Plasma and Urinary Amino Acids in Children with Primary Hyperoxaluria and in Normal Children

DOROTHY A. GIBBS, C. J. THOMPSON*, and R. W. E. WATTS

From the Medical Professorial Unit, St. Bartholomew's Hospital, London

The demonstration of an abnormal aminoaciduria or aminoacidaemia may provide a genetic marker in the study of inherited diseases as well as help to elucidate the biochemical abnormalities that are present. The urinary excretions of oxalate and glycollate are increased in primary hyperoxaluria, and there is evidence that the metabolic lesion involves the glycine-glyoxylate pathway of glycine metabolism (Frederick, Rabkin, Richie, and Smith, 1963; Hockaday, Clayton, and Smith, 1965; Dean, Griffin, and Watts, 1966). The present investigation was undertaken to determine if patients with primary hyperoxaluria have any detectable changes in their over-all pattern of amino acid metabolism, as judged by the plasma and urinary concentrations and the renal clearances of the main physiologically important amino acids.

Except for one small series of normal prepubertal children published after this work was completed (Scriver and Davies, 1965), the available data on normal plasma and urine amino acid concentrations refer to infants and adults. We therefore studied 5 normal children to establish that there were no gross changes in the plasma and urinary amino acid levels peculiar to the age-group with which we are concerned.

Methods and Subjects

Amino acid analyses. Fasting blood and 24-hour urine samples were collected and prepared for analysis as described elsewhere (Crawhall, Thompson, and Bradley, 1966; Crawhall, Scowen, Thompson, and Watts, 1967), a Technicon† amino acid analyser being used for the determinations (Crawhall *et al.*, 1966).

Urinary oxalate was determined by isotope dilution analysis (Dean and Griffin, 1965).

Subjects. *Hyperoxaluric 1*, was a 12-year-old boy, height 133 cm., weight 30.7 kg., previously reported as

'Case 4' in 'family 4' (Hall, Scowen, and Watts, 1960). He had a right renal calculus, blood urea 32 mg./100 ml., urinary oxalate excretion 162–319 mg. $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ /24 hr., on 10 successive days.

Hyperoxaluric 2 was a girl aged 7 years, with bilateral renal calculi. Height 119 cm., weight 22.8 kg., blood urea 62 mg./100 ml., urinary oxalate excretion 121–208 mg. $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ /24 hr., on 10 successive days. Her parents were unrelated, and one sib, who had renal calculi, died in uraemia, and oxalosis was demonstrated at necropsy.

Hyperoxaluric 3 was a 14-year-old girl, height 153 cm., weight 46.5 kg., referred to as 'subject 3' in a previous study (Crawhall and Watts, 1962). She had bilateral renal calculi, blood urea 34 mg./100 ml., urinary oxalate excretion 154–194 mg. $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ /24 hr. on successive days.

Control 1 was a 7-year-old boy, height 115.6 cm., weight 20.9 kg., urinary oxalate excretion 27.8 mg. $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ /24 hr. He was studied immediately before being discharged from hospital after an attack of acute encephalitis.

Control 2 was a 9-year-old boy, height 139.7 cm., weight 31.8 kg., urinary oxalate excretion 39.5 mg. $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ /24 hr.

Control 3 was an 8-year-old girl, height 129.5 cm., weight 27.9 kg., urinary oxalate excretion 29.4 mg. $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ /24 hr.

Control 4 was a 7-year-old girl height 121 cm., weight 20.6 kg., urinary oxalate excretion 22–30 mg. $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ /24 hr. on 6 successive days.

Control 5 was an 8-year-old girl, height 125 cm., weight 19.6 kg., urinary oxalate excretion 23–30 mg. $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ /24 hr. on 6 successive days. All the control subjects were free from evidence of metabolic, endocrine, or renal disease.

Results

The 24-hour amino acid excretions, plasma amino acid concentrations, and 24-hour renal clearances of amino acids were the same in the hyperoxaluric and control subjects (Tables I and II). No abnormal ninhydrin reacting substances were detected in any of the plasma and urine samples. Similar amounts of ethanolamine were present in some specimens from both the hyper-

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* Present address: The Department of Pharmacology, School of Pharmacy (University of London), Brunswick Square, London W.C.1.

† Technicon Instruments Ltd., Chertsey, Surrey, England.

TABLE I

Urinary Excretion and Plasma Concentration of Amino Acids in Patients with Primary Hyperoxaluria and in Non-hyperoxaluric Controls

Amino Acid	Urine (μ mole/min. 1.73 sq. m.)					Plasma (μ mole/ml.)						
	Primary Hyperoxaluria Patients			Controls		Primary Hyperoxaluria Patients			Controls			
	1	2*	3	Mean	Extreme Range	1	2	3	Mean	Extreme Range		
Taurine	—	0.957	—	0.097	0.598	0.147–0.952 (5)†	0.036	0.036	0.181	0.031	0.066	0.024–0.125 (5)†
Aspartic acid	—	0.087	—	0.058	0.170	0.046–0.295 (5)	0.031	0.013	0.014	0.009	0.017	0.017–0.019 (3)
Threonine	0.214	0.066	—	0.267	0.152	0.078–0.306 (4)	0.076	0.104	—	0.013	0.088	0.043–0.107 (4)
Serine	0.366	0.323	0.214	—	0.309	0.166–0.585 (4)	0.061	0.053	0.070	0.120	0.063	0.046–0.082 (5)
Glutamic acid	0.109	0.047	0.053	0.059	0.113	0.086–0.164 (3)	0.050	0.077	0.095	0.074	0.099	0.082–0.147 (5)
Proline	—	—	—	—	—	—	0.250	—	0.177	—	0.111	0.070–0.140 (3)
Glycine	0.981	0.758	0.329	0.719	1.46	0.459–2.60 (5)	0.200	0.200	0.218	0.280	0.193	0.170–0.208 (5)
Alanine	0.261	0.225	0.139	0.307	0.240	0.147–0.426 (5)	0.370	0.224	0.215	0.360	0.233	0.205–0.268 (5)
Valine	—	0.041	0.019	—	0.049	0.042–0.055 (2)	—	0.168	0.184	0.150	0.136	0.087–0.171 (5)
Cystine	0.058	0.042	0.026	0.027	0.030	0.024–0.034 (3)	0.031	0.022	0.059	0.046	0.031	0.024–0.039 (5)
Methionine	—	—	—	—	0.042‡	—	0.019	0.015	0.014	0.037	0.018	0.012–0.023 (5)
Isoleucine	—	—	—	—	0.030	0.022–0.037 (2)	0.070	0.049	0.043	0.049	0.040	0.038–0.043 (3)
Leucine	0.055	0.035	0.046	—	0.056	0.048–0.068 (3)	0.170	0.094	0.095	0.079	0.078	0.058–0.098 (3)
Tyrosine	0.138	0.074	0.047	0.035	0.104	0.063–0.197 (4)	0.082	0.046	0.039	0.059	0.053	0.045–0.068 (5)
Phenylalanine	0.065	0.078	0.065	0.036	0.071	0.015–0.132 (3)	0.053	0.049	0.046	0.062	0.046	0.033–0.053 (5)
Ornithine	0.120	—	—	0.027	0.316	—	0.058	0.043	0.060	0.042	0.046	0.032–0.056 (5)
Lysine	—	0.158	0.351	0.172	0.536	0.358–0.629 (4)	0.200	0.123	0.121	0.125	0.111	0.069–0.144 (5)
Histidine	1.144	0.480	0.242	0.182	0.555	0.085–1.600 (4)	0.084	0.090	0.064	0.078	0.094	0.063–0.176 (5)
Arginine	—	—	—	—	0.046‡	—	0.098	0.072	0.042	0.084	0.070	0.053–0.083 (5)

* Duplicate values shown for hyperoxaluric Patient 2 were obtained on separate 24-hour urine collections and blood samples.

† Number of observations on which the mean and extreme range are based.

‡ Single value.

A dash — indicates the amino acid concerned was either absent or if present the peak on the amino acid tracing was too small to be integrated accurately.

oxaluric and control patients, but we were unable to study this compound quantitatively.

Discussion

The present values for the plasma concentration and urinary excretion of the amino acids have been

expressed as μ mole/ml. and as μ mole/min. 1.73 sq.m. body surface area, in order to facilitate the comparison of our results with those of the other workers (Scriver and Davis, 1965), who have studied normal older children as opposed to infants or adults. The results for Patient 3 who was 14 years

TABLE II

24-hour Renal Clearances of Amino Acids in Patients with Primary Hyperoxaluria and in Non-hyperoxaluric Controls

Amino Acid	Renal Clearance (ml./min. 1.73 sq. m.)								
	Patients with Primary Hyperoxaluria				Non-hyperoxaluric Subjects				
	1	2*	3	Mean	1	2	3	4	5
Taurine	—	—	26.64	3.18	9.08	7.62	9.77	13.18	6.14
Aspartic acid	—	6.09	—	6.15	9.01	17.77	7.25	—	—
Threonine	2.82	—	—	1.99	—	2.85	1.12	—	0.76
Serine	6.05	3.06	6.14	—	3.31	7.13	2.01	—	0.72
Glutamic acid	—	0.56	0.60	0.79	0.90	1.88	0.59	—	—
Glycine	4.93	1.52	3.72	2.52	2.29	4.73	8.93	1.26	0.89
Alanine	0.70	0.65	0.94	0.84	0.56	2.00	1.07	0.91	0.78
Valine	—	0.10	0.25	—	—	—	0.26	—	0.68
Cystine	1.88	0.46	1.85	0.59	—	0.81	0.78	—	1.42
Methionine	—	—	—	—	—	2.27	—	—	—
Isoleucine	—	0.87	0.19	—	—	0.97	0.55	—	—
Leucine	0.31	0.50	0.37	—	—	0.81	0.67	—	—
Tyrosine	1.68	1.21	1.62	3.27	1.30	2.89	1.47	2.00	—
Phenylalanine	1.24	1.39	1.56	3.53	1.22	2.52	0.40	—	—
Ornithine	2.06	—	—	0.65	—	—	6.85	—	—
Lysine	—	2.89	1.29	1.37	5.15	—	4.88	—	7.00
Histidine	13.68	3.79	5.35	2.33	6.47	17.58	0.35	—	1.11
Arginine	—	—	—	—	—	—	0.78	—	—

* Duplicate values shown for hyperoxaluric Subject 2 were obtained in separate 24-hour clearance studies.

old are in general agreement with the younger members of the series, and the present data for 24-hour clearances agree with the findings of Scriver and Davies (1965) who used 3-hour clearance periods following an overnight fast. Age appears to have little effect on the plasma and urinary amino acid levels, the published values for infants and young children (e.g. O'Brien and Butterfield, 1963; Ghadimi and Pecora, 1964; Cockburn, Sherman, Ingall, and Klein, 1965; Dickinson, Rosenblum, and Hamilton, 1965), and for adults (e.g. Stein and Moore, 1954; Evered, 1956; Cusworth and Dent, 1960; Ackerman and Kheim, 1964; Iob, McMath, and Coon, 1963; Wehr and Lewis, 1966) being in general agreement with the present values.

Frederick *et al.* (1963) and Hockaday *et al.* (1965) reported decreased metabolism of glyoxylate to glycine and carbon dioxide in patients with primary hyperoxaluria, as judged by reduced incorporation of [$1-^{14}\text{C}$] glyoxylate into the urinary glycine and hippurate, and into respiratory carbon dioxide. Dean *et al.* (1966) found that glycine and carbon dioxide formation, from glyoxylate- C_1 , were grossly impaired in kidney tissue from two cases of primary hyperoxaluria. However, the hepatic metabolism of glyoxylate appears to be normal in the disease (Crawhall and Watts, 1962; Hockaday *et al.*, 1965; Dean, Watts, and Westwick, 1967). The metabolic abnormality in primary hyperoxaluria is not reflected in the plasma concentration or renal excretion of glycine or of the other amino acids (Tables I and II), indicating either that the transaminase system, which is defective in the disease, is unimportant from the point of view of glycine homeostasis, or that reduced activity of the pathway can be compensated by increased glycine synthesis. Ethanolamine is a possible precursor of glycine via glyoxylate (Arnstein, 1954), and it is of interest that the defective glyoxylate metabolism in primary hyperoxaluria does not lead to a gross accumulation of this substance.

The inborn metabolic errors in which there is a major defect in intermediary amino acid metabolism with overt changes in the concentrations of amino acids and their metabolites in the body fluids are often associated with gross clinical abnormalities. The absence of any detectable abnormality in the pattern of amino acid composition of the blood and urine in the present study is in accord with the clinical observation that children with primary hyperoxaluria develop normally and remain asymptomatic until urinary lithiasis and nephrocalcinosis occur.

Summary

The 24-hour excretions of amino acids, the plasma amino acid concentrations, and the renal clearances of the amino acids were studied by ion exchange chromatography in a group of children with primary hyperoxaluria and in control children of similar ages. There were no significant differences between the two groups. The results are discussed in relation to the published data on the amino acid concentrations in blood and urine in normal subjects, and to the current views concerning the aetiology of the disease.

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