Familial hyperargininaemia

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Terheggen, H. G., Lowenthal, A., Lavinha, F., and Colombo, J. P. (1975). *Archives of Disease in Childhood*, 50, 57. **Familial hyperargininaemia.** A third case of hyperargininaemia occurring in one family was studied from birth. In cord blood serum arginine concentration was only slightly raised, but arginase activity in red blood cell haemolysates was very low. In the urine on day 2 a typical cystinuria pattern was present. Arginine concentration in serum increased to 158 $\mu\text{mol}/100\text{ ml}$ on the 41st day of life. Later determinations of the arginase activity in peripheral blood showed values below the sensitivity of the method. Blood ammonia was consistently high, and cystinuria was present. The enzymatic defect was further displayed by intravenous loading tests with arginine.

Serum urea values were predominantly normal or near the lower limit of normal, suggesting the presence of other metabolic pathways of urea synthesis. In urine there was no excretion of guanidinosuccinic acid, while the excretion of other monosubstituted guanidine derivatives was increased, pointing to a connexion with hyperargininaemia.

Owing to parental attitude, a low protein diet (1.5 g/kg) was introduced only late. The infant developed severe mental retardation, athetosis, and spasticity.

In 1969 we demonstrated hyperargininaemia in two sibs. This was a new disorder of urea synthesis with arginase deficiency, the biochemical findings being argininaemia, hyperammonaemia, and serum urea concentrations near the lower normal limit. The urine showed a cystinuria pattern with marked argininuria. The clinical picture was characterized by psychomotor retardation, seizures, spastic palsy, ataxia, tremor, hepatopathy, and periodical vomiting. Investigations of the family showed that the parents and two other sibs were heterozygous for the disorder.

We now report a third child from this family, born in 1971 and showing the typical signs of hyperargininaemia.

Materials and methods

Amino acids and monosubstituted guanidine compounds. Amino acids in serum and urine were determined by column chromatography with an automatic amino acid analyser (Efron, 1965). Serum was deproteinized with a 5% sulphosalicylic acid solution (1:1 v/v). The monosubstituted guanidine compounds were determined in urine, acidified to pH 1-2 with HCl.

Amino acids and monosubstituted guanidine compounds were analysed simultaneously by splitting the column effluent and staining the monosubstituted guanidine derivatives with the reaction of Sakaguchi. Identification of the derivatives was by comparing with commercially available standards.

Arginase. Arginase was measured in red cell haemolysates. Preparation of haemolysates and enzyme determination were published earlier (Colombo *et al.*, 1967).

Diet and loading tests. Amino acids of serum and urine were determined while the patient was on low protein diet (1.5 g/kg), and intravenous loading tests with arginine (300 mg/kg) and ornithine (300 mg/kg) were done. During the arginine and ornithine loading test the monosubstituted guanidine compounds in the urine were also measured. For the loading test two children served as controls.

Other tests. Blood ammonia (Müller-Beissenhirtz and Keller, 1965), urea (Fawcett and Scott, 1960), and creatinine (Fauvert and Hartmann, 1962) were also determined.

Clinical progress

The mother's pregnancy, her seventh, was complicated by hypertension and proteinuria. The girl was born

TABLE I

Amino acids in serum ($\mu\text{mol}/100\text{ ml}$) and urine ($\mu\text{mol}/\text{g creatinine}$) before and after introduction of a low protein diet

	Normal values (± SD)	Protein supply (2·76–3·0 g/kg per 24 h)					Low protein diet (1·5 g/kg per 24 h)		
		Day of life					Day of life		
		1/2	8	25	41	54	81	102	130
<i>Serum</i>									
Citrulline	3·45 ± 0·92	1·17	1·24	2·01	4·72	3·39	3·07	2·00	2·09
Cystine	2·83 ± 2·17	8·78	1·85	0·87	3·38	7·74	Traces	Traces	Traces
Lysine	18·87 ± 3·83	37·77	16·08	28·83	23·56	26·90	12·26	9·10	8·63
Ornithine	10·86 ± 3·14	4·05	5·73	4·18	7·20	5·57	4·66	4·30	4·21
Arginine	8·17 ± 1·81	25·20	33·42	56·26	157·92	149·45	84·10	70·95	73·32
<i>Urine</i>									
Citrulline	9 ± 8	6	ND	102	1 401	—	18	8	Traces
Cystine	83 ± 18	352	126	685	2 323	—	331	255	138
Lysine	186 ± 77	471	224	2 677	10 649	—	409	430	356
Ornithine	35 ± 16	21	63	147	2 356	—	54	55	143
Arginine	33 ± 14	108	ND	500	17 787	—	301	319	302

ND, not determined.

after a normal delivery at term, weight 3 200 g, length 51 cm. The neonatal period was uneventful. The first metabolic investigations were made on cord blood, which showed a raised arginine level in serum (25 $\mu\text{mol}/100\text{ ml}$, Table I). The urine on day 2 showed a typical cystinuric pattern (Table I). Excretion of taurine, threonine, leucine, and ethanolamine was increased, that of histidine was decreased. Arginase activity in red blood cell haemolysates from cord blood was very low (63 $\mu\text{mol}/\text{h}$ per g haemoglobin; normal range 793–1 330). Later determinations in peripheral blood showed values below the sensitivity of the method (50 $\mu\text{mol}/\text{h}$ per g haemoglobin). The activity of the argininosuccinate cleavage enzyme was normal, 34.2 $\mu\text{mol}/\text{h}$ per g haemoglobin (normal range 18–40). From these enzyme activities the diagnosis of hyperargininaemia was likely, though the rise in serum arginine was not yet marked. Unfortunately the parents were not willing to hospitalize the child immediately, so that therapy was only belatedly instituted. At 5 months of age the girl showed obvious motor retardation, and at 7 months athetosis was evident. She is now 3 years old and shows marked psychomotor retardation, athetosis, and spasticity of the lower extremities. EEG and fundi of the eyes have been normal throughout.

Biochemical investigations

Amino acids in serum and urine. On day 25 the serum arginine concentration was unequivocally raised (56 $\mu\text{mol}/100\text{ ml}$), and further increased to 158 $\mu\text{mol}/100\text{ ml}$ on the 41st day and 149 $\mu\text{mol}/100\text{ ml}$ on the 54th day (Table I). Serum concentration of methionine was persistently increased, while the concentrations of taurine, glycine, and ornithine were decreased. The other amino acids were

present in normal concentration. In the urine, the pattern of cystinuria was persistently present.

Urinary monosubstituted guanidine derivatives (Table II). The excretion of guanidino-succinic acid, guanidinoacetic acid, N- α -acetyl arginine, γ -guanidinobutyric acid, and argininic acid

TABLE II

Excretion of monosubstituted guanidine compounds in urine ($\mu\text{mol}/\text{g creatinine}$)

Day of life	2	81	101*	Control subjects	
				a	b
Substance X†	789	882	775	—	—
Substance Y†	1 218	3 429	3 871	281	109
Guanidinosuccinic acid	150	—	—	88	81
Guanidinoacetic acid	1 249	2 668	1 946	657	145
N- α -acetyl arginine	226	1 285	657	22	31
γ -Guanidinobutyric acid	75	95	89	26	5
Argininic acid	55	419	439	—	—

*Collected over 24 hours.

†See text.

was measured in the patient and in 2 control subjects. The patient excreted a monosubstituted guanidine derivative (substance X) which was not detected in the controls and has not yet been identified. Another unidentified substance (Y) was found in the urine of the patient and of the controls. Except in the urine of day 2, guanidinosuccinic acid

was never excreted by the patient. Conversely, argininic acid was excreted only by the patient. All other monosubstituted guanidine derivatives were excreted in larger quantities by the patient than by the controls.

Amino acids in CSF. In CSF the arginine level was markedly increased (Table III). The concentrations of glutamine, citrulline, methionine, tyrosine, and histidine were also raised.

Ammonia determinations. Blood ammonia

TABLE III

Amino acid concentration in CSF on normal protein intake ($\mu\text{mol}/100\text{ ml} \pm \text{SD}$)

	Normal values	Day 37
Citrulline	0.21 ± 0.07	1.21
Cystine	Traces	—
Lysine	1.86 ± 0.64	1.51
Ornithine	0.84 ± 0.23	0.81
Arginine	1.42 ± 0.74	11.32

concentrations were high before a low protein diet was begun. Fasting values amounted to 237 and 329 $\mu\text{g}/100\text{ ml}$, respectively. 60 minutes after protein ingestion values rose to 356 and 590 $\mu\text{g}/100\text{ ml}$, respectively (Table IV). CSF ammonia was 203 $\mu\text{g}/100\text{ ml}$ in the fasting state and 426 $\mu\text{g}/100\text{ ml}$ 60 minutes after protein ingestion.

Loading tests. After intravenous arginine loading (Table V and VI) a marked rise in serum arginine concentration was noted. By comparison, ornithine concentration was only slightly raised. In urine a temporary increase of arginine excretion was seen, part of a typical cystinuria pattern. These changes in urine were accompanied by the appearance of Frimpter's disulphide and by an increased excretion of glutamine, glutamic acid, and citrulline. The urinary concentrations of all excreted monosubstituted guanidine compounds were persistently at a very high level (Table VII). Similar variations in serum and urine were seen after intravenous loading with ornithine.

In the 2 controls, intravenous loading with

TABLE IV

Ammonia in blood ($\mu\text{g}/100\text{ ml}$), urea ($\text{mg}/100\text{ ml}$), and arginine ($\mu\text{mol}/100\text{ ml}$) in serum in relation to protein intake

Day of life	Protein intake (2.76–3.0 g/kg per 24 h)					Low protein diet (1.5 g/kg per 24 h)						
	1	8	25	41	44	54	90	93	97	102	112	130
Fasting NH_3 in blood					237	329	171	177	143			
Postprandial NH_3 in blood					356	590		280				
Urea in serum	39.0	18.0	8.0	23.0		30.0			5.25	10.2	23.0	24.1
Arginine in serum	25.20	33.42	56.26	157.92		149.45			70.95			73.32

Normal values

Ammonia 45–110 $\mu\text{g}/100\text{ ml}$ (Müller-Beissenhirtz and Keller, 1965).

Urea 10–25 $\text{mg}/100\text{ ml}$ (Polonovski and Colin, 1963).

Arginine $8.17 \pm 1.81\text{ SD } \mu\text{mol}/100\text{ ml}$.

TABLE V

Serum arginine and ornithine levels ($\mu\text{mol}/100\text{ ml}$) after intravenous loading with arginine (300 mg/kg)

	Patient		Control a		Control b	
	Arginine	Ornithine	Arginine	Ornithine	Arginine	Ornithine
Before loading	70.95	4.30	10.00	7.24	8.63	5.80
After loading						
1 h	198.48	5.75	137.30	62.12	82.69	75.24
2 h	187.53	5.95	53.13	43.88	38.96	35.11
3 h	120.41	4.01	30.89	27.39	24.30	15.94
4 h	140.54	4.28	22.87	19.32	19.58	11.73

Normal values

Arginine $8.17 \pm 1.81\text{ SD}$.

Ornithine $10.86 \pm 3.14\text{ SD}$.

Amino acids in urine ($\mu\text{mol/g creatinine}$)

	Patient			
	Cystine	Lysine	Ornithine	Arginine
Before loading	—	—	—	—
After loading				
1 h	2 126	2 824	2 879	42 382
2 h	4 302	8 642	7 106	> 55 041
3 h	3 068	5 538	4 556	> 52 489
4 h	950	1 437	737	6 536

Normal values $\pm$ SDCystine 83 ± 18 Ornithine 35 ± 16 Lysine 186 ± 77 Arginine 33 ± 14

arginine caused similar metabolic changes, except that they were less pronounced and disappeared more rapidly.

Effect of a low protein diet (Tables I and IV). When the infant was 8 weeks old the daily protein intake was restricted to 1.5 g/kg body weight. The high blood ammonia concentrations fell to nearly normal. At the same time the arginine concentration in serum fell to approximately 80 $\mu\text{mol}/100$ ml.

Discussion

This newborn infant came to be investigated immediately after birth because of the known metabolic disorder in two of her sibs and the heterozygous state of disease in her parents (Terheggen *et al.*, 1969, 1970, 1972). In cord blood the serum arginine concentration was already slightly raised, and by the second day of life excretion of cystine, lysine, and arginine in the urine was high. Hyperargininaemia was proved by the very low activity of arginase in cord blood erythrocytes.

In the following weeks the diagnosis was confirmed by a continuous increase of the serum

arginine concentration up to approximately 150 $\mu\text{mol}/100$ ml, a permanent cystinuria pattern in the urine, and a high blood ammonia. The consistently high serum methionine level and low taurine and glycine levels were striking; the same pattern was present in the 2 other affected sibs.

Hyperargininaemia leads to an overflow amino-aciduria. The resulting cystine-lysinuria is due to competitive inhibition of tubular reabsorption of these amino acids by excess arginine.

Besides these changes in amino acid concentrations, evidence of an inborn defect in urea synthesis was the high blood ammonia concentration when the child was on a normal diet (protein approximately 3 g/kg) particularly when the measurement was made 60 minutes after protein intake. After reduction of protein administration to 1.5 g/kg body weight, the ammonia concentrations ranged slightly above the normal values. Hyperammonaemia is also found in other inborn errors of the urea cycle and so provides no information on the localization of the metabolic block, which requires investigation by loading tests. After intravenous arginine loading the serum arginine concentration was markedly increased,

TABLE VII

Excretion of monosubstituted guanidine derivatives in urine ($\mu\text{mol/g creatinine}$) after intravenous arginine load (300 mg/kg)

	Before loading	After loading (h)				
		1	2	3	4	5
Compound X*	280	263	384	422	472	444
Compound Y*	904	1 810	9 408	11 089	6 098	3 674
Guanidinosuccinic acid	—	—	—	—	—	—
Guanidinoacetic acid	1 182	1 675	4 031	3 387	1 533	1 595
N- α -acetyl arginine	649	1 884	7 020	5 264	1 987	1 768
γ -Guanidinobutyric acid	19	218	356	202	168	82
Argininic acid	61	79	350	321	178	228

*See text.

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after intravenous arginine load (300 mg/kg)

Control a				Control b			
Cystine	Lysine	Ornithine	Arginine	Cystine	Lysine	Ornithine	Arginine
53	63	29	22	45	61	7	8
4 540	7 517	16 182	59 017	2 013	10 432	34 065	87 266
1 236	3 529	6 881	8 927	637	3 151	4 872	6 784
186	444	781	1 027	120	395	230	477
74	97	122	147	103	164	61	116

while the values for ornithine and citrulline were unchanged. The lack of any rise in the ornithine concentration points to the defective cleavage of arginine into urea and ornithine.

After intravenous administration of arginine the cystinuria pattern in the urine became even more evident, arginine excretion in particular rising to high values. Frimpter's disulphide was also found. It may be concluded from this that cystinuria in hyperargininaemia is a secondary phenomenon and dependent on the quantity of filtered arginine. The secondary character of cystinuria is further shown by the fact that a transitory cystinuria was demonstrable in 2 healthy control persons after intravenous arginine loading.

The increased excretion of guanidinoacetic acid, N- α -acetyl arginine, γ -guanidinobutyric acid, and argininic acid in these cases, particularly pronounced after intravenous administration of arginine, points to a connexion with hyperargininaemia which requires further investigations.

Stein and co-workers (Stein, Cohen, and Kornhauser, 1969) noted increased elimination of guanidinosuccinic acid in uraemic patients and in rats treated with arginine. The fact that this substance is not excreted by patients with inborn defects of urea synthesis led them to postulate that arginine is a metabolite in guanidinosuccinic acid synthesis. Our findings are not in agreement with this suggestion, since in spite of high arginine concentrations no measurable amount of guanidinosuccinic acid was detected in the patient's urine.

In our patient serum urea values were either normal or near the lower limit of normal, despite the presence of an arginase deficiency. The metabolic pathway for urea synthesis as described by Cohen, Stein, and Bonas (1968), suggesting guanidinosuccinic acid as a precursor of urea, offers no explanation for this. Difficulties in accepting this alternative pathway have been outlined by Levin

(B. D. Cohen, personal communication, 1969), guanidinosuccinic acid is not excreted by the patient despite high arginine values.

A different metabolic pathway for urea synthesis was described by Scott-Emuakpor, Higgins, and Kohrman (1972) based on the earlier work of Ryan and Wells (1964). These authors interpret the normal ammonia and urea values in a case of citrullinaemia by formation of urea from lysine. Metabolites of this pathway are homocitrulline, homoargininosuccinic acid, and homoarginine. The authors designate this way of synthesis the 'lysine-urea cycle' in contrast to the ornithine-urea cycle of Krebs-Henseleit. Since arginase is deficient in hyperargininaemia, urea cannot arise from the splitting of homoarginine. Furthermore, it has been shown in human liver that no arginase activity could be measured when as substrate homoarginine was substituted for arginine (Colombo, 1971). An alternative urea cycle has also been questioned by Levin, Oberholzer, and Palmer (1973). They examined several patients with disorders of the urea cycle other than citrullinaemia and were unable to detect homocitrulline and homoarginine in the plasma.

von Murdaugh and co-workers (1958) showed that, in a state of low protein intake, the fraction of filtered urea excreted is decreased. The authors relate this finding to a functional change of the renal tubules. Some urea might also be ingested with food and a small quantity of it reabsorbed by the gut. Except for these last two possibilities we have no explanation for the normal urea concentration in the patient's serum.

After intravenous arginine administration, formation of ornithine in small amounts occurred in the patient, as had been observed in her two sibs. Several suggestions can be made for this phenomenon. Ornithine may originate from endogenous or exogenous proline. This reaction

was shown to occur in animal tissue (Smith, Benziman, and Strecker, 1967). Walker (1963) showed that ornithine and guanidinoacetic acid are formed by transamidation of glycine with arginine. The high urinary concentration of guanidinoacetic acid and its rise after arginine load might support this suggestion. In cerebral tissue ornithine may originate by transamidation of arginine with the abundantly available γ -aminobutyric acid (Pisano, Abraham, and Udenfriend, 1963). Finally the presence of an arginase functioning in tissues other than liver and erythrocytes cannot be excluded (Barej and Hill, 1970).

A low protein diet was instituted as a therapeutic measure, based on the suggestion that the chronic ammonia intoxication and possibly also the raised arginine concentrations are the major factors responsible for the cerebral damage in hyperargininaemia. Although the blood ammonia fell to values only slightly above the upper limit of normal, cerebral damage was not prevented. This raises the question whether other factors (arginine?) may play a part in the pathogenesis of brain injury, or whether brain injury results from starting the diet too late, or perhaps is congenital. Though ammonia is certainly detoxified by the mother in cases where there is a congenital enzyme defect, it is noteworthy that in one case of argininosuccinic aciduria the findings pointed to the cerebral damage having been prenatal (Baumgartner *et al.*, 1968).

One other case of hyperargininaemia was recently reported by Cederbaum, Shaw, and Valente (1973) who described a 5½-year-old patient with a similar clinical picture to that of our patients. Red blood cell arginase was less than 2% of normal; arginine plasma values were increased, though there was no hyperammonaemia. In the case described by Peralta Serrano (1965), hyperargininaemia was only shown in relation to epileptic crisis, and disappeared in the intervals; there was no cystinuria pattern, nor was serum arginine increased in the parents.

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