TABLE II. Blood Velocity and Volume Flow in Vessels (30 to 38 μ Diameter) of Hamster Cheek Pouch.

		Velocity, mm/sec			Vol flow, $mm^3 \times 10^{-3}$/sec			Diameter, μ		
Type of vessel	No.	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
Arterioles	18	4.8	2.78	1.2-12.9	4.08	2.22	.85-10.37	33.3	2.66	30-38
Venules	18	2 2	1.65	.4- 6.6	1.86	1.23	.10- 4.67	33.8	2.90	30-38

S.D. = Standard deviation.

cities in 82 arterioles (30 to 70 μ diam) and in 76 venules (30 to 70 μ diam) of the rabbit mesentery to be 6.0 and 3.6 mm/sec respectively. The results reported here (Table I) for vessels of the hamster cheek pouch are 4.4 mm/sec in arterioles and 2.6 mm/sec in venules comparable in size to those studied by Hugues. While the velocities in vessels of the cheek pouch appear to be somewhat lower than those in the rabbit mesentery, the ratios of mean velocity in venules to that in arterioles are approximately the same, 1:1.7, in both cases. If cheek pouch vessels of essentially the same size range are compared (Table II), the mean velocity in venules is 2.2 mm/sec and in arterioles, 4.8 mm/sec with a ratio of 1:2.2.

Neither these studies nor those of Hugues show any significant correlation between vessel size and blood velocity within the size ranges studied. However, it is possible that more refined high-speed microcinematographic techniques which permit measurement of velocity of individual blood cells within vessels at the capillary level(4) may reveal such relationships.

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Conversion of Phenylalanine to Phenethylamine in Patients with Phenylketonuria. (28256)

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In phenylketonuria there is a 20-40 fold increase in the blood levels of phenylalanine. Recent studies in this laboratory have shown that L-phenylalanine is decarboxylated by mammalian aromatic L-amino acid decarboxylase, an enzyme which is also present in brain(1). Since normal blood levels of phenylalanine are of the order of 10^{-4}M and the Michaelis constant for phenylalanine decarboxylation is greater than 10^{-2}M, one would expect to find an increased synthesis of phenethylamine in phenylketonurics.

In this investigation it was possible to show that production of phenethylamine is indeed markedly increased in patients with phenylketonuria. Also, it was found that synthesis can be appreciably reduced by administering the decarboxylase inhibitor, α-methyl-dopa*(1,2). The possible implications of increased synthesis of this biologically active amine in the brain are discussed.

Methods. The principal subjects of this investigation were 2 phenylketonuric patients (J.R., a 28 year old woman and D.P., a 22 year old man), hospitalized in the Clinical Center. In addition to clinical stigmata characteristic of the disease, the diagnosis had been confirmed by elevated fasting levels of plasma phenylalanine and by positive ferric

* α-Methyl-3,4-dihydroxy-DL-phenylalanine.

chloride tests of the urine. Monoamine oxidase (MAO) inhibitors were administered to these patients to block the metabolism of phenethylamine so that measurable quantities would be excreted in the urine. J.R. received nialamide 250 mg daily and D.P. received pargyline 75 mg daily throughout the study, beginning at least one week before any data was obtained. The decarboxylase inhibitor, α-methyl-dopa, was administered on one day during the study period. J.R. received DL-α-methyl-dopa 2.0 g and D.P. received 2.5 g, administered as a single dose 2 hours prior to the beginning of 8 hour urine collections. Eight hour collections of urine were obtained also during corresponding periods on days when the patient received only MAO inhibitor.

The non-phenylketonuric subjects were 2 normotensive and 4 hypertensive patients who had no gross impairment in renal function. They were treated with various monoamine oxidase inhibitors: β-phenylisopropyl hydrazine 25 mg daily, nialamide 300 mg daily, isocarboxazid 50 mg daily and pargyline 75 mg daily. Twenty-four hour urine collections were obtained for phenethylamine assay after the patient had been on the inhibitor for at least a week.

To determine what fraction of circulating phenethylamine was actually excreted in the urine during MAO inhibition, one of the normotensive non-phenylketonuric patients was infused with 5.1 mg of phenethylamine while being treated with pargyline 75 mg per day. The amine was given in 142 ml of 5% dextrose in water over a 2½ hour period, and urine was collected for 12 hours after beginning the infusion as well as on comparable periods on control days when he received only pargyline. The blood pressure was recorded every 5 minutes during the infusion and for 30 minutes prior to it.

Phenethylamine was extracted from urine in the following manner: Three to 50 ml of urine were made alkaline (*ca* pH 13) with 10 N NaOH, saturated with NaCl and extracted with 2 volumes of ether. The ether extract was washed with 5 ml of 0.1 N NaOH and the alkaline solution discarded. The ether layer was then shaken with 3 ml of 0.1 N HCl to re-extract the phenethylamine. The ether was discarded, and the acid layer was made alkaline by addition of 1 ml of 1 N NaOH and extracted with 10 ml of ether or benzene. The organic solvent layer was then transferred to another tube. A drop of 1% glacial acetic acid in benzene was added and the solvent evaporated to about 0.5 ml under a stream of nitrogen. When 10-20 μg of phenethylamine were carried through the extraction procedure recoveries at this stage were about 65-70%.

Since tryptamine is also present in the above extract, it was necessary to separate the phenethylamine by the following chromatographic techniques prior to its identification and assay:

Method A. Two-dimensional chromatography on Whatman #1 paper according to the procedure described by Smith(3). The first solvent was n-butanol:acetic acid:water (120:30:50); the second solvent was isopropanol:ammonia:water (200:10:20). The chromatograms were dipped in 0.2% ninhydrin in acetone containing several ml of pyridine. Upon drying these chromatograms were compared with several standards carried through the entire procedure.

Method B. One-dimensional chromatography on Whatman #1 paper buffered at pH 7.8 with 0.05 M phosphate buffer. The developing solvent was isopropanol:water (5:1). The chromatograms were dipped in a solution of 0.2% ninhydrin in acetone containing 2% acetic acid and were heated at 65° for 20 minutes. The phenethylamine spots were cut out, eluted with 1 ml of 50% ethanol for 20-30 min and the absorbancy measured in a spectrophotometer at 570 mμ. Comparisons were made with standards carried through the entire procedure.

With both chromatographic methods 10-20 μg of phenethylamine added to urine could be determined with a precision of about ± 25% based on standards carried through the entire procedure.

Gas chromatography of phenethylamine was carried out according to the technique described by Fales and Pisano(4), except

that 7% SE-30 siloxane polymer was used and the column temperature was 145°C. This procedure was used not only for identification but also for quantitative assay. The extraction procedure was the same as described for paper chromatography except that the final extraction was made into chloroform instead of benzene or ether. The chloroform was evaporated to 200 μl, and 4 μl aliquots were transferred to the gas chromatogram. With the MAO inhibitor, pargyline, gas chromatography was most useful since a metabolite of the drug (N-methyl benzylamine) interfered to some extent with the assay by paper chromatography.

Results. The phenethylamine in extracts of urine from phenylketonuric subjects receiving MAO inhibitors was identified in several ways. When authentic phenethylamine was added to the urine extract, its spot on the 2-dimensional chromatogram was found to be superimposed on that of phenethylamine from urine. The only other ninhydrin positive spot was that of tryptamine, which was completely separated from phenethylamine. In a similar manner, phenethylamine was identified by one-dimensional chromatography using paper buffered to pH 7.8 and isopropanol:H_2O as solvent. The Rf value of phenethylamine was 0.63 and that of tryptamine 0.52. The identity of urinary phenethylamine was confirmed by its mobility in the gas chromatogram (7 min retention time), which was identical with that of authentic phenethylamine.

As shown in Table I, the phenylketonuric patients excreted 190 to 810 μg of phenethylamine hourly during treatment with MAO inhibitors. Without inhibition of MAO, however, only trace amounts (<3 μg/hour) were detected. The non-phenylketonuric subjects excreted only trace amounts even when receiving MAO inhibitors.

TABLE I. Excretion of Phenethylamine by Phenylketonuric and Non-Phenylketonuric Subjects.

Patients	Urinary phenethylamine, μg/hr	
	Control	MAO inhibitor
Phenylketonuric J. R.	< 3	190
D. P.	< 3	450–810
Non-phenylketonuric (6 subjects)	< 3	< 4

TABLE II. Effect of α-Methyl-Dopa on Excretion Of Phenethylamine by Phenylketonuric Subjects.

Subject	Urinary phenethylamine (mg/8 hr)		
	MAO inhibitor	α-Methyl-dopa + MAO inhibitor	MAO inhibitor (post α-methyl-dopa)
J.R.*	1.5	.1-.2	1.5
D.P.*	3.5–6.0	1.0	—
D.P.†	3.6, 5.9, 6.5	1.0	—

* Analyses by Method A
† Analyses by Method B
} See text.

When 5.1 mg of phenethylamine was infused to a non-phenylketonuric subject† receiving a monoamine oxidase inhibitor, less than 10% of the administerd dose was excreted in the urine as phenethylamine.

When the decarboxylase inhibitor, α-methyl-dopa was administered to the phenylketonuric subjects receiving MAO inhibitors, a marked diminution of phenethylamine excretion occurred (Table II). Urinary phenethylamine was reduced to an average of 15% of that on the MAO inhibitor alone.

The neurological status of both patients was affected by MAO inhibitors. An increase in tremor which was slightly accentuated by intention, an ataxia without impairment of position sense, and easily induced ankle clonus all developed when J.R. received 25 mg per day of the MAO inhibitor, β-phenylisopropyl hydrazine. To a lesser degree, these changes also occurred on nialamide 200 mg per day. D.P. developed ataxia after 10 days on β-phenylisopropyl hydrazine 25 mg per day, although ataxia was not noted on pargyline 75 mg per day for 14 days. No clear cut alteration in clinical status was seen following administration of the single doses of α-methyl-dopa. To evaluate the clinical effects of more prolonged administration of α-methyl-dopa, patient J.R. was given DL-α-methyl-dopa 1 g every 8 hours for 22 days while receiving no other drugs. No change in neurological status was noted during this

† The infusion elevated average supine blood pressure from 125/65 to 170/100 mm Hg and produced a facial flush.

treatment period.

Discussion. These studies indicate that increased amounts of phenethylamine are synthesized by patients with phenylketonuria. While the extent of this pathway of phenylalanine metabolism is not precisely known, the small urinary recovery of infused phenethylamine suggests that the milligram quantities of phenethylamine excreted by phenylketonurics represent only a small fraction of that actually synthesized.

It has been shown in animal studies that the enzyme aromatic L-amino acid decarboxylase will decarboxylate several aromatic amino acids, one of these being phenylalanine(1). A unique feature of this enzyme is that it is inhibited by α-methyl-dopa *in vitro*(1) and *in vivo* in man(5,6). The finding that phenethylamine production in phenylketonuria is inhibited by α-methyl-dopa indicates that a similar decarboxylase is responsible for phenylalanine decarboxylation in phenylketonuria.

Since aromatic L-amino acid decarboxylase is found in mammalian brain, the synthesis of phenethylamine should be increased in the central nervous systems of patients with phenylketonuria. Since this amine is known to have vasoactive and convulsive properties (7), and is potent in its ability to release norepinephrine from binding sites(8), the possibility is raised that the central nervous system disease in phenylketonuria could be mediated to some degree by phenethylamine. Certainly, one or more of the amines derived from tryptophan seem to be responsible for the neurologic alterations produced by an excess of that amino acid(9). Thus, while demonstration of increased amounts of phenethylamine does not necessarily implicate it in the pathophysiology of phenylketonuria, investigation of its effects on the developing nervous system seem indicated.

Summary. 1. An increased production of phenethylamine was found in patients with phenylketonuria. 2. Phenethylamine synthesis was markedly reduced by α-methyldopa, an inhibitor of aromatic L-amino acid decarboxylase. This suggests that the synthesis of phenethylamine from phenylalanine in humans is catalyzed by this decarboxylase. 3. Since aromatic L-amino acid decarboxylase is present in brain, it is likely that increased amounts of phenethylamine are synthesized in the central nervous system of patients with phenylketonuria.

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