Effect of Phenylalanine Metabolites on Urinary Excretion of Indole-3-acetic Acid and 5-Hydroxyindole-3-acetic Acid in Man.* (25538)

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Increased urinary excretion of indole-3-acetic acid (IAA), indole-3-lactic acid, and indican, and decreased excretion of 5-hydroxyindole-3-acetic acid (5-HIAA) have been reported for the hereditary metabolic disease phenylketonuria(1-3). However, the relation between the increased concentration of phenylalanine and its metabolites in this disease and the disturbance in tryptophan metabolism has not been adequately explained. It has recently been found that the pyridoxine-requiring enzymes dihydroxyphenylalanine decarboxylase, 5-hydroxytryptophan decarboxylase, and glutamic acid decarboxylase can be inhibited *in vitro* by certain metabolites of phenylalanine(4-6). These findings suggest that the *in vivo* inhibition of tryptophan decarboxylase as well as 5-hydroxytryptophan decarboxylase might possibly underlie the alteration in tryptophan metabolism associated with phenylketonuria. *In vivo* evidence for the reversibility of this type of inhibition was demonstrated when it was shown that urinary 5-HIAA levels could be elevated to normal values in phenylketonurics when placed on low-phenylalanine diets or fed L-tryptophan (7,8), or reduced in patients with carcinoid tumors after ingestion of phenylacetic acid(9). Few data, however, have as yet been presented on the *in vivo* effects of specific phenylalanine metabolites on excretion of urinary indoles in healthy individuals. The present report describes the effect of phenylpyruvic acid, *o*-hydroxyphenylacetic acid, and phenylacetic acid on urinary excretion of IAA and 5-HIAA in normal human subjects.

Methods. The 5 adult males used as subjects were between 22 and 36 years of age. Two-hour control urine samples were taken from the test subjects prior to administration of the test compound. The orally administered dosages were: sodium phenylpyruvate† (20 mg/kg), phenylacetic acid† (20 mg/kg), and *o*-hydroxyphenylacetic acid‡ (2 mg/kg). Urine samples were then collected every 2 hours for the following 6-hour period. The urine samples were immediately assayed for indican (indoxylsulfate), IAA, and phenylpyruvic acid and then frozen for subsequent analysis. The frozen samples were assayed for *o*-hydroxyphenylacetic acid and 5-HIAA within 3 days. Indican was estimated by Parker's modification of the procedure of Askenstedt(10); no attempt was made to quantify these values which are presented as relative figures only. The colorimetric method of Berry and Woolf(11) was used for the phenylpyruvic acid assay, and a modification of the paper-chromatographic procedure of Armstrong *et al.*(12) for the *o*-hydroxyphenylacetic acid assay in which the spots were developed with 2,6-dichloroquinone chlorimide. IAA was measured by the colorimetric method of Weissbach *et al.*(13) and the 5-HIAA by the colorimetric technic of Udenfriend *et al.*(14). Because indolelactic acid is also extractable by the procedure used for the IAA assay, paper chromatograms were run on the urine extract as described by Weissbach *et al.*(13); no increase in indolelactic acid over control values was detectable. Even so, the contribution of indolelactic acid to the IAA values can not be completely discounted. The values for 5-HIAA were corrected for an estimated 85% recovery.

Results. Increases in the urinary excretion of IAA and 5-HIAA usually occurred within the first 2-hour period following administration of a specific phenylalanine metabolite. Increases in IAA excretion were in all cases greater than those of 5-HIAA.

Phenylpyruvic acid administration. Ingestion of phenylpyruvic acid resulted in a

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TABLE I. Urinary Excretion of Phenylpyruvic Acid (PPA), *o*-Hydroxyphenylacetic Acid (*o*-HPAA), 5-Hydroxyindoleacetic Acid (5-HIAA), Indoleacetic Acid (IAA), and Indican (IND) after Oral Administration of Sodium Phenylpyruvate (20 mg/kg Body Weight).

Hr after admin.	PPA	*o*-HPAA	IAA	5-HIAA	IND
			(μmoles)		
	Subject A (dose: 8.3 mmoles)				
0	0		1.7		3.6
2	1121		10.6		3.7
4	4.3		8.2		1.7
6	0		6.2		1.1
	Subject B (dose: 10.4 mmoles)				
0	0		2.3		2.4
2	1456		7.8		1.5
4	14.6		8.1		.9
6	0		3.3		.5
	Subject C (dose: 7.9 mmoles)				
0	0	.7	3.6	2.3	
2	904	12.2	16.6	8.2	
4	17.1	8.7	7.3	2.3	
6	0	4.3	6.6	2.9	

marked increase (3.5- to 6.2-fold) in the urinary excretion of IAA (Table I) and a 3.5-fold increase (one subject) in 5-HIAA. Since phenylacetic acid and possibly *o*-hydroxyphenylacetic acid(15) are derived from phenylpyruvic acid, the response to phenylpyruvic acid loading must be the result of the combined effect of all 3 metabolites. The low recovery (less than 14%) of administered phenylpyruvic acid attests to the efficient metabolism of this compound. No significant alteration in excretion of indican could be detected by the method employed.

TABLE II. Urinary Excretion of *o*-Hydroxyphenylacetic Acid (*o*-HPAA), Indoleacetic Acid (IAA), and 5-Hydroxyindoleacetic Acid (5-HIAA) after Oral Administration of *o*-Hydroxyphenylacetic Acid (2 mg/kg).

Hr after admin.	*o*-HPAA	IAA	5-HIAA
		(μmoles)	
	Subject D (dose: 2.1 mmoles)*		
0	1.5	2.9	1.4
2	814	19.5	2.4
4	503	14.3	4.3
6	425	4.7	1.7
	Subject A (dose: .9 mmole)		
0	.8	2.1	1.3
2	514	15.8	4.1
4	177	12.4	3.0
6	53	4.5	1.6
	Subject E (dose: 1 mmole)		
0		1.7	1.9
2	500	9.4	4.8
4	224	5.9	1.2
6	134	4.8	

* 4.5 mg/kg.

o-Hydroxyphenylacetic acid administration. The high recovery (greater than 83%) of the ingested *o*-hydroxyphenylacetic acid indicates that the alterations in the urinary excretion of IAA and 5-HIAA are due almost entirely to the unmetabolized compound. The sensitivity of tryptophan metabolism to *o*-hydroxyphenylacetic acid is borne out in Table II. Here, although the *o*-hydroxyphenylacetic acid dose was only 1/10 that of the phenylpyruvic acid test, IAA was excreted at higher levels (5.5- to 7.5-fold increase) than those produced by phenylpyruvic acid loading. The 2.5- to 3.1-fold increase in excretion of 5-HIAA was seemingly within the range induced by the phenylpyruvic acid test.

Phenylacetic acid administration. The 2- to about 4-fold increase in IAA (Table III) brought about by phenylacetic acid ingestion appeared to be similar to, or somewhat lower than, the increases resulting from phenylpyruvic acid administration. There is some indication of depression in excretion of 5-HIAA.

TABLE III. Urinary Excretion of *o*-Hydroxyphenylacetic Acid (*o*-HPAA), Indoleacetic Acid (IAA), and 5-Hydroxyindoleacetic Acid (5-HIAA) after Oral Administration of Phenylacetic Acid (20 mg/kg).

Hr after admin.	*o*-HPAA	IAA	5-HIAA
		(μmoles)	
	Subject A (dose: 10 mmoles)		
2	.6	10.3	.8
4	.7	7.0	1.3
6	.7	2.2	.8
	Subject E (dose: 11.5 mmoles)		
0		3.1	1.8
2		6.2	.5
4		3.4	.8
6		4.5	.9

Discussion. If we assume that the same metabolites that inhibit 5-hydroxytryptophan decarboxylase(5) can inhibit tryptophan decarboxylase as well, then the blocks in amine formation as outlined in Fig. 1 can be postulated. That tryptophan undergoes decarboxylation to tryptamine in mammalian tissues is supported by the recent findings of Weissbach *et al.*(13). Inhibition in formation of tryptamine and 5-hydroxytryptamine could have the effect of shunting an increased

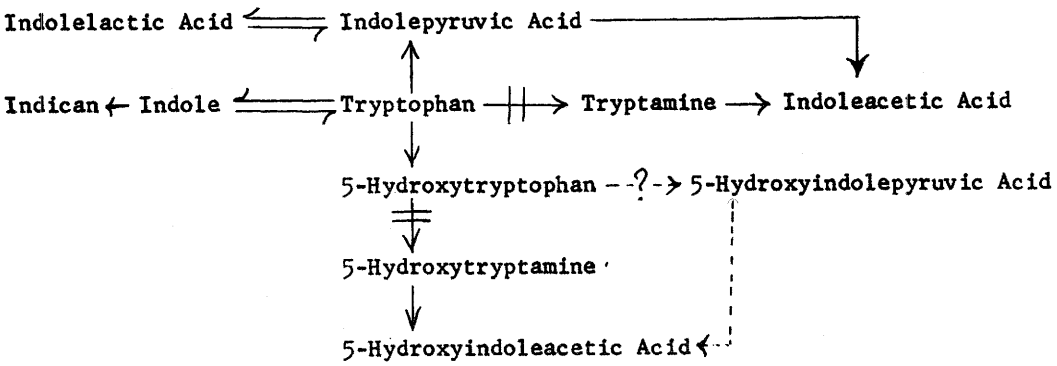

FIG. 1. Intermediary pathways in formation of indoleacetic acid, indolelactic acid, 5-hydroxyindoleacetic acid, and indican from tryptophan.

amount of tryptophan to the transamination route to indolepyruvic acid and IAA (Fig. 1). It is not clear, however, just why indolelactic acid is not also excreted in increased amounts. In this connection, it is also of interest that the increased levels of urinary indican reported in phenylketonurics(2) could not be experimentally induced by the PPA loads administered in the present study.

Because of the lack of sustained stress in the present work, the inhibition picture discussed above, although possibly applicable to the pathological state in phenylketonuria, would not necessarily apply to the results of the present study. An alternate explanation would be that these phenylalanine metabolites have the capacity, either directly or indirectly, to displace bound tryptamine and 5-hydroxytryptamine from tissues. These released amines then undergo conversion by amine oxidases to IAA and 5-HIAA. This could explain the paradox in the present study of an increase in excretion of 5-HIAA rather than the expected suppression resulting from inhibition of 5-hydroxytryptophan decarboxylase; for only when an equilibrium is reached and the tissue amine is depleted would the overall inhibitory effect become apparent. The ability of certain compounds to displace tissue 5-hydroxytryptamine has been attributed to their antimetabolite action(16).

The possibility remains that some 5-HIAA is formed *via* oxidative deamination of 5-hydroxytryptophan to 5-hydroxyindolepyruvic acid. If this route is operative, however, it does not appear to be an effective alternate pathway for the metabolism of 5-hydroxytryptophan. There is as yet no evidence for such a pathway in the mammal.

Summary. 1) Oral administration of phenylpyruvic acid, phenylacetic acid, and *o*-hydroxyphenylacetic acid to human subjects resulted in increase in urinary excretion of indoleacetic acid. A smaller increase in excretion of 5-hydroxyindoleacetic acid followed administration of phenylpyruvic acid and *o*-hydroxyphenylacetic acid. 2) *o*-Hydroxyphenylacetic acid was demonstrated to be more effective than either phenylpyruvic acid or phenylacetic acid in increasing excretion of indoleacetic acid. 3) The relation of these findings to inhibition of tryptophan decarboxylase and 5-hydroxytryptophan decarboxylase is discussed.

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Some Characteristics of Proteolytic System of a Marine Bacterial Species.* (25539)

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The desirability of increasing present limited knowledge of bacterial proteolytic enzymes may be inferred from work of previous authors(1-4), some of whom suggested potential usefulness of these enzymes in investigations of protein and enzyme structure and in industrial processes(3,4). Investigations of bacterial proteinases have dealt principally with enzymes from terrestrial species; marine organisms have been largely neglected, even though they have been reported more proteolytic than either soil or fresh water forms(5). In view of established proteolytic ability, marine bacteria appear to offer considerable promise in investigations designed to increase our overall information on bacterial proteolytic enzymes. This report describes characteristics of a proteolytic system of an organism isolated from a marine environment.

Materials and methods. The organism was a marine bacterial species isolated by Merkel and Traganza(6) from the alimentary canal of the marine isopod, *Limnoria,* and reported by them to be highly proteolytic.† Stock cultures were grown on slants consisting of 1% peptone and 2% agar in artificial sea water, and were transferred at monthly intervals. A transfer was made from stock culture to a slant identical to stock medium, except that artificial sea water diluted 1:10 with distilled water was used. After 12 to 24 hours incubation, a loop transfer was made to 50 ml Erlenmeyer flask containing 6 ml of growth medium (Table I); this subculture was incubated 12 to 24 hr before use to inoculate 1 liter of growth medium. Incubation was at 25°C with shaking. After 54 to 56 hours incubation, cells were removed by centrifugation and passage through a Seitz filter; the resulting culture filtrate was used as source of enzyme. One ml of enzyme solution was incubated at 37°C with 5 ml of denatured hemoglobin substrate.‡ At end of desired incubation time, reaction was stopped and residual protein precipitated by addition of 10 ml of 5% trichloroacetic acid. The mixture was filtered through Whatman No. 3 paper, and determination of acid-soluble split products was made by the method of Northrup *et al.* (7) by reading at 280 mμ in Beckman Model DU Spectrophotometer. Enzyme concentration and time of incubation were always chosen so that activity was proportional to

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‡ Prepared by suspending 5 g salt-free lyophilized hemoglobin (Mann Research Labs.) in 80 ml of water, adding 8 g urea, and incubating at 37° C for 45 to 60 minutes. Ten g urea and 125 ml of 0.2 M phosphate buffer (pH 8.0) were then added. Other pH values were employed where indicated.