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URINARY PURINES AND PYRIMIDINES IN PATIENTS WITH HYPERAMMONEMIA OF VARIOUS ORIGINS

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Summary

Excretion patterns of pyrimidines and purines in patients with various types of hyperammonemia have been investigated by 2-dimensional thin-layer chromatography and high pressure liquid chromatography (HPLC). For the quantitative analysis of pseudouridine, uracil and uridine a new procedure has been developed, consisting of pre-fractionation with Dowex 1 × 8, followed by dual column HPLC on a strong anion-exchanger and a reverse phase column. Thymine has also been analyzed in the pre-fractionated urine by a new HPLC method using the reverse phase column in combination with a strong cation-exchange column. Quantitative data for urinary pyrimidines and uric acid in hyperammonemia are given. In patients with a defect in one of the urea cycle enzymes, the level of pyrimidine excretion was found to depend on plasma ammonia concentrations. In other hyperammonemic patients, an increased excretion of orotic acid, uracil and uridine has only been found in one of the two patients with lysinuric protein intolerance, all other patients showing normal excretion patterns. Elevated uric acid excretions have been found frequently in our patients with hyperammonemia, but they did not always coincide with high plasma ammonia levels. A possible explanation for the difference in the excretion levels of the various pyrimidines is discussed.

Introduction

Hereditary enzyme defects causing severe hyperammonemia have been diagnosed with increasing frequency during the last decade. Apart from the well-known disorders of the urea cycle additional defects were discovered: hyperor-

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nithinemia combined with homocitrullinuria [1—3], hyperlysinemia associated with hyperammonemia [4,5], hyperlysinemia and homocitrullinuria associated with hyperammonemia [6,7], lysinuric protein intolerance [8—10]. Moreover, hyperammonemia is present in patients with Reye's syndrome [11—13], in acidemias like propionic acidemia [14] and methylmalonic acidemia [15,16], and in patients suffering from liver cirrhosis with collateral circulation and portocaval shunt. As has recently been found in tests on rats, dietary deficiency of arginine leads to hyperammonemia [17,18]. Normally, ammonia is for the greater part removed by conversion to urea via carbamylphosphate. A small amount of carbamylphosphate is used, however, for the synthesis of pyrimidines. In hyperammonemia due to a urea cycle defect, this route becomes important for the removal of the excess ammonia. The pyrimidines formed are excreted in the urine. Until now, increased orotic aciduria has been reported in patients with ornithine carbamyltransferase (OCT) deficiency [19, 20] and argininosuccinic acid (ASA) synthetase deficiency [21]. Increased urinary levels of uracil and uridine have also been reported in OCT deficiency [19,22,23]. So far, however, little information has been given in the literature about the pyrimidine excretory patterns in the other hyperammonemic conditions. Patients suffering from lysinuric protein intolerance have an increased orotic aciduria [10]. In hyperammonemia the synthesis of glutamine, a precursor of purines, is also increased. However, removal of ammonia by overexcretion of purines has not been described.

In this paper we describe urinary patterns of pyrimidines and purines in patients with various types of hyperammonemia.

Methods

Screening for abnormal urinary purines and pyrimidines was performed by two-dimensional thin-layer chromatography (TLC), after isolation of these compounds from the urine by anion-exchange column chromatography as described previously [24]. To detect N-carbamyl-β-alanine (N-C-β-ala), the chromatogram of fraction III of the pre-fractionated urine was sprayed with Ehrlich's reagent consisting of 100 ml absolute ethanol, 1 g paradimethylbenzaldehyde and 10 ml of conc. HCL. The chromatograms were then dried at 25°C for 5—10 min until a yellow spot appeared.

Quantitative high pressure liquid chromatography (HPLC)

Orotic acid and orotidine. These compounds were analyzed in fraction IV from the isolation procedure, using the dual HPLC-system described previously [25].

Pseudouridine, uracil and uridine. A high-resolution two-column system was developed. First analytical column: Nucleosil 10-SB, strong anion-exchanger, 3.9 mm × 30 cm or Partisil-10SAX, strong anion-exchanger, 4.6 mm × 25 cm, Chrompack Nederland B.V., Middelburg, The Netherlands. Second analytical column: μ Bondapak C_{18}, reverse phase 3.9 mm × 30 cm, Waters Associates, Inc., Milford, Mass., U.S.A. Mobile phase: methanol/KH_2PO_4 (0.002 mol/l,

pH 5.0) = 5/95 (v/v). A solvent flow rate of 1 ml/min was used and continuous monitoring of the eluent was performed at 254 nm. A 5 μl sample of fraction III was applied to the first column. After 3.6 min (Nucleosil) and 3.1 min (Partisil), respectively, just before pseudouridine, uracil and uridine were eluted from the first column, the balve (Valco CV-6UHPa-N60, Instruments Co., Houston, TX, U.S.A.) was switched in such a way that the second column was connected in series with the first. After 5.1 min (Nucleosil) and 4.2 min (Partisil), respectively, when elution of the compounds from the first column was completed, the valve was switched back to its original position. With this combination, however, thymine cannot be separated from xanthine.

Thymine. For analysis we used the reverse phase column in combination with a strong cation-exchange column, Partisil-10SCX, 4.6 mm × 25 cm (Chrompack). A 10 μl sample of fraction III was applied to the 3.9 mm × 30 cm μ Bondapak column (Waters) and eluted isocratically using the mobile phase described above, at a flow rate of 1 ml/min. Detection was at 254 nm.

After 6.8 min, just before thymine was eluted from the first column, the two columns were connected in series and after 8.4 min, when elution of thymine from the first column was completed, the valve was switched back to its original position.

Thymidine. Analyzed by using the reverse phase column only.

Calibration curves were prepared and a linear relationship between extinction peak area and concentration was found. For determination of the recoveries, the synthetic compounds were added to a pre-analyzed urine. For identification of the HPLC-peaks, a Perkin-Elmer model LC-55 UV/VIS spectrophotometric detector with scanning accessory (Perkin-Elmer Corp, Maywood, IL, U.S.A.) was used.

Plasma ammonia. Levels of ammonia were determined according to the method of Konitzer and Voigt [26].

Uric acid. This was determined using uricase.

Results

Analytical aspects

A complete separation of pseudouridine, uracil and uridine could not be achieved despite pre-fractionation, when only the reverse phase column or the anion-exchange column was used. The elution times for pseudouridine, uracil and uridine on the reverse phase column (μ Bondapak C_{18}) were 4.2 min, 4.5 min and 5.9 min, respectively. Pseudouridine and uracil are not completely separated and, as confirmed by "peak top UV-spectrometry", other compounds present in the urine interfered. Uric acid was eluted at 5.3 min, between uracil and uridine. When the concentration of uric acid in the urine was high it interfered. Hypoxanthine was not completely separated from uridine. Using the Partisil-10SAX anion-exchange column, elution times for pseudouridine, uridine and uracil were 3.3 min, 3.3 min and 3.6 min respec-

tively, and with the Nucleosil 10-SB column 3.9 min, 4.3 min and 4.3 min respectively. Uridine and hypoxanthine were completely separated. Uric acid was strongly retained and only eluted after 15.9 min. In Fig. 1 a chromatogram, using the dual-column system Partisil-10SAX/μBondapak C_{18}, of fraction III of a urine collected from a patient with OCT deficiency is shown.

Pseudouridine, uracil and uridine are well separated and, as confirmed by "peak top UV-spectrometry", there was no interference by other substances. In this system, pseudouridine, uracil and uridine eluted at 7.4 min, 8.1 min and 9.2 min respectively, and in the Nucleosil 10-SB/μ Bondapak C_{18} system these compounds were eluted at 8.1 min, 8.8 min and 10.2 min respectively. Hypoxanthine was completely separated from uridine. Uric acid did not appear because the columns were disconnected before this compound had been eluted from the first column. The overall recoveries of pseudouridine, uracil and uri-

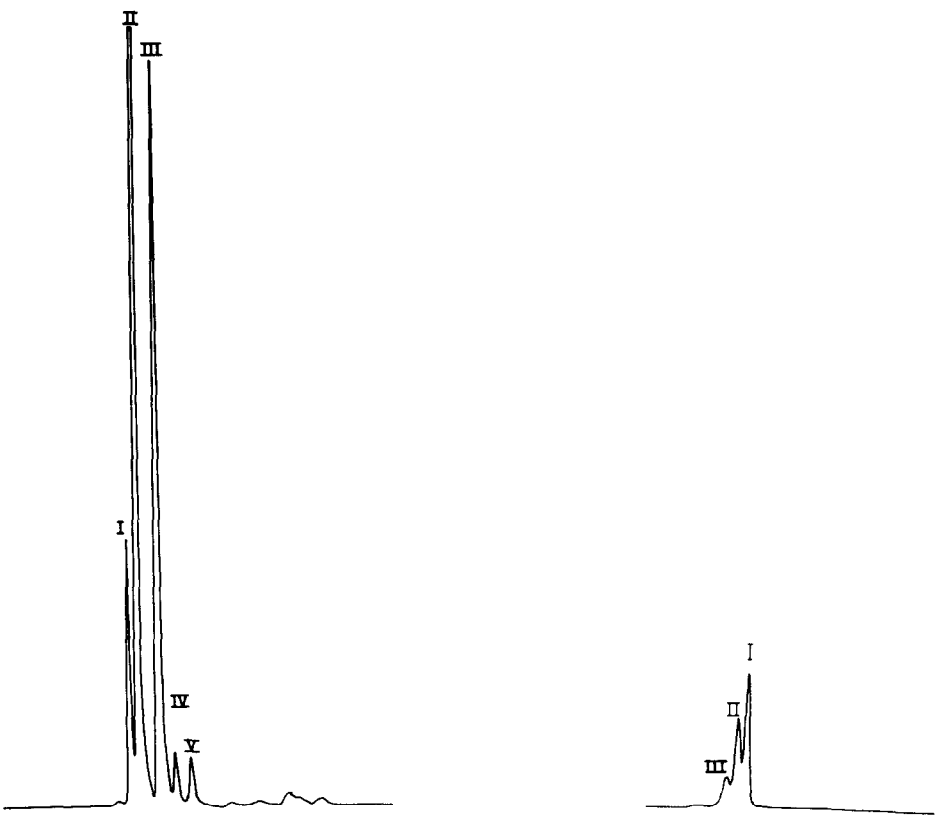

Fig. 1. Separation with dual-column HPLC of urinary pseudouridine (I), uracil (II), uridine (III), hypoxanthine (IV) and xanthine (V) in fraction III from a patient (A.v.d.R.) with OCT deficiency. The level of plasma ammonia was 92 μmol/l. Column I: Partisil-10-SAX, 4.6 mm × 25 cm. Column II: μ Bondapak C_{18}, 3.9 mm × 30 cm. Eluent: methanol/KH_2PO_4 (0.002 mol/l, pH 5.0) = 5/95 (v/v). Flow rate: 1.0 ml/min. UV-detection at 254 nm; detector sensitivity 0.1 Aufs.

Fig. 2. Separation with dual-column HPLC of urinary xanthine (I), thymine (II) and interfering compound (III) in fraction III from a patient (A.v.d.R.) with OCT deficiency. Level of plasma ammonia was 879 μmol/l. Column I: μ Bondapak C_{18}, 3.9 mm × 30 cm. Column II: Partisil-10 SCX, 4.6 × 25 cm. Other conditions: as in Fig. 1.

dine were 96%, 101% and 101% respectively (n = 10). Small losses occur during the pre-fractionation steps. The recovery from the dual-column system is complete. With the system described above, thymine co-eluted with xanthine at 11.2 min. However, with the combination of the reverse phase column and the cation-exchange column (μ Bondapak C_{18} and Partisil-10SCX), we obtained an adequate separation of thymine (11.4 min) and xanthine (10.8 min) and, as confirmed by "peak top UV spectrometry", there was no interference by other substances (see Fig. 2). The overall recovery was 100% (n = 5). Using the reverse phase column only and the mobile phase as described, thymidine was completely separated from the other pyrimidines and from the urinary purines and was eluted at 15.4 min. Recovery was 99.8% (n = 5).

Urinary values in patients with diseases associated with hyperammonemia

A. Carbamylphosphate synthetase (CPS) deficiency

Only one patient, N.A., a girl who died two days after birth, could be investigated. Plasma ammonia was as high as 2200 μmol/l. Plasma amino acids: glutamine, alanine and lysine were all strongly increased. Hepatic CPS amounted to 1% of normal activity (Dr. C. van der Heiden).

Two-dimensional TLC revealed a normal pyrimidine and purine excretion pattern. HPLC values were also normal; orotic acid, orotidine and uracil were present in trace amounts; uridine and pseudouridine amounted to 16 μmol/l and 858 μmol/l but thymine was absent. The level of uric acid was 1100 μmol/l, and of creatinine was 890 mg/l.

B. Ornithine carbamylphosphate transferase (OCT) deficiency

In the hemizygous (male) patients with OCT deficiency, high concentrations of urinary orotic acid and uracil, coinciding with increased levels of plasma ammonia, were found (Table I). Uridine was also excreted in high amounts. On the contrary, heterozygous carriers showed only slightly elevated excretions of orotic acid and uracil, but normal values for uridine. There were, however, two exceptions: patient E.C., an obligate heterozygous carrier, who had high concentrations of orotic acid, uracil and uridine, and patient N.G., who showed moderate excretions of orotic acid and uridine and a high concentration of uracil in the urine sample collected before treatment. Unfortunately, during urine collection in patient E.C., plasma ammonia levels were not established. However, the normal concentration of urinary glutamine in patient E.C. was not indicative of a high plasma ammonia level.

Increased excretion of orotidine calculated per gram creatinine was observed in only one patient (J.B.). Thymine was slightly elevated only in patients J.K. and N.G. Thymidine excretion was not detected at all using TLC, neither was any abnormality in the purine pattern.

Figures 3a and b show the course of urinary pyrimidines and uric acid in relation to fasting plasma ammonia levels in the male patient with OCT deficiency (A.v.d.R.), described elsewhere [27]. As can be seen, urinary orotic acid and uracil ran roughly parallel to plasma ammonia. High urinary concentrations of uridine coincided with high levels of plasma ammonia but, unlike orotic acid and uracil, uridine diminished drastically at low plasma ammonia

TABLE I

URINARY PYRIMIDINES AND URIC ACID IN 3 MALE (HEMIZYGOUS) PATIENTS WITH OCT DEFICIENCY, AND 5 FEMALE PATIENTS (HETEROZYGOUS CARRIERS)

Subject, age and plasma NH_3 (μmol/l)	Creatinine (mg/l)	Glutamine + glutamate (μmol/l)	Orotic acid (μmol/l)	Orotidine (μmol/l)	Uridine (μmol/l)	Uracil (μmol/l)	Thymine (μmol/l)	ψ Uridine (μmol/l)	Uric acid (μmol/l)
Male patients									
A.v.d.R., 0.4 y; NH_3: 296	145	154	1641	11	1210	955	14.0	129	798
J.B., 60 d; NH_3: 119	50	616	124	8.0	51	418	15.9	187	1500
P.L.,									
I 10.4 y, 13-9-'76; NH_3: 31	407	871	754	8.2	76	1154	8.9	151	1200
II 11.4 y, 12-1-'78; NH_3: 62	181	197	82	n.d.	9.6	50	±2.3	40	2900
III 11.4 y, 23-1-'78	1017	754	1389	23	11.0	574	n.d.	392	2000
IV 11.4 y, 26-1-'78	486	760	1210	10	5.2	443	n.d.	236	300
Female patients									
N.G., 2.1 y;									
I 4-7-'78; NH_3: 347	475	1491	281	trace	526	3530	26.8	261	2000
II 18-7-'78; NH_3: 150	226	415	26	trace	2	74	12.4	155	1500
J.K., 4 y									
I 1-3-'70	237	189	13	n.d.	7.2	146	n.d.	153	600
II 2-3-'70	249	210	44	n.d.	trace	267	21.3	254	2000
E.C., 4 y	848	1064	1829	14	2717	2998	n.d.	353	1700
D.C., adult									
I: fasting	1469	340	28	16.5	n.d.	389	n.d.	331	2200
II: post-protein	2068	218	150	8.0	n.d.	<603	n.d.	250	3300
D.B., adult	1616	1778	144	9.0	10	1050	trace	561	3900
6 control patients									
0.3—5.9 y	236—942	109—1399	3.9—20.3	9—26	<44	30—90	<15.3	<601	200—4800

n.d., not detectable with the method used.

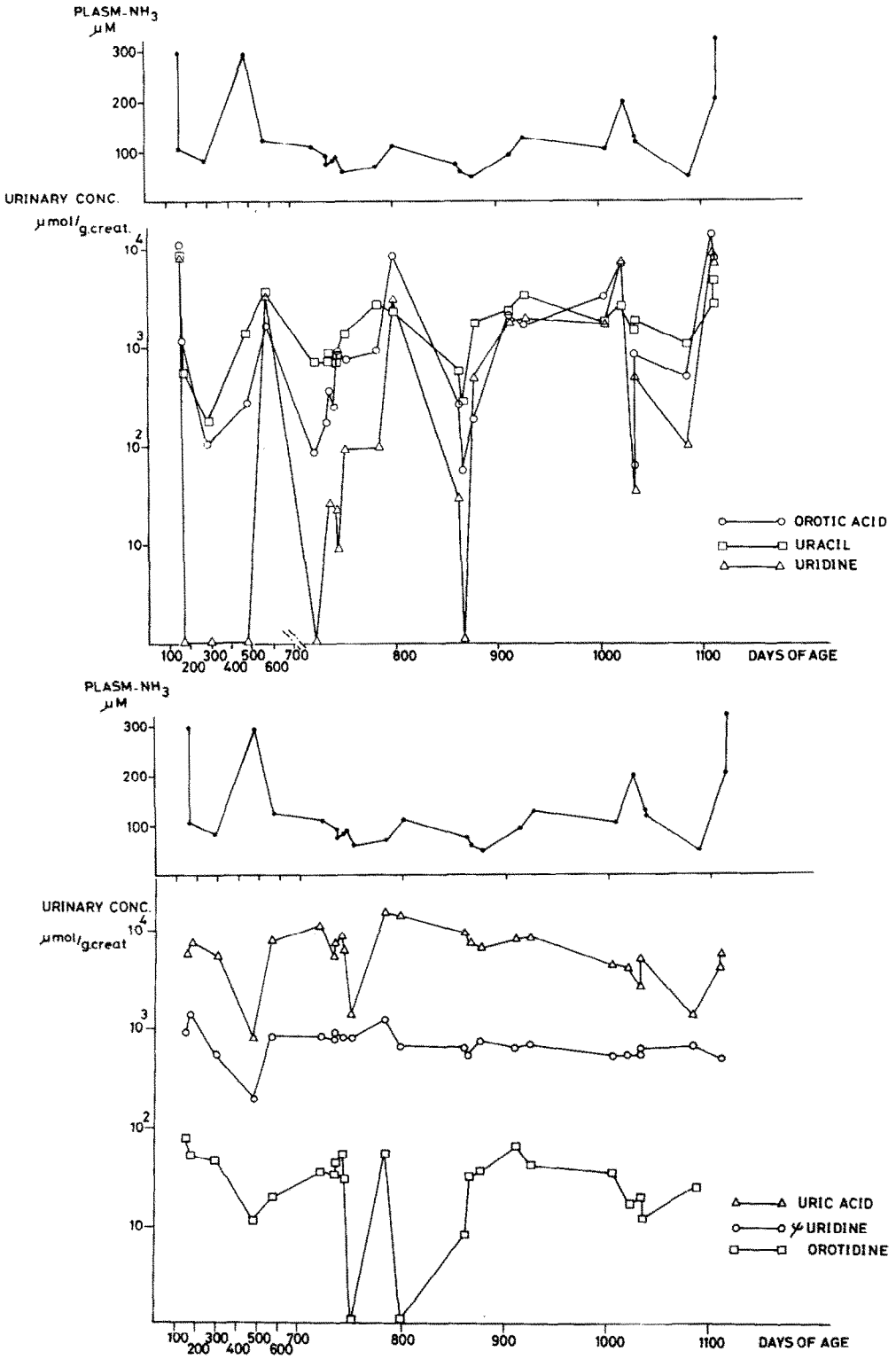

Fig. 3. The time course followed by (a) urinary orotic acid, uracil, uridine and (b) uric acid, pseudouridine and orotidine in relation to fasting plasma ammonia levels in a male patient (A.v.d.R.) with OCT deficiency. Urinary pyrimidines and uric acid are on a logarithmic scale, plasma ammonia on a linear scale.

levels. Increased urinary concentrations of N-C-β-ala were found by two-dimensional TLC of fraction III. N-C-β-ala appeared as a yellow spot, with Ehrlich's reagent, R_F values being 37 mm (R_F-I) and 69 mm (R_F-II) respectively. Urinary uric acid was also frequently elevated. Pseudouridine and orotidine were within the normal range. Only borderline elevated concentrations of thymine (up to 25.6 μmol/l) were found in some urine samples.

C. Argininosuccinic acid (ASA) synthetase deficiency

Table II presents levels of urinary pyrimidines and uric acid in relation to plasma ammonia, plasma or urinary glutamine and citrulline as far as available in 3 patients with ASA-synthetase deficiency, two of them (J.R. and B.H.) with the acute neonatal form, and one (P.S.) with benign citrullinemia [28]. Increased orotic acid, uracil and uridine coincided with hyperammonemia; orotidine and thymidine were normal. Thymine was normal as shown by two-dimensional TLC. Only in patient B.H. was uric acid increased. All other urinary purines were normal as shown by two-dimensional TLC. In patient P.S. uridine was normal, uracil increased on both successive days, but orotic acid only on the first day when citrulline and glutamine + glutamic acid were highest.

D. Argininosuccinic acid (ASA) lyase deficiency (ASA-uria)

In patients with ASA lyase deficiency only slightly elevated excretory levels of orotic acid were found (see Table III). Uracil, uridine and pseudouridine were within the normal range. Thymine was normal as shown by two-dimen-

TABLE II

URINARY PYRIMIDINES AND URIC ACID IN 3 PATIENTS WITH ASA-SYNTHETASE DEFICIENCY IN RELATION TO PLASMA AMMONIA, PLASMA GLUTAMINE OR URINARY GLUTAMINE + GLUTAMIC ACID AND CITRULLINE

Sample	Compound	Subject			
		J.R. ♀ age: 4 days	B.H. ♂ age: 1 day	P.S. ♂ age: 8 y 5 mo	
		μmol/l	μmol/l	date: 19-2-'78 p.m. μmol/l	date: 20-2-'78 μmol/l
Plasma	Ammonia	n.a.	2250	n.a.	n.a.
	Glutamine	1747	2356	n.a.	n.a.
	Citrulline	4943	1611	n.a.	n.a.
Urine	Gln + glu	10 087	3435	456	178
	Citrulline	33 909	7954	5712	3525
	Orotic acid	1160	1066	935	32
	Orotidine	trace	n.d.	9.6	10
	Uridine	77	40	71	n.d.
	Uracil	455	721	1190	674
	ψ Uridine	661	125	205	186
	Uric acid	2200	9400	2900	1200
	Creatinine	4425	1372	6018	5399

n.a. = not available; n.d. = not detected.

TABLE III

URINARY PYRIMIDINES AND URIC ACID IN 6 PATIENTS WITH ASA-LYASE DEFICIENCY IN RELATION TO PLASMA AMMONIA OR GLUTAMINE AND URINARY GLUTAMINE AND ASA

Sample	Compound	Subject					
		R.S. ♀; 10 y 5 mo *μmol/l*	S.D. ♀; 8 d *μmol/l*	X.B. ♀; 5 d *μmol/l*	V.Z. ♂; 4 d *μmol/l*	M.Z. ♀; 1 d *μmol/l*	S.M. ♀; 5 y 6 mo *μmol/l*
Plasma	Ammonia	55	533	strongly elevated	362	108	22
	Glutamine	n.a.	n.a.	strongly elevated	2466	3418	1050
Urine	Glutamine	195	6	2582	218	240	453
	ASA + ASA I + ASA II	12090	8525	4090	13651	19294	24408
	Orotic acid	44	11	5.4	22	<22	245
	Orotidine	18	3.8	n.d.	9.7	3.8	11
	Uridine	trace	n.d.	<84	n.d.	7.9	2.9
	Uracil	83	trace	<27	trace	trace	422
	ψ Uridine	309	160	<23	247	281	420
	Uric acid	3400	1399	300	4048	400	3300
	Creatinine	8274	1770	823	3761	3407	6372

n.a. = not available; n.d. = not detected.
ASA = arginino succinic acid; ASA I and ASA II: anhydrides of ASA.

sional TLC. Except for the increased uric acid excretion in patient V.Z., no abnormalities of urinary purines were found.

E. Lysinuric protein intolerance

Urine samples were analyzed from two patients with lysinuric protein intolerance, from a girl M.K. (aged 3 years and 9 months) and her brother R.K. (aged 3 years and 8 months). Both children had hepatomegaly and growth

TABLE IV

URINARY PYRIMIDINES AND URIC ACID IN RELATION TO URINARY GLUTAMINE, LYSINE AND ARGININE IN 2 SIBS WITH LYSINURGIC PROTEIN INTOLERANCE

Compound	Subject	
	R.K. ♂; 3 y 8 mo *μmol/l*	M.K. ♀; 3 y 9 mo *μmol/l*
Glutamine	545	591
Lysine	2534	2946
Arginine	457	141
Orotic acid	387	<42
Orotidine	11	trace
Uridine	38	7
Uracil	196	42
ψ Uridine	355	107
Uric acid	2900	300
Creatinine	3451	7655

TABLE V

URINARY PYRIMIDINES AND URIC ACID IN RELATION TO PLASMA AMMONIA IN 3 PATIENTS WITH REYE'S SYNDROME (A.B., E.C. AND K.W.), 2 PATIENTS WITH METHYLMALONIC ACIDEMIA DURING KETOTIC ATTACKS (S.G. AND A.G.) AND 1 PATIENT WITH PROPIONIC ACIDEMIA (S.)

Compound	Subject					
	A.B. ♂; 2 y 1 mo μmol/l	E.C. ♂; 10 y μmol/l	K.W. ♀; 3 mo μmol/l	S.G. ♀; 2 y 7 mo μmol/l	A.G. ♂; 3 y 1 mo μmol/l	S. ♀; 5 d μmol/l
Plasma ammonia	94	398	167	171	84	1220
Orotic acid	21	trace	(1)	<68	9	(1)
Orotidine	10	trace	13	6.5	9.7	42
Uridine	n.d.	1	n.d.	n.d.	n.d.	7
Uracil	37	78	7	n.d.	n.d.	trace
ψ Uridine	232	34	381	140	137	511
Uric acid	3200	200	5600	1600	800	1000
Creatinine	2655	3673	3451	1400	1800	—

n.d. = not detected. (1) interference with large amount of unknown product.

retardation. The urine samples were collected before therapy with arginine supplements was initiated. M.K. showed normal levels of excretion, but in R.K. urinary orotic acid, uracil and uridine were increased, orotidine, pseudouridine and uric acid being normal. Thymine was not detected by two-dimensional TLC. Plasma ammonia levels were not determined. However, the normal excretion of glutamine in all of the three patients indicated that highly increased levels of plasma ammonia were not likely (see Table IV).

F. Reye's syndrome and organic acidemia

Patients with Reye's syndrome have elevated concentrations of plasma ammonia early in the course of the disease. In our cases, slightly to moderately elevated plasma ammonia levels were found. However, the excretion of pyrimidines and uric acid was normal (see Table V). Thymine was not detected.

Two patients (A.G. and S.G.) with methylmalonic acidemia showed slightly (A.G.) and moderately (S.G.) increased hyperammonemia during ketotic attacks. A strongly elevated plasma ammonia (>1000 μmol/l) was present in a patient (S.) with propionic acidemia. HPLC of urine collected from these patients during the hyperammonemic period showed no abnormalities in urinary pyrimidine and uric acid (see Table V). Furthermore, two-dimensional TLC revealed normal excretion patterns.

Discussion

HPLC of urinary pyrimidines, using non-specific UV-detection, is complicated by the interference of other urinary constituents.

Several column chromatographic methods for the analysis of pyrimidines and purines in the urine have been described, including conventional cation-exchange chromatography [29,30], HPLC anion-exchange chromatography

[31—35] and HPLC using normal-phase [36] and reverse-phase [37,38] columns. The conventional cation-exchange methods have the capability of separating numerous pyrimidine and purine bases and nucleosides in urine, but on the other hand have the disadvantages of long run times and regeneration steps, and the use of acid buffers, causing decomposition of purine nucleosides and deoxynucleosides. With the HPLC procedures, using totally porous or pellicular anion-exchangers (31—35), the separations of the free bases and the nucleosides are limited. Moreover, the time required for these analyses makes them unsuitable for metabolic studies involving multiple samples. However, micro-particle normal-phase columns, the chemically bonded reverse-phase partition columns and the micro-particle chemically bonded ion-exchange columns allow short analysis times. In the method of Evans et al. [36], the separation of 13 pyrimidines, including pseudouridine, uracil, uridine, thymine, orotate and orotidine is rapidly accomplished on a LiChrosorb SI-100 normal phase column. When applying this method to urine, however, purines and other UV-absorbing compounds interfere despite pre-fractionation. Furthermore, the reverse-phase methods reported in the literature appeared not to be suitable for the analysis of the pyrimidines in which we were interested.

With the dual-column methods described here, the urinary excretion of pseudouridine, uracil, uridine and thymine can be measured quantitatively in the fractions obtained by the isolation method. The same fractions are used for pre-screening of the urine by two-dimensional TLC.

In man there are two carbamylphosphate synthetases (CPS): for the mitochondrial CPS I, ammonia is the nitrogen substrate; the enzyme is activated by acetylglutamine. For CPS II, found in the cytosol, glutamine is the normal substrate. CPS I provides carbamylphosphate (CP) for the urea cycle and CPS II for pyrimidine biosynthesis [39]. However, at least in rat liver, the two CP-pools are not completely separated [40] and the mitochondrial CPS contributes to hepatic pyrimidine synthesis [41].

In CPS I deficiency, ammonia cannot be converted into CP, but in spite of an increased glutamine pool apparently no increase of pyrimidine synthesis occurs. Consequently urinary pyrimidines are normal. However, in a defect of one of the urea cycle enzymes an accumulation of CP occurs leading to an overproduction of pyrimidines. From the results in our patients the conclusion seems to be justified that the level of pyrimidine excretion depends on plasma ammonia concentrations. In cases in which the defective enzyme is located further on in the urea cycle, the hyperammonemia and pyrimidine excretion seem to be less pronounced. This may be explained by the excretion of the accumulating nitrogen-containing metabolites of the urea cycle in these conditions, resulting in lower levels of CP. The excretion of ornithine, citrulline, argininosuccinate and arginine is equivalent to the waste of two, three, four and four nitrogen atoms, respectively. Levels of orotic acid and uracil roughly follow the plasma ammonia levels. However, urinary uridine is only elevated at high ammonia concentrations and high excretions are mainly found in OCT deficiency, which may be of diagnostic value. It seems likely that the excessive synthesis of pyrimidines results from overflow of CP being normally compartmentalized for the urea synthesis. However, this does not explain the difference in the amount of the various pyrimidines excreted. The accumulation of

uridine monophosphate (UMP), not used for de novo nucleotide synthesis, may inhibit the orotidine-5-phosphate-orotate phosphoribosyltransferase enzyme complex [42,43]. This inhibition may lead to orotic aciduria. On the other hand UMP will be catabolized via uridine, uracil, dihydrouracil and N-C-β-ala.

We speculate that N-C-β-ala is not converted into β-ala, CO_2 and NH_3 because of the inhibition of ureidopropionase by the excess of ammonia present. This hypothesis is supported by the fact that an increased urinary N-C-β-ala has been observed by Oberholzer et al. [44]. We could confirm this finding in patient A.v.d.R. with OCT deficiency. Increased excretions of thymidine, pseudouridine, thymine and N-carbamyl-β-aminoisobutyric acid are not expected because these compounds result from a nucleotide breakdown. Urinary thymine concentrations in A.v.d.R. did not correlate with plasma ammonia.

In Reye's syndrome, the hepatic mitochondrial enzymes CPS-I and OCT have been found to be drastically reduced (at least during the first days of the clinical symptoms), the cytoplasmatic enzymes of the urea cycle being normal [11,45—48]. Severe hyperammonemia apparently results from excess waste nitrogen overwhelming the capacity of the reduced CPS and OCT. Occasionally isolated OCT deficiency has been described [49] in patients with Reye's syndrome and in these cases extremely elevated orotic acid excretions have been found. In patients with combined OCT and CPS deficiency, one expects urinary orotic acid to be dependent on the residual activity of CPS-I. Unfortunately, values for the activities of CPS and OCT in our patients were not available. However, the moderate hyperammonemia and normal urinary pyrimidines in our patients suggest a not too great reduction in CPS and OCT.

The cause of hyperammonemia in patients with organic acidemias is not known. In propionic acidemia, inhibition of N-acetyl-glutamate synthetase by elevated propionyl-CoA has been postulated [50]. In a patient with methylmalonyl-CoA mutase deficiency and severe neonatal hyperammonemia, hepatic CPS activity was not detectable, OCT and ASA synthetase activities were greatly reduced and the activity of arginase was moderately depressed [51]. On the other hand, severe hyperammonemia has been described in a newborn infant with methylmalonyl-CoA-mutase deficiency, liver CPS and OCT being normal [52]. In our patients with organic acidemia and hyperammonemia, CPS and OCT were not determined. However, the normal values for plasma urea and for urinary pyrimidines argue against a much reduced CPS and OCT.

Elevated uric acid excretions, frequently present in our patients with hyperammonemia, did not always coincide with high plasma ammonia levels. However, at least in patient A.v.d.R. (Fig. 3), urinary uric acid followed, roughly speaking, urinary pseudouridine, indicating tissue breakdown as a possible cause of the hyperuricosuria.

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References

1 Shih, V.E., Efron, M.L. and Moser, H.W. (1969) Amer. J. Dis. Child. 117, 83
2 Shih, V.E. and Schulman, J.D. (1970) Clin. Chim. Acta 27, 73
3 Fell, V., Pollitt, R.J., Sampson, G.A. and Wright, T. (1974) Amer. J. Dis. Child. 127, 752
4 Colombo, J.P., Richterich, R., Donath, A., Spahr, A. and Rossi, E. (1964) Lancet i, 1014
5 Colombo, J.P., Burgi, W., Richterich, R. and Rossi, E. (1967) Metabolism 16, 910
6 Oyanagi, K., Sogawa, H., Sato, S., Orii, T., Nakao, T. and Fujita, S. (1976) Tohoku J. Exp. Med. 120, 105
7 Sogawa, H., Oyagani, K. and Nakao, T. (1977) Pediat. Res. 11, 949
8 Kekomaki, M., Visakorpi, J.K., Perheentupa, J. and Saxon, L. (1967) Acta Paediat. 56, 617
9 Simell, O., Perheentupa, J., Rapola, J., Visakorpi, J.K. and Eskelin, L. (1975) Amer. J. Med. 59, 229
10 Carson, N.A.J. and Redmond, O.A.B. (1977) Ann. Clin. Biochem. 14, 135
11 Reye, R.D.K., Morgan, G. and Baral, L. (1963) Lancet ii, 749
12 Huttenlochter, P.R., Schwartz, A.D. and Klatskin, G. (1969) Pediatrics 43, 443
13 Snodgrass, P.J. and DeLong, G.R. (1976) N. Engl. J. Med. 294, 855
14 Candes, R.D., Avery, G.B., Walker, F.A. and Hsia, I.E. (1972) Pediat. Res. 6, 394
15 Kang, E.S., Snodgrass, P.J. and Gerald, P. (1972) Pediat. Res. 6, 875
16 Packman, S., Mahoney, M.J., Tanaka, K. and Hsia, I.E. (1978) J. Pediat. 92, 769
17 Morris, J.G. and Rogers, Q.R. (1978) Science 199, 431
18 Milner, J.A. and Visek, W.J. (1974) Proc. Soc. Exp. Biol. Med. 147, 754
19 Levin, B., Abraham, J.M., Oberholzer, V.G. and Burgess, E.A. (1969) Arch. Dis. Child. 44, 152
20 Levin, B., Oberholzer, V.G. and Sinclair, L. (1969) Lancet ii, 170
21 Lis, E.W., Lis, A.W. and deHackbeil, K.F. (1970) Clin. Chem. 16, 714
22 Russell, A., Levin, B., Oberholzer, V.G. and Sinclair, L. (1962) Lancet ii, 699
23 Levin, B., Dobbs, R.H., Burgess, E.A. and Palmer, T. (1969) Arch. Dis. Child. 44, 162
24 Van Gennip, A.H., Van Noordenburg-Huistra, D.Y., De Bree, P.K. and Wadman, S.K. (1978) Clin. Chim. Acta 86, 7
25 Van Gennip, A.H., Grift, J., De Bree, P.K., Zegers, B.J.M., Stoop, J.W. and Wadman, S.K. (1979) Clin. Chim. Acta 93, 419
26 Konitzer, K. and Voigt, S. (1963) Clin. Chim. Acta 8, 5
27 Van der Heiden, C., Bakker, H.D., Desplanque, J., Brink, M., De Bree, P.K. and Wadman, S.K. (1978) Eur. J. Pediatr. 128, 261
28 Burgess, E.A., Oberholzer, V.G., Semmens, J.M. and Stern, J. (1978) Arch. Dis. Childh. 53, 179
29 Wadman, S.K., De Bree, P.K., Van Gennip, A.H., Stoop, J.W., Zegers, B.J.M., Staal, G.E.J. and Siegenbeek-van Heukelom, L.H. (1977) 2nd Int. Symposium on Purine Metabolism in Man, Baden near Vienna, Austria 1976, in Purine Metabolism in Man II: Regulation of Pathways and Enzyme Defects (Müller, M.M., Kaiser, E. and Seegmiller, J.E., eds.) Plenum Press, New York
30 Mills, G.C., Goldblum, R.M., Newkirk, K.E. and Schmalstieg, F.C. (1978) Biochem. Med. 20, 180
31 Scott, C.D., Attrill, J.E. and Anderson, N.G. (1967) Proc. Soc. Exp. Biol. Med. 125, 181
32 Scott, C.D. (1968) Clin. Chem. 14, 521
33 Pitt, W.W., Scott, C.D., Johnson, W.F. and Jones, Jr., G. (1970) Clin. Chem. 16, 657
34 Burtis, A.C., Munk, M.N. and MacDonald, F.R. (1970) Clin. Chem. 16, 667
35 Chilcote, D.D. and Mrocheck, J.E. (1973) Analytical Letters 6, 531
36 Evans, J.E., Tieckelman, H., Taylor, E.W. and Guthrie, R. (1979) J. Chromatog. 163, 29
37 Kuo, K.C., Gehrke, C.W. and McCune, R.A. (1978) J. Chromatog. 145, 383
38 Knudson, E.J., Lau, Y.C., Veening, H. and Dayton, D.A. (1978) Clin. Chem. 24, 686
39 Levin, R.L., Hoogenraad, N.J. and Kretchmer, N. (1974) Pediat. Res. 8, 724
40 Natale, P.J. and Tremblay, G.C. (1969) Biochem. Biophys. Res. Commun. 37, 512
41 Colombo, J.P., Berüter, J., Bachmann, C. and Peheim, E. (1977) Enzyme 22, 391
42 Blair, D.G.R. and Potter, V.R. (1961) J. Biol. Chem. 236, 2503
43 Brown, G.K. and O'Sullivan, W.J. (1977) Biochem. Pharmacol. 26, 1947
44 Oberholzer, V.G. and Palmer, T. (1976) Clin. Chim. Acta 68, 73
45 Thaler, M.M., Hoogenraad, N.J. and Boswell, M. (1974) Lancet ii, 438
46 Brown, T., Hug, G., Bove, K., Brown, H. and Lansky, L. (1974) Lancet ii, 716
47 Sinatra, F., Yoshida, T., Applebaum, M., Mason, W., Hoogenraad, N.J. and Sunshine, P. (1975) Pediat. Res. 9, 829
48 Brown, T., Hug, G., Lansky, L., Bove, K., Scheve, A., Ryan, M., Brown, H., Schubert, W.K., Partin, J.C. and Lloyd-Still, J. (1976) N. Engl. J. Med. 294, 861
49 Thaler, M.M. and Beideman, B. (1977) Pediat. Res. 11, 522
50 Shafai, T., Sweetman, L., Weyler, W., Goodman, S.I., Fennessey, P.V. and Nijhan, W.L. (1978) J. Pediat. 92, 84
51 Shapiro, L.J., Bocian, M.E., Raijman, L., Cederbaum, S.D. and Shaw, K.N.F. (1978) J. Pediat. 93, 986
52 Packman, S., Mahoney, M.J., Tanaka, K. and Hsia, J.E. (1978) J. Pediat. 92, 769