BIKEN JOURNAL
Preliminary Report

Liver Kynureninase Activity of Tumor Bearing Animals

In the course of our investigations on the tumor-host relationship it was observed that tumor bearing animals showed a considerable disturbance in tryptophan metabolism. Previously we reported that in the liver of rhodamine fibrosarcoma bearing rats and also toxohormone -- the tumor extract -- injected rats, the increase of tryptophan pyrrolase activity after tryptophan administration was much less than that in the normal rats, while the amounts of kynurenine detectable in the liver and urine of rhodamine fibrosarcoma bearing rats after tryptophan administration were found to be much larger than those of normal rats.[1,2] From these results, it was supposed that some catalytic pathway of kynurenine might be suppressed in the liver of tumor bearing animals. The present work deals with the activity of liver kynureninase in tumor bearing animals, showing that kynureninase activity decreases in the liver of both tumor bearing and toxohormone administered animals.

Male Wistar strain rats, 170-200 gr in weight, transplanted with rhodamine fibrosarcoma, ascites hepatoma AH-130 and Walker carcinosarcoma-256, and male ddO stock mice, 20-25 gr in weight, transplanted with actinomycin-induced ascites sarcoma[3] were used for these experiments.

Toxohormone was prepared in the form of an alcohol precipitate according to the method of Nakahara and Fukuoka, with a slight modification, using rat rhodamine fibrosarcoma[4].

For assaying the activity of liver kynureninase, a slight modification of the method of Knox based on kinetic measurement, was employed.[5] Thus liver tissue removed from exsanguinated animals was homogenized, and the homogenate was incubated with pyridoxal phosphate and kynurenine. The reaction was stopped with 1 per cent boric acid in ethanol. The initial and final amounts of kynurenine in the supernatant of the incubation medium were measured spectrophotometrically at 365 mμ.* In preliminary experiments, it was observed that the increment in anthranilic acid, which was determined by the method of Mason and Berg, was proportional to the decrease in kynurenine[6]**.

* Kynureninase activity is expressed in arbitrary units, as μmoles of kynurenine per hour per gr of fresh tissue, since about 20 per cent of the values represent kynurenine not disappearing by kynureninase action.

** Anthranilic acid was assayed by the technique of diazotization and coupling with N-(1-naphthyl)-ethylenediamine with a one hour incubation period at 37° between addition of ammonium sulfamate and the coupling component. Tabone and Magis have reported that the diazonium salt of kynurenine is decomposed completely by incubation for an hour at 36°.[7] We have confirmed that kynurenine in the solution does not disturb the assay of anthranilic acid.

The results are shown in Fig. 1 and also in Tables 1 and 2.

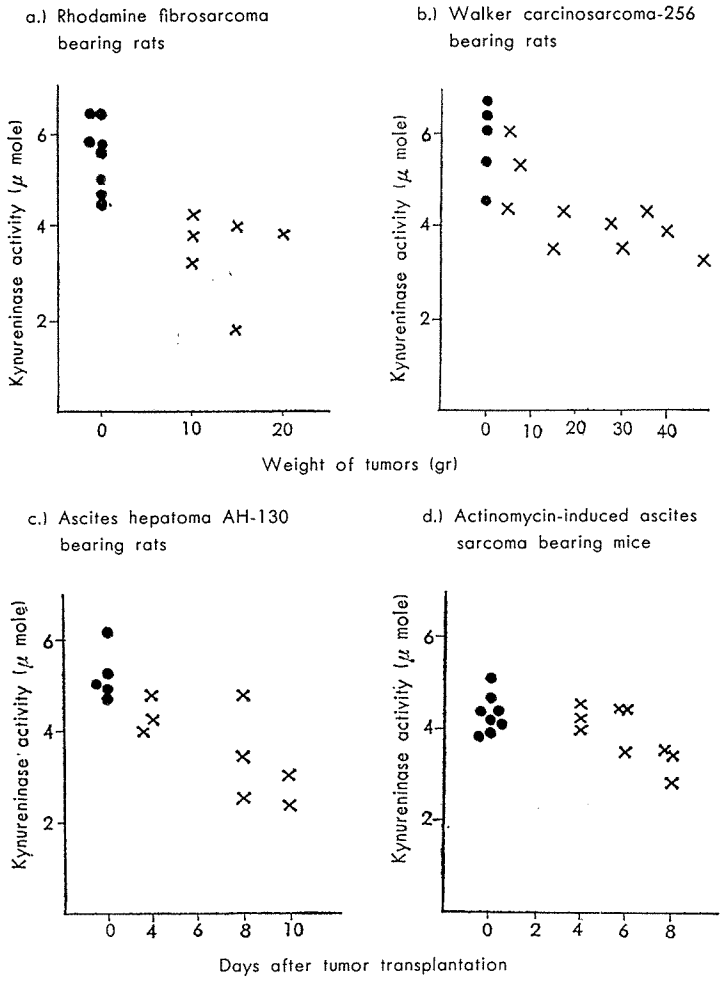

Fig. 1 Liver Kynureninase Activity* of Tumor Bearing Animals

* μ moles of kynurenine / hr. / gr of fresh tissue

The kynureninase activity of normal rats was 5.8 μmoles per hour per gr of fresh tissue, whereas those of rhodamine fibrosarcoma and Walker carcinosarcoma-256 bearing rats was reduced to only 60-70 per cent of that of normal rats when the tumors had grown to 10-20 gr in weight. A decrease in Kynureninase activity was observed in the liver of ascites hepatoma AH-130 bearing rats on the 4th day after tumor transplantation, and the activity of the enzyme was found to decrease greatly later. A decrease to half the original enzyme activity was observed on

the 10th day. Liver kynureninase activity of normal mice was 4.1 μmoles per hour per gr of fresh tissue. In actinomycin-induced ascites sarcoma bearing mice, however, there was a 24 per cent decrease in liver kynureninase activity in comparison with control mice on the 8th day after tumor transplantation.

Liver kynureninase activity of toxohormone administered rats, which were injected intraperitoneally with 200 mg of toxohormone in 5 ml of saline 24 hours before sacrifice, was about two thirds of that in control rats. In order to test

Table 1. Liver Kynureninase Activity of Tumor Bearing Animals

Animal	Tumor		Kynureninase activity*	No. of animals
rat	control	——	5.8	10
	rhodamine fibrosarcoma	10-20 gr	3.7	6
	Walker carcinosarcoma -256	10-50 gr	3.8	7
	ascites hepatoma AH-130	10 days	2.6	2
mouse	control	——	4.1	8
	actinomycin-induced ascites sarcoma	8 days	3.2	3

* μ-moles of kynurenine /hr./gr of fresh tissue

0.4 ml of liver homogenate, containing 80 mg of liver tissue, was incubated for 15 min. at 38° C with 0.1 ml of 20 mg % pyridoxal phosphate and 0.5 ml of 0.2 M phosphate buffer, pH 8.0. Then 0.2 ml of 0.0075 M kynurenine was added and a second incubation was carried out for 40 min. at 38° C. The incubation medium was deproteinized by adding 5.0 ml of 1 % boric acid in ethanol and the optical density was measured spectrophotometrically at 365 mμ.

Table 2. Effect of Toxohormone on the Kynureninase Activity of Rat liver

Treatment	Kynureninase activity*
control	5.4
toxohormone (200 mg / rat)	3.5
alcohol precipitate of normal rat liver (500 mg / rat)	5.3

* μ moles of kynurenine / hr. / gr of fresh tissue

whether this low enzyme activity was due to the specific action of toxohormone, 500 mg of alcohol precipitate, extracted from normal rat liver by the same method as that used for toxohormone preparation, was administered to normal rats. However, there was no difference in the activity of liver kynureninase in treated and control animals.

The results suggest that the increase in kynurenine in the liver and urine of tumor bearing animals after adminstration of tryptophan, may be partially accounted for by the reduced activity of kynureninase. Moreover, the fact that toxohor-

mone administration lowers the kynureninase activity of normal rat liver to the same degree as that of tumor bearing animals may indicate that the low activity of liver kynureninase in tumor bearing animals is apparently not due to hemorrhage, infection or malnutrition accompanying the tumors but to the action of toxohormone released from the tumors.

However, the intrinsic mechanism underlying the suppressive action of toxohormone on liver kynureninase must await further biochemical investigation.

REFERENCES

1) Dohi, J. (1961). Studies on the enzymatic formation in tumor bearing animals; adaptive formation of tryptophan pyrrolase in the livers of tumor bearing and toxohormone administered rats. *J. Nara Med. Assoc.* **12,** 635-649.
2) Shiba, S., Terawaki, A., Miyatake, M. and Matsuyoshi, H. (1962). Protein metabolism in tumor-bearing animals, III. On tryptophan metabolism. *Proc. Jap. Cancer Assoc., The 21st General Meeting* p. 60.
3) Kawamata, J., Nakabayashi, N., Kawai, A., Fujita, J., Imanishi, M. and Ikegami, R. (1959). Studies on the carcinogenic effect of actinomycin. *Bikens' J.* **2.** 105-112.
4) Nakagawa, S., Kosuge, T. and Tokunaka, H. (1955). Purification of the liver catalase-reducing substance occurring in cancer tissues with special reference to its activity. *Gann* **46,** 585-596.
5) Knox, W.E. (1955). Liver and bacterial kynureninase. In *Methods in enzymology, Vol.* **2,** 249-253.
6) Mason, M.M. and Berg, C.P. (1952). The metabolism of D- and L-tryptophan and D- and L-kynurenine by liver and kidney preparations. *J. Biol. Chem.* **195,** 515-524.
7) Tabone, J. and Magis, C. (1946). Etudes chimiques sur les arylamines biologiques. IV. Caractères analytiques de la cynurénine: sa differenciation d'avec les acides aminobenzoiques biologiques. *Bull. soc. chim. biol.* **28,** 744-747; V. Nouvelles dennées analytiques sur l'arylamine du muscle. Son isolement sous forme d'azoique. *Bull. soc. chim. biol.* **28,** 747-750.

SHIGERU SHIBA
ASAHARU TERAWAKI
YOSUKE TOMIYAMA*

*Surgical Division, Department of Clinical Research and *Department of Chemotherapy, The Research Institute for Microbial Diseases, Osaka University, Osaka*

Received on March 12, 1963