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Diagnostic Value of Orotic Acid Excretion in Heritable Disorders of the Urea Cycle and in Hyperammonemia due to Organic Acidurias*

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Abstract. Orotic acid excretion in urine is increased in ornithine transcarbamylase deficiency, citrullinemia and argininemia; it is barely increased in argininosuccinic aciduria and normal in carbamylphosphate synthetase deficiency and in hyperammonemia due to organic aciduria. The determination of orotic acid excretion is useful in differentiating the causes of hyperammonemia and reduces the need for enzymatic assays on tissue biopsies for decisions on therapy. The data indicate that orotic acid does not merely reflect ammonia concentration in plasma, but depends on carbamylphosphate concentration. Arginine could play a key role in the regulation of ammonia detoxication.

Key words: Orotic acid – Urea cycle – Metabolism, inborn errors – Hyperammonemia – Organic aciduria.

Orotic acid is an intermediary metabolite of pyrimidine synthesis. It originates from aspartate and carbamylphosphate (Fig. 1). An elevation of urinary orotic acid excretion has been described in primary orotic aciduria [15] and in some isolated cases of congenital defects of the urea cycle enzymes [1, 13, 18, 20, 22, 24].

We investigated to what extent the determination of urinary orotic acid excretion is useful in the diagnosis of primary defects of the urea cycle. In addition, because in our experience hyperammonemia appears to be a predominant finding in the organic acidurias, we analyzed urine samples from patients with propionic acidemia, methylmalonic aciduria and combined propionic and 3-methylcrotonic aciduria (biotin dependent) in order to assess whether orotic acid excretion can be used to differentiate these latter defects from disorders of the urea cycle.

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Methods

Orotic acid in urine was determined by anion-exchange chromatography, as described elsewhere [1, 4]. Creatinine was analysed with a modified Jaffé reagent [10].

The determinations of urea cycle enzymes in liver or other tissues as indicated in Table 1 (except patient S.W.) were performed in our laboratory according to Short et al. [23] or modified from Brown and Cohen [6]. Reference ranges are given in Table 3.

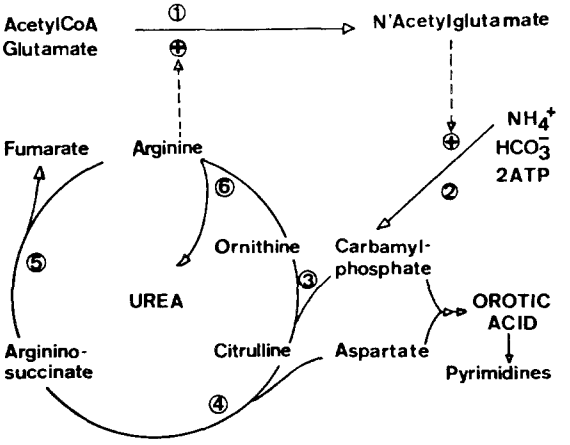

Fig. 1. Urea cycle. *1* N-Acetylglutamate synthetase, *2* Carbamylphosphate synthetase (*CPS*), *3* Ornithine transcarbamylase (*OTC*), *4* Argininosuccinate synthetase, *5* Argininosuccinate lyase, *6* Arginase. N-acetylglutamate activates CPS and arginine stimulates N-acetylglutamate synthetase. If an excess of carbamylphosphate is formed in the mitochondria it is shunted into the extramitochondrial pyrimidine pathway

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Table 1. Orotic acid in urine from patients with congenital defects of the urea cycle

	Age	Sex	NH_3 (plasma) μmol/l	Enzyme activity %[a]	Tissue[b]	Orotic acid		Ref.
						μmol/l	μmol/g creatinine	
Carbamylphosphate synthetase (CPS) deficiency								
K.G.	5d	m	>1760	16	L	3.1	12.9	
Ornithinetranscarbamylase (OTC) deficiency								
J.L.	5d	m	n.m.[c]	n.m.[c]	–	4330	9220	[1]
R.H.	4d	m	1554	n.d.[c]	L	2667	3887	
A.G.	10d	m	Increased	0.6	L	469	2468	
C.P.	1.5y	f	494	17	L	125	162	
V.B.	9m	f	245	20	L	451	1840	
C.D.	3y	f	147–435	23	L	638	3890	
S.B.	12y	f	95–180	26	L	140	224	
A.C.	10m	f	68–500	71	L	7.7	96	
P.J.	4y	f	152	n.m.	–	512	1451	
K.B.	1y	f	Increased	n.m.	–	70	244	
S.W.	3m	f	350	20	WBC	2226	7668	[16]
S.G.	11y	f	141–390	n.m.	–	1856	4789	
Asymptomatic mothers of								
R.H.						49	21	
A.G.						Trace	Trace	
V.B.						2.1	4.6	
C.D.						3.6	4.7	
S.B.						Trace	Trace	
S.W.						87	77	[16]
C.P.						Trace	Trace	
Citrullinemia								
W.R.	4d	m	>1500	14	L	3557	5988	[25]
W.M.	1y	m	100–500	5.5	L	472	4369	
P.R.	7m	m	58	70	F	0.91	5.3	[25]
Argininosuccinic aciduria								
W.M.	64y	f		13	E	5.6	10.3	
				1.7	L			
P.St.	12y	m		10	E	16.9	8.3	
C.St.	3y	f		36	E	2.3	7.0	
P.V.	5y	f	18	42	E	1.5	5.3	
M.S.	9m	f	83	10	E	3.7	68	
Argininemia								
I.W.	6y	f	84–100	8	E	1249	7310	[9]
W.M.	9y	f		n.d.	E	1327	5720	[9]

[a] % of lower reference limit given in Table 3
[b] L: Liver; E: Erythrocytes; WBC: White blood cells; F: Fibroblasts
[c] n.m.: not measured; n.d.: not detectable

Table 2. Urinary orotic acid in patients with hyperammonemia in connexion with organic aciduria

		NH_3[a] (plasma) µmol/l	Orotic acid[a] (urine)	
			µmol/l	µmol/g creatinine
Propionic acidemia				
G.C.	3d	435	0.07	0.19
L.	14m	200	2.6	0.06
M.D.	10m	382	nd	nd
S.Z.	7d	490	2.1	11.4
Methylmalonic acidemia				
N.M.	2y	176	nd	nd
Biotin-dependent propionic and methylcrotonic acidemia				
D.H.	6m	89	1.1	2.6

[a] Reference values cp. Table 3

Table 3. Reference values (range) [4]

	µmol/l	µmol/g creatinine
Orotic acid (urine):		
Neonates ($n=8$)	0.7–8.0	6.0 –29
1 month – 1 year ($n=10$)	0.4–7.8	1.7 –34
1 year – 7 years ($n=8$)	0.5–6.4	0.7 – 3.9
Adult females ($n=22$)	<0.2–1.6	<0.3 – 2.3
	µmol/h/g	µmol/h/mg protein
Urea cycle enzymes: Liver ($n=10$)		
Carbamylphosphate synthetase	110– 356	0.66– 2.07
Ornithinetranscarbamylase	2495– 5700	9.5 –33.3
Argininosuccinatesynthetase	38– 80	0.09– 0.41
Argininosuccinatelyase	128– 294	0.72– 2.10
Arginase	5340–14550	35.4 –99.1
	µmoles/h/g hemoglobin	
Erythrocytes ($n=22$)		
Argininosuccinatelyase	5.3– 9.6	
Arginase	2225 –6230	
Ammonia (plasma, enzymatic method)		
Neonates (day 5)	<140 µmol/l	
Children	< 50 µmol/l	

Short chain fatty acids in plasma (where available) and organic acids in urine were analyzed by gas chromatography mass spectrometry [3].

Hyperammonemic patients confront the physician with an emergency situation and rapid diagnostic procedures are needed. Thus, the urines sent to us were mostly aliquots. The reference values have also been done on spot urines and related to creatinine excretion. The urines were usually obtained from the patients during exacerbations of their illnesses which had led to diagnostic studies. The tissue and urine samples were sent frozen on dry ice to our laboratory.

Results

The orotic acid excretion in urine from 23 patients with congenital defects of the urea cycle is shown in Table 1. We also include data on 7 asymptomatic mothers of patients with OTC deficiency. Table 2 shows data on five patients with organic acidemias presenting with hyperammonemia.

In disorders of the urea cycle marked differences of orotic acid excretion were found. *CPS deficiency* did not lead to over excretion. In *OTC deficiency* three populations could be differentiated. In this X-linked disorder homozygous males have a very marked overexcretion. In female patients who have varying amounts of residual enzyme activity (as expected from the Lyon hypothesis) [1, 19] the urinary orotic acid overexcretion is generally less than in homozygous males.

Asymptomatic mothers of patients with OTC-deficiency excrete traces or slightly increased amounts of orotate.

In *citrullinemia* (argininosuccinate synthetase deficiency) the orotic acid excretion closely reflects the clinical expression of the disease. It is very pronounced in the neonatal form, increased in the classical type, while a patient with the benign course shows no abnormal elevation. All the patients with *argininosuccinic aciduria* had moderate hyperammonemia, but orotic acid excretion was barely or not increased at all in the patients although the disease was clinically manifest. In contrast, *argininemia* patients [9] in whom blood ammonia elevation was comparable to that found in the patients with argininosuccinic aciduria showed a very marked increase of orotic acid in their urine.

Despite high plasma ammonia concentrations, patients with *organic acidurias* did not have any increase of orotic acid excretion (Table 2).

Discussion

Hyperammonemia is caused by congenital disorders of the urea cycle and it is frequently found with the organic acidurias and in several aminoacidopathies [1]. The aminoacid analysis in plasma and urine allows the diagnosis of citrullinemia, argininosuccinic aciduria, argininemia, ornithinemia (ornithine-aminotransferase deficiency), lysinuric protein intolerance, and of nonketotic hyperglycinemia (cerebrospinal fluid). In these disorders the enzymatic assay in tissue samples is not

necessary for treatment. However, the aminoacid pattern is nonspecific in CPS deficiency, in OTC deficiency and in the organic acidurias. Our data show that among this latter group of diseases the elevation of orotic acid is useful for identifying OTC deficiency. If orotic acid is not elevated, organic acidurias should be searched for by determining short chain fatty acids and organic acids in plasma and urine [3].

The comparison of orotic acid excretion with residual enzyme activity in our series of patients, which encompasses all types of the known urea cycle enzymes, allows an insight into the regulation of the urea cycle in vivo.

We consider enzymatic activities to be more relevant to the understanding of our data than ammonia values. The latter reflect momentary situations and are prone to sampling and methodological variations [2].

Furthermore, ammonia levels were not available for the period of urine collection in all instances and had been obtained with different methods.

Accumulation of carbamylphosphate and a diversion into the pyrimidine pathway is probably responsible for the orotic acid over excretion [1, 22]. Therefore, if carbamylphosphate formation is impeded (patient K.G.) there is no increase of orotic acid. In OTC deficiency the utilisation of carbamylphosphate is reduced. This leads to an overflow into the pyrimidine pathway and to an accumulation of orotic acid at a rate limiting step [1]. This is more pronounced in homozygous males than in females with residual enzyme activity. Some asymptomatic mothers, including one with a history suggesting a carrier status (A.G., affected male offspring), did not have elevated orotic acid excretion. The orotic acid determination for the detection of carriers should be performed after a protein challenge for better discrimination [11–14] and not, as here, in morning urine specimens.

The excretion of orotic acid found in citrullinemia, indicates that carbamylphosphate accumulates in the clinically manifest types of this disease. Our finding that orotic acid excretion is barely elevated in argininosuccinic aciduria also fits the calculations of carbamylphosphate concentrations in liver by Kuchel et al. [17] using computer simulation of the urea cycle. In this model only four enzymes in the cycle were considered. N-acetylglutamate synthetase and CPS were not taken into account. Using this model Kuchel et al. [17] computed no change of carbamylphosphate concentration in argininemia.

This is in contradiction with our findings. Kuchel et al. [17] did not take into account the ornithine depletion due to decreased tubular reabsorption in argininemia [9]. If in this disorder carbamylphosphate accumulates solely because of ornithine depletion (substrate limitation for OTC) as in lysinuric protein intolerance or severe argininosuccinic aciduria [7], one would expect a concordant rise of ammonia. However, in all reported cases of argininemia the plasma ammonia was only mildly elevated, if at all. In our cases it reached the level found in patient M.S. who had argininosuccinic aciduria, while in contrast orotic acid excretion in argininemic patients is of the magnitude found in homozygous males with OTC deficiency or in severe citrullinemia (ammonia at least 400–800 µmol/l). Thus orotic acid excretion does not merely reflect ammonia accumulation and an additional mechanism regulating carbamylphosphate formation must be taken into account to explain our findings. Arginine is a potent stimulator of N-acetylglutamate synthetase in rats [21]. This enzyme catalyzes the formation of N-acetylglutamate, an activator of carbamylphosphate synthetase (Fig. 1). If arginine is increased the conversion of ammonia to carbamylphosphate will be enhanced. In argininemia the utilisation of this latter compound might however be limited because ornithine is depleted (product of the deficient enzyme and increased urinary excretion) [9]. In our opinion the increased orotic acid excretion in argininemia results from both decreased utilisation and increased formation of carbamylphosphate. Our data probably give the first indirect evidence in man that arginine modulates the detoxification of ammonia by its action on acetylglutamate synthetase. This mechanism should be included in computer simulations.

The absence of orotic acid elevation in propionic and methylmalonic acidemia, as well as in biotin-dependent multiple carboxylase deficiency, supports our speculation [1] that the mechanism leading to hyperammonemia involves a decreased activity of CPS, most likely secondary to the inhibition of acetylglutamate formation by propionyl-CoA or formation of propionylglutamate, a much less potent activator of CPS [21]. Arginine therapy might therefore be useful in these organic acidurias. Another mechanism whereby intramitochondrial ATP depletion leads to a diminished carbamylphosphate formation, as recently put forward by Catelineau et al. [8], should also be considered.

Treatment with arginine has also been used in urea cycle defects [7]. Although arginine is probably only slightly diminished in these disorders it might help provide enough ornithine for OTC and enhance the formation of carbamylphosphate. However, as long as we are not sure that this latter compound is less toxic than ammonia we would advocate care during substitution therapy.

Our data show that orotic acid determination would probably fail to detect cases of argininosuccinic

aciduria and CPS deficiency if used solely for the screening of urea cycle defects. Urinary orotic acid determination is a useful tool for further differentiating the hyperammonemic disorders which cannot be readily diagnosed by aminoacid chromatography and reduces the need for enzyme determinations in tissue biopsies.

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