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Phenylalanine metabolism in uremic and normal man

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Phenylalanine metabolism in uremic and normal man. The metabolism of phenylalanine and tyrosine was evaluated in six normal men, five chronically uremic men, and three men undergoing maintenance hemodialysis. Phenylalanine, tyrosine, and 13 acidic metabolites of those amino acids were measured in plasma postabsorptively and in plasma and urine after a phenylalanine load of 100 mg/kg. In addition, five normal subjects and five dialysis patients ingested L-[^{14}C]-phenylalanine (uniformly labeled) with the load. In uremic and dialysis patients, plasma phenylalanine rose higher and fell more gradually after the load, and tyrosine rose more slowly. The 24-hr urinary concentrations of phenylalanine and tyrosine were similar in the three groups. At 24 hr, cumulative expiration of $^{14}CO_2$ was 20.2% in the dialysis patients and 28.4% in the normal subjects. Plasma phenylalanine levels and $^{14}CO_2$ expiration varied with protein intake in normal subjects. In uremic and dialysis patients, plasma phenyllactic acid, *p*-hydroxyphenylacetic acid, and *p*-hydroxybenzoic acid were elevated, the last one markedly so. Moreover, plasma phenylpyruvic acid (PPA) and mandelic acid were detected only in dialysis patients. After the phenylalanine load, plasma conjugated phenylacetic acid rose in uremic patients, and PPA increased transiently in some dialysis patients. In urine of dialysis patients, concentrations of benzoic acid and conjugated *o*-hydroxyphenylacetic acid were decreased, and PPA was sometimes increased. The data suggest a mild impairment in the hydroxylation of phenylalanine which does not result in marked changes in plasma or in urinary metabolites after a phenylalanine load.

Métabolisme de la phénylalanine chez l'homme normal et l'homme urémique. Le métabolisme de la phénylalanine et de la tyrosine a été étudié chez six sujets normaux, cinq malades atteints d'insuffisance rénale chronique et trois hommes soumis à l'hémodialyse itérative. La phénylalanine, la tyrosine et 13 métabolites acides de ces acides aminés ont été dosés dans le plasma après absorption et dans le plasma et l'urine après une charge de phénylalanine (100 mg/kg). De plus cinq sujets normaux et cinq malades en hémodialyse ont ingéré de la L-[^{14}C]-phénylalanine (*ul*) en même temps que la charge. Chez les malades urémiques et en dialyse la concentration plasmatique de la phénylalanine augmente plus et diminue plus lentement après la charge alors que la tyrosine augmente plus lentement. L'excrétion urinaire de 24 hr de phénylalanine et de tyrosine est semblable dans les trois groupes. A 24 hr le $^{14}CO_2$ expizé cumulé est de 20,2% chez les malades en dialyse et de 28,4% chez les sujets normaux. Les concentrations plasmatiques des acides phényllactique, *p*-hydroxyphénylacétique et *p*-hydroxybenzoïque sont élevées, surtout pour ce dernier. De plus, les acides phénylpyruvique et mandélique ne sont détectés que dans le plasma des sujets en dialyse. Après la charge, l'acide phénylacétique conjugué du plasma augmente chez les malades urémiques et l'acide phénylpyruvique augmente transitoirement chez quelques malades en dialyse. Dans l'urine des malades en dialyse les acides benzoïques et *o*-hydroxyphénylacétique conjugué sont diminués et l'acide phénylpyruvique parfois augmenté. Ces résultats suggèrent une altération modérée de l'hydroxylation de la phénylalanine qui n'entraîne pas de modifications importantes des métabolites plasmatiques ou urinaires après une charge en phénylalanine.

The ratio of tyrosine to phenylalanine in plasma has been shown by many investigators to be reduced in renal failure. Moreover, in renal failure, plasma phenylalanine and tyrosine respond abnormally to a phenylalanine load, suggesting that the conversion of phenylalanine to tyrosine is impaired [1, 2]. The metabolism of tyrosine in chronic renal failure appears to be normal, as indicated by *in vivo* kinetic studies using tyrosine labeled with radioactive carbon (^{14}C) [3]. The clinical significance of these findings is unclear, and some investigators have questioned whether the accumulation of phenylalanine metabolites other than tyrosine may contribute to uremic toxicity [4, 5].

The present study was undertaken to investigate further phenylalanine metabolism in normal subjects, patients with chronic uremia, and patients undergoing maintenance hemodialysis. Phenylalanine, tyrosine, and certain of their metabolites were measured in plasma and urine in the postabsorptive state and after ingestion of a phenylalanine load. In some normal subjects and hemodialysis patients, a tracer dose of L-phenylalanine uniformly labeled with carbon-14 was administered with the phenylalanine load. Expiration of $^{14}CO_2$, urinary excretion of carbon-14, and the quantity of carbon-14 in plasma proteins were measured.

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Methods

Subjects. Eleven normal men, five men with chronic uremia, and eight men undergoing maintenance hemodialysis were studied. Their characteristics are given in Table 1. The patients with chronic uremia and the patients undergoing dialysis were clinically stable, and none had diabetes mellitus, liver disease, or inflammatory or catabolic diseases other than uremia.

Four normal subjects lived in a metabolic research unit and ingested rigidly controlled diets that provided 40 g/day of protein of primarily high biological value for 21 ± (SD) 7 days (range, 11 to 27 days) prior to the study. Seven normal subjects ingested their usual diets *ad lib*. One normal subject was studied both after eating 40 g of protein and his usual 90-g protein diet. The uremic and dialysis subjects were outpatients. One chronically uremic patient was restudied after he had received dialysis therapy for 4 months. Mean calculated dietary protein intake of these seven normal subjects and the chronically uremic and dialysis patients were 98 ± 21, 56 ± 11, and 91 ± 17 g/day, respectively.

Load tests. Subjects fasted from midnight until approximately 8:30 A.M. and then drank a suspension of L-phenylalanine (Ajinomoto, Inc.), 100 mg/kg of body wt, in 400 ml of water. The container was then rinsed with 300 ml of water which the subjects drank. Blood was collected 5 min before administering the phenylalanine and 30, 60, 90, 120, 180, and 240 min afterwards. In some subjects, blood samples were also obtained 24 hr following the load, after a 9-hr fast. Subjects voided 5 or 10 min before drinking the phenylalanine suspension, and urine was then collected for the next 24 hr. After ingesting the load, subjects were fasted until blood sampling was completed at 240 min. They then were encouraged to eat their usual total daily food intake, including breakfast. Dialysis patients received the load test either 15 hr (three patients) or 61 hr (five patients) after the termination of a dialysis treatment.

Five normal and five hemodialysis subjects received tracer L-[^{14}C]-phenylalanine (uniformly labeled), 0.3 μCi/kg of body wt, with the phenylalanine suspension. Immediately afterwards, each subject sat in an easy chair and wore a plastic helmet that formed an almost air-tight seal. Air was pumped into the helmet and was evacuated at a slightly greater rate (10 liters/min) to create a small degree of negative pressure within the helmet. The evacuated air, which included carbon dioxide expired from the subject, flowed through an ion chamber (Cary Instruments) and a carbon dioxide analyzer (Godart Capnograph®). Ion currents generated by carbon-14 in the ion chamber were detected by a vibrating reed electrometer (Cary Instruments). Expiration of total carbon dioxide and $^{14}CO_2$ was monitored continuously for the first 4 hr and periodically for the next 20 hr. Data from the present and previous studies indicate that the times of measurement of total carbon dioxide and $^{14}CO_2$ were sufficient to determine the 24 hr expiration of these gases.

General analytical procedures. Plasma samples were deproteinized for amino acid analysis by mixing them with sulfosalicylic acid (SSA), 45 mg per ml of plasma, and centrifuging them at 3,000 rpm for 10 min. Urine samples were not deproteinized, but the pH was adjusted to 2.2. Phenylalanine and tyrosine were analyzed with an amino acid analyzer (Beckman 120 HP or 121 M). Urea and creatinine were measured with an autoanalyzer (Technicon).

Total radioactivity in urine, in deproteinized plasma, and in plasma proteins was measured with a liquid scintillation counter (Beckman LS 3150T) using Biofluor® (New England Nuclear) scintillation liquid. For measurement of radioactivity in plasma proteins, the SSA precipitate was dissolved in a two percent aqueous solution of unlabeled phenylalanine to wash out any labeled phenylalanine noncovalently bound to protein. The proteins were reprecipitated with concentrated hydrochloric acid, reconstituted in 0.5 N sodium hydroxide, and added to the Biofluor® scintillation liquid. Because of chemiluminescence, several days of dark adaptation were necessary before the counts stabilized. To calculate the quantity

Table 1. Clinical characteristics of subjects[a]

	No. of subjects	Age *yr*	Wt *kg*	Serum urea nitrogen *mg/dl*	Serum creatinine *mg/dl*	Urea clearance *ml/min*	Creatinine clearance *ml/min*
Normal	11	43 ± 10	76 ± 10	15 ± 7	1.0 ± 0.4	48 ± 14	135 ± 40
Chronically uremic	5	49 ± 10	83 ± 11	55 ± 7	8.9 ± 2.0	6 ± 2	15 ± 6
Hemodialysis	8	53 ± 5	82 ± 15	60 ± 28	14.7 ± 4.6	1(0–5)[b]	2(0–6)[b]

[a] Except where indicated by footnote, values represent the mean ± SD.
[b] Mean (range); distribution not Gaussian.

of radioactivity in total plasma protein, the radioactivity was first determined in the protein present in each milliliter of plasma. Total plasma volume was estimated by multiplying body weight by 0.045.

For measurement of $^{14}CO_2$ expiration, the area under the activity-time curve was measured by a disc integrator. The rate of total carbon dioxide expiration was similar in all subjects and did not vary greatly during the study.

Gas chromatographic analyses of metabolites in plasma and urine. Ten-milliliter aliquots of either plasma or urine were acidified, saturated with sodium chloride, and extracted with ethyl acetate. Plasma extracts were then shaken with 2 N aqueous sodium hydroxide. The aqueous portion was separated, acidified, saturated with sodium chloride, and extracted with ethyl acetate. The ethyl acetate extracts of plasma or urine were dried under a stream of air. Deproteinized plasma, 0.5 ml, was mixed with 0.5 ml of concentrated hydrochloric acid and heated in a sealed glass tube for 12 hr at 90° C. Five-milliliter aliquots of urine were mixed with 5 ml of concentrated hydrochloric acid and heated for 12 hr at 90° C. Extraction of hydrolyzed plasma or urine was carried out as with unhydrolyzed specimens. For derivatization, dried residues of the extracts of plasma or urine were dissolved in 50 μl of N,O-Bis-(trimethylsilyl)-trifluoracetamide (BSTFA, Pierce Chem. Co.), allowed to stand for 15 min at room temperature, and then injected into the gas chromatograph.

Analyses were carried out with a gas chromatograph (Hewlett-Packard Model 700) for phenylacetic acid (PAA), benzoic acid (BA), phenyllactic acid (PLA), phenylpyruvic acid (PPA), mandelic acid (MA), *o*-hydroxyphenylacetic acid (OHPAA), *m*-hydroxyphenylacetic acid (MHPAA), hippuric acid, *p*-hydroxyphenylacetic acid (PHPAA), *p*-hydroxybenzoic acid (PHBA), *p*-hydroxyphenylpyruvic acid (PHPPA), *p*-hydroxyphenyllactic acid (PHPLA), and homogentisic acid. The chromatographic conditions were similar to those used by Wadman et al [6]. The volumes injected were 5 μl of plasma extract and 1 μl of urine extract. Linear calibration curves were obtained for each compound assayed. All standards gave a single peak.

O-chlorobenzoic acid (OCBA) was used as an internal standard, added prior to extraction. Recoveries from plasma for the compounds assayed were greater than 90% except for PPA. This latter compound was partially decomposed by extraction in 2 N sodium hydroxide, resulting in an average recovery of 52%. Upon repeated injections of the same sample, the coefficient of variation for most of the compounds was 5% or less. For PAA, however, the coefficient of variation was 20%.

Chromatographic peak assignments in three plasma specimens were independently analyzed by gas chromatography/mass spectrometry (GC/MS). With the exception of PHPPA, all peak assignments were confirmed. A large peak having the same retention time as PHPPA was found to contain no PHPPA. The peak initially identified as PHPAA was found to be a mixture of PHPAA and PHBA. PHPAA and PHBA were found to have approximately equal detector responses on our instrument. Therefore, all data derived from this peak are reported as the sum of the concentrations of PHPAA and PHBA and are referred to as PHPAA + PHBA. These compounds were separated on the column used in the GC/MS system (*vide infra*).

Informed consent was obtained from all subjects. Statistical analyses were performed with Student's *t* test, paired *t* test, linear regression analysis, and Wilcoxon Rank Sum test.

Results

Plasma phenylalanine. Fasting phenylalanine concentrations and the rates of rise in concentration during the first 30 min after the load were similar in the three groups (Fig. 1). The plasma concentration of phenylalanine then rose higher in the uremic and dialysis patients and in each group was significantly greater than it was in normal subjects at 120 and 240 min. Phenylalanine concentrations in the uremic and dialysis patients did not differ from each other. The time from ingestion of the phenylalanine load until attainment of the highest plasma values was 55 ± 23 min in the normal subjects and tended to be longer in the uremic patients (84 ± 33 min, $0.05 < P < 0.1$) and dialysis patients (94 ± 34 min, $P < 0.01$). After the maximum plasma concentration was reached, the mean half-life (min) of plasma phenylalanine was 140 ± 21 in the normal subjects, and significantly greater in the uremic patients (194 ± 62, $P < 0.02$) and the dialysis patients (203 ± 52, $P < 0.005$). Plasma phenylalanine concentrations after the load tended to be higher in the normal subjects ingesting 40 g of protein/day as compared to those eating 80 to 120 g of protein/day (Fig. 2).

Plasma tyrosine. Fasting plasma tyrosine concentrations were significantly lower in the uremic ($P < 0.02$) and dialysis patients ($P < 0.0001$) as compared with normal subjects (Fig. 3). The fasting plasma tyrosine concentrations in the uremic and dialysis patients taken together correlated directly with the glomerular filtration rate (GFR) ($r = 0.595$, $P < 0.05$).

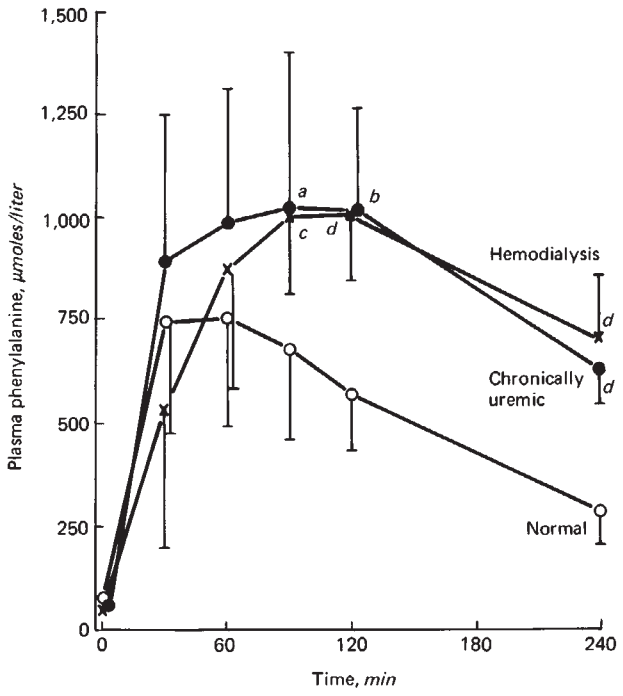

Fig. 1. *Mean plasma phenylalanine concentrations in normal subjects (open circles, N = 11), chronically uremic patients (closed circles, N = 5), and hemodialysis patients (x's, N = 8) during fasting and after a phenylalanine load.* Vertical lines indicate standard deviations. Superscripts *a,b,c,d* denote values that were significantly different from those of normal subjects: $P < 0.05$, $P < 0.01$, $P < 0.005$ and $P < 0.0001$, respectively.

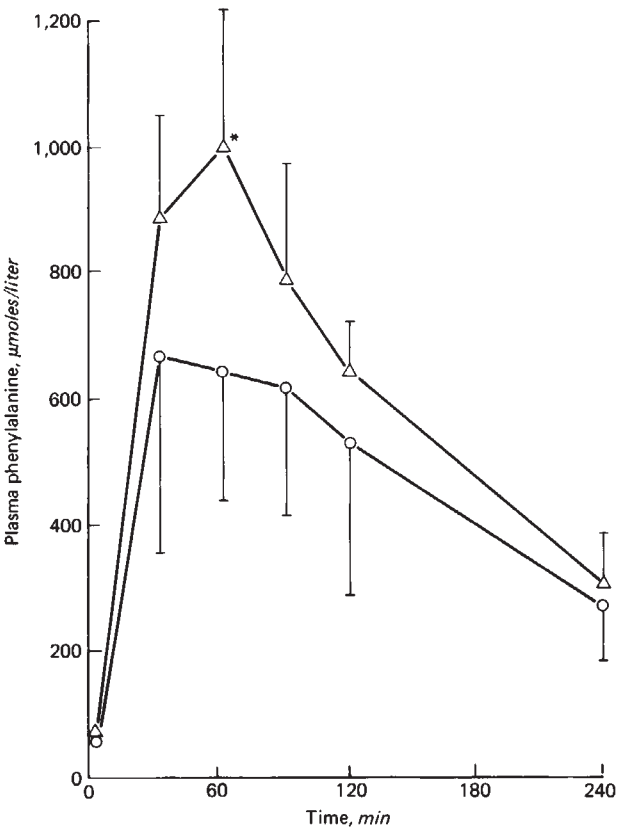

Fig. 2. *Mean plasma phenylalanine concentrations during fasting and after a phenylalanine load in normal subjects ingesting 80 to 120 g of protein/day (open circles, N = 7) or 40 g of protein/day (triangles, N = 4). Vertical lines* indicate standard deviations. *Asterisk* indicates that the value was significantly different from that of subjects ingesting 80 to 120 g of protein: $P < 0.05$.

After ingestion of the phenylalanine load, plasma tyrosine concentrations rose approximately twice as rapidly in the normal subjects as they did in the uremic patients and three times as fast as they did in the dialysis patients (Fig. 3). Tyrosine concentrations were significantly different at most times between any two groups. Plasma tyrosine concentrations in the normal subjects ingesting unregulated diets and 40-g protein diets were similar.

Plasma tyrosine concentrations 24 hr after the phenylalanine load were 43 and 38 μmoles/liter in two normal subjects and 34 ± 8 in four dialysis patients. These values did not differ from each other or from values prior to ingestion of the load in the same subjects. The plasma tyrosine/phenylalanine ratios at each sampling before and after the load were significantly lower in the uremic and dialysis patients than they were in normal subjects. During fasting, the ratios in the combined group of uremic and dialysis patients correlated directly with the GFR ($r = 0.565$, $P < 0.05$).

Radioactivity in plasma. The percent of ingested carbon-14 present in the total plasma pool of SSA-precipitable proteins was 2.3 ± 1.2 in the normal subjects and 1.9 ± 1.5 in the dialysis patients at 4 hr and 5.4 ± 3 and 5.6 ± 1.4, respectively, at 24 hr. The

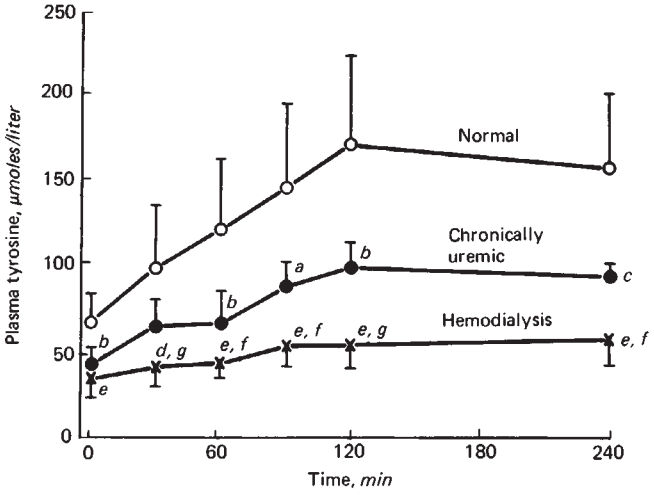

Fig. 3. *Mean plasma tyrosine concentrations in normal subjects (open circles, N = 11), chronically uremic patients (closed circles, N = 5), and hemodialysis patients (x's, N = 8) during fasting and after a phenylalanine load.* Vertical lines indicate standard deviations. Superscripts *a,b,c,d,e* denote values that were significantly different from normal subjects: $P < 0.05$, $P < 0.02$, $P < 0.01$, $P < 0.005$, and $P < 0.0001$, respectively. Superscripts *f* and *g* denote values that were significantly different from chronically uremic patients: $P < 0.02$ and $P < 0.01$, respectively.

radioactivity in the protein-poor plasma filtrate at 24 hr was 0.2 ± 0.1% and 2.2 ± 1.8% of the ingested dose in the normal and dialysis subjects ($P < 0.01$). Thus, the portion of the ingested label retained in plasma at 24 hr was approximately 6% in the normal subjects and 8% in the dialysis patients.

Expiration of $^{14}CO_2$. During the first four hours after the load, the cumulative expiration of $^{14}CO_2$ rose more than twice as rapidly in normal subjects as it did in dialysis patients (Fig. 4). After the first four hours, the rate of $^{14}CO_2$ expiration was only slightly greater in the normal subjects. The total $^{14}CO_2$ expired during the 24-hr period was 28.4 ± 3.5% of the ingested label in the normal subjects and 20.2 ± 2.8% in the dialysis patients ($P < 0.005$). Ingestion of meals was not associated with significant changes in $^{14}CO_2$ expiration (Fig. 4).

Cumulative expiration of $^{14}CO_2$ in three normal subjects receiving 40 g/day of protein, as compared with two normal subjects ingesting unregulated diets, was usually significantly less during each of the first 10 hr after the load. Subsequently, cumulative expiration, although lower in the former subjects, did not differ significantly from the normal individuals eating unregulated diets. The cumulative expiration of $^{14}CO_2$ in normal subjects ingesting the 40-g protein diet was significantly greater at each measurement than it was in the dialysis patients.

In the dialysis patients, the total amount of $^{14}CO_2$ expired during the 24 hr was directly correlated with the GFR ($r = 0.77$, $P < 0.05$). The cumulative $^{14}CO_2$ expiration at 24 hr was significantly greater, 21.8 ± 2.5%, in the three patients studied approximately 15 hr after dialysis as compared to that of 17.9 ± 0.3% in the two patients evaluated 61 hr after dialysis ($P < 0.05$).

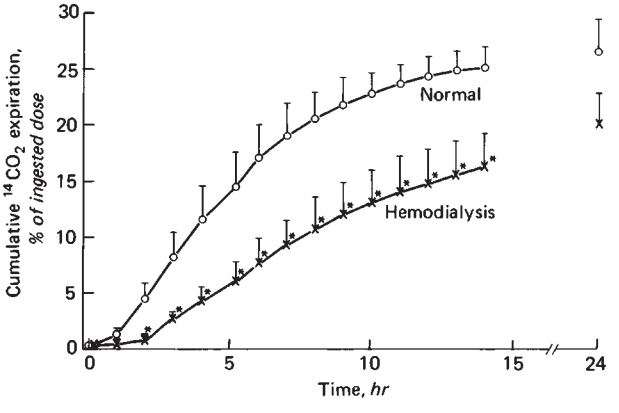

Fig. 4. *Mean cumulative expiration of $^{14}CO_2$ in normal subjects (open circles, N = 5) and hemodialysis patients (x's, N = 5) after an oral load of phenylalanine and a dose of* L-[^{14}C]*-phenylalanine (uniformly labeled).* Statistically significant differences between the groups are indicated by *asterisks* ($P < 0.05$). Standard deviations are denoted by vertical lines.

Urinary excretion of phenylalanine, tyrosine, and carbon-14. The urinary excretion of phenylalanine and tyrosine during the 24-hr period after the load did not differ significantly among the three groups, although the mean level for each amino acid was lowest in the dialysis patients (Table 2). Also, the tyrosine/phenylalanine ratios in urine were similar for the three groups.

Urinary excretion of the carbon-14 label during this 24-hr period was 2.6 ± 0.6% of the ingested dose in the normal subjects and lower, 0.23% (range, 0.001 to 0.87), in the dialysis patients ($P < 0.01$). In the dialysis patients, urinary excretion of the carbon-14 label was directly correlated with the GFR ($r = 0.995$, $P < 0.001$).

Metabolic products of phenylalanine in plasma. In each group of subjects, plasma concentrations of free and conjugated PAA during fasting and after the phenylalanine load were greater than those of all other phenylalanine metabolites combined (Table 3). Free PAA was observed in every sample, and conjugated PAA was identified in most plasma specimens from each of the three groups. Plasma free PAA concentrations were similar in the three groups at all times, and after the load they did not change in any group. Mean levels of conjugated PAA tended to rise more after the load, particularly in the uremic patients (Table 3), and the increment in plasma conjugated PAA at 240 min was greater in the uremic patients as compared to that in normal subjects ($P = 0.05$). Plasma conjugated PAA was significantly greater in the dialysis patients than it was in normal subjects at 120 and 240 min.

Free BA, PAA, PLA, and MA were found only occasionally in the subjects both before and after the load. When identified, these metabolites tended to be higher in the dialysis and uremic patients. There was no significant change in their concentrations after the load. Free OHPAA and MHPAA were not found in any plasma specimen from any subject. PAA was the only metabolite in plasma that increased after hydrolysis.

Metabolic products of tyrosine in plasma. The PHPAA + PHBA peak was not detected in plasma of any normal subject during fasting, but after the load it was identified after 60 min in one man (Table 4). In contrast, this peak was observed in every specimen from each uremic and hemodialysis patient. The fasting concentrations of PHPAA + PHBA in the combined groups of uremic and dialysis patients ranged from 6.1 to 66.2 μmoles/liter and were inversely correlated with the GFR ($r = -0.83$, $P < 0.05$, Fig. 5). Also, there was an inverse correlation between the ratio of the PHPAA + PHBA peak to the plasma tyrosine concentrations and the GFR

Table 2. Urinary excretion of phenylalanine and tyrosine and their metabolites[a]

Compound	Normal subjects	Chronically uremic patients	Hemodialysis patients
Phenylalanine	124 ± 80	325 (10–1088)	88 ± 87
Metabolites of phenylalanine			
phenylpyruvic acid	0[b]	0[b]	14(0–57)[c]
phenyllactic acid	42 ± 21	6(0–18)[e]	37 ± 22
o-hydroxyphenylacetic acid			
free	45 ± 21	79 ± 53	33 ± 21
conjugated	328 ± 203	412 (4–1464)	88 (9–250)
total	373 ± 196	491 (39–1493)	120 ± 103[d]
phenylacetic acid			
free	180 ± 148	59 (0–139)	116 (34–341)
conjugated	1304 ± 398	1253 ± 825	719 ± 447
total	1483 ± 439	1312 ± 817	836 ± 489
benzoic acid			
free	83 ± 72	19 (0–95)	25 (0–59)
conjugated	2167 ± 724	2220 ± 917	910 ± 660
total	2200 ± 732	2239 ± 94	935 ± 678[d,f]
Total phenylalanine metabolites			
free	350 ± 181	158 ± 84[d]	216 ± 171
conjugated	3748 ± 901	3886 ± 1714	1761 ± 1156[d]
grand total	4143 ± 1050	4088 ± 1744	1990 ± 1222[d]
Tyrosine	113 ± 86	126 (20–333)	45 ± 43
Metabolites of tyrosine			
p-hydroxyphenylacetic acid + *p*-hydroxybenzoic acid			
free	170 ± 103	174 ± 69	120 ± 67
conjugated	181 ± 110	319 ± 149	219 ± 200
total	350 ± 55	492 ± 208	339 ± 256

[a] Values represent the mean ± SD; or if distribution is not normal, the values represent the mean and range. Values are expressed as μmoles/24 hr.
[b] Not identified in any subject.
[c] Found in one subject only.
[d] Differs from normal, $P < 0.05$.
[e] Differs from normal, $P < 0.01$.
[f] Differs from chronically uremic, $P < 0.05$.

($r = -0.92$, $P < 0.01$). The PHPAA + PHBA peak was significantly greater in the dialysis patients as compared to the uremic subjects during fasting and 30 min after the load. After the load, the PHPAA + PHBA peak did not change in the uremic patients but fell significantly at 180 and 240 min in the dialysis patients. In all specimens which were subjected to mass spectrometry, PHBA accounted for about 90% of the total concentration of PHBA and PHPAA. There was no increase in concentration of PHPAA + PHBA after hydrolysis.

PHPPA, PHPLA, and homogentisic acid were not identified in plasma of any subject. There were more unidentified peaks in the chromatograms of plasma from the uremic and dialysis patients as compared to the normal subjects.

Metabolites of phenylalanine in urine. BA accounted for about 50% of the metabolites of phenylalanine in the urine of each group during the 24 hr after the load (Table 2). Urinary excretion of total BA was significantly less in hemodialysis patients than it was in normal subjects ($P < 0.05$) or uremic patients ($P < 0.05$). In every subject, over 90% of total urinary BA was present in the bound form. PAA was the second most abundant metabolite in urine; more than 85% of total PAA was excreted in the bound form in each group of subjects. Urinary OHPAA was significantly less in dialysis patients than it was in normal subjects ($P < 0.05$). The fraction of total OHPAA which was conjugated tended to be higher in uremic patients ($0.05 < P < 0.1$) and in dialysis patients ($P < 0.02$) as compared to normal subjects. Urinary excretion of PLA in the normal subjects was similar to that in hemodialysis patients, but it was greater than that in uremic patients ($P < 0.01$). PPA was detected in urine of only one hemodialysis patient and not in any normal subject or uremic patient. MHPAA was not found in urine of any subject.

The urinary excretion of both conjugated and total metabolites of phenylalanine in the normal subjects was not different from that in the uremic patients, but it was significantly greater in both groups than it was in the hemodialysis patients. The calculated dietary intake of phenylalanine in the normal, uremic,

Table 3. Plasma concentrations of phenylalanine metabolites before and after a phenylalanine load[a]

Time[b] *min*	Subjects	Phenylacetic acid, *μmoles/liter* Free	Conjugated[c]	Total[c]	Benzoic acid	Phenylpyruvic acid *μmoles/liter*	Phenyllactic acid *μmoles/liter*	Mandelic acid
0	Normal	74 ± 32	12(2–31)	91 ± 54	11(0–32)	0	0	0
	Chronically uremic	59(13–173)	74(0–201)	105 ± 73	2(0–9)	0	2(0–10)	0
	Hemodialysis	58 ± 37	58(4–143)	136 ± 88	11 ± 10	12 ± 12	1 ± 1	1 ± 1
30	Normal	77 ± 44			6(0–26)	0	0	0
	Chronically uremic	56(12– 54)			2(0–10)	0	2(0–9)	0
	Hemodialysis	57 ± 40			10 ± 5	16(0–34)	7(0–28)	2(0–6)
60	Normal	76 ± 65			5(0–20)	0	0	0
	Chronically uremic	57 ± 50			2(0–10)	0	2(0–9)	0
	Hemodialysis	73 ± 62			5(0–15)	18 ± 14	0	4(0–14)
90	Normal	119(32–154)			1(0–6)	2(0–10)	0	0
	Chronically uremic	69 ± 54			1(0–5)	0	2(0–10)	0
	Hemodialysis	62 ± 41			9(0–27)	23(0–140)	0	2(0–6)
120	Normal	94 ± 78	30 ± 24	116 ± 69	2(0–10)	0	0	0
	Chronically uremic	56 ± 40	95(0–267)	141 ± 116	2(0–6)	1 ± 1	2(0–8)	0
	Hemodialysis	37 ± 25	110 ± 49[e]	147 ± 65	16(0–48)	16 ± 14	0	0.3(0–1)
180	Normal	77 ± 41			2(0–12)	0	0	0
	Chronically uremic	53 ± 33			3(0–13)	1 ± 1	2(0–10)	0
	Hemodialysis	36 ± 26			27 ± 25	52(0–144)	0	3(0–9)
240	Normal	68 ± 38	22±17	81± 50	2(0–10)	0	0	0
	Chronically uremic	57 ± 38	138(0–346)[f]	180 ± 130[f]	1(0–3)	0	2(0–12)	0
	Hemodialysis	35 ± 24	124 ± 51[d]	162 ± 27[d]	15(0–42)	30(0–78)	1 ± 1	0

[a] Concentration values represent the mean ± SD, or the mean and range when distribution was not normal.
[b] 0 time refers to blood collected immediately before ingestion of the load.
[c] In a few subjects, conjugated phenylacetic acid was not measured; data from these subjects were excluded from calculation of the total phenylacetic acid.
[d] Differs from normal values obtained at the same time; $P < 0.05$.
[e] Differs from normal values obtained at the same time, $P < 0.01$.
[f] Differs from values obtained at zero time (paired t test), $P < 0.05$.

and dialysis subjects during the 24 hr after the load was 33 ± 10, 15 ± 3, and 28 ± 4 mmoles, respectively. The percentage of the total intake, including the load, which was excreted as phenylalanine metabolites during the 24 hr in the normal, uremic, and dialysis subjects was 6.2 ± 1.5, 7.6 ± 2.4, 3.0 ± 2.1, respectively (dialysis vs. normal or uremic subjects, $P < 0.05$ and $P = 0.05$, respectively).

Tyrosine metabolites in urine. The only metabolites of tyrosine identified in urine were PHPAA + PHBA (Table 2). Free, conjugated, and total PHPAA + PHBA excretion were similar in the three groups.

Table 4. Plasma concentrations of tyrosine metabolites before and after a phenylalanine load

Time[a] *min*	Subjects	*p*-Hydroxyphenylacetic acid + *p*-hydroxybenzoic acid[b] *μmoles/liter*
0	Normal	0
	Chronically uremic	17 ± 8
	Hemodialysis	58 ± 11[c]
30	Normal	0
	Chronically uremic	18 ± 13
	Hemodialysis	52 ± 21[c]
60	Normal	0
	Chronically uremic	22 ± 21
	Hemodialysis	63 ± 35
90	Normal	1 (0–5)
	Chronically uremic	24 ± 20
	Hemodialysis	45 ± 20
120	Normal	2 (0–10)
	Chronically uremic	24 ± 18
	Hemodialysis	34 ± 9[d]
180	Normal	1 (0–7)
	Chronically uremic	23 ± 17
	Hemodialysis	36 ± 8[d]
240	Normal	1 (0–6)
	Chronically uremic	22 ± 21
	Hemodialysis	34 ± 10[d]

[a] 0 time refers to blood collected immediately before ingestion of the load.
[b] Values represent the mean ± SD or the mean and range if distribution was not normal.
[c] Differs from uremic patients at the same time, $P < 0.05$.
[d] Differs from fasting level, $P < 0.05$.

Discussion

Phenylalanine, an essential amino acid, participates in a number of metabolic pathways. The major one is irreversible hydroxylation to form tyrosine. Three other pathways of phenylalanine metabolism, normally of minor quantitative importance, are transamination to phenylpyruvic acid (PPA), decarboxylation to form β-phenylethylamine, and acetylation of the amino group. PPA may be reduced to phenyllactic acid (PLA) or may undergo oxidative decarboxylation to phenylacetic acid (PAA) which may then be converted to benzoic acid (BA). PAA

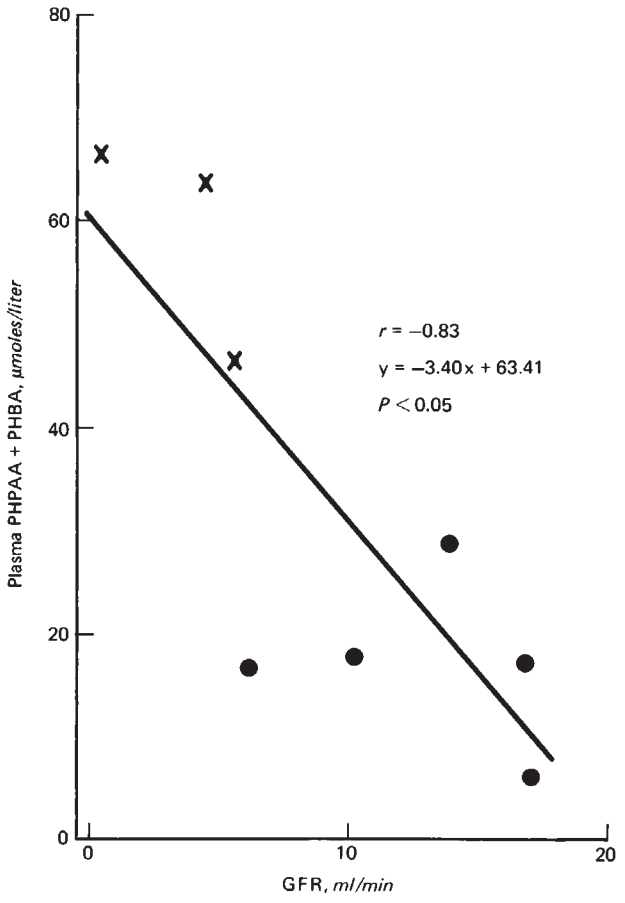

Fig. 5. *The relationship of postabsorptive plasma p-hyroxyphenylacetic acid (PHPAA) + p-hydroxybenzoic acid (PHBA) to the GFR in chronically uremic patients (indicated by closed circles) and hemodialysis patients (indicated by x's).*

may also be derived from β-phenylethylamine by oxidation. Tyrosine may be hydroxylated to form dihydroxyphenylalanine (DOPA), iodinated to form triiodothyronine (T_3), or decarboxylated to form tyramine. The major metabolic pathway for tyrosine, however, is transamination to *p*-hydroxyphenylpyruvic acid (PHPPA). Most PHPPA is converted to homogentisic acid; the phenyl ring is then cleaved to give fumaric and acetoacetic acid. PHPPA may also be converted to *p*-hydroxyphenylacetic acid (PHPAA) and then to *p*-hydroxybenzoic acid (PHBA) [7]. There is some evidence that PHPAA and PHBA may also be derived from phenylalanine via hydroxylation of β-phenylethylamine to tyramine and oxidation of the latter compound [8]. Thus, phenylalanine and tyrosine are both glycogenic and ketogenic, and during normal metabolism they are primarily converted to fat, carbohydrate, or carbon dioxide. Many metabolic products, however, are not completely oxidized, and in normal subjects they are excreted in the urine.

Our findings demonstrate that in patients with chronic uremia and in those on hemodialysis, as compared to normal subjects, *1)* plasma phenylalanine was greater after a phenylalanine load and fell more slowly, *2)* plasma tyrosine and the tyrosine/phenylalanine ratio were lower before and after the load, and *3)* in the dialysis patients who ingested ^{14}C-phenylalanine, expiration of $^{14}CO_2$ was decreased. These observations indicate that in renal failure, there is a block in the conversion of phenylalanine to tyrosine. This block, in association with reduced renal clearance, theoretically could cause potentially toxic metabolites of phenylalanine to accumulate in plasma.

The tracer studies may provide more information concerning the metabolic fate of phenylalanine after the load. It is recognized that these studies were carried out under nonsteady-state conditions, and there was little information regarding pool size. Differences in free phenylalanine pools, however, were probably overwhelmed by the large magnitude of the load, 42 mmoles/70 kg, compared to the estimated total free phenylalanine pool of 2 to 3 mmoles [9].

Approximately 5% of the ingested carbon-14 was incorporated into plasma proteins, presumably as phenylalanine and tyrosine. Grümer, Koblet, and Woodard [10] estimated from phenylalanine turnover studies that plasma protein turnover represents less than 10% of total daily protein turnover. Thus, almost certainly, a large percentage of the ingested carbon-14 was incorporated into body protein as a result of normal protein turnover. There was probably little if any net synthesis of protein during the study, and the labeled phenylalanine and tyrosine that entered protein were replaced by unlabeled phenylalanine and tyrosine released from protein. Some of the label may have been incorporated into carbohydrate or fat.

In the normal and dialysis subjects 24 hr after the load, 0.2% and 2.2% of ingested carbon-14, respectively, was in the nonprotein portion of plasma, and, hence, and 1% and 11% of the ingested dose was in the nonprotein fraction of extracellular fluid. Urinary excretion of carbon-14 during this 24 hr was small in both groups but greater in the normal subjects (2.6% vs. 0.2% of the ingested dose). The nonprotein-bound label in extracellular fluid and urine was present in phenylalanine, tyrosine, and their metabolites.

About 28% of the ingested label appeared as $^{14}CO_2$ during the first 24 hr in the normal subjects, as

compared to 20% in the dialysis patients. Since much of the labeled phenylalanine and tyrosine were exchanged in protein for the nonlabeled amino acids, these values of 28% and 20% must represent lower limits to the fraction of phenylalanine which was oxidized.

The foregoing considerations suggest that the degradation of phenylalanine via tyrosine to carbon dioxide was somewhat greater in the normal subjects as compared to the dialysis patients. In the dialysis patients as well as the normal subjects, however, the hydroxylation of phenylalanine to tyrosine and its subsequent degradation to carbon dioxide was still the primary route for utilization of the load. These observations are in direct contrast to the response of phenylketonuric patients to a phenylalanine load where most of the ingested load is excreted in the urine either unchanged or as non-para-hydroxylated metabolites [6].

The finding that after the load $^{14}CO_2$ expiration was lower in the dialysis patients who were studied an average of 61 hr as compared to those studied 15 hr after the end of a dialysis therapy suggest that a dialyzable uremic toxin may adversely affect phenylalanine or tyrosine metabolism. These conclusions are tentative because of the small number of patients studied.

The present study also provides the first evidence for a relationship between protein intake and the rate of phenylalanine degradation in normal man. In the normal subjects ingesting 40-g protein diets as compared to those eating their regular unrestricted diets, there was a greater rise in plasma phenylalanine and a lower rate of expiration of $^{14}CO_2$ after the load. These results suggest that the combined rates of hydroxylation of phenylalanine and oxidation of its metabolites were decreased in subjects receiving the lower protein diet. These findings are not unexpected. Wang et al [11] and Young and Parsons [12] have shown that in liver of both chronically uremic and control rats, activity of phenylalanine hydroxylase is directly related to protein intake. Also, kinetic studies in chronically uremic humans suggest that tyrosine degradation is directly related to protein intake [3].

Even when the normal subjects ingesting the 40-g protein diets were compared to the uremic and dialysis patients who were eating similar or greater quantities, the differences in phenylalanine and tyrosine levels and $^{14}CO_2$ expiration persisted but were not as marked. The results of this study therefore suggest two acquired conditions which affect phenylalanine metabolism in man: *1)* renal failure and *2)* dietary protein intake. Since uremic patients usually ingest low protein diets, it is possible that both of these factors contribute to abnormal phenylalanine metabolism in renal failure.

A surprising finding was the relatively low plasma levels of phenylalanine metabolites in the patients with renal failure. PLA and possibly MA were increased slightly in the uremic or dialysis patients before and/or after the phenylalanine load. PPA rose markedly after the load in one hemodialysis patient but in other patients was increased slightly or not at all. The metabolities PAA, BA, OHPAA, and MHPAA were not increased in any uremic or dialysis patient. The tyrosine metabolites, PHPPA, PHPLA, and homogentisic acid, were not detected in plasma or urine from any subject.

A striking observation, however, was the large chromatographic peak for the phenolic acids, PHPAA + PHBA, in plasma of uremic and dialysis patients but not in that of normal subjects. The values, which ranged from 6 to 66 μmoles/liter, were sometimes as high as the fasting levels of phenylalanine and tyrosine, and these were the only free metabolites of either amino acid which were markedly elevated in these patients. The preliminary results from mass spectrometry indicate that PHBA accounted for about 90% of the total concentration of the two metabolic products of tyrosine. It is of interest that plasma values for PHPAA + PHBA were similar to the levels of free phenolic acids (7 to 102 μmoles/liter) reported by other investigators in uremic patients [13, 14]. Hence, PHBA and/or PHPAA are probably the major free phenolic compounds which accumulate in renal failure. It is not clear why the ratio of plasma PHPAA + PHBA to tyrosine was inversely correlated with the GFR. Progressive impairment in urinary clearance of PHPAA + PHBA with falling GFR may be a cause. Increased conversion of tyrosine to PHPAA and PHBA could also explain this finding.

It was also surprising that no free metabolite of phenylalanine or tyrosine increased significantly in any of the three groups after the phenylalanine load, although values in individual patients sometimes rose substantially. By comparison, plasma phenylalanine increased 10 to 15 times, and tyrosine doubled or tripled. Conjugated PAA, which tended to be elevated in the fasting state in both groups of patients, increased significantly in the uremic patients and became greater than normal in the dialysis patients after the load. The increased conjugated PAA in the uremic and dialysis patients after the load may reflect enhanced production or delayed renal excre-

tion (Table 2). The conjugated PAA was probably primarily phenylacetylglutamine, which is the most abundant conjugate of PAA in normal people [15]. Frimpter, Thompson, and Luckey reported elevated plasma phenylacetylglutamine levels in acutely and chronically uremic patients who were not fasting [16].

It is of interest that the urinary excretion of every measured free and conjugated metabolite of phenylalanine and tyrosine was normal in the uremic and dialysis patients despite their markedly reduced GFR's. A comparison of the plasma and urinary levels of these compounds to the GFR indicates that there may be increased fractional excretion of these metabolites. This may be due to decreased renal tubular reabsorption. Since the clearance for some compounds, however, may have exceeded the GFR, tubular secretion may also be present. This could account for the relatively high clearance of PLA and conjugated PAA in the dialysis patients and possibly of conjugated PAA in the uremic subjects. Evidence for renal tubular secretion of phenylpyruvic acid [17] and phenylacetylglutamine [18] has been reported.

In the three groups of subjects, urinary PPA was identified only in the dialysis patient who manifested the highest plasma PPA both before and after ingesting the phenylalanine. Giordano et al reported increased urinary PPA after a phenylalanine load in uremic patients but not in normal subjects [19]. Pickford, McGale, and Aber, however, also found no increase in PPA excretion after a phenylalanine load in either uremic or normal subjects [1]. The plasma and urinary data in the present study are consistent with relatively minor increased production, and/or decreased degradation of PPA in renal failure.

The results of this study indicate that in renal failure the conversion of phenylalanine to tyrosine is impaired, and the degree of the block is related to the severity of renal failure. The magnitude of impairment, however, appears small. Even when the uremic or dialysis patient is stressed with a phenylalanine load, the great fraction of phenylalanine is metabolized through normal pathways, albeit somewhat more slowly than normal. The metabolites of phenylalanine in plasma were only slightly increased, if at all, and this appeared to be primarily due to decreased renal clearance rather than increased synthesis through metabolic shunts. An exception to this was the elevated PPA levels in some patients. In fact, the highest plasma levels of metabolites found in the patients were for PHPAA + PHBA, which were probably derived from tyrosine. The present observations also indicate that in normal subjects the magnitude of protein intake will affect the rate of degradation of phenylalanine.

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