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CHANGES OF ASPARTATE AMINOTRANSFERASE AND ALANINE AMINOTRANSFERASE ACTIVITY IN VITAMIN B_6 DEFICIENT RAT

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SUMMARY

The aspartate aminotransferase and alanine aminotransferase activities in vitamin B_6 deficient rats were assayed. The activities of aspartate aminotransferase and alanine aminotransferase were reduced in the supernatant fraction, while their activities were preserved in the mitochondrial fraction. With the addition of pyridoxal phosphate *in vitro* to the liver or kidney of vitamin B_6 deficient rats, the supernatant aspartate aminotransferase recovered its activity to a respectable degree, while alanine aminotransferase not so much. The addition of pyridoxal phosphate *in vitro* had no influence upon the activity of mitochondrial aspartate aminotransferase and alanine aminotransferase. The results of these experiments indicate that these supernatant transaminases are affected by the diminution of vitamin B_6 content.

INTRODUCTION

In order to explain the mechanism of increased excretion of xanthurenic acid in vitamin B_6 deficiency, Ogasawara *et al.* in this laboratory reported a series of experimental results[1]. In vitamin B_6 deficient rats, the kynurenine aminotransferase of the supernatant fraction showed obviously reduced activity, while the activity of the mitochondrial kynurenine aminotransferase remained constant. The authors also found that the vitamin B_6 content in liver and kidney decreased in the supernatant fraction more markedly than in the mitochondrial fraction isolated from vitamin B_6 deficient rats.

In the present study, it is demonstrated how the activity of aspartate aminotransferase (EC. 2. 6. 1. 1) and alanine aminotransferase (EC. 2. 6. 1. 2) with pyridoxal phosphate as coenzyme change in vitamin B_6 deficient rats, and how aspartate aminotransferase and alanine aminotransferase obtained from vitamin B_6 deficient rats are influenced by the addition of pyridoxal phosphate *in vitro*.

EXPERIMENTAL MATERIALS

Young albino rats, weighing 50～60 g, were fed with a vitamin B_6 free diet.

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This diet for a rat was composed of 3 g of vitamin free casein, 3 g of dextrose, 1.0 ml of purified salad oil, 0.5 g of salt mixture (product of Tanabe pharmaceutical Co., Osaka), and vitamin mixture (which consisted of thiamine 40 μg, riboflavin 60 μg, *P*-aminobenzoic acid, 500 μg; nicotinic acid 200 μg, calcium pantothenate 200 μg, choline hydrochloride 5 mg, biotin 1 μg, vitamin B_{12} 0.1 μg, folic acid 2.5 μg, inositol 1.5 mg, vitamin A 300 IU and vitamin D 30 IU). The control rats were fed with the same diet to which 100 μg of vitamin B_6 was added. After 5 or 6 weeks, these rats were used for the experiments after checking the increased xanthurenic acid excretion.

FRACTIONATION OF CELL COMPONENTS

After the rats were killed by decapitation and exsanguination, the kidney and liver were removed quickly and 1.0 g of each organ was put immediately in 10 volumes of ice cold 0.25 M sucrose solution. The tissues were homogenized in a teflon homogenizer. The fractionation of cell components was carried out according to Hogeboom's method with slight modification[2]. The homogenate was centrifuged for 10 min. at 700 × g. The supernatant fluid was again centrifuged for 15 min. at 9,000 × g. This supernatant fraction was kept in the cold state, and the precipitate was washed twice with 5 volumes of 0.25 M sucrose solution by centrifugation at 9,000 × g for 15 min.. The authors used this precipitate as mitochondrial fraction. After suspending the mitochondrial fraction in 5 volumes of 0.25 M sucrose solution it was sonicated for 10 min. at 10 KC by a sonic oscillator. The mitochondrial and supernatant fractions were used as enzyme solution throughout this study.

ENZYME ASSAY

The activity of aspartate aminotransferase was assayed by the modified method of Tonhazy[3]. The incubation mixture contained 20 μmoles of DL-aspartate, 10 μmoles of α-ketoglutarate, 25 μg of pyridoxal phosphate, 0.1 ml of 1/10 M potassium phosphate buffer, pH 7.4 and 0.1 ml of enzyme solution in a total volume of 1.5 ml. After preincubation for 5 min. at 37°C, DL-aspartate was added, and the mixture was further incubated for 10 min.. The reaction was stopped by adding 0.1 ml of 100% trichloracetic acid. The control reaction mixture was deproteinized at zero time. The incubation mixture to which added 0.1 ml of aniline citrate was then incubated for 5 min. To each reaction tube, 1.0 ml of 2, 4-dinitrophenylhydrazine reagent was added. The reaction was allowed to proceed for 5 min. at 37°C. Two ml of toluene were added. They were shaken for 3 min. with a shaker and centrifuged for 5 min. at 2,500 rpm. One ml of the toluene layer was taken out and was mixed with 5.0 ml of an alcoholic solution of potassium hydroxide. The optical density was meas-

ured at 490 mμ after 5-10 min.

The activity of alanine aminotransferase was determined by the modified Felix Wroblewski's method[4] in a volume of 1.5 ml containing 20 μmoles of DL-alanine, 10 μmoles of α-ketoglutarate, 25 μg of pyridoxal phosphate, 0.1 ml of 1/10 M potassium phosphate buffer of pH 7.4 and 0.1 ml of enzyme solution. After preincubation for 5 min. at 37°C, the incubation was continued for 10 min. at 37°C with the addition of DL-alanine. The reaction was stopped by adding 0.1 ml of 100% trichloracetic acid. To the control reaction mixture was added 0.1 ml of 100% trichloracetic acid at zero time. After 20 min. at room temperature (22°C), one ml of 2,4-dinitrophenylhydrazine was added, and incubation was carried out for 5 min. at 37°C. The subsequent step for measuring the activity of alanine aminotransferase was the same as that for aspartate aminotransferase. Its optical density was measured at 490 mμ. The activity of these enzymes was proportional to the concentration of enzyme solution and increased linearly with time for 10 min.. The activity of aspartate aminotransferase and alanine aminotransferase was determined by mμmoles of pyruvate produced per 10 min. per mg of protein. The protein concentration was measured by biuret method.

RESULTS AND DISCUSSION

The activity of aspartate aminotransferase and alanine aminotransferase in the liver and kidney of vitamin B_6 deficient rats is shown in Table 1. It was observed that the activity of aspartate aminotransferase and alanine aminotransferase was decreased in the supernatant fraction of kidney or liver, whereas the activity of these enzymes was preserved in the mitochondrial fraction. As shown in Fig. 1, the activity of aspartate aminotransferase decreased by 45% in the liver supernatant fraction but by 17% in the kidney supernatant fraction compared with that of control animals. On the other hand, the decrease of aspartate aminotransferase activity in the mitochondrial fraction was much less, *i.e.*, 10% in the liver, and 6% in the kidney.

The activity of alanine aminotransferase was also reduced by 35% of its activity in the liver supernatant fraction and by 10% in the kidney supernatant fraction, whereas in the liver mitochondrial fraction, the alanine aminotransferase activity decreased only by 10% and in the kidney mitochondrial fraction alanine aminotransferase activity remained constant.

Table 2 shows the activities of aspartate aminotransferase and alanine aminotransferase in the presence of 25 μg of pyridoxal phosphate using the same enzymes as in Table 1. The increase in aspartate aminotransferase activities was observed in the supernatant fraction from vitamin B_6 deficient rats by the

TABLE 1. Changes of aspartate aminotransferase and alanine aminotransferase activities from vitamin B_6 deficient rats.

Tissue	Enzyme	Activity PAL-P(−) mμmoles/10min./mgprotein GOT		GPT	
	Fraction	Super.	Mito.	Super.	Mito.
Liver	Control	244±46	1140±215	110±22	22±4.5
	Vitamin B_6 Deficiency	136±18	1030±207	75±18	19±4.1
Kidney	Control	150±26	215± 38	14±2.7	11±2.3
	Vitamin B_6 Deficiency	124±17	200± 31	13±2.6	11±2.5

Aspartate aminotransferase and alanine aminotransferase were assayed in the supernatant and mitochondrial fractions from the liver and kidney.

Activity : amount of pyruvate produced mμmoles/10 min./mg protein

Abbreviation used; Mito : mitochondria
Super : supernatant
PAL-P : pyridoxal phosphate
GOT : aspartate aminotransferase
GPT : alanine aminotransferase

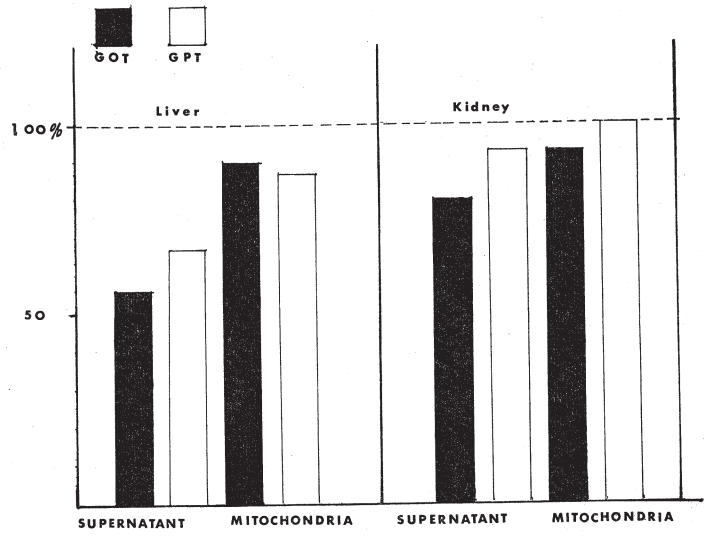

FIG. 1. Diminution ratio of aspartate aminotransferase and alanine aminotransferase activities from vitamin B_6 deficient rats.

This figure shows the decrease of aspartate aminotransferase and alanine aminotransferase activities against the control which expresses to be 100% in each fraction from vitamin B_6 deficient rats.

GOT : aspartate aminotransferase
GPT : alanine aminotransferase

TABLE 2. Effect of pyridoxal phosphate on the activities of aspartate aminotransferase and alanine aminotransferase.

Tissue	Enzyme	Activity PAL-P(+) mμmoles/10min./mgprotein GOT		GPT	
	Fraction	Super.	Mito.	Super.	Mito.
Liver	Control	256±48	1151±218	113±23	23±4.7
	Vitamin B_6 Deficiency	165±24	1020±205	85±22	20±4.3
Kidney	Control	157±28	209± 41	15±3.0	10.1±2.9
	Vitamin B_6 Deficiency	146±24	194± 41	15±31	10.7±2.4

This table corresponsd to each value of table 1. The same enzyme was used in both experiments.

PAL-P (25 μg/0.1 ml) was added *in vitro*.
Activity : amount of pyruvate produced mμmoles/10 min./mg protein
Abbreviation used ; Mito : mitochondria
Super : supernatant
PAL-P : pyridoxal phosphate
GOT : aspartate aminotransferase
GPT : alanine aminotransferase

addition of sufficient amounts of pyridoxal phosphate to the reaction mixture. But the restoration of alanine aminotransferase activities by the addition of pyridoxal phosphate was not so distinct. However, the effect of the addition of pyridoxal phosphate on the activities of aspartate aminotransferase and alanine aminotransferase was not observed in the mitochondrial fraction.

Henley *et al.*[5] and Ichihara *et al.*[6] observed that when cells were dispersed, many enzymes, such as malic dehydrogenase, alanine aminotransferase, tryptophan pyrrolase, serine dehydrase and threonine dehydrase leaked out from rat liver cells. Katsunuma *et al.*[7][8] found that aspartate aminotransferase and alanine aminotransferase existed in both soluble and mitochondrial fractions, and that the enzymes in these two fractions had different properties. Ichihara *et al.*[6] reported that in the dispersed cells, alanine aminotransferase in the soluble fraction leaked out from the cell, whereas the mitochondrial alanine aminotransferase stayed in the particles, and that 90% of aspartate aminotransferase remained in the dispersed cell. In the previous paper, the authors reported that in vitamin B_6 deficient rats, the decrease of the vitamin B_6 level in the cell components of liver and kidney was more significant in the supernatant fraction than in the mitochondrial fraction. From the above mentioned results, it is speculated that these transaminases existing in the mitochondria may be protected by the mitochondria membrane and may not leak out from the

cell and not decrease their activity, while the enzymes in the supernatant fraction are apt to be affected by the environmental condition, such as the condition of the cell membrane and the change of vitamin B_6 content.

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