ABNORMAL TYROSINE AND PHENYLALANINE METABOLISM IN PATIENTS WITH TYROSYLURIA AND PHENYLKETONURIA; GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF URINARY METABOLITES

S. K. WADMAN, C. VAN DER HEIDEN, D. KETTING AND F. J. VAN SPRANG
Laboratory of the Wilhelmina Kinderziekenhuis, Nieuwe Gracht 137, Utrecht (The Netherlands)

SUMMARY

Gas–liquid chromatographic methods have been developed for the analysis of: urinary phenylalanine metabolites (I) in patients with phenylketonuria, tyrosine metabolites (II) in patients with a disturbed tyrosine metabolism at the level of *p*-hydroxyphenylpyruvate hydroxylase, and homogentisic acid in alkaptonuria.

Metabolites I include: phenylpyruvic, -lactic, -acetic (free and conjugated), -mandelic, *o*-hydroxyphenylacetic and benzoic (free and conjugated) acids. Metabolites II include: *p*-hydroxyphenylpyruvic, -lactic, -acetic and *p*-hydroxymandelic acids.

Urinary excretions of phenylalanine and its waste metabolites I in patients with phenylalanine hydroxylase deficiency, at high and moderate loads are given. In 3 patients with classical phenylketonuria the total excretion of phenylalanine and its waste metabolites were found to be 90, 81 and 82% of the phenylalanine intake. In 2 other patients, with a higher phenylalanine tolerance, 19 and 34% was found. The excretion intake ratio is proposed to be a better parameter for phenylalanine hydroxylation capacity than is plasma phenylalanine.

Urinary excretions of tyrosine and its waste metabolites in 2 patients with (inherited) liver disease and tyrosyluria were studied and compared with the urinary findings in a patient who presumably had a primary *p*-hydroxyphenylpyruvate hydroxylase deficiency. In one of the patients with liver disease the excretion/load ratio was determined. It is suggested that this ratio is a better clinical parameter than is plasma tyrosine.

INTRODUCTION

In the last few years gas–liquid chromatographic (GLC) techniques have been appearing for the analysis of phenylalanine and tyrosine metabolites in patients with phenylketonuria and tyrosyluria respectively[1–4]. Previously[5], we presented a communication about the GLC determination of urinary phenylpyruvic, phenyllactic, phenylacetic (free and conjugated), *o*-hydroxyphenylacetic, mandelic, and benzoic

(free and conjugated) acids. Some results in patients with phenylketonuria were given. Also tyrosyluria was analyzed by gas chromatography in our laboratory. We determined urinary *p*-hydroxyphenylpyruvic, *p*-hydroxyphenyllactic, *p*-hydroxyphenylacetic and *p*-hydroxymandelic acids in an exceptional case of presumed inborn *p*-hydroxyphenylpyruvic acid hydroxylase deficiency[6].

In the meantime, our methods have been improved: isothermal analysis was replaced by a temperature gradient technique and a high performance stationary phase was applied, both resulting in a better separation. Automatic integration improved accuracy. The new chromatographic procedure is suited for the separation of all above-mentioned compounds as they appear in the urine of such patients. Furthermore other urinary phenolic and phenyl acids, formed by intestinal bacteria from non-absorbed tyrosine and phenylalanine respectively can be analyzed now[7,8]. Also urinary homogentisic acid in patients with alkaptonuria can be determined.

In the present paper this method is described as well as are the preliminary steps necessary for the isolation of the compounds from the urine and their conversion into volatile derivatives. A few results in patients with phenylketonuria and tyrosyluria is given and the clinical applicability of such parameters is discussed.

METHODS

Metabolites in phenylketonuria (PKU)

In Scheme 1 the analytical steps are given. Free acids: phenylpyruvic acid (PPA), phenyllactic acid (PLA), *o*-hydroxyphenylacetic acid (*o*-OHPAA) and mandelic acid (MA) are extracted with ethyl acetate. The extract also contains some free benzoic acid, some free phenylacetic acid, hippuric acid and many other aromatic and aliphatic compounds. Hippuric acid does not interfere with typical PKU metabolites in the chromatograms. Most other substances generally occur in amounts small enough to justifiy their neglection when interfering at all with main metabolites. However, preliminary inspection by two-dimensional paper or thin-layer chromatography of the phenolic acids remains necessary. Free salicylic acid and *β-m*-hydroxyphenylhydracrylic acid (which is excreted in relatively large amounts by some patients on a free diet) interfere with the internal standard I and II respectively. Therefore prior to urinary collection all medication must be stopped and preferably the patient should be given a simple plant-free diet. Alternatively other internal standards can be chosen (*e.g.* phenylpropionic instead of phenylbutyric acid). A guarantee for the absence of compounds interfering with internal standards can be obtained from a "standard-free" analysis.

Step A 1. Extraction. To 5 ml of cold urine is added: 10 mg ascorbic acid (for

SCHEME 1.

ANALYTICAL STEPS A AND B FOR PHENYLALINE METABOLITES IN PHENYLKETONURIA. ANALITICAL STEPS A FOR *p*-HYDROXYPHENYL ACIDS IN TYROSYLURIA.

A	B
1. Extraction of *unconjugated* acids with ethyl acetate.	1. Removal of phenylpyruvic acid with dinitrophenyl hydrazine.
2. Conversion to volatile TMS derivatives.	2. Alkaline hydrolysis of *conjugated* acids.
3. Gas chromatography.	3. Extraction, etc. as in A 1, A 2, A 3.

protection of PPA against oxydation), 1.0 mg phenylbutyric acid (internal standard I) and 1.0 mg *p*-hydroxyphenylbutyric acid (internal standard II). The pH is adjusted to 2.0 with conc. HCl, 5 ml of saturated NaCl solution is added and the mixture is extracted twice with 20 ml of ethyl acetate. The extract is dried on Na_2SO_4 and the solvent is evaporated in vacuum (Rotavapor) below 30°.

Step A 2. Trimethylsilylation. Trimethylsilylation according to Klebe *et al.*[9] was preferred to methylation. By the former method single ether/ester derivatives are obtained in a good yield; reactions are rapid at low temperature. Even PPA gives rise to a single TMS derivative appearing as a symmetrical peak in the chromatogram.

The residue obtained from step A 1 is dissolved in 1 ml of chloroform containing bis(trimethylsilyl)acetamide (1:3 v/v). The solution is left at 30° in a paraffin bath for 20 min and is then ready for injection on the column.

Steps B 1 and B 2. Phenylacetic acid (PPA) and benzoic acid (BA) mainly occur conjugated with glutamine and glycine respectively. The glutamine conjugate is not easily extractable with ethyl acetate. Total PAA and total BA can be extracted after hydrolysis. However, PPA gives rise to various volatile products when subjected to hydrolysis and is removed before by precipitation as a dinitrophenyl hydrazone. The excess of dinitrophenyl hydrazine is eliminated as acetone dinitrophenyl hydrazone. Hydrolysis is effectuated by NaOH. Acid hydrolysis seemed less suited as PLA can decompose into cinnamic acid and other products in strong acids.

B 1: To 5 ml urine 5 ml 2,4-dinitrophenyl hydrazine (0.2% in 2 *N* HCl) is added. The mixture is heated at 37° for 30 min. Then 0.2 ml acetone is added and again the mixture is heated at 37° for 30 min. After centrifugation the supernatant is collected quantitatively.

B 2: To the supernatant 10 ml of 11 *N* NaOH and 1.0 mg phenylbutyric acid (internal standard) are added. The mixture is heated at 100° for 3 h in a closed tube. It is then cooled below 5° and acidified to pH 1.5 with concentrated HCl.

After steps B 1 and B 2 proceed as in A 1 and A 2.

Metabolites in tyrosyluria

Urinary p-hydroxyphenylpyruvic (*p*-OHPPA), *p*-hydroxyphenyllactic (*p*-OHPLA), *p*-hydroxyphenylacetic (*p*-OHPAA) and *p*-hydroxymandelic (*p*-OHMA) acids occur as free acids. For these compounds the steps A 1 and A 2 can be followed. As from PPA also from *p*-OHPPA a single TMS compound is formed emerging as a symmetrical peak at the end of the chromatogram.

Homogentisic acid in alkaptonuria

For homogentisic acid (HGA) the steps A 1 and A 2 can be followed. The TMS derivative of HGA coincides with that of hippuric acid in the chromatogram. Yet, the influence of the latter can be neglected at the level of dilution used for injection on the column. The excretion of HGA considerably exceeds that of hippuric acid and its TMS derivative gives a much greater response in the flame ionization detector.

Step A 3. Gas chromatography. The F & M 810 instrument equipped with a Hewlett–Packard Integrator 3370 A was used. Columns: stainless steel, 8 ft × 1/8 inch. Stationary phase: 5% SE52 on Chromosorb W AW DMCS; 100–120 mesh. Temperatures: over 100–220° (2°/min); injection port 190°; detector 220°. Gas flows:

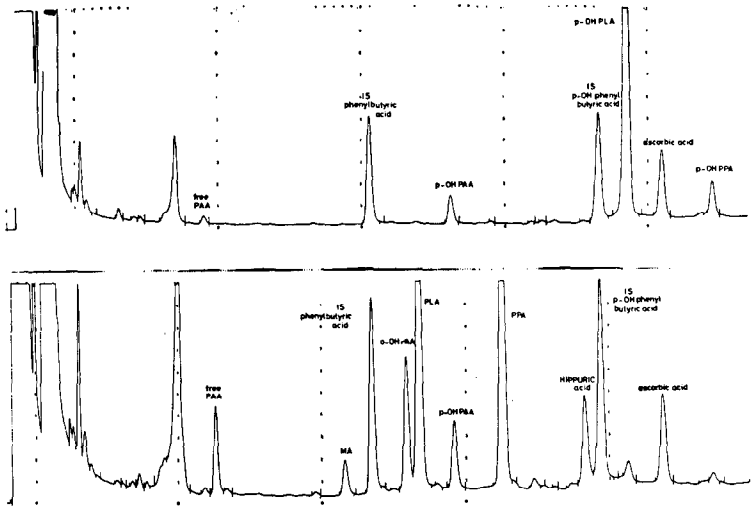


Fig. 1. Chromatograms in tyrosyluria (upper) and phenylketonuria (lower).

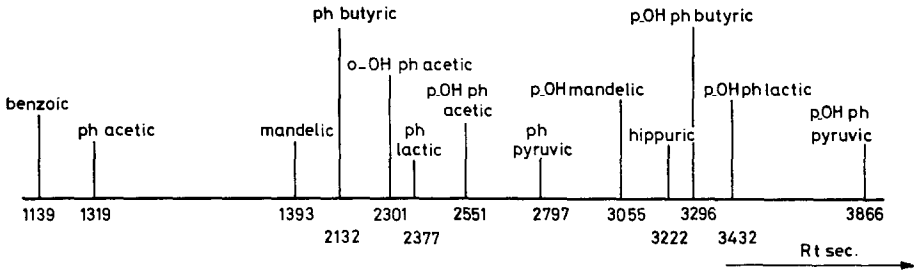


Fig. 2. Rt values of phenyl and *p*-hydroxyphenyl compounds.

N_2 27 ml/min; H_2 28 ml/min; air 450 ml/min. Range: 10^2. Attenuation: 8. Chart speed 1/2 inch/min.

For calculations a calibration curve for each compound is used. Such curves are practically linear up to 3 μg, except in the very low ranges here not used. Compensation for methodical variations is obtained by using internal standards.

Additional specifications and remarks

The gas-chromatographic determination of urinary phenylalanine metabolites in phenylketonuria and tyrosine metabolites in tyrosyluria and alkaptonuria is greatly facilitated by the great excess of the typical metabolites as compared with other excretory products, allowing for the application of a single separatory method for all compounds. The addition of ascorbic acid prevents the oxidation of PPA, *p*-OHPPA and HGA, although this addition gives rise to an extra peak in the chromatogram between *p*-OHPLA and *p*-OHPPA.

In Fig. 1 characteristic chromatograms are shown. Rt values can be read from Fig. 2. In Table I the results from reproducibility and recovery experiments are given. The data were obtained from urine to which known amounts of all compounds were added. PPA and *p*-OHPPA are more susceptible to losses than are the other acids.

TABLE I

REPRODUCIBILITIES (S, %) AND MEAN RECOVERIES

N = 5.

	S. % of mean	*Mean recovery %*
Free phenylacetic acid	1.2	91.5
Mandelic acid	0.8	101
o-Hydroxyphenylacetic acid	2.9	100
Phenyllactic acid	3.0	94.5
Phenylpyruvic acid	4.8	92
p-Hydroxyphenylacetic acid	5.2	110.5
p-Hydroxymandelic acid	1.0	96.5
p-Hydroxyphenyllactic acid	2.3	108
p-Hydroxyphenylpyruvic acid	3.2	86
Total benzoic acid*	2.9	105
Total phenylacetic acid*	3.4	95

* For these compounds the values given in ref. 8 are listed.

From aqueous solutions containing ascorbic acid PPA and *p*-OHPPA can be recovered completely but, when dissolved in urine, their recovery is somewhat lower. For these compounds step A 1 is probably the most critical one. Evaporation of the ethyl acetate must be done at a temperature as low as possible and A 2 has to be performed without delay. When dissolved in urine and stored at −20° *p*-OHPPA is stable.

Collection of urine samples

Drug-free 24-h urine samples are collected by freezing each portion in a polythene stock bottle at −20°. For a quantitative interpretation of the analytical data patients must be on a constant well-known protein intake for some days. Preferably a plant-free diet should be given in order to reduce the excretion of exogenous aromatic products as much as possible.

CLINICAL APPLICATIONS AND COMMENTS

In this section we shall give some results of the analysis here described from which its use for clinical purposes may appear. The main features of the metabolism of phenylalanine and tyrosine have been known for many years. An excellent review was given by Woolf[10] and since then comparatively little new basic information has been added. A survey of main metabolic routes is given in Scheme 2.

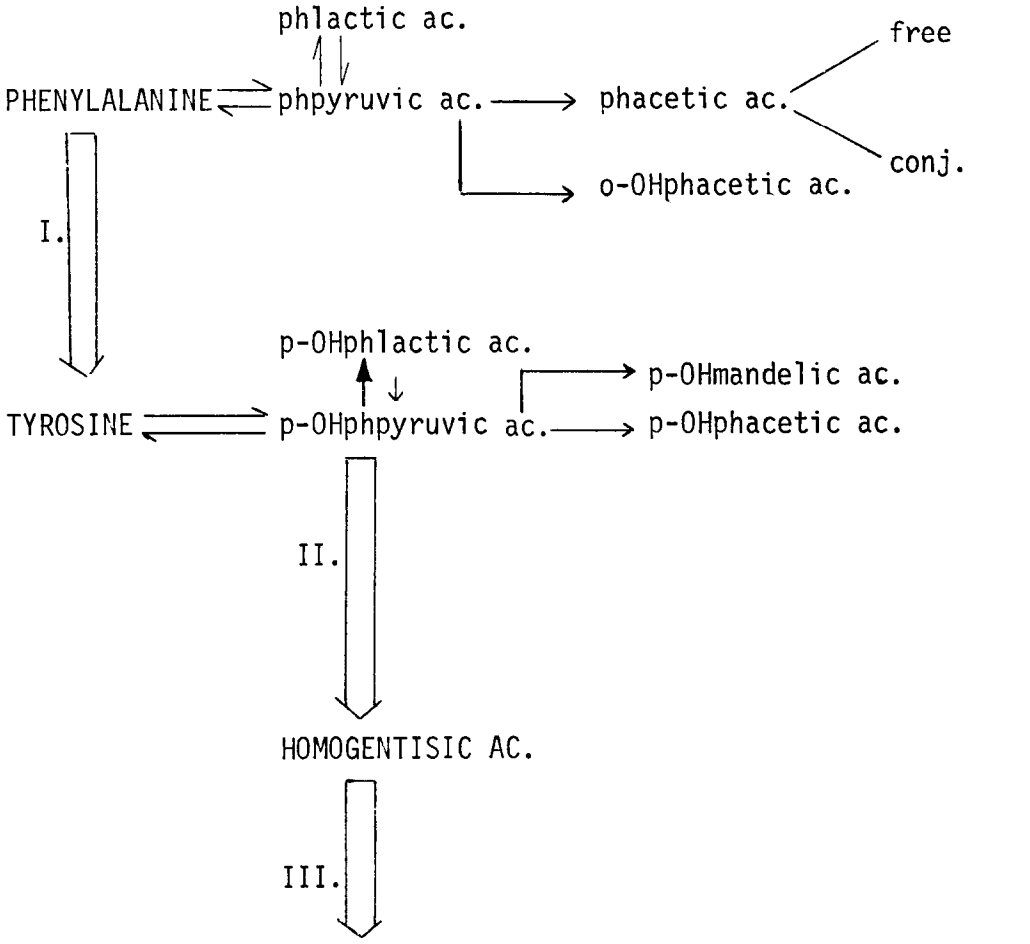
Abnormal phenylalanine metabolism

The urinary excretion pattern of patients with phenylalanine hydroxylase deficiency is dependent on the degree of phenylalanine overflow. This can be seen from Table II. At plasma phenylalanine concentrations of 10 mg/100 ml and lower the PPA formed is converted quantitatively into PPA and *o*-OHPAA, the latter being a minor metabolite. At a higher plasma phenylalanine, between 10 and 20 mg/100 ml, the exact level being somewhat different in one patient or the other, the oxidative decarboxylation of PPA is apparently not rapid enough to compensate for its formation. Then PPA and PLA begin to accumulate. At a still higher plasma phenylalanine

SCHEME 2

PHENYLALANINE AND TYROSINE METABOLISM

Main endogenous metabolic routes. I. Block in phenylketonuria
II. Block in tyrosyluria.
III. Block in alkaptonuria.



concentration, PPA and PLA are main excretory products, their amounts being about equal. Then well-known picture of phenylketonuria is present.

Total benzoic acid varies greatly, even in the same patient. This is thought to be a bacterial metabolite formed from non-absorbed phenylalanine in the intestinal

TABLE II

URINARY EXCRETION OF PHENYLALANINE METABOLITES (mmoles/g CREATININE) AND FASTING SERUM PHENYLALANINE (mg/100 ml) IN PATIENTS WITH PHENYLKETONURIA

For abbreviations of urinary metabolites see text. PA = phenylalanine.

Patient	*BA*	*PAA*	*MA*	*o-OHPAA*	*PLA*	*PPA*	*PA (urine)*	*PA (serum)*
Ku.	1.67	8.70	0.27	1.14	10.3	18.6	2.51	32.0
	1.11	1.85	0.07	0.46	0.68	1.21	1.46	15.5
Wo.	2.38	18.6	0.36	1.67	11.1	17.1	4.48	49.5
	1.06	4.43	0.05	0.23	0.41	0.38	1.36	23.1
Wi.	3.56	4.59	0.15	0.74	0.82	0.95	1.00	13.8
	3.07	4.77	0.14	0.83	1.46	1.20	0.99	18.8

TABLE III

EXCRETION OF PHENYLALANINE AND METABOLITES COMPARED WITH PHENYLALANINE INTAKE AND FASTING SERUM PHENYLALANINE

	Intake		*Excretion*		*Serum phal. mg%*
	mmoles/24 h	*mg/kg*	*mmoles/24 h*	*%*	
PKU patients					
Ku.	8.8	121	7.9	90	28.1
$2^0/_{12}$ y.	8.8	121	7.9	90	27.7
Wo.	9.5	185	7.7	81	52.8
$1\ ^1/_{12}$ y.					
Wi.	15.5	57	12.7	82	19.3
$19\ ^4/_{12}$ y.					
Variant forms?					
Pa.	1.6	89	0.3	19	29.7
10 days					
Cr.	24.2	220	8.2	34	21.5
$6\ ^4/_{12}$ y.	24.2	220	8.4	35	27.5

lumen. Also PAA may partly arise from exogenous bacterial phenylalanine metabolism[7].

Frequently, a small amount of mandelic acid was found[5] as was also observed by Blau[2]. It increased and decreased together with plasma phenylalanine. Presumably this compound is a metabolite of PPA.

The picture here described resulted from a study of metabolic events in several patients with phenylketonuria who, after some days, were put on a low phenylalanine diet. Urinary excretions and plasma phenylalanine were analyzed at regular times according to [5] and [11] before and after institution of the diet.

In classical phenylketonuria phenylalanine hydroxylase activity in the liver is practically zero. Yet some phenylalanine (about 1/15 of the normal level) can be converted into tyrosine as Udenfriend and Bessman[12] demonstrated in experiments with ^{14}C-labeled phenylalanine. In Table III the total 24-h excretion of phenylalanine+metabolites is compared with the intake in three presumably well-equilibrated classical patients. Excretions amounted to 90, 81 and 82% of the intake, suggesting a severely diminished hydroxylating capacity.

In Table III, also data of two other patients Pa. and Cr. are shown, who only eliminated 19 and 34.5% of the load as urinary phenylalanine+metabolites.

Patient Pa. (second child of the family) was a boy of 10 days, whose sister (first child) had phenylketonuria. Having been on a diet of 37 mg phenylalanine per kg for

7 days his serum phenylalanine remained invariably at 29 mg/100 ml. On the first day of analysis, before treatment was started, his excretion was 19% of the intake, the excretory pattern being of the phenylketonuric type at a low level. After 3 days PPA and PLA had disappeared from the urine. Serum phenylalanine became normal when the intake was decreased to 21 mg phenylalanine/kg. Then the intake was gradually increased up to 53 mg/kg without a significant effect on serum phenylalanine concentration. For safety's sake, after discharge from hospital, the infant has been treated with a low phenylalanine diet (varying from 18 to 47 mg/kg). Now, at the age of 2 years and 4 months, his mental development is normal. During treatment at home his fasting serum phenylalanine levels were occasionally elevated (up to 9.3 mg/100 ml), indicating a persisting low phenylalanine tolerance.

The simplest hypothesis is that in this patient phenylalanine hydroxylase deficiency improved somewhat in the postnatal period. Further investigations are necessary for definitely establishing the enzymatic status of this patient.

Patient Cr.* was a mentally retarded girl of 6 years and 4 months with comparatively moderate serum phenylalanine concentrations at a high and moderate phenylalanine intake. She had an excretory pattern as occurs in phenylketonuria but at a lower level. Her 24-h excretion was about 34% of the calculated phenylalanine intake, suggesting a partial defect of phenylalanine hydroxylase. However, phenylalanine hydroxylase of the liver, enzymatically determined,** was negligible. Thus the partial phenylalanine tolerance in this patient remains unexplained. Until now there is slight evidence for the existence of other catabolic routes for phenylalanine nor for the existence of extra-hepatic phenylalanine hydroxylation to any appreciable extent.

We suggest that quantitative evaluation of the excretion of phenylalanine and its metabolites in relation to the intake can contribute to a better appreciation of decreased phenylalanine tolerance. The output/intake ratio, adequately standardized for the level of intake and the time of observation is a better parameter for phenylalanine hydroxylation capacity than is plasma phenylalanine. In the latter the contribution of hydroxylation and removal via the transamination pathway are not separated. In addition, plasma phenylalanine is greatly time dependent.

Abnormal tyrosine metabolism

Deficiency of *p*-OHPPA hydroxylase can easily be recognized with paper or thin-layer chromatography of phenolic acids. Some quantitative results obtained by GLC are given in Table IV.

Patient La. was a boy of 1 year and 5 months with unidentified chronic liver disease and gross tyrosyluria. His liver was enlarged and cirrhotic. P.a. inspection of liver biopsy was suspectible of glycogenosis. Serum tyrosine*** and methionine*** were increased.

Patient Vr., a boy of 1 year and 3 months was a case of so-called tyrosinosis or hereditary tyrosinaemia. For references see[13]. He had a greatly enlarged liver, gross

* Prof. Dr. J. Brodehl, Universitäts-Kinderklinik und Poliklinik, Bonn; present address: Department for Pediatric Nephrology, Medizinische Hochschule, Hannover.

** Dr. S. Kaufman, Laboratory of Neurochemistry, National Institute of Mental Health, Bethesda, Maryland 20014.

*** Serum and urinary amino acids were determined by column chromatography (Auto Analyzer Method).

TABLE IV

URINARY *p*-HYDROXYPHENYL COMPOUNDS mmoles/g CREATININE IN LIVER DISEASE AND IN PRESUMED PRIMARY *p*-HYDROXYPHENYLPYRUVIC ACID HYDROXYLASE DEFICIENCY

	Liver disease		*Primary p-OHPPA hydroxylase deficiency*	
	La. $1^5/_{12}$ *y.* *26/27-2-'68*	*Vr.* $1^3/_{12}$ *y.* *8/9-12-'68*	*Ma.* $16^{11}/_{12}$ *y.* *23-3-'66*	*Ma.* $17^1/_{12}$ *y.* *31-5-'66*
Tyrosine	2.0	3.8	1.37	0.96
p-OHPLA	21.1	50.5	11.7	9.9
p-OHPPA	5.3	7.2	5.2	3.4
p-OHPAA	6.0	5.9	2.8	2.5
p-OHMA	0.2	0.4	0.8	0.5
Load tyr+phal mmoles/24 h/kg	2.0	1.95	1.14	1.14
Serum tyr mg/100 ml	4.60	7.31	11.0	9.1
Serum me mg/100 ml	4.78	3.22	0.40	0.32

tyrosyluria and increased serum tyrosine and methionine concentrations. A younger brother died of the same disease and so did an older girl of the family. (Amino acid metabolism had not been studied in the latter).

We believe that *p*-OHPPA hydroxylase deficiency is rather a secondary phenomenon in such patients. Also methionine intolerance is probably secondary[13]. As can be seen from Table IV, *p*-OHPLA is the main urinary metabolite, with *p*-OHPPA and *p*-OHPAA following. The excretion of tyrosine is relatively low; *p*-hydroxymandelic acid is a minor metabolite.

For comparison, also the excretion pattern is given of a 17-year-old mentally retarded girl Ma., described elsewhere[6] with severe automutilation and cataracts developed at a later age, but without any sign of liver disease. She had persistent gross tyrosyluria, her serum tyrosine was elevated whereas methionine was perfectly normal. Presumably she had a primary *p*-OHPPA hydroxylase deficiency. As compared with *p*-OHPLA she excreted more *p*-OHPPA than the former patients did, the ratio being 0.44 and 0.34 in Ma., 0.26 in La. and 0.14 in Vr., indicating less reductive metabolism in the liver. Patient Ma. was loaded with tyrosine. The results are given in Table V. In 11 h, nearly 42% of the load was recovered as urinary *p*-hydroxyphenyl

TABLE V

PATIENT MA: EXCRETION OF TYROSINE AND p-HYDROXYPHENYL METABOLITES (as 10^{-4} mole) AFTER ORAL ADMINISTRATION OF L-TYROSINE (5.2 g = 285×10^{-4} mole)
L.M., 52 kg; protein-free diet during the expriment. Start at 8.00 a.m. Total excretion 118.7×10^{-4} mole.

Collection time	*Creatinine mg*	*Tyrosine* 10^{-4} *moles*	*p-HPPA* 10^{-4} *moles*	*p-HPLA* 10^{-4} *moles*	*p-HPAA* 10^{-4} *moles*	*p-HMA* 10^{-4} *moles*	*Total*
8.00–8.45 a.m.	72.0	0.75	3.15	5.1	1.9	—	10.9
8.45–9.30 a.m.	34.7	0.55	2.7	2.7	1.55	—	7.5
9.30–12.00 a.m.	78.4	1.25	7.8	11.15	3.4	0.1	23.7
12.00–2.00 p.m.	74.7	1.15	7.95	11.55	2.9	0.65	24.2
2.00–4.00 p.m.	84.3	1.0	8.55	11.45	2.3	—	23.3
4.00–6.00 p.m.	64.0	0.9	6.1	10.35	3.0	—	20.35
6.00–7.00 p.m.	35.2	0.65	1.85	4.5	1.65	—	8.75
Total excretion in 11 hours	443.3	6.35	38.1	56.8	16.7	0.75	118.7 41.6%

compounds, suggesting that the enzyme block was not total. This experiment yielded a still higher ratio of *p*-OHPPA/*p*-OHPLA: 0.67.

La. and Vr. were treated with a low phenylalanine, tyrosine and methionine diet. Clinically they responded well, presumably as a consequence of relief of the metabolically overcharged diseased liver. However, the metabolic capacity for tyrosine remained low, indicating that the primary liver disease had not been cured. In Table VI some data of quantitative tyrosine metabolism in La. are shown. We calculated to which extent dietary tyrosine and phenylalanine were excreted as *p*-hydroxyphenyl compounds. Thus urinary *p*-hydroxyphenyl compounds (intake of phenylalanine + tyrosine − phenylalanine metabolites) × 100 is given. Before treatment was started this ratio increased from 20.7 to 31.5% in 11 days at a constant load of 0.36 mmoles/kg/day, indicating deterioration of the metabolic condition of the liver. After having been on a load of 0.36 mmoles/kg/day or even less for 2 years, the load was increased tentatively to 0.73 and again 21.8% was excreted as *p*-hydroxyphenyl compounds, indicating little metabolic improvement. Thereafter the dose was reduced to 0.32, whereupon the urinary excretion of *p*-hydroxyphenyl compounds decreased to 1/3 of the 0.73 -level.

TABLE VI

TYROSINE METABOLISM IN PATIENT La. WITH LIVER DISEASE

	15/16-2 1968	*19/20-2 1968*	*26/27-2 1968*	*4/5-1 1970*	*29/30-11 1970*
Intake mmoles/24 h					
Tyrosine	8.8	8.8	8.8	4.5	2.4
Phenylalanine	10.3	10.3	10.3	6.1	2.9
Total	19.1	19.1	19.1	10.6	5.3
Total mmoles/kg/24 h	2.0	2.0	2.0	0.73	0.32
Excretion mmoles/24 h					
Tyrosine + metab.	3.8	4.9	5.7	1.9	0.6
Phenylalanine + metab.	0.7	0.6	1.0	1.9	—
p-Hydroxyphenyl compounds as % of (intake minus phal + metab.)	20.7	26.5	31.5	21.8	—
Serum tyrosine mg/100 ml	—	—	4.6	4.7	3.0
Serum methionine mg/100 ml	—	—	4.8	0.16	0.27

As can be seen from Table VI the response of fasting serum tyrosine is but slightly proportional to alterations of the intake. Apparently secondary catabolism and renal removal of *p*-hydroxyphenyl metabolites are important compensatory mechanism.

Theoretically a tyrosine loading experiment with frequent blood sampling can provide more information than fasting tyrosine alone. But even then, the effect of secondary metabolism will remain unknown.

Therefore increased levels of fasting serum tyrosine, may reveal *p*-hydroxyphenylpyruvate hydroxylase deficiency. However, the degree of its increase, even in relation to the intake, gives little information about the degree of the enzyme defect. For the quantitative measurement of the capacity of tyrosine metabolism, the level of *p*-OHPPA hydroxylase determination of the excreted *p*-hydroxyphenyl compounds is required. This quantity, related to the intake, is recommended as a clinical parameter in patients with liver disease of the type here described.

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