

THE ACTIVITIES OF SOME ENZYMES OF TRYPTOPHAN METABOLISM IN FETAL, NEONATAL AND ADULT RAT LIVER AND KIDNEY

I. KYNURENINASE AND KYNURENINE AMINOTRANSFERASE

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Kynureninase activity in fetal tissue of rat is two thirds the level of the adult, while kynurenine aminotransferase (L-kynurenine: 2-oxoglutarate aminotransferase, EC. 2.6.1.7) activity could not be observed at all in fetal tissue. The latter began to arise after birth and reached the adult level at 50 g of body weight. This fact suggests that anthranilic acid in fetal tissue might have some special significance. It is also suspectable that some hormones regulate these enzymes which catalyze tryptophan metabolism.

The administration of estradiol-17 β inhibited the activities of these two enzymes *in vivo*, especially kynurenine aminotransferase. Progesterone did not show such an effect.

Since Lepkovsky reported that the excretion of xanthurenic acid in urine was much more in vitamin B₆ deficient rat than normal¹⁾, there has been many publications on the relationship between vitamin B₆ and tryptophan metabolism²⁾⁻⁵⁾. Ogasawara *et al.* explained that the excretion of large amount of xanthurenic acid was due to the difference between the distribution of kynureninase and that of kynurenine aminotransferase in the cell⁶⁾.

On the other hand, a similar phenomenon to the vitamin B₆ deficiency in the rat was observed in normal pregnant women⁷⁾. Though the administration of pyridoxine returned tryptophan metabolism to almost normal in these women, some metabolites remained slightly elevated. Therefore, the workers speculated that any other factors such as hormonal control to the enzyme activities might affect on tryptophan metabolism of pregnant women⁸⁾. Mason *et al.* reported the effects of hormones on kynurenine aminotransferase, and the results revealed a relationship between enzymes of tryptophan metabolism and hormones⁹⁾.

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In order to clarify the difference between the distribution of kynurenine aminotransferase and that of kynureninase, the authors examined changes of the enzyme activities of tryptophan metabolism in fetal, neonatal and adult rat, and also the effects of hormones on those two enzymes. The results obtained are mentioned below.

MATERIALS AND METHODS

Materials

L-Kynurenine sulfate monohydrate was purchased from California Corporation for Biochemical Research. Pyridoxal phosphate was obtained from Chugai Pharmaceutical Co., Tokyo. Progesterone and estradiol-17 β were obtained from Teikoku Zoki Pharmaceutical Co., Tokyo. All other reagents were the commercially available reagent grade.

Preparation of Mitochondria

After rats were sacrificed by stunning and decapitation, their liver and kidney were removed quickly and chilled in ice-cold 0.25 M sucrose solution. The tissues were homogenized with 5 volumes of ice-cold 0.25 M sucrose in a Potter-Elvehjem type homogenizer, and the homogenates were centrifuged for 15 min. at $700 \times g$. The precipitates were washed once with a small amount of the same sucrose solution. Then, both supernatants were collected and centrifuged for 15 min. at $10,000 \times g$. The precipitates were washed once and suspended in 0.25 M sucrose solution. These precipitates and supernatants were used as mitochondria and supernatant fractions, respectively, throughout this study.

Enzyme Assay

The activity of kynurenine aminotransferase was measured according to Knox⁵. The reaction mixture contained the following compounds in 0.5 ml: 1.0 μ mole of L-kynurenine, 3.0 μ moles of α -ketoglutaric acid, 50 μ g of pyridoxal phosphate and 10 μ moles of potassium phosphate; pH 6.8.

For the determination of the activity of kynureninase, 1.0 ml of a solution which contained 1.0 μ mole of L-kynurenine, 25 μ g of pyridoxal phosphate and 100 μ moles of potassium phosphate; pH 8.0. Anthranilic acid formed was determined by the method of Price *et al.*¹⁰.

Protein concentrations were measured by the biuret method.

Administration of Hormones

A daily dose of 2.7 mg of progesterone or 30 μ g of estradiol-17 β , dissolved in olive oil, per 100 g of body weight was administered subcutaneously for 1 week to one group and for 2 weeks to the other.

RESULTS AND DISCUSSION

The activity of kynureninase in fetal rat liver is shown in Fig. 1. On the

21st fetal day, the activity was two thirds of normal adult level. The postnatal period activity was equal to the adult.

Figs. 2 and 3 show the activity of kynurenine aminotransferase in both the supernatant and mitochondrial fractions as a function of body weight. The kynurenine aminotransferase activity on the 21st fetal day was nearly zero. After birth, this enzyme activity began to increase and reached that of the adult at 50 g of body weight. This change in activity was much different from that of kynureninase. The above results were the

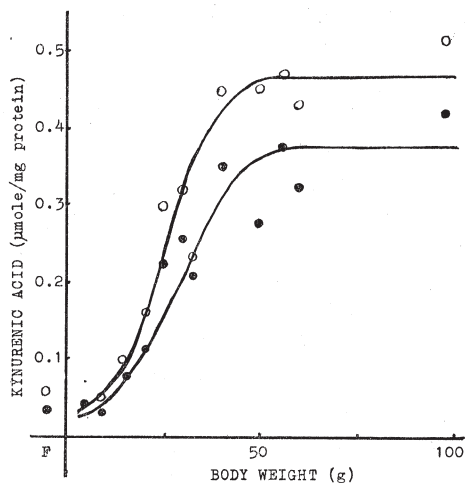


FIG. 2. The activity of mitochondrial kynurenine aminotransferase in fetal, neonatal and adult rat liver.

—○—, with pyridoxal phosphate
—●—, without pyridoxal phosphate
F, fetal tissue.

Kynurenine aminotransferase activity in fetal and neonatal period was nearly zero.

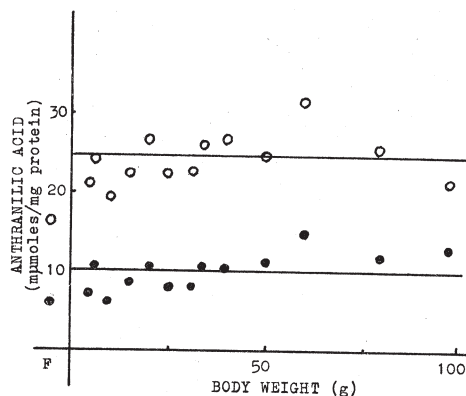


FIG. 1. The activity of kynureninase in fetal, neonatal and adult rat liver.

—○—, with pyridoxal phosphate
—●—, without pyridoxal phosphate
F, fetal tissue.

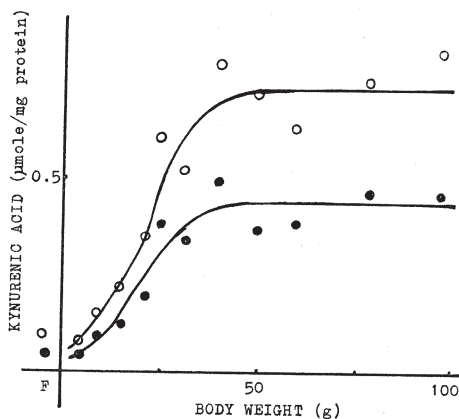


FIG. 3. The activity of supernatant kynurenine aminotransferase in fetal, neonatal and adult rat liver.

—○—, with pyridoxal phosphate
—●—, without pyridoxal phosphate
F, fetal tissue.

The change in this enzyme activity was similar to that of mitochondrial fraction.

same in both cases of with and without pyridoxal phosphate.

From the above mentioned results, there was no activity of the fetal kynurenine aminotransferase, but kynureninase was present to the extent of

two thirds the level of the adult. The lack of kynurenine aminotransferase activity from the fetal period to the neonatal is considered that this enzyme is not synthesized at that time. Thus some hormones, especially female hormones, might affect the synthesis of the enzyme during that period.

The latter speculation was suggested from the facts that, even after the administration of pyridoxine hydrochloride, the elevated excretion of kynurenine, 3-OH-kynurenine, and pyridone (N-methyl-2-pyridone-5-carboxamide) did not return to normal level in normal pregnant women⁸). Mason *et al.* reported the facts that estradiol disulfate and diethylstilbestrol disulfate inhibited the kynurenine aminotransferase activity at levels of as low as $5 \times 10^{-7}M$ ⁹). Porter *et al.* showed that adrenalectomized rats excreted a small amount of kynurenic acid even after given tryptophan. When cortisone was administered to these rats, the excretion of kynurenic acid returned to the normal level¹¹).

The authors examined the effects of progesterone and estradiol-17 β on the kynureninase and kynurenine aminotransferase activities *in vivo*.

As shown in Figs. 4 and 5, the kynurenine aminotransferase activity was inhibited to the extent of from 40 to 50% and the kynureninase activity was

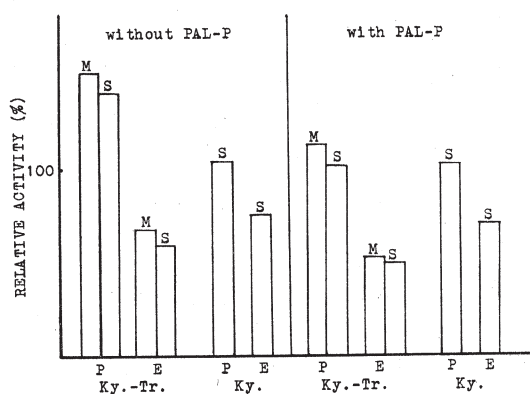


FIG. 4. Activities of kynurenine aminotransferase and kynureninase after administration of progesterone or estradiol-17 β for one week.

The figure shows the percentages of the specific activities in hormone-treated rats to those in the control. Female albino rats weighing about 100 g were used in this experiment.

Ky.-Tr., kynurenine aminotransferase; Ky., kynureninase; P, progesterone; E, estradiol-17 β ; M, mitochondrial fraction; S, supernatant fraction.

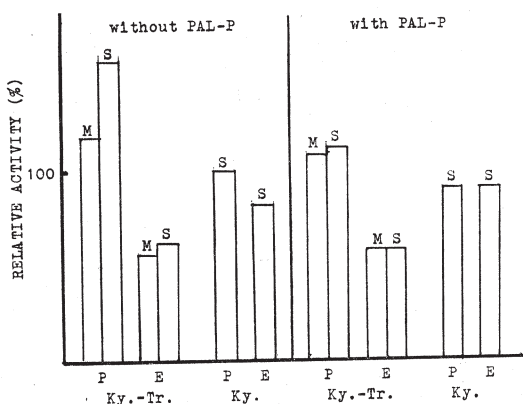


FIG. 5. Activities of kynurenine aminotransferase and kynureninase after administration of progesterone or estradiol-17 β for two weeks.

The figure shows the percentages of the specific activities in hormone-treated rats to those in the control.

decreased to 80% by the daily administration of 30 μ g of estradiol-17 β . But after the administration of 2.7 mg of progesterone there was little change.

It is very interesting that there is some difference between the change of the kynurenine aminotransferase activity in vitamin B₆ deficiency and in estradiol-17 β administration to the rat. That is, the kynurenine aminotransferase activity of the supernatant fraction decreased remarkably, but that of mitochondrial fraction remained almost the same as normal in vitamin B₆ deficient rat. On the other hand, in estradiol-17 β administration the activities of both fractions decreased. It is considered that there is some difference in the action of enzyme between vitamin B₆ deficiency and administration of estradiol-17 β .

From the above mentioned facts of this study the possibility is considered that anthranilic acid derived from kynurenine has some special significance in fetal tissue, and also that some hormones act as regulatory factors. Unfortunately, the data presented in this communication is not sufficient enough to draw further conclusion concerning to the regulatory mechanism between the enzymes of tryptophan metabolism and related hormones.

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