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mental retardation
phenolic acids
tyrosine aminotransferase deficency
tyrosinemia
tyrosyluria

Metabolic Studies in a Patient with Hepatic Cytosol Tyrosine Aminotransferase Deficiency

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Extract

Metabolic studies on a patient having multiple congenital anomalies and defective hepatic soluble tyrosine aminotransferase activity are presented. The activities of hepatic mitochondrial tyrosine aminotransferase and soluble *p*-hydroxyphenylpyruvate hydroxylase were normal. When untreated, levels of tyrosine in plasma rose as high as 62 mg/100 ml. He had phenolic aciduria (3.1–4.9 mg/mg creatinine) with *p*-hydroxyphenylpyruvic acid (0.6–1.8 mg/mg creatinine), *p*-hydroxyphenylacetic acid (0.7–1.2 mg/mg creatinine), *p*-hydroxyphenyllactic acid (1.0–1.7 mg/mg creatinine), and *N*-acetyltyrosine (0.9–1.6 mg/mg creatinine). These metabolites were identified by gas chromatography-mass spectrometry. Excretion of *p*-tyramine was grossly elevated (17–44 μg/mg creatinine) and was not reduced by orally administered antibiotics. Oral tolerance tests showed rapid conversion of phenylpyruvic acid to phenylalanine and of phenylalanine to tyrosine.

Speculation

The results described suggest the need to reexamine subcellular distribution of enzymes both in normal subjects and in patients with inborn errors of metabolism. When methods are developed to fractionate tyrosine aminotransferase, it may become apparent that there are several isozymes under individual genetic control. Accumulation of an intermediary metabolite should not be taken as conclusive evidence of a primary abnormality of its catabolism. The similarity of the findings in the urine of a patient with hepatic soluble tyrosine aminotransferase deficiency and in tyrosyluric neonates suggests that delayed maturation of soluble tyrosine aminotransferase rather than *p*-hydroxyphenylpyruvate hydroxylase might be present in at least some of these infants.

Introduction

Tyrosinosis with tyrosyluria was first described by Medes in 1932 [28]. No enzyme defect was reported in that study, but more recently a distinct form of tyrosinemia associated with deficiency of *p*-hydroxyphenylpyruvate hydroxylase activity in liver has been recognized [35]. In our report we describe some metabolic studies on a patient with a different form of tyrosinemia. The clinical symptoms of this patient have been briefly reported elsewhere [6], and a detailed case study is in preparation [5].

In our patient, hepatic soluble tyrosine aminotransferase activity was absent, but the hepatic mitochondrial tyrosine aminotransferase activity was normal and hepatic *p*-hydroxyphenylpyruvate hydroxylase activity was intact [12]. Fasting levels of tyrosine in plasma were high, generally 20–50 mg/100 ml, and sev-

Table I. Tyrosine levels in plasma and urine

Diet	Plasma		Urine	
	Mean	Range	Mean	Range
	mg/100 ml		mg/24 hr	
Normal[1]	38.7(22)[2]	11.0-62.5	64.6(16)	8.8-201
Low tyrosine[3]	4.8(21)	1.1-12.7	5.4(13)	0.9-13.9

[1] Containing 190–380 mg/kg tyrosine and 190–440 mg/kg phenylalanine/24 hr.
[2] Number in parentheses indicates number of samples.
[3] Product 3200-AB (Mead Johnson) with added phenylalanine was used to provide 15–30 mg/kg tyrosine and 45–60 mg/kg phenylalanine/24 hr.

eral phenolic acids (*p*HPPA: *p*-hydroxyphenylpyruvic acid; *p*HPAA: *p*-hydroxyphenylacetic acid; *p*HPLA: *p*-hydroxyphenyllactic acid; NAT: *N*-acetyltyrosine) were excreted in large quantities in the urine.

In view of the paradox of elevated levels of phenolic acids in the urine in the face of normal *p*-hydroxyphenylpyruvate hydroxylase activity, these studies are reported in detail.

Case History

The patient was a 5-year-old white male; tyrosinemia and tyrosyluria were first found at 19 months of age. He showed multiple congenital anomalies including microcephaly, cleft lip and palate, talipes equino-varus, and absence of one kidney. He was severely mentally retarded. Fasting levels of tyrosine in plasma (Table I) were not affected by intramuscular administration of cortisone, 100 mg/24 hr for 10 days or oral doses of pyridoxine, 500 mg/24 hr for 2 months, folic acid, 20 mg/24 hr for 1 week, or ascorbic acid, 1.0 g/24 hr for 2 months. Dietary restriction of tyrosine and phenylalanine caused a prompt fall in the tyrosine level in plasma to 2–6 mg/100 ml. Fasting levels of phenylalanine in plasma were never elevated. Histological examination of liver showed no hepatocellular damage other than mild fatty infiltration.

Methods

All urine samples were collected on ice in dark containers with 2 ml concentrated hydrochloric acid/24-hr sample. All samples were stored at −20° until analyzed. Creatinine was determined by the Jaffé reaction. "Total phenols" were determined by the Millon reaction as described by Medes [28]. Phenolic acids, used as quantitative standards, were obtained commercially [43, 44].

Amino Acids

Amino acids were measured by ion exchange chromatography using spherical bead resin P.A. 28 [45] with a modified one-column procedure [4]. Tyrosine and phenylalanine in plasma were measured on a 20-cm column at 65° with 0.2 N-citrate buffer, pH 3.28. Plasma samples were precipitated with sulfosalicylic acid, 65 mg/ml, and separated by centrifugation.

Total tyrosine (free + conjugated) was determined after hydrolysis in 6 N hydrochloric acid at 110° for 23 hr.

The percentage of renal tubular reabsorption of amino acids was calculated using inulin clearance [31] to measure glomerular filtration rate.

Phenolic Acids

Two-dimensional thin layer chromatography (TLC) of ethyl acetate/ether extracts of urine was performed on silica gel plates [46]. The system most suitable for the separation of the major metabolites, including NAT, was isopropanol-ammonia-water (8:1:1) in the first dimension and the organic phase of chloroform-acetic acid-water (2:2:1) in the second dimension [9]. Following chromatography, the plates were dried and sprayed with diazotized sulfanilic acid [36].

Several methods for quantitative assay of *p*HPPA were attempted [11, 16, 42], including the enol-borate method of Lin *et al.* [26]. All quantitative results of *p*HPPA found in urine and reported in this study refer to values obtained by the method of Lin *et al.* Phenylpyruvic acid and *p*HPPA in serum were determined by the same procedure, using the modification of Gentz *et al.* [13].

To measure *p*-tyramine levels in urine, the amines were first concentrated from urine on Dowex 50-X2 according to the method of Kakimoto and Armstrong [22]. *p*-Tyramine was then quantitated by two-dimensional paper chromatography [34, 47].

Gas Chromatography

Urinary acids were extracted at pH 1 into ethyl acetate and ethyl ether. Methyl ester and methyl ester-trimethylsilyl ether (ME/TMSi) derivatives were prepared from the dried extract by first treating the extract with an excess of an ethereal solution of diazomethane for 1 min and then with pyridine-bis-trimethylsilylacetamide-trimethylchlorosilane (3:2:1), at room temperature overnight [7, 17, 20].

Serum acids were isolated by ion exchange [21], using DEAE-Sephadex. After conversion of ketoacids to the methoxime derivative, the residue was treated with bis-trimethylsilyltrifluoroacetamide to form the TMSi derivatives [19].

Separations by gas chromatography (GC) were carried

out using a chromatograph [48] fitted with a hydrogen flame ionization detector. The columns were stainless steel coils, 12 ft × 1/8 in, packed with 5% SE-30 on acid-washed silanized Gas Chrom P (80–100 mesh). Nitrogen was used as carrier gas at a flow rate of 30 ml/min, and separations were carried out by temperature programming at 1.8 or 2°/min.

Individual peaks were characterized by their methylene unit values [39] obtained by cochromatography with a reference mixture of hydrocarbons.

Quantitation of levels of phenolic acids in urine was carried out with hexadecane as an internal standard. This was added after formation of the methyl ester derivatives in order to avoid loss of the standard by evaporation under nitrogen. Response factors were determined using commercial standards. Peak areas were calculated as height × width at half height and converted to mass using the appropriate response factors. The recovery of phenolic acid standards, added to urine, ranged from 95–103%.

Identification of metabolites in serum and urine was carried out by combined gas chromatography-mass spectrometry (GC-MS) [49]. The column was of glass, 9 ft × 4 mm, packed with 1% SE-30 on acid-washed, silanized Gas Chrom P (80–100 mesh). The ion source temperature was 250°, the accelerating voltage 70 ev, and the current 60 μa.

Hepatic Enzyme Assay

A liver sample was obtained by open biopsy. *p*-Hydroxyphenylpyruvate hydroxylase (EC. 1.14.2.2.) activity, measured on the day following the biopsy by the method of Knox and Pitt [23], was normal. Tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC. 2.6.1.5) activity in supernatant and mitochondrial fractions of liver was assayed by Fellman and co-workers, using two different methods. These results, previously reported [12], are shown in Figure 1.

An outline of aromatic amino acid metabolism is shown in Figure 2.

Results

Loading Studies

Figure 3 shows the levels of phenylalanine and tyrosine in plasma in the patient following three phenylalanine tolerance tests with different loading doses and following a phenylpyruvic acid tolerance test.

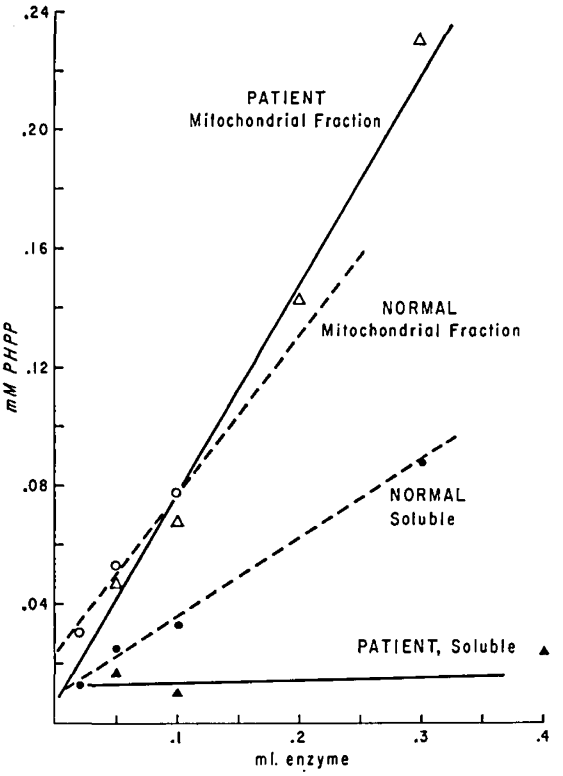

Fig. 1. Comparison of tyrosine aminotransferase activity determined by radiochemical assay of liver fractions from the patient (△) and from normal control (○). Volumes of soluble and mitochondrial fractions, designated by solid and open symbols, respectively, were varied between 0.025 and 0.4 ml, and results were expressed as millimoles of *p*-hydroxyphenylpyruvate formed in 30 min determined by radiochemical technique. Figure 7 in Fellman *et al.* [12].

Amino Acids in Plasma and Urine

Mean tyrosine values found in plasma and urine are given in Table I. Renal tubular reabsorption was 99.7% and 99.8% for tyrosine and phenylalanine, respectively.

Levels of all other amino acids, including phenylalanine and methionine, were consistently normal in both urine and plasma as was the percentage tubular reabsorption. 3,4-Dihydroxyphenylalanine and *o*-tyrosine excretion in urine was less than 1 mg/24 hr and that of δ-aminolevulinic acid less than 2 mg/24 hr in all samples examined.

When the fasting level of tyrosine in plasma was 62 mg/100 ml, the 24-hr urine sample contained 48 mg free tyrosine and 221 mg bound tyrosine. *N*-Acetyltyrosine accounted for 81% of the bound tyrosine.

Identification and Investigation of p-Hydroxyphenylpyruvic Acid

p-Hydroxyphenylpyruvic acid was identified by electrolytic reduction of the 2,4-dinitrophenylhydrazone

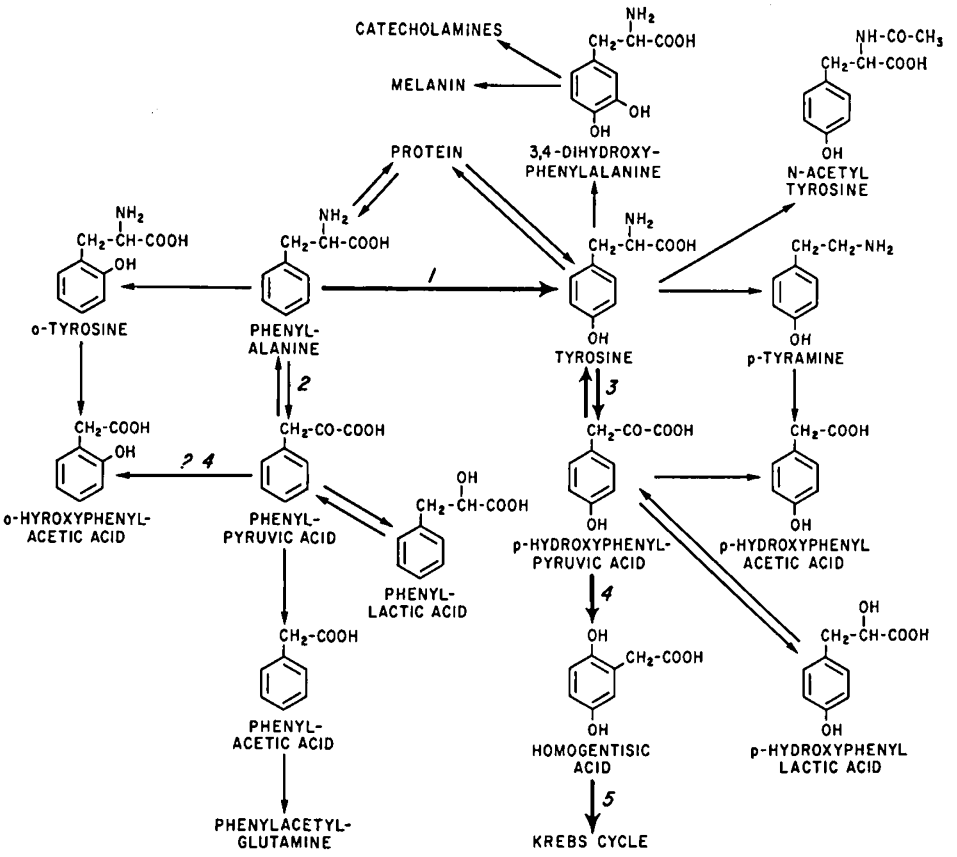

Fig. 2. Outline of aromatic amino acid metabolism: *1*) phenylalanine hydroxylase; *2*) phenylalanine aminotransferase; *3*) tyrosine aminotransferase; *4*) *p*-hydroxyphenylpyruvate hydroxylase; *5*) homogentisic oxidase.

derivative [30] of an ethyl acetate extract which was free of amino acids. Identification of the tyrosine formed was then established by column chromatography. A similar result, through formation of the oxime, was obtained by Dr. K. N. F. Shaw [50]. The identity of *p*HPPA was confirmed by GC and GC-MS. The mass spectrum obtained was in agreement with the expected structure of the derivative and with the standard material.

The *p*HPPA standard was not stable during preparation for GC, decomposing and producing a number of unidentified peaks that eluted soon after the solvent front. *p*-Hydroxyphenylacetic acid was also produced and accounted for 10–20% of the standard, whether or not the extract was taken to dryness [13]. Two additional products were occasionally seen; these had the same methylene units as *p*-hydroxymandelic acid (17.23) and a peak seen occasionally in the patient's urine (17.87), which had a mass spectrum consistent with the structure of *p*-hydroxyphenylacetaldehyde.

Identification of N-Acetyltyrosine

N-Acetyltyrosine was one of the major tyrosine metabolites present in the patient's urine. On TLC it cochromatographed with authentic standard in two different solvent systems. On GC, the ME/TMSi derivative had a methylene unit of 20.02, which was identical with that of the standard. When subjected to GC-MS, the spectrum obtained was consistent with the structure of NAT.

The possibility that NAT could have arisen from tyrosine during ethyl acetate extraction was excluded in two ways. (*1*) Tyrosine was added to normal urine at a concentration of 0.6 mg/ml and the ethyl acetate extract examined by TLC and by GC. No NAT was detected by either procedure. (*2*) A sample of the patient's urine, adjusted to pH 1 with 6 N hydrochloric acid, was extracted with 3 volumes of butanol saturated with 0.1 N hydrochloric acid. The butanol extract was dried and the residue was derivatized and examined by GC in the usual way. *N*-Acetyltyrosine was

present in approximately the same concentration as in the ethyl acetate-ether extract.

Phenolic Acid Excretion in Urine

Thin layer chromatograms of the phenolic acid excretion pattern of the patient fed a plant-free diet and of a premature infant with tyrosyluria are shown in Figure 4.

Gas chromatography was performed upon several urine extracts. Methylene units of standard compounds were reproducible within 0.02 and most values agreed within 0.04 with values obtained under similar conditions in other laboratories [13, 18]. A typical chromatogram of urine, obtained while the patient was fed a plant-free diet, and after oral administration of neomycin and sulfathalazole for 3 days, is shown in Figure 5.

The identification of phenyllactic acid, *p*HPAA, homovanillic acid, *p*-hydroxymandelic acid, *p*HPLA, vanillylmandelic acid, *p*HPPA, NAT, and *p*-cresol glucuronide was confirmed by GC-MS. A number of other minor peaks (A-E, Fig. 5) were also examined; their methylene units are given in Table II. Some of these

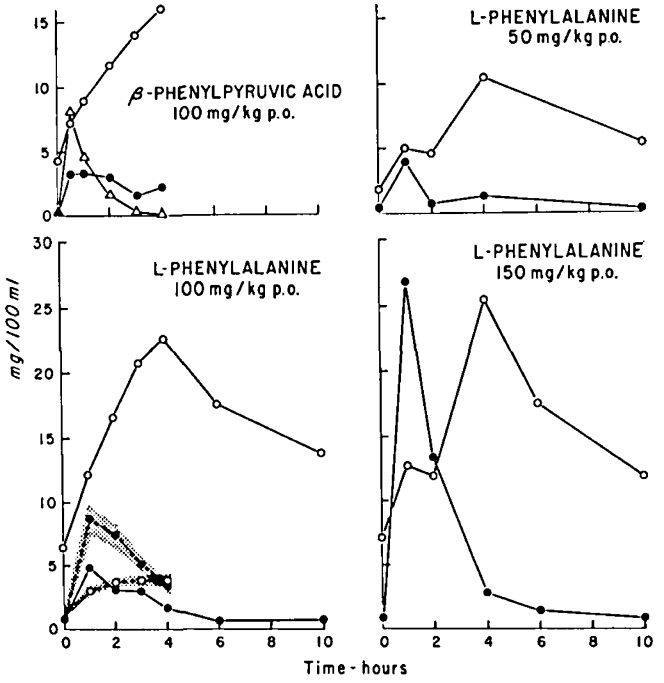

Fig. 3. Oral tolerance tests with L-phenylalanine and phenylpyruvic acid performed at age 2.5 years. ○——○: Serum tyrosine; ●——●: serum phenylalanine; △——△: serum phenylpyruvic acid. The normal controls (mean ±SE) are from Wang *et al.* [41]. ○- - -○: Tyrosine; ●- - -●: phenylalanine.

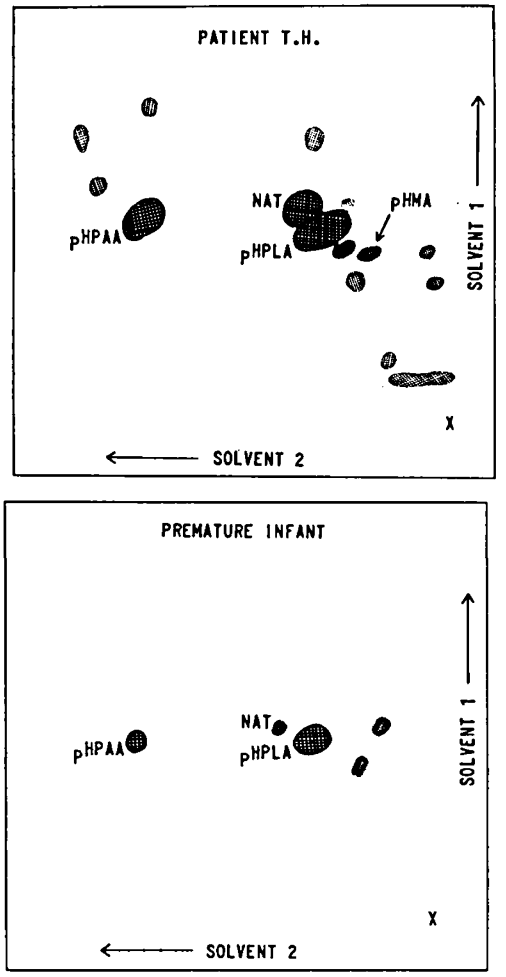

Fig. 4. Thin layer chromatograms of urine containing 30 μg creatinine from the patient and from a premature infant with tyrosyluria.

appear to be tyrosine derivatives by GC-MS, but they have not been identified.

Quantitative results of phenolic acids in urine when the patient was on a plant-free diet and a low tyrosine diet are given in Table III. The range of excretion of these metabolites in three premature infants with tyrosyluria is shown in Table IV. The results are expressed in terms of the creatinine excretion because of the difficulty of obtaining reliable 24-hr urine volumes.

Effect of Increasing Dietary Phenylalanine

To determine the effect of tyrosine levels in plasma on excretion of tyrosine metabolites in urine, the patient was fed a diet containing daily increasing supplements of phenylalanine over a period of 6 days. Blood was obtained daily [51] and 24-hr urine collections

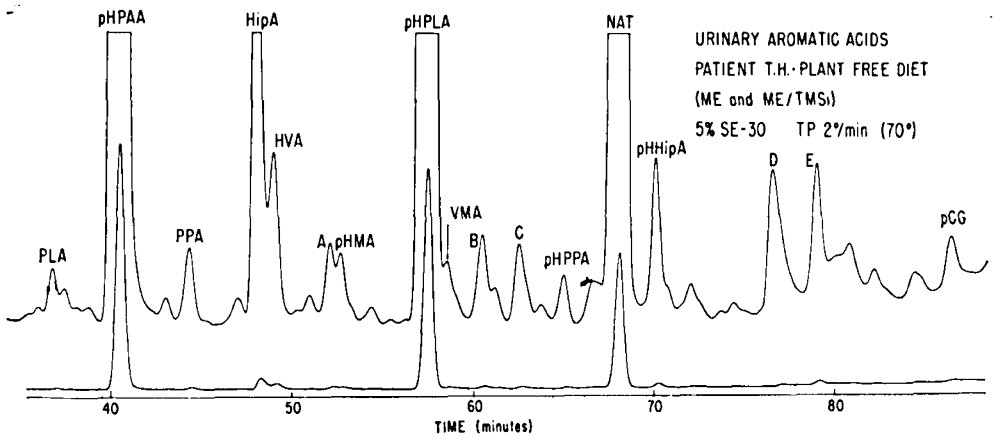

Fig. 5. Gas chromatogram of excretion of aromatic acids in urine by the patient on a plant-free diet, after 3 days of orally administered neomycin and sulfathalazole. For conditions, see *Methods* section.

Table II. Methylene units of methyl ester and methyl ester-trimethylsilyl ether derivatives of standard aromatic acids and some unidentified peaks in the patient's urine

Standard	Methylene unit
Phenylacetic acid	11.43
o-Hydroxyphenylacetic acid	14.51
Phenyllactic acid	14.63
p-Hydroxybenzoic acid	14.73
p-Hydroxyphenylacetic acid	15.19
Phenylpyruvic acid	15.85
Hippuric acid	16.38
Homovanillic acid	16.58
3,4-Dihydroxybenzoic acid	17.11
p-Hydroxymandelic acid	17.23
3,4-Dihydroxyphenylacetic acid	17.29
Homogentisic acid	17.50
p-Coumaric acid	17.59
p-Hydroxyphenyllactic acid	18.01
Vanillylmandelic acid	18.28
p-Hydroxyphenylpyruvic acid	19.46
m-Hydroxyhippuric acid	19.78
N-Acetyltyrosine	20.02
p-Hydroxyhippuric acid	20.47
p-Cresol glucuronide	24.26
Unidentified peaks in patient's urine	
A	17.11
B	18.61
C	19.00
D	21.95
E	22.46

were made during this period. Levels of tyrosine in plasma and phenolic acid values in urine were determined within 1 week of collection. These results are presented in Table V.

The excretion of a number of other aromatic acids increased while the patient was receiving daily increasing supplements of phenylalanine. The amount of phenylpyruvic acid increased approximately 3-fold during this period while that of phenyllactic acid increased more than 20-fold. There was no increase in levels of *o*-hydroxyphenylacetic acid. Two other peaks (A and E, Table II, Fig. 5) showed a consistent rise during this time; the methylene unit of peak A is the same as that of 3,4-dihydroxybenzoic acid. No other peak showed a parallel, consistent increase. Excretion of phenylacetic acid in urine was variable, as was that of *m*-hydroxyhippuric acid, *p*-hydroxyhippuric acid, and *p*-cresol glucuronide. Levels of homovanillic acid, *p*-hydroxymandelic acid, and vanillylmandelic acid showed little variation. Excretion of indoles did not increase over the 6 days.

Phenylpyruvic acid and phenyllactic acid were notably increased in the urine sample from the tyrosyluric infant with the highest blood tyrosine level; *o*-hydroxyphenylacetic acid was not detectable.

Effect of Oral Antibiotics

The effect of orally administered antibiotics on the excretion of *p*-tyramine in urine was studied in the patient on two separate occasions. These results, along with corresponding tyrosine levels in plasma and tyrosine levels and "total phenols" (as tyrosine equivalents) in urine are presented in Table VI.

Examination of Phenolic Acids in Serum

The organic acid fraction from serum was examined by GC-MS at a time when the tyrosine level in plasma was 62 mg/100 ml. The sample had been stored frozen for 1 week. A small peak of *p*HPLA was detected, and its identity was established by the mass spectrum.

In a second sample of serum, obtained when the

Table III. Excretion of tyrosine and tyrosine metabolites in urine by patient fed a plant-free diet and a low tyrosine diet

Drugs	Vol/24 hr, ml	Creatinine, mg/100 ml	Urine: Tyr[1]	*p*HPPA[2]	*p*HPAA	*p*HPLA	NAT	Tyrosine equiv[2]
			mg/mg creatinine					
Normal control, aged 4 yrs (plant-free diet)[4]								
None	350	55		0.02	0.04	0.005	<0.01	
Patient, aged 4 yrs (plant-free diet)[4]								
None	265	57	0.32	0.60	0.72	1.10	1.09	4.9
N + S,[5] 2 days	290	57	0.51	0.63	1.13	1.04	0.93	4.7
N + S, 3 days	355	60	0.94	0.74	1.23	1.10	1.15	4.4
Patient, aged 2.5 yrs (low tyrosine diet)								
None	380	20	0.02	<0.005	0.04	0.01	<0.01	0.07

[1] See text for abbreviations.
[2] Measured after storage at −20° for 3–5 months.
[3] Millon reaction.
[4] A plant-free diet was prepared according to Perry [32].
[5] N: neomycin, 100 mg/kg/24 hr *per os;* S: sulfathalazole, 150 mg/kg/24 hr *per os.*

Table IV. Excretion of tyrosine and tyrosine metabolites in three premature infants with tyrosyluria

Plasma	Urine				
Tyr, mg/100 ml	Tyr[1]	*p*HPPA	*p*HPAA	*p*HPLA	NAT
	mg/mg creatinine				
9.2–29.5	0.14–0.29	<0.2–1.4	0.24–0.85	0.44–1.26	0.03–0.22

[1] See text for abbreviations.

time. The deficiency of hepatic soluble tyrosine aminotransferase activity prompted Fellman *et al.* to search for alternative pathways of tyrosine metabolism and led to a demonstration of a mitochondrial tyrosine aminotransferase which was distinct from the enzyme in the cellular supernatant [12, 24].

The normal hepatic mitochondrial enzyme activity in the patient could account for the production of the

Table V. Effect of increasing dietary phenylalanine on the level of tyrosine in plasma and on the excretion of tyrosine and tyrosine metabolites in urine by the patient

Day	Diet: Phe mg/24 hr[1]	Diet: Tyr	Plasma: Tyr[2], mg/100 ml	Urine: Vol/24 hr, ml	Creatinine, mg/100 ml	Tyr[3]	*p*HPPA	*p*HPAA	*p*HPLA	NAT
						mg/mg creatinine				
1	760	260	3.3	208	59	0.05	0.005	0.25	0.06	<0.01
2	1500	81	4.5	463	24	0.12	0.03	0.13	0.05	<0.01
3	2600	100	14.4	328	40	0.32	0.25	0.25	0.14	0.08
4	3600	115	27.3	150	67	0.47	0.79	0.60	0.56	0.20
5	5700	200	39.8	128	78	0.44	1.63	0.59	0.79	0.52
6	5600	90	51.4	256	64	0.54	1.82	1.21	1.68	1.60

[1] Weight: 12.5 kg.
[2] Plasma phenylalanine levels were all within normal limits.
[3] See text for abbreviations.

tyrosine level was over 50 mg/100 ml, *p*HPPA was 0.36 mg/100 ml by the enol-borate method.

Discussion

The patient who is the subject of this communication exhibited a rare, if not unique, disorder of tyrosine metabolism. The finding of gross elevations of both substrate and product of a missing enzyme by conventional assay appeared to be without parallel at the tyrosine metabolites. Alternatively, the kidney might possess another tyrosine aminotransferase isozyme which would presumably be intact. The finding of small amounts of *p*HPPA and *p*HPLA in the plasma would be consistent with either hypothesis. Renal biopsy has not been done since the patient has congenital absence of one kidney. Two explanations of the failure of subsequent phenolic acid metabolism seem possible. The first is that a change in the normal site of tyrosine degradation within the cell could cause *p*HPPA

Table VI. Effect of orally administered antibiotics on the excretion of *p*-tyramine and "total phenols" in patient[1]

Drugs[2]	Plasma	Urine				
	Tyr, mg/100 ml	Vol/24 hr, ml	Creatinine, mg/100 ml	Tyr	*p*-Tyramine (free base)	Tyr equiv[3]
				mg/mg creatinine		
None	—	315	57	0.05	0.017	3.1
N, 5 days	24	234	49	0.18	0.025	3.9
None	51	340	61	0.51	0.031	3.6
None	—	252	70	0.26	0.024	3.7
N + S, 5 days[4]	—	198	61	0.83	0.041	4.5
N + S, 6 days[5]	29	248	68	0.31	0.044	3.1

[1] Patient was 3 years of age and was given a normal diet.
[2] N: neomycin, 100 mg/kg/24 hr *per os;* S: sulfathalazole, 150 mg/kg/24 hr *per os*.
[3] Millon reaction.
[4] Stool culture showed a scanty growth of *Streptococcus faecalis* and a few unidentified anaerobes.
[5] Stool culture showed a scanty growth of *Escherichia coli* and a few unidentified anaerobes.

accumulation in mitochondria which lack *p*-hydroxyphenylpyruvate hydroxylase activity [12]. If this were so, *p*HPPA might leach from the mitochondria and escape from the cells before conversion to homogentisic acid. Alternatively, high levels of tyrosine in plasma could inhibit the oxidase system in the liver or elsewhere and cause *p*HPPA and its metabolites to appear in the urine. A likelier explanation for urinary *p*HPPA may be a low renal tubular Tm *p*HPPA in the presence of elevated plasma levels released from tissues which have transaminase but lack *p*-hydroxyphenylpyruvate hydroxylase activity.

In the case of tyrosinosis reported by Medes, enzyme studies were not performed, but it has been suggested that a defect of tyrosine aminotransferase activity might have been present [25]. Our finding of accumulation of *p*HPPA in the presence of normal *p*-hydroxyphenylpyruvate hydroxylase activity is consistent with a defect of one form of tyrosine aminotransferase activity in Medes' patient.

Another patient with tyrosinemia but without hepatorenal damage has been reported [40]. This patient had findings in urine similar to those found in our patient, but no enzyme studies were made. In two other patients with tyrosinemia [10, 27] a partial deficiency of tyrosine transaminase activity together with a deficiency of *p*-hydroxyphenylpyruvate hydroxylase activity has been suggested. Both of these patients had liver and kidney damage and phenolic aciduria without elevated levels of *p*HPPA. An alternative explanation in these cases might be deficiency of hepatic, but intact renal, *p*-hydroxyphenylpyruvate hydroxylase activity.

In our patient, the phenylalanine level in plasma was not elevated in over 50 samples. Moreover, the phenylalanine load tests suggested a normal conversion of phenylalanine to tyrosine at all doses regardless of the sustained high tyrosine levels. This was unexpected since in infants with tyrosyluria and in patients with tyrosinemia the phenylalanine levels in plasma are often elevated. This has been thought to be secondary to the raised tyrosine levels [2]. Our finding argues against the suggestion that the hyperphenylalaninemia found in infants with tyrosyluria is secondary only to tyrosine accumulation.

The phenylpyruvic acid tolerance test produced a rise in phenylalanine levels in plasma and a prolonged rise in tyrosine levels, similar to that produced by the same dose of phenylalanine. This confirms that phenylalanine aminotransferase activity was present in this patient.

In normal subjects, *o*-hydroxyphenylacetic acid is thought to be produced from *o*-tyrosine whereas the large amounts excreted by patients with phenylketonuria are thought to be formed from phenylpyruvic acid by the action of phenylpyruvate oxidase [29]. The identity of this enzyme with *p*-hydroxyphenylpyruvate hydroxylase has been suggested [38] (Fig. 2). This is supported by the absence of both these enzyme activities in liver and kidney from a patient with tyrosinosis [37] and our finding of increased phenylpyruvic and phenyllactic acid and normal *o*-hydroxyphenylacetic acid excretion in infants with tyrosyluria. Similar findings in the urine of our patient suggest that the failure of phenylpyruvic acid metabolism is caused by the same mechanism that inhibits oxidation of *p*HPPA.

During TLC [14] and GC, *p*HPPA decomposes to *p*HPAA and a number of other compounds. The *p*HPPA was therefore quantitated spectrophotometrically. Degradation to *p*HPAA means that up to 30% of the *p*HPAA measured by GC could have been formed in this way.

Levels of *p*HPPA, *p*HPAA, *p*HPLA, and NAT excreted in urine were extremely high in the patient fed an unrestricted or a normal protein, plant-free diet (Table III). The *p*HPPA values (Table III) are low since the urine samples had been stored at −20° for 3–5 months and *p*HPPA deteriorates at a rate of approximately 5%/week under these conditions [13].

The phenolic acid values were not grossly affected by the administration of oral antibiotics to the patient, indicating that intestinal bacteria played at most a

minor role in the production of these compounds from tyrosine. When the patient was fed a low tyrosine diet, however, the excretion of phenolic acids fell to normal levels.

p-Hydroxyphenylpyruvic acid, *p*HPAA, and *p*HPLA are excreted in large amounts in the urine of infants with tyrosluria and patients with inherited tyrosinemia associated with deficiency of *p*-hydroxyphenylpyruvate hydroxylase activity. Excretion of NAT in urine does not appear to have been studied in these cases but has been reported in some patients with tyrosyluria [9, 10, 40]. In the present study, NAT was identified in the urines of three infants with tyrosyluria as well as in the patient's urine. The values in Table V indicate that NAT appeared in the patient's urine when the tyrosine level in plasma was between 4.5 and 14.4 mg/100 ml. The lowest tyrosine value in the plasma of three premature infants was 9.2 mg/100 ml. The finding of NAT in the urine of patients with tyrosinemia and of *N*-acetylphenylalanine in urine of patients with phenylketonuria [15] suggests that *N*-acetylation of aromatic amino acids is a normal, albeit minor, metabolic pathway. The fact that NAT cochromatographs with *p*HPLA in several different solvent systems may explain why it has not been detected more often in the past.

The figures in Table V indicate that with rising tyrosine levels in plasma, the excretion of tyrosine as a percentage of total urinary phenolic acids fell from 33 to 8%. This was presumably due to the maintenance of normal tubular reabsorption of tyrosine in the face of less efficient renal conservation of its metabolites. This mechanism may explain the different tyrosine/phenolic acid ratios seen in urine of patients with tyrosinemia and neonatal tyrosinemia.

The excretion of *p*-tyramine in normal children is 0.1–0.5 μg/mg creatinine [34]. In the patient's urine, this compound was increased more than 100-fold and was not lowered by treatment with oral antibiotics (Table VI). The possible increase during antibiotic therapy argues against the theory that *p*-tyramine in urine is normally of bacterial origin from the gut [33], although it is possible that antibiotics decreased tyrosine absorption from the bowel, leaving more substrate for surviving intestinal bacteria to decarboxylate. Diminution of intestinal flora, after antibiotic therapy, accompanied by increased excretion of amines was also observed by DeQuattro and Sjoerdsma [8], and increased levels of *p*-tyramine in urine along with normal fecal levels of this substance were reported in premature infants with tyrosyluria [3]. Our results appear to confirm the conclusion of these authors that *p*-tyramine found in urine is largely of tissue, and not bacterial, origin.

The absence of indoluria in our patient and in infants with tyrosyluria contrasts with that seen in patients with phenylketonuria. This suggests that, unlike phenylalanine, neither tyrosine nor its metabolites interfere with tryptophan transport in the gut or in its subsequent metabolism [1].

The cause of the hepatorenal damage in tyrosinemia associated with *p*-hydroxyphenylpyruvate hydroxylase deficiency is not known. Since liver biopsy on this patient showed no cellular destruction and renal tubular reabsorption of amino acids was normal [5], we believe that tissue damage is not caused by high tyrosine levels. Development of the different cellular forms of tyrosine aminotransferase has been studied in fetal monkeys [24], and it has been shown that soluble enzyme is normally not active until just before birth. For this reason, we suggest that the multiple congenital anomalies present in our patient were not causally related to the enzyme defect.

Summary

Until recently, hepatic tyrosine aminotransferase activity has been thought to reside mainly in the soluble fractions of cell homogenates. Our finding of gross phenolic aciduria in a patient with deficiency of this enzyme led us to examine the metabolism of tyrosine and the pattern of the urinary phenolic acids in detail. *p*-Hydroxyphenylpyruvic acid, *p*-hydroxyphenyllactic acid, *p*-hydroxyphenylacetic acid, and *N*-acetyltyrosine were the major phenolic compounds identified in the urine. All these compounds were also present in the urines of three infants with tyrosyluria. The reason for the failure of *p*-hydroxyphenylpyruvic acid oxidation in the patient in the presence of normal *p*-hydroxyphenylpyruvate hydroxylase activity is not proven.

Phenylalanine levels were never elevated, and load studies showed rapid conversion of phenylalanine to tyrosine and of phenylpyruvic acid to phenylalanine. Levels of *p*-tyramine excreted in urine were grossly elevated and were not reduced by oral antibiotics.

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49. Model 9000, LKB Instruments, Rockville, Md.
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