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Studies on Inhibition of 5-Hydroxy Tryptophan Decarboxylase by Phenylalanine Metabolites.* (27956)

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The conversion of 5-hydroxy tryptophan (5 HTP) to 5-hydroxy tryptamin (5 HTA) is catalyzed by the enzyme 5 HTP decarboxylase(1) and requires pyridoxal phosphate as a cofactor(2,3). Davison and Sandler(4) have shown by *in vitro* studies that phenylpyruvic acid, phenyllactic acid, and phenylacetic acid inhibit 5 HTP decarboxylase.

The present communication describes experiments carried out to determine the mechanism of inhibition of 5 HTP decarboxylase by phenylalanine metabolites.

Materials and methods. For the *in vivo* studies, weanling rats of Sprague-Dawley strain were made phenylketonuric using high phenylalanine diets described by Wang and Waisman(5). Two series of experiments were carried out. In the first, 5 HTP decarboxylase was determined weekly in groups of 10 control and experimental rats fed 3.75% each of DL-phenylalanine and L-tyrosine for a period of 2 months. In the second, the enzyme was assayed at 4 weeks of age in groups of control and experimental rats fed on 2 dietary regimes: 1) 4% L-phenylalanine and 4% DL-phenylalanine, and 2) 7% L-phenylalanine alone.

Serum phenylalanine was determined by the spectrofluorimetric method of McCaman and Robins(6) and urine phenylpyruvate by the procedure of Berry and Woolf(7). All the animals were killed by a sharp blow on the head and bled from the neck. The kidneys were removed and decarboxylation carried out in Warburg flasks using the technic previously described(8). Studies were carried out both with and without supplementation with pyridoxal phosphate in the incubation mixture.

The *in vitro* inhibition studies were carried out using the same procedure as described above. For each inhibitor, littermates of albino rats weighing about 200 g on a normal diet were used. The inhibitor was dissolved in the buffer and incubation carried out under 3 sets of conditions: 1) without pyridoxal phosphate, 2) with 0.1 μM pyridoxal phosphate added at the start, and 3) with 0.5 μM pyridoxal phosphate added from the side arm of the Warburg flask one hour after incubation and permitting the incubation to continue for another hour. For the kinetic studies 0.1 μM pyridoxal phosphate was added in all experiments, and at least 2, and more usually 5 or more runs were made for each substrate concentration. The mean values were plotted and eye-fitted curves were drawn for Lineweaver-Burk analysis(9).

Results. The weekly values of 5 HTP decarboxylase of rats fed 3.75% each of DL-phenylalanine and L-tyrosine are given in Fig. 1. When the incubation mixture was

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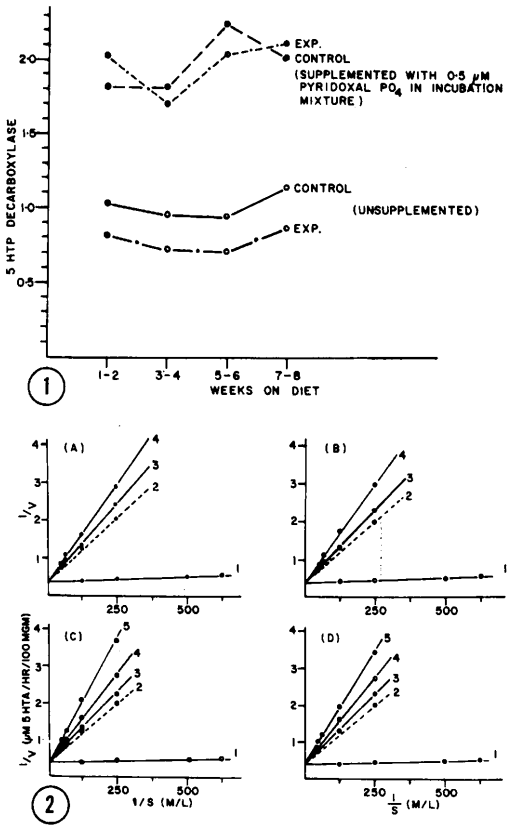

FIG. 1. Kidney 5 HTP decarboxylase (expressed as μM 5 HTA formed per 100 mg dry wt per hr) of control and experimental rats fed 3.75% each of DL-phenylalanine and L-tyrosine.

FIG. 2. Inhibition of 5 HTP decarboxylase by phenylalanine and its derivatives. The calculated $K_i \times 10^{-4}$ are as follows: phenylalanine 12.5 ± 2.5, phenylpyruvic acid 2.7 ± 0.5, phenyllactic acid 2.5 ± 0.5, and phenylacetic acid 2.2 ± 0.2.

A. Phenylalanine
1. Control ($Y = 1.95 \times 10^{-4} + .39$)
2. Without PP ($Y = .81 + .39$)
3. $.7 \times 10^{-2}$ M
4. 1.1×10^{-2} M

B. Phenylpyruvic acid
1. Control
2. Without PP
3. $.8 \times 10^{-2}$ M
4. 1.7×10^{-2} M

C. Phenyllactic acid
1. Control
2. Without PP
3. $.8 \times 10^{-3}$ M
4. 1.3×10^{-2} M
5. 1.7×10^{-2} M

D. Phenylacetic acid
1. Control
2. Without PP
3. $.7 \times 10^{-2}$ M
4. 1.0×10^{-2} M
5. 1.5×10^{-2} M

not supplemented with pyridoxal phosphate, the experimental animals showed lower enzyme activity than did the controls. However, addition of 0.5 μM of cofactor to the incubation mixture resulted in an increase of 5 HTA formation so that the levels in the experimental and control animals were the same. Similarly, addition of 0.002% pyridoxine hydrochloride to the experimental diet for 4 to 8 weeks was found to be sufficient to prevent the inhibition of 5 HTP decarboxylase by phenylalanine metabolites.

A second series of experiments using higher dietary loads of phenylalanine for 4 weeks was carried out next. This resulted in a marked increase of serum phenylalanine and urine phenylpyruvate levels (Table I). This was accompanied by poor weight gain and the dermatitis seen in pyridoxine deficiency. When sacrificed, there was a significant decrease of 5 HTP decarboxylase activity in the phenylketonuric rats as compared with controls. In those fed 4% L-phenylalanine plus 4% DL-phenylalanine, this effect could be overcome by supplementation with both 0.5 and 1.0 μM pyridoxal phosphate. In those fed 7% L-phenylalanine, 1.0 μM pyridoxal phosphate was required to overcome the decrease.

The *in vitro* inhibition of 5 HTP decarboxylase by phenylalanine metabolites is shown in Table II. The data indicate that phenylalanine is the least active and phenylacetic acid the most active inhibitor, with phenylpyruvic acid and phenyllactic acid in between. These data from rats are similar to those based on guinea pig kidney homogenates as reported by Davison and Sandler (4). The inhibition was present under all 3 sets of experimental conditions and could not be reversed by supplementation with pyridoxal phosphate as illustrated in Table III.

Finally, kinetic studies indicate that inhibition of 5 HTP decarboxylase by phenylalanine and its metabolites is competitive and substrate-dependent at pH 8 (Fig. 2). The K_m for 5 HTP decarboxylation is estimated to be 5.12×10^{-4}.

Discussion. Buzard and Nytch(2,3) have shown that with a pyridoxine intake adequate for normal growth, the apoenzyme of rat kidney 5 HTP decarboxylase is not saturated with the coenzyme under experimental conditions. Furthermore, addition of excess pyridoxal phosphate will overcome the inhibition of this enzyme system by hydroxylamine.

Data from the present study suggest that

TABLE I. Kidney 5 HTP Decarboxylase Activity of Control and Experimental Rats Fed on Experimental Diets Containing Increased Phenylalanine.

Group*	No.	Serum phenylalanine (10^{-3} M)	Urine phenylpyruvate (μM/24 hr)	5 HTP decarboxylase†		
				Without PP	With .5 μM PP	With 1.0 μM PP
I	7	.13 ± .07	5 ± 1	1.13 ±.08	2.55 ±.40	2.44 ±.41
II	8	3.06 ± 2.28‡	220 ± 53‡	.97 ±.06‡	2.48 ±.35	2.26 ±.09
III	8	2.25 ± 1.98‡	45 ± 39‡	.68 ±.16‡	1.25 ±.34‡	2.25 ±.27

* Group I, control; II, 4% L-phenylalanine + 4% DL-phenylalanine; III, 7% L-phenylalanine.

† Expressed as μM 5 HTA formed per 100 mg dry wt per hr.

‡ Significant at .01 probability.

the inhibition of 5 HTP decarboxylase by phenylalanine and its derivatives may involve two mechanisms. At lower inhibitor concentrations, the primary effect appears to be upon the coenzyme. This is shown by the fact that experimental animals with serum phenylalanine levels of 3×10^{-3}M show decreased 5 HTP decarboxylase activity. However, addition of pyridoxal phosphate to the reaction mixture or supplementation of the cofactor in the high phenylalanine diet overcomes this inhibition. Finally, at these concentrations it is not possible to demonstrate inhibition *in vitro* when pyridoxal phosphate is present in excess. At higher concentrations, the inhibitor appears to compete with the substrate for the enzyme. This is shown by the fact that the inhibitor is competitive and substrate-dependent at pH 8. Also, the inhibitory effect is irreversible when cofactor is added after pre-incubation with 3.3×10^{-2}M of inhibitor for one hour.

In phenylketonuria, the decrease of 5 HTA in the blood and 5-hydroxyindoleacetic acid in the urine has been attributed to the inhibition of 5 HTP decarboxylase by the aromatic acid metabolites of phenylalanine(10). The ability to correct these abnormalities by use of monoamine oxidase inhibitors(11) and by diets low in phenylalanine content(10) would be compatible with the concept that the inhibition caused by the levels of serum phenylalanine and its metabolites in phenylketonuria is not sufficient to affect the apoenzyme in an irreversible manner. However, evidence that the low phenylalanine diet is effective in bringing about near-normal mental development only if initiated during the first few months of life(12) emphasizes the need for further investigation of the mechanisms involved in development of aromatic L-amino acid decarboxylation in the immature individual(8,13-16).

TABLE II. *In vitro* Inhibition of 5-Hydroxy tryptophan Decarboxylase by Phenylalanine Metabolites (50% Inhibition).

	Conc $\times 10^{-2}$ M
Phenylalanine	7.5
Phenylpyruvic acid	2.4
Phenyllactic "	2.4
Phenylacetic "	1.7

Each flask contained: 2.4 ml 0.1 N sodium pyrophosphate buffer pH 8, inhibitors in 0.3 ml buffer, 10 μM 5 HTP, and 0.3 ml kidney homogenate.

TABLE III. Irreversibility of Pyridoxal Phosphate to *in vitro* Inhibition of 5 HTP Decarboxylase by Phenylalanine Metabolites.

	5 HTP decarboxylase*	
	Without supplementation	0.5 μM pyridoxal phosphate added
Control	1.25	3.31
Phenylalanine (5×10^{-2} M)	.90	3.00
Phenylpyruvate (3.3×10^{-2} M)	.25	.65
Phenyllactic acid (3.3×10^{-2} M)	.62	1.38
Phenylacetic acid (3.3×10^{-2} M)	.24	.75

* Expressed as μM 5 HTA formed per 100 mg dry wt per hr. Each flask contained 2.2 ml 0.1 N sodium pyrophosphate buffer, pH 8.0, inhibitors in 0.5 ml buffer, 10 μM 5 HTP, and 0.3 ml kidney homogenate. After incubation for 1 hr, 0.5 μM pyridoxal phosphate was added from side arm of flask and incubation continued for another hour.

Summary. Administration of high phenylalanine diets to weanling rats resulted in serum phenylalanine levels of 3×10^{-3}M. At these concentrations, the decrease of kidney 5 HTP decarboxylase can be overcome

by supplementation with excess pyridoxal phosphate. However, if incubation was carried out with 3×10^{-2}M inhibitor, the inhibitor effect can no longer be reversed by supplementation with cofactor. The inhibition of phenylalanine and its derivatives, phenylpyruvic, phenyllactic, and phenylacetic acid, is competitive and substrate-dependent at pH 8. The K_m for 5 HTP decarboxylase is estimated to be 5.12×10^{-4}.

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"Bound" Growth Inhibitor in Raw Soybean Meal.* (27957)

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The poor growth of experimental animals fed raw soybean(1) as a source of protein, when compared with cooked soybean, would suggest the existence of specific growth inhibitor(s) in the raw soybean. Repeated attempts in this laboratory to prepare such a factor have yielded erratic and inconclusive results. Working with various soybean fractions, Rackis *et al.*(2) have recently concluded, "All raw fractions contained widely different levels of trypsin inhibitor activity as measured chemically; and, there appears to be no direct relationship between trypsin inhibitor activity, growth inhibition and pancreatic hypertrophy." Generally, the results of Birk and Gertler(3), Rackis *et al.*(2), and Saxena *et al.*(4) suggested that a water extracted soybean residue was more growth inhibitory than the soluble fraction. These reports and the results obtained in our laboratory suggested that the soybean growth inhibitor may exist in a "bound" or insoluble form. This hypothesis was examined by digesting raw soybean oil meal with papain, later by merely soaking in water. In this connection, McGinnis and Menzies(5) reported that papain digestion improved growth over raw soybean while Desikachar and De (6) reported no improvement from papain digestion.

Methods. The raw soybean meal used in this study was prepared by the solvent extraction process. Heated meal was prepared by autoclaving the raw meal at 15 lb pressure for 30 minutes. The raw or heated soybean meal was suspended in 10 ml water per g of meal and maintained at 37° with stirring for the indicated period. Commercial crude papain, when used, was added at a level of 50 mg per g of meal. The "digests,"

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