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The Synthesis of Taurine from Sulfate
VI. Vitamin B_6 Deficiency and Taurine Synthesis in the Rat[1] (38450)

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There are two metabolic pathways for the biosynthesis of taurine by the animal. The first, which involves the oxidation of cysteine to cysteic acid (CA) or to cysteine sulfinic acid (CSA) followed by decarboxylation, is referred to as the organic pathway. The second, which involves the formation of taurine from serine and 3′-phosphoadenosine-5′-phosphosulfate (PAPS), is referred to as the inorganic pathway or the P_1 system (1). The P_1 enzyme system also possesses CA decarboxylase activity and has been shown to require pyridoxal phosphate (PLP) as its coenzyme (1). Thus, the only known reactions leading to the biosynthesis of taurine require PLP-dependent decarboxylases.

After partially characterizing the P_1 system for chick tissue (1, 2) and demonstrating its presence in rat tissues (3), it was desirable to compare the activities of the two pathways. The tissue taurine concentration is maintained at a constant value in the rat even in stress conditions such as fasting (4) or receiving a vitamin B_6-deficient diet (5). These authors indicated significantly reduced urinary taurine in the PLP-deficient rats and suggested that taurine was conserved by reduced renal clearance. Hepatic CA/CSA decarboxylase has been shown to essentially disappear after the rat has consumed a B_6-deficient diet for 2 weeks (6). If rats were fed a B_6-deficient diet for longer periods causing a reduction or loss of hepatic CA/CSA decarboxylase activity, then an increasingly higher proportion of the taurine synthesized would have to originate from the inorganic pathway.

To test this hypothesis, the experiments reported herein were conducted to ascertain the influence of a vitamin B_6 deficiency on the change in enzyme activity of the two pathways of taurine biosynthesis.

Materials and Methods. The rats used in these experiments were young albino males from an inbred colony maintained by the Agricultural Biochemistry faculty at WVU. The rats, approximately 70 g initial weight, were fed the complete purified diet (3) for a 7-day preliminary period, then randomly divided into two groups of 20. One group was fed the complete purified diet (PLP adequate) and the other received the same diet with vitamin B_6 omitted from the vitamin premix (PLP deficient).

At weekly intervals all of the rats were weighed and four from each dietary group were randomly selected for sacrifice. The experiment was completed when the last four rats of each group were sacrificed after 5 weeks. These conditions were the same for both experiments.

In Experiment 1, each rat was subcutaneously injected with 20 μCi $^{35}SO_4^{2-}$ 4 hr prior to sacrifice and the livers and hearts from the rats of each dietary group were pooled. Total taurine was determined (7) from the 25% aqueous homogenate of the pooled livers and hearts. Taurine - ^{35}S was determined from an aliquot of each sample following the ion exchange step of the taurine procedure, purified by paper chromatography with 80% aqueous phenol and counted by liquid scintillation. Taurine specific activity was then calculated from each pooled sample.

In Experiment 2, the liver of each rat was excised, chilled and prepared for individual enzyme analysis. These rats were not injected with $^{35}SO_4^{2-}$ prior to weekly sacrifice. The tissues were homogenized in distilled water (25%) and were divided into four fractions by sequential ammonium sulfate precipitation. These fractions, F_1, F_2, F_3, and F_4, refer to protein precipitated by 0.25, 0.5, and 0.7 saturation with

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$(NH_4)_2SO_4$, and the resulting supernatant, respectively. The CSA/CA decarboxylase activity (8), reported as μl CO_2/hr/mg protein, is comparable to P_1 activity (1), reported as taurine-^{35}S formed/min/μg protein, since each is the product of enzymatic decarboxylation. The protein concentration (9) of each fraction was also determined. The PAP ^{35}S used in the P_1 assay was enzymatically synthesized from $^{35}SO_4^{2-}$ incubated with rat liver F_2 (10).

Results. The ammonium sulfate precipitation of liver proteins provided two distinct enzyme fractions with decarboxylase activity (Table I). The CSA/CA decarboxylase activity was highest in F_2 while the P_1 activity was highest in F_4, verifying the earlier reports on CSA decarboxylase (11) and on P_1 activity (1). Davison (11) had also reported hepatic decarboxylase to be more specific for CSA than for CA. This was tested with F_2 protein from mouse and rat liver and the following specific activities obtained: rat F_2 enzyme with CSA, 11.79, and with CA, 0.63; mouse F_2 enzyme with CSA, 4.30, and with CA, 0.24. Having verified the substrate specificity of the F_2 decarboxylase, CSA was used in the enzyme assay of Experiment 2.

In Experiment 1, the rats receiving the B_6-deficient diet had a significantly lower growth rate than the rats fed the vitamin (Table II). Although the B_6-deficient rats lost weight after 3 weeks none died, indicating that some vitamin B_6 was available, probably from the dietary soybean protein and intestinal microbial synthesis. The deficiency was severe enough to cause a marked decrease in liver taurine concentration. The taurine concentration of the heart was relatively constant throughout the experiment and no dietary difference was observed.

The most significant observation of Experiment 1 was the large increase in taurine specific activity in the heart tissue of rats fed the B_6-deficient diet. With increasing time on the diet the taurine concentration of the livers from rats fed each diet was similar. It appeared, therefore, that the rate of taurine synthesis from the organic pathway (the B_6-dependent decarboxylase of F_2) was decreased while the inorganic pathway contribution was increased with time in the deficient animals.

TABLE I. CSA Decarboxylase and P_1 Activities of Rat Liver Fractions Resulting from Sequential Precipitation with $(NH_4)_2SO_4$.

	Liver fractions			
Enzyme assay	F_1	F_2	F_3	F_4
CSA decarboxylase[a]	2.49	7.50	1.69	1.35
P_1 activity[b]	496	478	374	1651

[a] μl CO_2/hr/mg protein.

[b] Counts per minute (cpm) taurine—^{35}S produced from PAPS ^{35}S/μg protein.

Experiment 2 was then conducted to measure the CSA decarboxylase activity of F_2 and the P_1 activity of F_4 from the individual livers during the 5-week feeding period (Table III). A highly significant decrease in CSA decarboxylase activity was observed in the livers of the B_6-deficient rats by the first week and the activity declined significantly in each succeeding week ($P < 0.01$). No change was observed in the hepatic CSA decarboxylase activity in rats fed the control diet. The P_1 activity was constant during the entire experiment and the activity of each dietary group was equal.

Discussion. The biosynthesis of taurine by the organic and inorganic pathways appears to be interregulated and possibly controlled by a switchover mechanism (2). Both metabolic pathways are terminated with PLP-dependent decarboxylases and each of these enzymes appears to possess different properties. The CSA/CA decarboxylase is precipitated by 0.5 saturation with ammonium sulfate, has a high specificity for CSA, and is known to disappear from the liver of rats fed a vitamin B_6-deficient diet for two weeks (8, 12). The decarboxylase activity of the P_1 system is CA specific (1), inhibited by CSA (2), remains soluble after the homogenate is 0.7 saturated with ammonium sulfate (1), and does not lose activity after the rats are fed a B_6-deficient diet for 5 weeks. Jacobsen *et al.* (13) reported a decarboxylase more specific for CA than CSA in chick liver and that CSA was inhibitory (13, 14).

Since each pathway of taurine biosynthesis requires PLP and the F_2 enzyme is lost during a vitamin B_6 deficiency (8), it appears that the P_1 system either requires a smaller quantity of cofactor for activity or that the available PLP is more tightly bound. Furthermore, the CSA decarboxylase activity of mammals appears to be tissue specific and in most species it appears only in liver, brain, and kidney (15). In contrast, the inorganic pathway (P_1) has been shown in all tissues tested of the chick (16) and the rat (3). Of

TABLE II. Growth and Tissue Data for Rats fed Diets with (+) or Without (−) Supplemental Vitamin B_6.[a]

	Supplemental Vitamin B_6	Weeks fed diets 1	2	3	4	5
Average body wt. (g)[b]	−	107	116	125	117	110
	+	145	192	220	242	249
Liver taurine (μg/g)[c]	−	43	45	39	37	43
	+	76	78	111	109	105
Heart taurine (μg/g)[c]	−	194	180	230	216	226
	+	189	195	220	228	240
Liver taurine Specific Activity[d]	−	37	15	9	11	10
	+	14	8	1	1	3
Heart taurine Specific Activity[d]	−	29	45	37	81	141
	+	24	28	39	37	46

[a] All rats were weighed, injected with 20 μCi $^{35}SO_4^{2-}$ and sacrificed after 4 hr.
[b] Average body weights for 20 rats at week 1 and then four less for each succeeding week.
[c] Taurine concentrations = μg/g, duplicate determinations/pooled sample.
[d] Taurine Specific Activity = cpm taurine $-^{35}S/\mu$g taurine, duplicate determinations/pooled sample.

significance is the finding that the P_1 system is present in heart tissue, while little or no heart CSA decarboxylase activity has been reported in any mammalian species (15). The increased taurine specific activity observed in the heart (Table II) after 3 weeks on the B_6-deficient diet is probably a result of active absorption and biosynthesis by the P_1 system. This increased specific activity is probably due to a greater percentage of the taurine arising from the stable P_1 system during the period that F_2 CSA decarboxylase is losing activity. The conditions for the acceleration of the inorganic pathway would be enhanced in the B_6-deficient animal: decreased production of cysteine would favor optimal PAPS synthesis, shown to enhance P_1 taurine synthesis (3); decreased consumption of L-serine in cystathionine biosynthesis (17) thereby providing increased substrate for the P_1 system; and increased methionine which has been shown to enhance the *in vivo* synthesis of taurine from sulfate in chicks (18, 19). The exact mechanism of the P_1 system enhancement due to methionine is not known.

Taurine is excreted in the urine and may be second only to glycine as to the concentration of free amino acids. The urinary excretion of taurine in man and experimental animals is well known and its reduced elimination during a B_6 deficiency is amply documented (20, 21). The urinary taurine concentration was not a criterion of these experiments, but random samples of

TABLE III. CSA Decarboxylase Activity of F_2 and P_1 Activity of F_4 from Livers of Rats After Varying Periods of Vitamin B_6 Deficiency.

Enzyme assay	Dietary vitamin B_6	Weeks fed purified diets 1	2	3	4	5
CSA decarboxylase[a,b]	−	5.00 ± 0.82	4.65 ± 0.69	4.02 ± 0.55	3.69 ± 0.63	2.50 ± 0.79
	+	8.12 ± 1.23	8.42 ± 0.93	8.24 ± 0.42	8.94 ± 1.02	6.87 ± 0.70
P_1 activity[a,c]	−	59.7 ± 0.76	57.5 ± 0.93	59.9 ± 1.07	59.9 ± 0.86	60.5 ± 0.30
	+	58.7 ± 0.63	58.2 ± 0.76	59.9 ± 0.70	59.6 ± 0.97	60.1 ± 1.36

[a] Each value is the mean ± SD of duplicate determinations from each of four livers.
[b] CSA specific activity = μl CO_2/hr/mg protein.
[c] P_1 activity = cpm taurine-^{35}S formed/min/μg protein.

urine from deficient and control rats were analyzed for total taurine and taurine specific activity. Again, the B_6-deficient rats had a much lower urinary taurine concentration and its specific activity was much higher.

These data support the significance of the inorganic pathway of taurine biosynthesis in the animal, and indicate that under certain conditions this alternate pathway may make a major contribution to maintaining body taurine.

Summary. An enzyme system capable of catalyzing the biosynthesis of taurine in vitamin B_6-deficient rats has been demonstrated. This activity was constant in liver even after rats had been fed the B_6-deficient diet for 5 weeks. The CSA decarboxylase of rat liver was shown to be significantly reduced after 5 weeks of B_6-deficient feeding. The significance of this enzyme system to the animal is discussed.

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