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Phenylpyruvic Acid as a Possible Precursor of *o*-Hydroxyphenylacetic Acid in Man

Abstract. The oral administration of phenylpyruvic acid to human subjects results in increased urinary excretion of *o*-hydroxyphenylacetic acid. This demonstrates that phenylpyruvic acid may act as a precursor for *o*-hydroxy derivatives of phenylalanine and suggests that the formation of *o*-tyrosine is not necessary to account for the excretion of *o*-hydroxyphenylacetic acid in phenylketonuria.

o-Hydroxyphenylacetic acid (*o*-HPAA) has been found to be the major hydroxy metabolite of phenylalanine excreted in the urine of patients with the hereditary metabolic disorder known as phenylketonuria (*1–3*). Such a conversion could theoretically occur by the *o*-hydroxylation of phenylalanine, phenylpyruvic acid, or phenylacetic acid. Armstrong and Shaw (*4*) found that the excretion of *o*-HPAA followed the oral administration of *o*-tyrosine in man, and postulated that *o*-tyrosine was a probable intermediate in the formation of *o*-HPAA in phenylketonurics. Mitoma *et al.* (*5*) suggested that the overproduction of *o*-tyramine, the decarboxylation product of *o*-tyrosine, was perhaps responsible for the mental defect in phenylketonuria. The presence of *o*-tyrosine or *o*-tyramine has not as yet been demonstrated in the tissues of normal or phenylketonuric individuals. It has been reported, however, that beef adrenals normally contain free *o*-tyrosine (*6*), and it is possible that the normal excretion of *o*-HPAA in man could originate from such a source.

When Berry *et al.* (*7*) applied phenylalanine tolerance tests (*8*) to individuals heterozygous for phenylketonuria (0.1 g of L-phenylalanine per kilogram of body weight), they found that *o*-HPAA but not phenylpyruvic acid was excreted in the urine in increased amounts. Cullen and Knox (*9*) recently confirmed these findings and also showed that a dose of at least 0.13 g of L-phenylalanine per kilogram is usually required before any increased *o*-HPAA can be detected in the urine of normal subjects.

In the course of experiments in this laboratory (*10*), it was found that ingestion of the D-isomer of phenylalanine in man in amounts as low as 0.015 g/kg regularly results in the urinary excretion of phenylpyruvic acid and of increased amounts of *o*-HPAA. This raised the question whether D-phenylalanine or one of its metabolites might be the substrate for *o*-hydroxylation.

In order to test for the possible *o*-hydroxylation of phenylpyruvic acid or its metabolites, the sodium salt of phenylpyruvic acid (Nutritional Biochemicals Corp.) was orally administered to three normal, adult male subjects. Each urine sample following the ingestion of phenylpyruvic acid was immediately assayed for phenylpyruvic acid by a modification of the procedure of Berry and Woolf (*11*), and the collection of urine was continued until no further phenylpyruvic acid could be detected. The individual urine samples in which phenylpyruvic acid was present were then pooled and assayed for *o*-HPAA by a modification of the paper-chromatography technique of Armstrong *et al.* (*2, 12*) in which the chromatogram was developed with 2,6-dichloroquinone chlorimide.

The results (Table 1) show that the ingestion of phenylpyruvic acid causes an increased excretion of *o*-HPAA and indicates that phenylpyruvic acid may be a precursor for *o*-HPAA. However, since no isotope study was carried out with labeled phenylpyruvic acid, the possibility remains that the administration of phenylpyruvic acid might be indirectly increasing the excretion of *o*-HPAA.

Because the evidence presented here shows that the ingestion of phenylpyruvic acid can cause an increased urinary excretion of *o*-HPAA, it is not necessary to assume an increased production of *o*-tyrosine in phenylketonuria in order to account for the increase of *o*-hydroxy derivatives of phenylalanine (*13*).

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Table 1. Urinary excretion of phenylpyruvic acid (PPA) and *o*-hydroxyphenylacetic acid (*o*-HPAA) following the oral administration of sodium phenylpyruvic acid.

PPA ingested (mmole)	Total PPA excreted (μmole)	Collection time (hr) (*X*)	*o*-HPAA excreted (μmole/24 hr) (control)* (*Y*)	Total *o*-HPAA excreted during *X* (μmole) (*Z*)	*Z* less estimated normal excretion of *o*-HPAA during *X* (μmole) [(*Z* − *XY*)/24]
			Subject A		
0			2.6		
2.5	185	3.0		8.5	8.2
5.0	711	6.5		42	41
			Subject B		
0			5.0		
5.0	598	4.5		19	18
			Subject C		
0			10.6		
5.0	581	7.0		51	48

* The normal daily excretion of *o*-HPAA for seven subjects, including subjects A, B, and C, ranged from 2.0 to 10.6 μmole (mean, 5.6 μmole).

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