female mice of the SMA strain weighing 20 to 30 g were fed mainly on solid mouse food (NMF) prepared by the Oriental Yeast Co., Japan. Several other kinds of food which differ in their protein components were also used to meet the purpose of the individual experiments (Table 1). Intraperitoneal in-

佐野峯雄
Received for publication December 21, 1970.

TABLE 1. Composition of the Diets Used in the Present Study

	Protein-free diet	Low protein diet	High protein diet
Casein	0 (g)	5 (g)	60 (g)
Corn starch	70	65	10
Sucrose	11.5	11.5	11.5
Oil*	10	10	10
Salt mixture*	5	5	5
Vitamin mixture*	0.85	0.85	0.85
Choline chloride	0.15	0.15	0.15
Carboxymethyl cellulose	2.5	2.5	2.5

* Supplied by Tanabe Amino Acids Research Foundation.

oculation of 5×10^6 of Ehrlich ascites tumor cells was performed to produce tumor-bearing mice. The mean survival time of the mice after the inoculation was about 3 weeks. Mice whose ascites became bloody at the terminal stage were excluded from the present study. Solid tumors were produced by intramuscular transplantation of 10^7 ascites tumor cells into the back of the mice, and 6 to 8 weeks later they were used for the experiments. Under anesthesia with 1.5 mg of Nembutal, the adrenals were extirpated through bilateral subcostal incision. After surgical operation, the animals were given physiological saline instead of fresh water. Immediately after exsanguination of a mouse by decapitation, the liver was removed and weighed. Exactly 250 mg of liver of wet weight was homogenized in 4.75 ml of ice-cold distilled water with a teflon homogenizer in the standardized manner. The 5 per cent homogenate was used for all enzyme assays. Activities of arginase (EC 3.5.3.1) and ornithine transcarbamylase (EC 2.1.3.3) were assayed. In addition, the over all reaction system[7] of arginine synthetase and argininosuccinate cleavage enzyme[4)-8)] (EC 4.3.2.1) were also assayed according to Schimke's method[9]. All assays were performed in duplicates at 37°C. Enzyme activities were expressed in micromoles (μ moles) of reaction product per hour per 100 g of the carcass weight. The carcass weight was measured after the solid tumor or ascites was removed.

The determination of urea was made with α-iso-nitrosopropiophenone reagent according to Ratner's[7] modification of Archibald's method[27] and 2.5 ml of a mixture of sulfuric and phosphoric acid solutions was used for the development of color. The spectrophotometer was utilized for the determination at 540 mμ. Citrulline was spectrophotometrically measured at 490 mμ bv Archibald's method[28] employing diacetylmonoxime reagent. The material was heated in the dark, in colored test tubes to prevent it from light-induced discoloration.

Mice were kept in metabolic cages to collect the 24-hour urine in a bottle

containing 5 ml of 10 per cent trichloroacetic acid (TCA) to prevent decomposition. They were given food and water *ad libitum*. The metabolic cages were thoroughly rinsed with water. The washings together with the urine collected were diluted to 1 liter with distilled water. After deproteinization with TCA, the fluid was centrifuged and the supernate was used for the determination of urea.

RESULTS

Figures in parentheses immediately after mean values indicate standard error of the mean.

Serial changes in the activity of hepatic urea cycle enzymes in tumor-bearing mice

Serial changes in the hepatic arginase activity were analyzed in mice with Ehrlich ascites tumor killed 2 to 16 days after the inoculation of tumor cells. As shown in Table 2 and Fig. 1, there was no significant variation in the activity of the enzyme in the tumor-free control mice kept under the same condition as the tumor-bearing mice except for the presence of tumor. On the other hand, in tumor-bearing mice the activity began to rise 8 days after inoculation. Sixteen days after inoculation, it reached a very high level, showing a highly significant difference in the mean between the tumor-bearing and control mice (1,338,000 (60,000) vs. 635,000 (7,000) μmoles) ($p < 0.001$). The average

TABLE 2. Hepatic Arginase Activity in Mice with Ehrlich Ascites Tumor

Days after inoculation		No. of mice	Carcass weight (g)	Liver weight (g)	Arginase activity*
2	Tumor-bearing	5	23.6±0.7	1.20±0.08	601,000±18,000
	Tumor-free	5	23.4±0.8	1.08±0.05	610,000±36,000
4	Tumor-bearing	5	24.5±0.3	1.32±0.04	699,000±31,000
	Tumor-free	5	25.3±0.3	1.38±0.02	643,000± 9,800
6	Tumor-bearing	5	25.5±0.8	1.38±0.13	688,000±28,000
	Tumor-free	5	25.5±1.1	1.31±0.06	652,000±15,000
8	Tumor-bearing	6	24.0±0.7	1.42±0.04	784,000±23,000
	Tumor-free	4	25.6±0.4	1.32±0.04	683,000±18,000
11	Tumor-bearing	6	23.9±0.6	1.48±0.08	989,000±42,000
	Tumor-free	5	24.7±0.9	1.21±0.07	656,000± 9,800
16	Tumor-bearing	5	23.1±0.9	1.47±0.05	1,338,000±60,000
	Tumor-free	5	25.2±0.6	1.25±0.03	635,000± 7,000

Values in the table indicate the mean and its standard error.
* Micromoles of urea formed / 100 g body weight / hour.

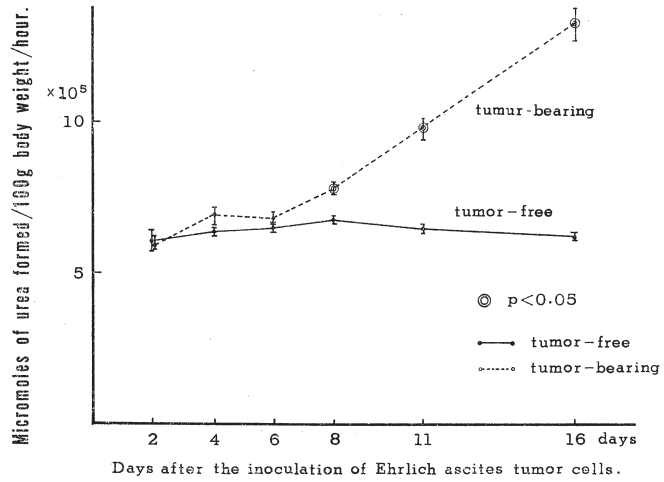

FIG. 1. Hepatic arginase activity in mice with Fhrlich ascites tumor. Vertical bars indicate the standard error of the mean.

carcass weight of the control mice was 25.2 (0 6) g, while 16 days after inoculation the value for tumor-bearing mice was reduced to 23.1 (0.9) g. On the contrary, the average liver weight was 1.25 (0 03) g in tumor-free mice and increased to 1.47 (0.05) g in tumor-bearing mice.

Figure 2 shows that the weight of the solid tumor was positively correlated with the level of the hepatic arginase activity ($r = 0.64$ $p < 0.001$). In mice

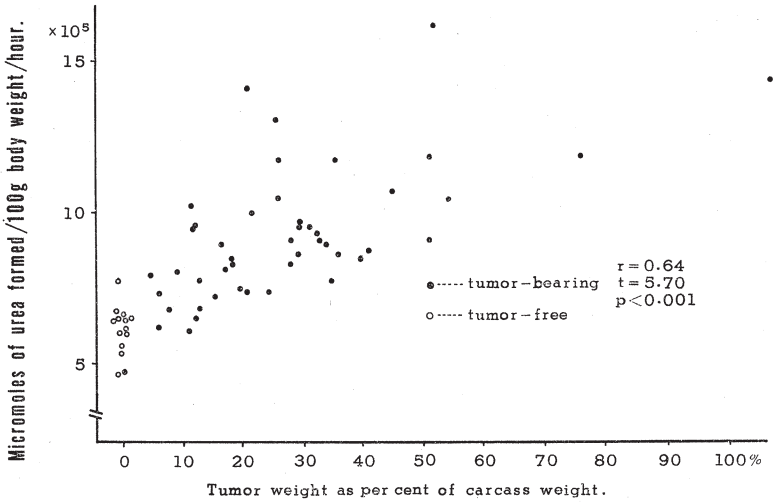

FIG. 2. Correlation between tumor weight and hepatic arginase activity in mice with solid tumor. Determined 6 to 8 weeks after the intramuscular inoculation of Ehrlich ascites tumor cells.

TABLE 3. Hepatic Arginase Activity in Mice with Solid Tumor

	Tumor weight as per cent of carcass weight	No. of mice	Arginase activity*
Tumor-bearing ☆	Less than 10%	7	670,000±43,000 $p<0.4$
	10~20%	13	821,000±35,000 $p<0.001$
	20~30%	12	1,008,000±61,000 $p<0.001$
	30~40%	8	938,000±42,000 $p<0.001$
	More than 40%	8	1,196,000±96,000 $p<0.001$
Tumor-free		13	630,000±22,000

Values in the table indicate the mean and its standard error.
* Micromoles of urea formed / 100 g body weight / hour.
☆ Determined 6 to 8 weeks after the intramuscular inoculation of Ehrlich ascites tumor cells.

with tumor weight of less than 10 per cent of the carcass weight, no significant difference was noted in the activity between tumor-bearing and control mice (Table 3). When the tumor weight was more than 10 per cent of the carcass weight, a significant rise was noted in the hepatic arginase activity ($p<0.001$). In mice whose tumor was more than 40 per cent of the carcass weight, the average enzyme activity was elevated to a high value of 1,196,000 (96,000) μmoles as compared with 630,000 (20,000) μmoles in tumor-free mice ($p<0.001$). Table 4 shows that in tumor-bearing mice at the terminal stage, the activities of hepatic arginase, ornithine transcarbamylase, arginine synthetase (over all reaction), and argininosuccinate cleavage enzyme were all en-

TABLE 4. Activities of Hepatic Urea Cycle Enzymes in Mice with Ehrlich Ascites Tumor

	Tumor-bearing	Tumor-free
No. of mice	8	6
Carcass weight (g)	20.4 ±0.4	23.1 ±0.4
Liver weight (g)	1.48±0.3	1.10±0.04
Arginase activity*	1,209,000±54,000 $p<0.001$	648,000±33,000
Ornithine transcarbamylase activity*	1,455,000±48,000 $p<0.05$	1,283,000±66,000
Arginine synthetase activity (Over all reaction)*	1,108±31.0 $p<0.001$	869±24.7
Cleavage enzyme activity*	5,180±108 $p<0.001$	2,876±99.2

Values in the table indicate the mean and its standard error.
* Micromoles of product / 100 g body weight / hour.
Determined 15 days after the intraperitoneal inoculation of Ehrlich ascites tumor cells.

hanced as compared with those in tumor-free mice. In other words, all the urea cycle enzymes tended to increase their activity at the terminal stage. The degree of the increased activities was not uniform in individual enzymes. The elevated activity of arginase and argininosuccinate cleavage enzyme was quite noteworthy, being nearly doubled as compared with the control value ($p<0.001$). However, the ornithine transcarbamylase and arginine synthetase activity rose only slightly, the former being about 1.1 times and the latter about 1.3 times as high as those in tumor-free mice ($p<0.05$).

The relation between food intake and urea excretion in mice with Ehrlich ascites tumor.

Eight mice were raised in metabolic cages for 7 days and then inoculated with Ehrlich ascites tumor cells. Their urine was collected every 2 to 3 days to evaluate the average amount of urea excreted in 24 hours. The intake of food was also determined. As shown in Fig. 3, urea excretion by the tumor-bearing mice decreased moderately during the period of 6 to 12 days after inoculation and was reduced to 96 mg / 24 hours / mouse from the pre-inoculation

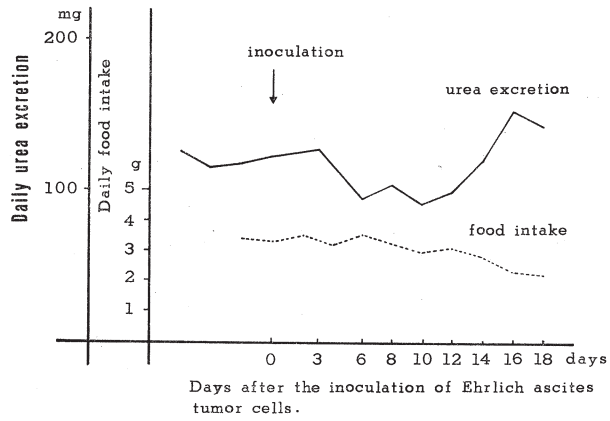

FIG. 3. Urea excretion and food intake in mice with Ehrlich ascites tumor.

level of 120 mg. Subsequently, it tended, however, to increase and reached a significantly higher level of 147 mg/24 hours/mouse 16 days after inoculation. The intake of food by the tumor-bearing mice began to decrease 10 days after inoculation, being reduced to about 60 per cent of the pre-inoculation amount. For analysis of the effects of fasting in tumor-free animals, a group of mice was subjected to starvation for 4 days but was allowed free intake of water. Since the hepatic arginase activity is affected by the protein content in food[9], protein-free food was fed to the control mice for 4 days. As shown in Table

TABLE 5. Effects of Fasting on the Hepatic Arginase Activity in Mice

	No. of mice	Carcass weight (g)	Liver weight (g)	Arginase activity*
Fasting for 4 days	5	18.4±0.7	0.71±0.05	619,000±20,000 $p<0.001$
Protein-free diet for 4 days	6	19.8±0.5	0.76±0.02	349,000±19,000

Values in the table indicate the mean and its standard error.
* Micromoles of urea formed / 100 g body weight / hour.

5, the hepatic arginase activity of the fasting group was, on the average, 619,000 (20,000) μmoles which was significantly higher than the 349,000 (19,000) μmoles for the control group ($p<0.001$).

Effects of dietary protein content on hepatic arginase activity in mice with Ehrlich ascites tumor

Tumor-bearing and tumor-free mice were fed on three different foods; standard, low protein and high protein diets. Thus, the hepatic arginase activity was compared in 6 different groups. Table 6 summarizes the results of this experiment. The tumor-free group on low protein diet showed the lowest activity of the enzyme among the 6 groups. The mean value for this group was significantly different from those of the 2 other tumor-free groups ($p<0.001$). In contrast, the tumor-bearing groups on high protein and standard

TABLE 6. Effects of Dietary Protein on the Hepatic Arginase Activity in Mice with Ehrlich Ascites Tumor

		No. of mice	Carcass weight (g)	Liver weight (g)	Arginase activity*
Standard diet※	Tumor-bearing	6	25.9±0.6	1.68±0.04	1,289,000±44,000 $p<0.001$
	Tumor-free	5	28.7±0.6	1.37±0.05	564,000±13,000
Low protein diet	Tumor-bearing	6	26.9±0.9	0.82±0.01	721,000±47,000 $p<0.001$
	Tumor-free	6	26.0±0.7	0.96±0.03	343,000±28,000
High protein diet	Tumor-bearing	5	26.2±1.9	1.36±0.12	1,286,000±37,000 $p<0.001$
	Tumor-free	6	26.1±0.9	1.26±0.06	801,000±31,000

Values in the table indicate the mean and its standard error.
* Micromoles of urea formed / 100 g body weight / hour.
Determined 15 days after the intraperitoneal inoculation of Ehrlich ascites tumor cells.
※ Mouse food prepared by Oriental Yeast Co., Ltd. (NMF).

diets showed the highest level of enzyme activity as compared with all the other 4 groups ($p<0.001$). Each tumor-bearing group showed a significantly higher activity than the corresponding tumor-free group ($p<0.001$).

Effect of glucocorticoid on the hepatic arginase activity in tumor-free mice

A group of tumor-free mice was given subcutaneously a single daily dose of 1 mg of prednisolone hemisuccinate for 3 successive days and was studied for the hepatic arginase activity 24 hours later. The enzyme activity showed a mean of 863,000 (52,000) μmoles, which was significantly higher than that of the control group ($p<0.001$). However, no increase in activity was noted in mice which received only a single subcutaneous dose of 1 mg of the drug (Table 7).

TABLE 7. Effect of Glucocorticoid on the Hepatic Arginase Activity in Tumor-free Mice

	No. of mice	Carcass weight (g)	Liver weight (g)	Arginase activity*
Glucocorticoid administered for 3 successive days※	6	17.7±1.0	0.96±0.04	863,000±52,000 $p<0.001$
Single dose of glucocorticoid administered※	7	19.6±0.4	1.01±0.02	692,000±17,000
Control	6	24.3±0.9	1.25±0.05	653,000±24,000

Values in the table indicate the mean and its standard error.
* Micromoles of urea formed / 100 g body weight / hour.
※ Prednisolone hemisuccinate 1 mg / day was administered by subcutaneous injection.

Effect of adrenalectomy on the hepatic arginase activity in tumor-bearing mice

Adrenalectomy was performed in 5 tumor-bearing and 5 tumor-free mice, and a sham operation in control tumor-bearing and tumor-free groups, each group consisting of 6 mice. Tumor-bearing mice were operated 3 days after the Ehrlich ascites tumor implant into the abdominal cavity. The hepatic arginase activity was determined 6 days after operation in each group. Table 8 indicates that the average enzyme activity in tumor-bearing and tumor-free mice with sham operation was 720,000 (27,000) and 622,000 (18,000) μmoles, respectively, which were significantly different ($p<0.05$). As compared with the sham operation groups, adrenalectomy significantly lowered the enzyme activity in both tumor-bearing and tumor-free mice, with a very similar average value of approximately 284,000 μmoles. Similar experiments were performed to evaluate the effects of adrenalectomy on the hepatic arginase activity in mice with

TABLE 8. Effect of Adrenalectomy on the Hepatic Arginase Activity in Mice with Ehrlich Ascites Tumor

		No. of mice	Carcass weight (g)	Liver weight (g)	Arginase activity*
Adrenalectomy★	Tumor-bearing☆	5	25.9±1.3	1.07±0.07	284,000± 6,200
	Tumor-free	5	25.4±0.8	1.05±0.03	283,000±13,000
Sham operation★	Tumor-bearing☆	6	27.0±1.2	1.29±0.03	720,000±27,000
	Tumor-free	6	28.6±0.6	1.28±0.08	622,000±18,000

Values in the table indicate the mean and its standard error.
* Micromoles of urea formed / 100 g body weight / hour.
☆ Determined 9 days after the intraperitoneal inoculation of the Ehrlich ascites tumor cells.
★ Determined 6 days after the operation.

TABLE 9. Effect of Adrenalectomy on the Hepatic Arginase Activity in Mice with Solid Tumor

		No. of mice	Carcass weight (g)	Liver weight (g)	Tumor weight (g)	Arginase activity*
Adrenalectomy★	Tumor bearing☆	6	24.6±1.2	1.66±0.10	5.03±1.18	525,000±19,000
	Tumor free	5	21.7±0.8	1.32±0.07		559,000±19,000
Sham operation★	Tumor bearing☆	7	24.0±1.1	1.60±0.07	4.58±1.29	850,000±65,000
	Tumor free	6	22.2±0.5	1.14±0.04		666,000±25,000

Values in the table indicate the mean and its standard error.
* Micromoles of urea / 100 g body weight / hour.
☆ Determined 6 to 8 weeks after the intramuscular inoculation of Ehrlich ascites tumor cells.
★ Determined 3 days after the operation.

a solid tumor of similar size. As shown in Table 9, the average activity in the sham operation groups was 850,000 (65,000) μmoles in tumor-bearing mice and 666,000 (25,000) μmoles in tumor-free ones, the former being significantly higher than the latter ($p<0.05$). On the other hand, each group of adrenalectomized mice showed a significantly lower enzyme activity than the corresponding group with sham operation ($p<0.001$). No significant difference was noted between the tumor-bearing and tumor-free mice.

DISCUSSION

There have been many reports[18)-26)] on the activity of hepatic arginase in experimental animals with transplantable tumor. Greenstein[18)-21)] noted a sig-

nificant reduction in the hepatic arginase activity in fast-growing hepatoma 31 of rats. Weil[22] stated that the enzyme activity decreased in rats with transplansplanted sarcoma. Fujiwara[23] also reported a decrease in the activity in tumor-bearing mice. In contrast, Greenberg and Sassenrath[24] stated that there was no statistical difference in the hepatic arginase activity between tumor-bearing and control mice. Abreu and Abreu[25] reported that, in Ehrlich ascites tumor-bearing mice, the activity of hepatic arginase remarkably increased at the terminal stage. Thus, there has been considerable disagreement on the behavior of the hepatic arginase activity in tumor-bearing animals. The results of the present study agree with the observations made by Abreu and Abreu[25].

Only a few investigators have studied hepatic urea cycle enzymes other than arginase in tumor-bearers. Greenstein *et al.*[19] stated that no significant difference was found in hepatic urea synthesis between tumor-bearing and control rats. Nakata *et al.*[26] also noted no change in the level of urea cycle enzymes in tumor transplanted mice. In the present study, however, the activity of the individual enzymes in the cycle was increased. This disagreement may be attributed partly to the fact that activities of the hepatic arginase and other urea cycle enzymes do not rise until the terminal stage in tumor-beaing animals[25], and partly to the difference in the manner of expressing enzyme activities. In view of the increase in the liver weight and also of the marked decrease in the body weight in tumor-bearing animals, enzyme activities should be corrected by both of the two factors, the total liver weight and body weight instead of unit weight of protein or nitrogen, for proper evaluation of the change in enzyme activities[10,29]. The present experiments demonstrated that the activity of hepatic urea cycle enzymes was elevated in association with the increased urea excretion by the host at the terminal stage. These results agreed with the reports that urea excretion in urine was in proportion to the activity of hepatic arginase[30] or to the total urea cycle enzymes[9]. Urinary excretion of urea is said to be affected by catabolism of body protein and intake of protein[9,30]. Consequently, the observation that mice with ascites tumor showed an increased urea excretion at the terminal stage in spite of decreased food intake, could be attributed to increased catabolism of body protein. It was observed in the present study, that fasting or administration of a large dose of glucocorticoid enhanced the hepatic arginase activity. This could suggest that the enhancement of the activity is related to increased protein catabolism. Schimke had made a similar observation[10,31]. Tumor-bearing mice showed a higher hepatic arginase activity than the tumor-free ones independent of protein intake. This result would imply that the higher level of activity is induced by increased catabolism of protein in the tumor-bearing mice at the terminal stage.

Because of increased consumption of glucose[32] by the tumor cells and in-

creased energy expenditure by the tumor-bearing animals[33], the host is considered to develop carbohydrate deficiency, which, in turn, accelerates protein catabolism. Mider *et al.*[34,35] stated that as the tumor grows, hosts consume lipid which is a storage of energy, and at the terminal stage, they utilize protein as source of energy, with a consequent negative nitrogen balance developing.

Adrenal hyperfunction has commonly been recognized in tumor-bearing hosts. Balls and Samuels[36] reported adrenal hypertrophy in rats with Walker 256 tumor. Savard[37] also made a similar observation in mice with sarcoma 180. Nadel and Burstein[38] reported an increase in urinary corticosteroid excretion in guinea pig with liposarcoma and leukemia. Hilf *et al.*[39], and Peric-Golia and Jones[40] also reported an increased plasma corticosterone level in experimental animals with malignant neoplasm. It was also shown that adrenal hyperfunction was responsible for the increased activity of steroid-dependent tryptophan pyrrolase[16,41] and tyrosine aminotransferase[17] in tumor-bearing animals. In the present experiments, it was noted that adrenalectomy kept the hepatic arginase activity at a low fixed level in mice with ascites tumor, and decreased the enzyme level which had already become higher in mice with solid tumor. These would suggest that adrenal function is closely related to the hepatic arginase activity in tumor-bearing mice.

When metabolic abnormalities in a tumor-bearing host is viewed in terms of protein metabolism, several factors such as nitrogen trap[34], anorexia, and accelerated protein catabolism and gluconeogenesis under adrenal hyperfunction for correcting carbohydrate deficiency are all considered to play an important role in developing malignancy-induced cachexia. Thus, it seems that increased activities of urea cycle enzymes are one of the expressions of the host's adaptive mechanism to maintain its internal environment.

SUMMARY

Female SMA mice inoculated with Ehrlich ascites tumor were studied for the activity of urea cycle enzymes (arginase, ornithine transcarbamylase, arginine synthetase and argininosuccinate cleavage enzyme) as well as for urinary urea excretion. Analyses were made to evaluate the effects of food intake, especially protein content in the diet, and adrenal function on the hepatic arginase activity in tumor-bearing mice at the terminal stage.

1) In mice with transplanted Ehrlich ascites tumor, the hepatic arginase activity began to rise 8 days after inoculation and it rose to about 2.1 times the value for the control group 16 days after inoculation.

2) In mice bearing a solid tumor, significantly positive correlation was found between the size of tumor and the hepatic arginase activity. As the tumor weight increased beyond 10 per cent of the carcass weight, the activity tended

to rise. In mice whose tumor weight was more than 40 per cent of the carcass weight, the enzyme activity was about 1.9 times as high as the control value.

3) In mice bearing Ehrlich ascites tumor, activities of ornithine transcarbamylase, arginine synthetase (over all reaction), and argininosuccinate cleavage enzyme were also elevated at the terminal stage.

4) In mice bearing Ehrich ascites tumor urinary excretion of urea increased at the terminal stage.

5) The hepatic arginase activity was elevated in tumor-bearing mice at the terminal stage, irrespective of protein content in food.

6) Adrenalectomy, which was performed 3 days after the intraperitoneal inoculation of Ehrlich ascites tumor cells, suppressed an expected elevation of the hepatic arginase activity. It also lowered the enzyme activity which had been elevated in mice with a solid tumor prior to adrenalectomy.

7) The rise in the hepatic arginase activity of tumor-bearing mice at the terminal stage was concluded to be closely related to increased protein catabolism induced by adrenal hyperfunction.

REFERENCES

1) Krebs, H. A. und Henseleit, K., Untersuchungen über die Harnstoffbildung im Tierkörper, *Z. Physiol. Chem.*, **210**, 33, 1932.
2) Jones, M. E., Spector, L. and Lipmann, F., Carbamyl phosphate, the carbamyl donor in enzymatic citrulline synthesis, *J. Amer. Chem. Soc.*, **77**, 819, 1955.
3) Grisolia, S. and Cohen, P. P., The catalytic role of carbamyl glutamate in citrulline biosynthesis, *J. Biol. Chem.*, **198**, 561, 1952.
4) Ratner, S. and Petrack, B., Biosynthesis of urea IV. Further studies on condensation in arginine synthesis from citrulline, *J. Biol. Chem.*, **200**, 161, 1953.
5) Ratner, S. and Petrack, B., The mechanism of arginine synthesis from citrulline in kidney, *J. Biol. Chem.*, **200**, 175, 1953.
6) Schuegraf, A., Ratner, S. and Warner, R. C., Free energy changes of the argininosuccinate synthetase reaction and of the hydrolysis of the inner pyrophosphate bond of adenosine triphosphate, *J. Biol. Chem.*, **235**, 3597, 1960.
7) Ratner, S., *In* Methods in Enzymology Vol. II. Edited by S. P. Colowick and N. O. Kaplan, Academic Press Inc., New York, 1955, p. 356.
8) Rochovansky, O. and Ratner, S., Biosynthesis of urea IX. Further studies of mechanism of argininosuccinate synthetase reaction, *J. Biol. Chem.*, **236**, 2254, 1961.
9) Schimke, R. T., Adaptive characteristics of urea cycle enzymes in the rat, *J. Biol. Chem.*, **237**, 459, 1962.
10) Schimke, R. T., Differential effects of fasting and protein-free diets on levels of urea cycle enzymes in rat liver, *J. Biol. Chem.*, **237**, 1921, 1962.
11) Greenstein, J. P., Jenrette, W. V. and White, J., The liver catalase activity of tumor-bearing rats and the effect of extirpation of the tumors, *J. Nat. Cancer Inst.*, **2**, 283, 1941.
12) Lucké, B., Berwick, M. and Zeckwer, I., Liver catalase activity in parabiotic rats with one partner tumor-bearing, *J. Nat. Cancer Inst.*, **13**, 681, 1952.

13) Lan, T. H., A study of D-amino acid oxidase, uricase, and choline oxidase in the liver and in isolated liver cell nuclei of rats bearing transplanted tumors, *Cancer Res.*, **4**, 37, 1944.
14) Greenberg, D. M. and Sassenrath, E. N., Tumor-host relationships II. Tolerance to injection of amino acids, *Cancer Res.*, **15**, 620, 1955.
15) White, J. M., Osawa, G., Ross, G. A. L. and McHenry, E. W., An effect of neoplasma on glutamic acid metabolism in the host, *Cancer Res.*, **14**, 508, 1954.
16) Wood, S., Rivlin, R. S. and Knox, W. E., Biphasic changes of tryptophan peroxidase level in tumor-bearing mice and in mice subjected to growth hormone and stress, *Cacer Res.*, **16**, 1053, 1956.
17) Kato, Y., Factors elevating liver tyrosine: 2-oxoglutarate aminotransferase activity in tumor-bearing mice, *Nagoya J. Med. Sci.*, **30**, 211, 1967.
18) Greenstein, J. P., Biochemistry of Cancer, Academic Press Inc., New York, 1954, p. 542.
19) Greenstein, J. P. and Leuthardt, F. M., F. M., Enzymatic activity in primary and transplanted rat hepatomas, *J. Nat. Cancer Inst.*, **6**, 211, 1946.
20) Greenstein, J. P., Leuthardt, F. M., Note on the enzymatic activity of normal and neoplastic tissues of the rat, *J. Nat. Cancer Inst.*, **6**, 317, 1946.
21) Greenstein, J. P., Jenrette, W. V., Mider, G. B., and White, J., Chemical studies on the components of normal and neoplastic tissues, V. The relative arginase activity of certain tumors and normal control tissues, *J. Nat. Cancer Inst.*, **1**, 687, 1941.
22) Weil, L., The activation of arginase, *J. Biol. Chem.*, **110**, 201, 1935.
23) Fujiwara, H., Über den Argininstoffwechsel bei Tuberkulose und Carcinom, *Z. Physiol. Chem.*, **185**, 1, 1929.
24) Greenberg, D. M. and Sassenrath, E. N., Lack of effect of high potency arginase on tumor growth, *Cancer Res.*, **13**, 709, 1953.
25) Abreu, L. A. and Abreu, R. R., Hepatic arginase activity in mice bearing Ehrlich ascites carcinoma, *Arch. Geschwulstforsch.*, **31**, 1, 1968.
26) Nakata, Y. and Sakamoto, Y., Protein metabolism of the tumor-bearing host, *Sougou Igaku*, **20**, 785, 1963 (in Japanese).
27) Archibald. R. M., Colorimetric determination of urea, *J. Biol. Chem.*, **157**, 507, 1945.
28) Archibald, R. M., Determination of citrulline and allantoin and demonstration of citrulline in blood plasma *J. Biol. Chem.*, **156**, 121, 1944.
29) Knox, W. E. and Greengard, O., *Advances Enzym. Regulat.*, **3**, 247, 1965.
30) Ashida, K. and Harper, A. E., Metabolic adaptation in higher animals, VI. Liver arginase activity during adaptation to high protein diet, *Proc. Soc. Exp. Biol. Med.*, **107**, 151, 1961.
31) Shimke, R. T., Studies on factors affecting the levels of urea cycle enzymes in rat liver, *J. Biol. Chem.*, **238**, 1012, 1963.
32) Del Monte, U. and Rossi, C. B., Glucose supply by the living host and glycolysis of Yoshida ascites hepatoma *in vivo*, *Cancer Res.*, **23**, 363, 1963.
33) Mider, G. B., Fenninger, L. D., Haven, F. L. and Morton, J. J., The energy expenditure of rats bearing Walker carcinoma 256, *Cancer Res.*, **11**, 731, 1951.
34) Mider, G. B., Some aspects of nitrogen and energy metabolism in cancerous subjects: A review, *Cancer Res.*, **11**, 821, 1951.
35) Mider, G. B., Sherman, C. D. Jr. and Morton, J. J., The effect of Walker carcinoma 256 on the total lipid content of rats, *Cancer Res.*, **9**, 222, 1949.
36) Ball, H. A. and Samuels, L. T., Adrenal weights in tumor-bearing rats, *Proc. Soc. Exp. Biol. Med.*, **38**, 441, 1938.
37) Savard, K., Adrenal changes in animals bearing transplanted tumors, *Science*, **108**, 381, 1948.
38) Nadel, E. M. and Burstein, S., Urinary excretion of corticosteroids in guinea pigs

with malignant neoplastic disease, *J. Nat. Cancer Inst.*, **17**, 213, 1956.
39) Hilf, R. Burnett, F. F. and Borman, A., The effect of sarcoma 180 and other stressing agents upon adrenal and plasma corticosterone in mice, *Cancer Res.*, **20**, 1389, 1960.
40) Peric-Golia, L. and Jones, R. S., Corticosterone in lymphoma bearing rats, *Proc. Soc. Exp. Biol. Med.*, **113**, 317, 1963.
41) Kawachi, T., Fujii, S., Uesaki, N., Suzuki T. and Yamamura, Y., Studies on tryptophan pyrrolase of tumor-bearing rats with special reference to the function of adrenal glands, *Gann*, **52**, 219, 1961.