Metabolism of Guanidoacetic Acid in Tumor-Bearing Rats.* (25544)

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We outlined(1) observations on some phases of creatine metabolism in rats bearing Walker carcinoma 256 and showed that there was a net decrease in creatine synthesis in these animals. Creatine synthesis may be considered in 2 steps: (a) formation of guanidoacetic acid (GAA) from arginine and glycine, and (b) methylation of GAA to give creatine. Obviously, an impairment in either one or both of these synthetic steps can result in reduction of creatine synthesis. Therefore formation and excretion of GAA in tumor-bearing rats were first studied to determine if rate of GAA production could be a limiting factor in creatine synthesis in these rats.

Materials and methods. Male rats of both Sprague-Dawley strain and Wistar strain (Carworth Farms) were used. No difference in data obtained with these 2 strains of animals was noticeable. Hence, results have been pooled and averaged. Animals weighing 150-300 g were paired by weight and fed a balanced semi-synthetic diet containing neither creatine nor GAA. One member of each pair received a subcutaneous transplantation of Walker carcinoma 256 and ate the diet *ad lib.*, the other member served as control and was pair-fed to its tumor-bearing mate. The animals were housed in individual metabolism cages to facilitate collection of urine samples. Food intake was recorded daily. Body weights were determined every fourth day and *in vivo* estimation of tumor weights(2) was made at same time interval. Urine collections were made for 2 successive days in each 4-day interval throughout the experiment. Thymol was used as a preservative. At various stages of tumor growth, animal pairs were sacrificed under ether anesthesia. Blood samples were withdrawn from the abdominal aorta and heparinized. Liver and kidneys were excised, weighed, and immediately frozen in liquid nitrogen. Frozen tissues were dried by freeze-dry technic. Dry tissues were weighed, ground to fine powder, and stored in deep freeze until analyses were made. Before analysis, the kidney powder was passed through a 60-mesh sieve, to give a more uniform product. The enzyme, transamidinase, which catalyzes the formation of GAA from arginine and glycine, was first found in the kidney(3). In our study, the activity of this enzyme was assayed as follows: To 25 mg of kidney powder were added 3 ml of M/15 phosphate buffer, pH 7.4, and 1.5 ml each of glycine (20 μmoles/ml) and of L-arginine (20 μmoles/ml). After thorough mixing, an aliquot of 2 ml of the suspension serving as control was taken, adjusted to pH 6, and kept in boiling water bath for 10 minutes. Upon cooling, the boiled tissue suspension was filtered. The protein-free filtrate was used for determination of GAA by chromatographic-colorimetric method(4). This method was used also for determination of GAA in plasma, tissues and urine. Another aliquot of 2 ml of kidney tissue suspension was pipetted into a small Erlenmeyer flask and incubated in Dubnoff shaking incubator at 37° for one hour. At end of incubation, the contents of flask were adjusted to pH 6 and subsequently treated in the same manner as the control sample. The difference in GAA values between control sample and incubated sample represents the amount of GAA synthesized under conditions employed. When liver was used in determination of GAA, the recovery of added GAA was not always satisfactory. The recovery was less with larger samples of liver. Presumably, this is due to presence in liver of certain substances interfering with the Sakaguchi reaction used for determination of GAA. However, if amount of liver used is small, quantitative recovery of added GAA can be obtained. It was satisfactory to use a protein-free filtrate prepared as for the kidney and equivalent to no more than 70 mg of dry liver for determination of GAA. Plasma

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TABLE I. Kidney Transamidinase Activity of Tumor-Bearing Rats. All values expressed as μmoles of guanidoacetic acid synthesized/g of dry kidney/hr.

Type animal	Tumor size		
	Small	Medium	Large
Control	58.4 (48.7-65.1)† (3)*	52.3 ± 8.5‡ (6)	42.4 ± 5.4 (8)
Tumor-bearing	48.6 (30.6-61.0)	36.5 ± 14.6§	12.3 ± 2.8§

* No. of pairs of animals used. † Range. ‡ Value following ± sign is stand. error of mean. § Difference in mean values between control and exp. groups is significant by the "t" test.

was deproteinized with 4% trichloroacetic acid, final concentration, and the protein-free filtrate was used for the determination.

Results. For the purpose of correlating biochemical findings with different stages of tumor growth, an arbitrary classification based on tumor weight expressed as per cent of total body weight (carcass plus tumor) has been adopted. Animals with tumors weighing less than 10% of total body weight were grouped under "Small," those with tumors weighing between 10 and 30% were grouped under "Medium," and those with tumors weighing more than 30% were grouped under "Large."

Transamidinase activity of the kidney was lower in tumor-bearing rats than in controls (Table I). Furthermore, lowering in enzyme activity was more marked in rats with larger tumors. Thus, transamidinase activity in rats bearing large tumors was only one-fourth that in rats bearing small tumors. Although a depletion of protein intake resulted in reduction of this enzyme activity in the rat(5), and inanition usually develops in animals with large tumors, the use of the pair-feeding technic has ruled out possible implications of dietary restriction on our observations in tumor-bearing rats. Actually, the difference in enzyme activity between control animals pair-fed to animals bearing large tumors and those pair-fed to animals bearing small tumors, was not great, so that any effect of dietary restriction must have been slight. It may be concluded, therefore, that kidney transamidinase activity was decreased as a result of tumor growth.

A decrease in enzyme activity in a tissue measured *in vitro* does not necessarily mean a decrease in rate of a synthetic reaction catalyzed by the enzyme *in vivo*(6,7). This raised the question whether rate of production of GAA was really decreased in tumor-bearing animals which showed reduced transamidinase activity as studied *in vitro*. No direct evidence is available to answer this question. In an effort to gain information on *in vivo* production of GAA, a study of concentrations of this substance in plasma, kidney and liver and its excretion in urine was undertaken. Fig. 1 shows urinary excretion of GAA for a control and a tumor-bearing rat throughout the period of tumor growth. The data for this Figure were obtained from a single pair of animals, but they are typical of the 7 pairs of animals whose urinary pattern of GAA excretion has been studied. In none of these animals was there noted significant difference between amount of GAA excreted by the tumor-bearing and control rat. In sharp contrast to the large increase in urinary excretion of creatine observed in the pre-terminal stage of tumor growth(1), urinary excretion of GAA was unchanged at this time. In other words, there was creatinuria without a correspondingly high level of GAA excretion. This situation is similar to that observed with experimental avitaminosis E(8). Whether the principles underlying creatinuria in these 2 cases are the same remains to be seen.

Concentrations of GAA in plasma and kidney of tumor-bearing rats were the same as control values (Table II). Its concentration in liver, however, was increased in rats with

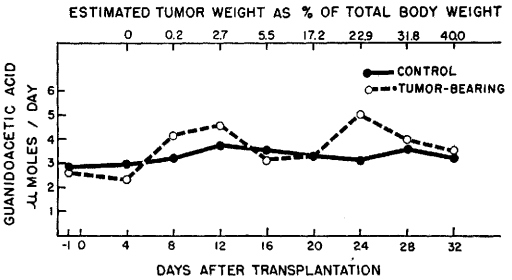

FIG. 1. Urinary excretion of guanidoacetic acid by a control and a tumor-bearing rat at different stages of tumor growth.

TABLE II. Guanidoacetic Acid in Plasma, Kidney and Liver of Tumor-Bearing Rats. Values expressed as μmoles of guanidoacetic acid/100 ml of plasma or /g of dry tissue.

Type animal	Plasma*	Kidney*	Liver Tumor size Small	Medium	Large
Control	4.2 ±.4‡ (5)†	5.4 ±.9 (17)	1.34 ±.34 (5)	1.32 ±.20 (5)	1.38 ±.25 (5)
Tumor-bearing	4.3 ±.5	5.2 ±.7	1.67 ±.32§	1.74 ±.23§	2.09 ±.42§

* Values in plasma and kidney were obtained from animals with medium and large tumors, and averaged.

† No. of pairs of animals used.

‡ Value following ± sign is stand. error of mean.

§ Difference in mean values between control and exp. groups is significant by the "t" test.

tumors. The increase appears to be greater in animals with larger tumors. This indicates that ability of liver to concentrate GAA was not impaired by tumor growth. The fact that concentration of GAA actually was increased in the liver of tumor-bearing animals suggests that there was a decrease in utilization of GAA for creatine synthesis. This suggestion is consistent with our previous observation of decreased creatine synthesis in these animals (1). Since concentrations of GAA in plasma and kidney and its excretion in urine were unchanged, and its concentration in liver was somewhat elevated in tumor-bearing rats, it appears probable that the synthesis of GAA proceeded at a rate comparable to that in control animals. However, the possibility that rate of GAA synthesis could have been reduced to be commensurate with decreased creatine synthesis and still result in the observed levels of GAA in tissues, plasma and urine, cannot be excluded. This possibility will be tested further.

Summary. Transamidinase activity in the kidney of rats bearing Walker carcinoma 256 was lower than that of the control. However, concentrations of guanidoacetic acid in plasma and kidney and its excretion in urine of tumor-bearing rats were not changed. In the liver, concentration of this compound was increased with tumor growth. These findings seem to indicate that rate of guanidoacetic acid synthesis is not a limiting factor in synthesis of creatine by the tumor-bearing rat.

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