

### 11.1 Introduction

Hyperammonemia (systemic venous or arterial plasma ammonia  $>80$  in newborns or  $>50$   $\mu\text{mol/L}$  after 28 days postnatally) is due either to an increased production exceeding the capacity to detoxify (as in colonization with urease containing microorganisms in an intestinal loop, a neurogenic bladder or with a ureterosigmoidostomy), or to a decreased detoxification capacity. Among the latter causes are primary or secondary defects of enzymes involved in ammonia detoxification or a deficiency of intermediates needed as substrates for a functional urea cycle, such as a nutritional, enzyme, or transport defect, or to interference with portal circulation so that portal blood does not reach the hepatocytes (a portacaval bypass or a patent ductus), which can cause “transient hyperammonemia of the premature”. Ammonia detoxification is reduced in deficiencies of urea cycle enzymes, transport proteins (estimated incidence 1:30 000 newborns, [1]) in conditions where glutamate or acetyl CoA is decreased (valproate therapy and organic acidurias), with carnitine and CoA (sequestered by pathological acyl moieties) and defects of mitochondrial beta-oxidation or carnitine metabolism. These lead to a deficient formation of N-acetylglutamate (NAG), an obligate activator of the first step of ammonia detoxification, and thus to a functional NAGS deficiency. An acetyl CoA deficiency further reduces pyruvate carboxylase, which blocks gluconeogenesis. These two effects of an acetyl CoA deficiency lead to a Reye syndrome. Today, because more specific etiological diagnoses can be made, the Reye Syndrome is disappearing.

The actual enzyme activity in urea cycle disorders (UCD) in vivo is only partially assessed by in vitro assays (artificial conditions). It is a problem and must always be viewed in respect to the nitrogen load entering the pathway (Fig. 11.3). This depends as well on the exogenous nutritional supply or bacterial ammonia production in the gut as on the endogenous balance or imbalance between protein synthesis and catabolism. The clinical heterogeneity of the disorders and any prognostic predictions will thus only partly depend on the genetic background if residual protein is present. “Mild” leaky variants (unstable enzymes in vitro or residual enzyme activ-

ity) may lead to severe hyperammonemic crises if protein catabolism predominates (e.g. major weight loss in newborns, viral infections etc.).

Hyperammonemia is toxic to the brain. It exerts reversible (mostly serotonergic) and irreversible effects. Blood ammonia ( $\text{NH}_3$ ) concentrations exceeding  $180 \mu\text{mol/L}$ , or a coma lasting more than 2–3 days appear to be associated with irreversible defects which worsen with the duration of the coma. Thus, ammonia should be assayed in any sick newborn as a “stat” analysis together with a sepsis work-up, or with the suspicion of an intracerebral hemorrhage which is not confirmed. If hyperammonemia is found, it should be confirmed by a second “stat” assay with samples obtained for a complete laboratory evaluation (plasma and simultaneous spot urine). A diagnosis should be made as rapidly as possible and not later than 12–24 h in order to initiate specific treatment. Among the non-artefactual hyperammonemias, 2/3 are due to urea cycle defects, and 1/3 to organic acidemias and other defects which can not be distinguished by the extent of the hyperammonemia. Blood gas analyses and anion gap determinations are often not helpful since secondary lactic acidosis is often present in UCD patients with circulatory failure. Since the specific treatment used for a UCD can be deleterious to patients with organic acidurias (e.g., amino acid mixtures containing high isoleucine or valine and to some extent benzoate and phenylbutyrate, especially in an MCAD dehydrogenase deficiency and vice versa), a rapid diagnosis is necessary. If a decision to treat is made, emergency therapy (see below) prolonged beyond 24 hours will lead to low essential amino acids and impaired protein synthesis with all the ensuing risks and complications (coagulation problems, hemodialysis and or hemofiltration). This can be avoided or minimized by a rapid and complete laboratory evaluation. The laboratory workload should not be underestimated. Besides the initial diagnostic studies, frequent monitoring is required during treatment. In a UCD, treatment consists of measures for reducing the nitrogen load (restricting natural protein intake, gut acidification with lactulose) and providing the substrates which are rate limiting due to the restriction of natural nutrients or due to the enzyme block. These would be arginine or citrulline, citric acid in the case of ASA, essential amino acids in calculated amounts, and adequate calcium, phosphate, iron, trace elements and vitamins. Also if needed, substrates for alternate pathways such as benzoate, with proper controls in neonates. One must be very cautious with the chronic use of phenylbutyrate because of its long term side effects, which include its interference with cell replication and farnesylation. The above treatment should only be instituted after a definite diagnosis is made and it would be contraindicated in an organic aciduria. Whatever treatment variation is used, it must be carefully controlled, especially in order to avoid chronic malnutrition due to a deficiency of essential amino acids. Dietary management is actually more of a challenge, in practice, than is the hyperammonemia. Because of the expertise and experience

needed in managing patients with UCD's, the transfer of patients into a center with experienced clinicians and a laboratory is recommended.

Brain ammonia toxicity depends upon the level of blood ammonia, which crosses membranes in its undissociated form ( $pK\ 9.05$  at  $37^{\circ}C$ ). Increased brain ammonia is considered to augment the synthesis of glutamate and glutamine, the intercellular transport moiety. This in turn increases the transport capacity of large neutral amino acids (including tryptophan) at the blood brain barrier ( $\gamma$ GT dependant) and elicits an increased serotonin secretion [2]. The increased glutamate released by neurons and its decreased re-uptake is probably exotoxic. The accumulation of glutamine in astrocytes has been shown, under extreme conditions, to lead to astrocytic swelling which may be responsible for terminal brain edema. The mechanisms affecting the energy pathways in brain are still controversial. Since the major portion of brain glutamate is synthesized within the brain, it is not at all clear if plasma glutamine plays any pathogenic role in the brain's toxicity. It is, however, an indicator of ammonia detoxification in the peri-central part of the hepatic lobules (urea cycle enzymes are periportal), or of its release by muscle or other tissues. When managing patients, one must also know that arginine, a semi-essential amino acid, is mainly synthesized in the kidneys from citrulline, which in turn is formed predominantly in the intestine (see Table 11.2). Argininosuccinate synthetase and lyase and the arginine transporters (CAT), additionally, play a role in the recycling of citrulline to arginine (e.g. for NO synthesis in kidneys, intestine and brain [3]).

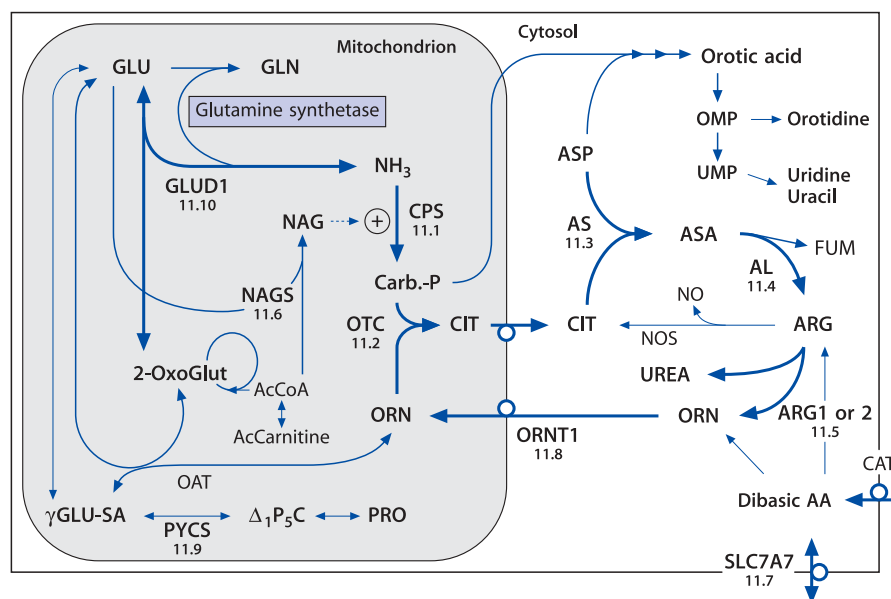
The inherited enzyme deficiencies listed in Table 11.2 lead to the accumulation of substrates and deficiencies of products. For correct interpretation of laboratory results, one need be aware that substrate accumulation can affect the prior enzyme in the pathway (e.g. increased carbamyl phosphate inhibits CPS). A deficiency of urea cycle intermediates (transport or enzyme products or dietary substances) e.g. arginine or ornithine, is often rate limiting. It can initiate a vicious cycle, which worsens the urea synthetic capacity in the cytosol (e.g. by limiting protein synthesis), or in the mitochondria (deficient stimulation of NAGS and of substrate for OTC). Measured plasma values reflect cytosolic metabolite concentrations, not those of mitochondria. Protein catabolism contributes to the plasma amino acid values. Thus, the interpretation of results for plasma arginine, proline and lysine must be done within the context of the pattern found for all of the amino acids. Urea concentrations depend upon the arginine in the cytosol originating from protein catabolism, urea cycle synthesis, and therapeutic applications.

## 11.2 Nomenclature

No.	Disorder	Expression	Chromosome	MIM
11.1	Carbamylphosphate synthetase (CPS 1)	Mitochondrial: liver (periportal → pericentral; all urea cycle enzymes) and much less intestine (enzyme not expressed in red or white blood cells or fibroblasts)	2q35	237300
11.2	Ornithine transcarbamylase (OTC, OCT)	Mitochondrial: liver and much less intestine. Mosaicism in heterozygote females (not expressed in red or white blood cells or fibroblasts)	Xp21.1	311250
11.3	Argininosuccinate synthetase (AS); Citrullinemia Type 1	Cytosolic: Liver, kidney (cortical proximal tubule); intestine, myenteric neurons, ileal and colonic muscles; CNS: not in astrocytes (selective neurons in neocortex, and midbrain), brainstem, diencephalon, cerebellar molecular and granular layer; eye. Fibroblasts	9q34	215700
11.4	Argininosuccinate lyase (AL); Argininosuccinic aciduria	Cytosolic: Liver; kidney (cortical proximal tubule); intestine, myenteric neurons, ileal and colonic muscles; CNS: cerebrum ubiquitous, cerebellum (not in cerebellar white matter); eye: Red cells, fibroblasts	7cen-q11.2	207900
11.5	Arginase 1	Arginase 1 cytosol (liver) Arginase 2 (mitochondria: small intestine, kidney (outer medulla and partly cortex), CNS: ubiquitous; eye: solely retina. Red cells, Fibroblasts	6q23	207800
11.6	N-Acetylglutamate synthetase (NAGS)	Mitochondrial: liver ≫ intestine ≫ spleen. Intestine (enzyme not expressed in red or white blood cells or fibroblasts)	unknown	237310
11.7	Solute carrier family 7 A7 (SLC7A7); Lysinuric protein intolerance	Basolateral membrane Liver, intestine, kidney	14q11.2	222700
11.8	Solute carrier family 25 A15 (SLC25A15, ORNT1); Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome	Mitochondrial membrane: Fibroblasts	13q14	238970
11.9	Δ <sup>1</sup> -Pyrroline-5-carboxylate synthetase, PYCS	Mitochondrial	10q24.3	138250

No.	Disorder	Expression	Chromosome	MIM
11.10	Glutamate dehydrogenase 1, GTP binding site mutations (GLUD1); Hyperinsulinism-Hyperammonemia syndrome	Mitochondrial	10q23.3	138130
11.11	Citrullinemia type 2 (SLC25A13 gene) Citrin deficiency	Mitochondrial membrane, liver (not kidney) with secondary AS deficiency	7q21.3	603471

### 11.3 Metabolic Pathway



**Fig. 11.1.** Metabolites: *GLU*, glutamate; *2-Oxo-Glut*, 2-oxoglutarate; *NAG*, N-acetylglutamate; *NH<sub>3</sub>*, ammonia; *Carb.-P*, carbamylphosphate; *ORN*, ornithine; *CIT*, citrulline; *ASP*, aspartate; *ASA*, argininosuccinate; *FUM*, fumarate; *ARG*, arginine; *Dibasic AA*, dibasic amino acids (lysine, ornithine, arginine);  $\gamma$ *Glu-SA*, gammaglutamyl semialdehyde;  *$\Delta$ 1P5C*, pyrroline-5-carboxylate; *PRO*, proline; *GLN*, glutamine; *OMP*, orotidine 5'-monophosphate; *UMP*, uridine 5'-monophosphate. Enzymes: *OAT*, ornithine-oxoacid aminotransferase; *NOS*, nitric oxide synthetases; *CAT*, cationic amino acid transporters ( $\gamma$ +); others as listed in the table in Sect. 11.2. Glutamate and 2-oxoglutarate are key metabolites for the interconnection of the Krebs cycle (shown as cycle) and the urea cycle; they are also important substrates for transamination reactions (e.g. *ASAT*, *ALAT*) including mitochondrial aspartate synthesis which is transported to the cytosol by the aspartate/glutamate carriers (citrin).

## 11.4 Signs and Symptoms

### (Common to all urea cycle disorders except argininemia)

Central nervous system	Loss of appetite, vomiting Aversion of high protein containing food (Protein intolerance) with consequent malnutrition Lethargy, somnolence, coma Seizures (neonates, infants) Hyperpnea (up to 6 months) Hypo-/hyperthermia (new-borns) Muscular hypo-/hypertonus Ataxia, irritability, sleep disturbance (children) Asterixis, delusions, psychotic behavior (>10 years) Mental retardation	Metabolic alkalosis Growth retardation, osteoporosis, Vit B12, Zn deficiency Terminally: cerebral edema Increased glutamine release Respiratory alkalosis
Eye	Scotomas, visual hallucinations	Papilledema
Circulation	Circulatory failure	Metabolic acidosis
Kidney	Renal failure	Metabolic acidosis
Lung	Hemorrhage	
Liver	Hepatomegaly	Cytolysis (ALAT, ASAT increased); reduced protein synthesis: coagulation defect
Hair & skin	Fibrosis, cirrhosis (chronic) Fragility of hair/trichorrhexis nodosa	Low urea (P) (not always) Arginine deficiency (including iatrogenic)

## ■ Disease “Specific” Signs and Symptoms

Disease	
Citrullinemia type 2 (decreased AS activity, citrin deficiency)	Cholestatic jaundice, hepatic steatosis and siderosis Hyperammonemia not obligate
Argininosuccinic aciduria	Facies with epicanthic fold, depressed nasal bridge (saddle nose) as newborn
Argininemia	Nervous system: increased irritability and muscle tone. Progressive loss of motor and mental skills and increasing spasticity of the lower extremities. Seizures; ataxia, atheto- sis, dysarthria
Lysinuric protein intolerance	Lungs: proteinosis, interstitial pneumonia (white lung disease) Hematology: hemolysis (increased LDH, ferritin), lympho- histiocytic autophagocytosis Kidney: glomerulonephritis Bones: osteopenia
HHH syndrome	Immunity: decreased response to varicella immunization Neurological: pyramidal signs in absence of decerebration (C. Dionisi-Vici, personal communication) Hematology: factor VII & X deficiency
Pyrroline 5 carboxylate synthetase deficiency (few patients, possibly ascertainment bias):	Eye: cataracts Bone and skin: hyperlaxity and increased skin elasticity Hyperammonemia (mild) only preprandial!
Glutamate dehydrogenase mutations	Fasting hypoglycemia noticed mostly in infants or later (hyperinsulinism) Growth failure, variable mental retardation

## 11.5 Reference Values

Analyte	<28 days mature	4 months	1–12 months	2–14 years	Adult men	Adult women	Remarks
Ammonia (P) enzymatic ( $\mu\text{mol/l}$ )	<80	<50					postprandially 30–60 $\mu\text{mol/l}$ higher depending on time and N load
Ammonia (P) microdiffusion ( $\mu\text{mol/l}$ )	21–95		18–74	17–68	21–71	19–63	
Amino acids (P) ( $\mu\text{mol/l}$ )	Range: sampling >3.5 h after end of last feed (p.p.: 1–3.5 h after feed)						
Arginine	77–165 (65–200)	41–190 (60–190)	10–65		35–140	25–125	
Arginino-succinate	<2						
Citrulline	17–41 (13–45)	11–32 (8–36)	10–30		20–55	15–55	In contrast to other amino acids Citrulline is higher pre- than postprandially
Ornithine	55–120 (55–420)	28–150 (40–125)	10–110		30–100	20–90	
Lysine	110–290 (115–330)	60–230 (75–275)	45–145		135–260	115–250	
Glutamine	380–660 (380–710)	200–720 (265–650)	60–470		550–830	440–810	
Alanine	200–490 (185–645)	110–480 (190–550)	100–310		240–600	200–550	
Proline	120–260 (130–310)	64–272 (120–260)	50–190		100–380	70–270	
Amino acids (U) (fractional tubular reabsorption, %)							
Lysine	>95%						
Ornithine & Arginine							
Orotate (mmol/mol creatinine)	0.7–3.3		0.2–3.8	0.08–0.44		0.035–0.26	Increase in 6 h urine after protein load of 1 g/kg; <0.7 mmol/mol cr

No reference values can be given for enzyme assays, since the results depend upon the method used. The reference ranges must be obtained from the testing laboratory which should be contacted for correct sampling and transport conditions.



## 11.6 Pathological Values/Differential Diagnosis

The extent of increase varies widely depending on mutation and especially internal and external nitrogen load.

No.		NH <sub>4</sub>	Arg (P)	ASA (U)	Cit (P/U)	OROT (U)	Homo- citrul- line (U)	Pro (P)	FTR DibAA	Gln/ NH <sub>4</sub> <sup>+</sup>	Orn (P)
11.1	CPS 1 def.	↑-↑↑	↓-n	nd	↓-n	↓-n		n-↑	n	>1.6	
11.2	OTC def.	n-↑↑	↓	nd	↓-n	↑-↑↑	(↑)	n-↑	n	>1.6	
11.3	Citrulline- mia I	↑↑	↓↓	nd	↑↑↑	↑-↑↑	↑	n-↑	n		
	Citrulline- mia II	↑	n-↑	↑	↑				n		
11.4	Arginino- succinic aciduria	↑-↑↑	↓	↑↑↑	↑	n-↑↑				>1.6	
11.5	Argininemia	n-↑	↑↑↑	↑	↑	↑-↑↑			↓	>1.6	Exclude Arg load
11.6	NAGS def.	↑-↑↑	↓	nd	↓-n	↓-n	nd				
11.7	LPI	↑-↑↑	↓	nd	↑	↑-↑↑		↑	↓↓		↓
11.8	HHH syndrome	↑-↑↑	n		n	↑	↑↑				↑ (not in neo- nates)
11.9	PYCS def.	↑ <sup>a</sup>			↓-n			↓			↓
11.10	Hyperinsu- linism-hy- perammone- mia (HIHA) syndrome	↑-↑ <sup>b</sup>	n	nd	n	n				<1.6 DD Bypass of liver	Hypo- glycemia

<sup>a</sup> Only fasting not postprandial.

<sup>b</sup> Unchanged by protein load or restriction. Non-responsiveness to benzoate or phenylbutyrate treatment.

FTR, fractional tubular reabsorption of dibasic amino acids compared to creatinine; n, normal; nd, not detectable; ASA, argininosuccinate; OROT, orotic acid.

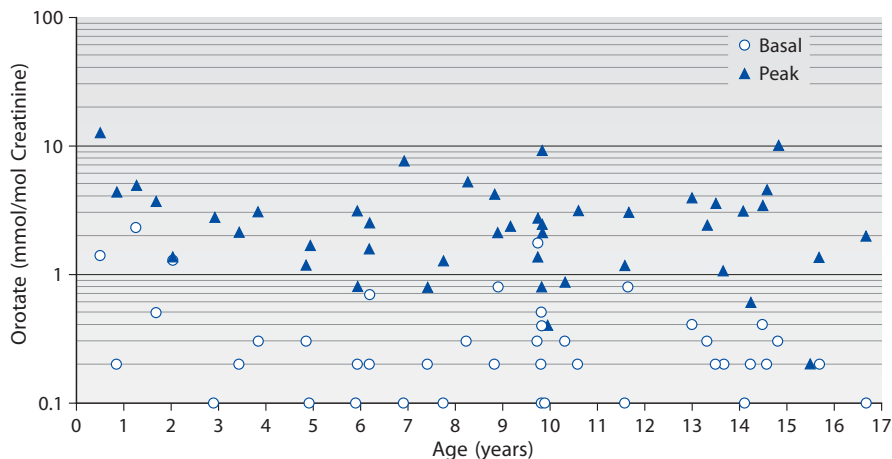
## 11.7 Loading Tests

The goal of loading tests is to unmask a functional defect where there is residual activity. In heterozygotic females with OTC deficiency (x-chromosomal) it can only be used to confirm, but never to exclude a carrier status because of mosaicism (Lyon hypothesis) which might be strongly skewed towards the wild type and thus overlap normal values.

Protein loading tests bear the risk of eliciting hyperammonemia. However, the loading dose can be adapted. Regardless, these tests should only be used when a diagnosis is uncertain and after establishing a daily ammonia profile (preprandial and 1 and 2 hours postprandially) so that a safe tolerated protein intake regime can be calculated prior to the load. Postprandial hyperammonemia levels determined during the profile allows one to estimate the risk of a protein loading dose.

The popular Allopurinol test does not take into account variations of the flux through the pyrimidine synthetic pathway, be it from the carbamyl-phosphate load, due either to endogenous protein breakdown or exogenous protein and nucleotides or to tissue regeneration (CPSII). Furthermore, the regulation of the first multifunctional enzyme is generally ignored. A phosphoribosylpyrophosphate deficiency (as in the Lesch-Nyhan syndrome) also leads to increased orotate. The interpretation of results is not as straightforward as one would wish. False positive and false negative tests have been described. The diagnostic value of orotate (orotic acid) vs an orotidine assay is an ongoing debate.

Procedure: After collecting a baseline urine sample, urine is collected in 4 sequential 6 hour periods and stored frozen after the oral administration



**Fig. 11.2.** Basal orotate decreases with age: the variation of orotate after allopurinol challenge is shown (adapted from Burlina et al. [10])

of a single dose of allopurinol (children >6 y: 100 mg, 6–10 y: 200 mg, >10 y: 300 mg).

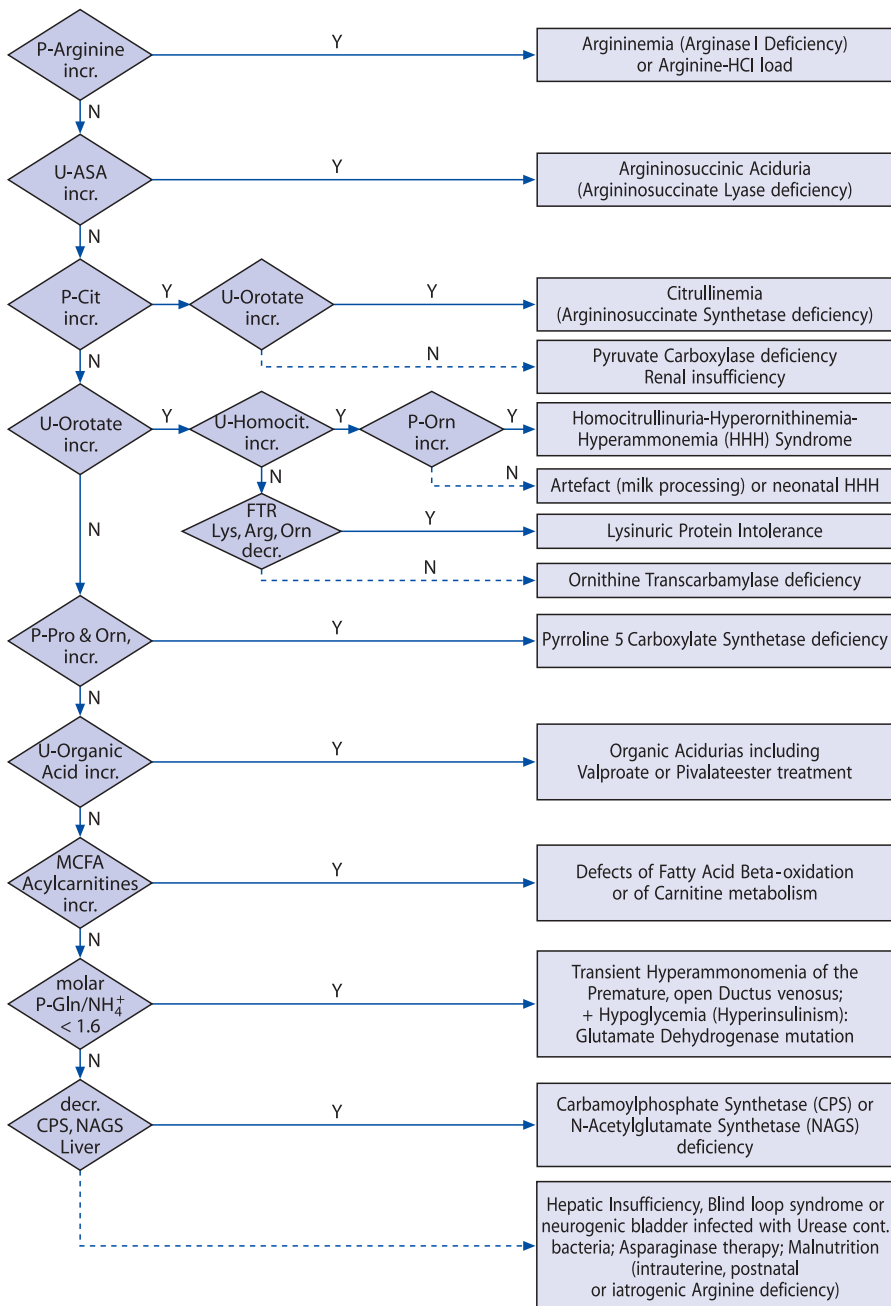
The upper limits or maximal orotate excretion after a load is [10]: 6 mo–6 y: 13.0 mmol/mol Cr; 6–10 y: 9.3 mmol/mol Cr; 10–17 y: 10.2 mmol/mol Cr (see Fig. 11.7). For orotidine, the limit of decision is 8 mmol/mol creatinine [11].

**Protein load:** A protein load is done when a diagnosis is unclear or for heterozygote detection in OTC deficiencies. After one has determined a daily profile for pre- and postprandial ammonia and the amino acids in a self chosen diet, the protein content should be estimated per meal. The patient should not be in a catabolic but steady state for at least 4 days. For women, the test should be avoided around the period of menstruation. The protein load is, in contrast to the allopurinol test, also useful for assessing protein tolerance. False negatives have been described in conjunction with OTC heterozygote testing; skewed toward a predominance of wild-type OTC.

**Procedure:** After a breakfast of mainly carbohydrates, a baseline urine (approximately 4 hours) is collected. After the last voiding, a high protein meal (lean meat, poultry, cottage cheese, etc.) containing 1 g/kg body weight (bw) is given as “a load”. The dose should be reduced, if, by history and/or experience, a protein intolerance to such a dose is suspected. Urine is quantitatively collected during the 6 hours after the end of the meal and cumulatively frozen. It is assayed for orotate by HPLC after alkalisation.

In adult women, the upper limit of normal for orotate 6 hours post load, after a 1 g/kg bw load, is 0.7 mmol/mol creatinine.

## 11.8 Diagnostic Flow Chart



**Fig. 11.3.** Top down algorithm for the differential diagnosis of hyperammonemia based on the results of amino acids and creatinine (P+U), U-urate and organic acids, glucose, acylcarnitines or medium chain fatty acids in plasma. Abbreviations: *incr.*, increased; *decr.*, decreased; *Homocit.*, homocitrulline; *FTR*, fractional tubular re-absorption ( $1 - [(P\text{-creatinine} \times U\text{-amino acid}) / (U\text{-creatinine} \rightarrow P\text{-amino acid})]$ ); *MCFA*, medium chain fatty acids; other abbreviations as in Fig. 11.1

## 11.9 Specimen Collection

Test	Precondition	Material	Handling	Pitfalls
Ammonia	At least 4 h after end of the last meal or stopping intravenous AA supply from a central vein or artery	EDTA blood	Immediately put on ice and centrifuge (4 °C) not later than 15 min after sampling, plasma decanted and frozen at -20 °C Stable at -20 °C up to 48 h  Avoid any dilution of sample before assay (as distilled water, or other acid solution contain ammonia trapped from air)	Capillary sampling increases ammonia concentrations  Muscle hyperactivity liberates ammonia as does a prolonged garrot or hemolysis High ALT or $\gamma$ GT increase ammonia  False low values are found with pyruvate concentrations >200 $\mu$ M False high values in micro-diffusion methods by osmotic hemolysis and glutamine breakdown
Amino acids		Plasma	Centrifuge within 15 min	Contamination by intracellular fluid (capillary blood).
		Urine (spot)	Deproteinize with sulfosalicylic acid Keep frozen and at pH 2 for accurate glutamine results Freeze rapidly to avoid bacterial interference	Glutamine release by muscle activity (e.g. seizures)
Orotate		Urine 5–10 ml	Store at -20 °C	Use HPLC method or isotope dilution only. Orotidine seems to have a limited stability
Benzoate, phenylacetate, phenylbutyrate		Lithium heparinate plasma		
Hippurate, phenylacetylglutamine, phenylacetate	No change of dose during the last 5 days	24 hour urine collection	Cumulatively frozen	For checking therapeutic compliance Indicate total 24 h urine volume and total daily dose of benzoate, phenylacetate or phenylbutyrate

Test	Precondition	Material	Handling	Pitfalls
NAGS, CPS, OTC	Specify appr. protein intake in the last 3 days	Liver biopsy (30 mg)	Blot and freeze in liquid nitrogen, store immediately at $-80^{\circ}\text{C}$ , send with ample dry ice	Activity dependent on protein intake. Only optimized NAGS assays (with arginine) should be used. Gene expression of the urea cycle enzymes in liver is down regulated to 10% by lipopolysaccharides
HHH, ORNT 1		Fibroblasts		Frozen material cannot be used for ornithine incorporation assay

### 11.10 Prenatal Diagnosis

The laboratory should be contacted before collecting/sending specimens.

	DNA	Protein (activity)	Metabolite (amniotic fluid)	Comment
NAGS def.	Gene (RFLP/mutation) Not feasible	Not feasible	Not feasible	
CPS def.	Not in all instances, only known mutations	Fetal liver <sup>a</sup>	Not informative	
OTC def.	Many private mutations	Fetal liver <sup>a</sup>	Not informative	Not predictive in female fetus
AS (citrullinemia I)	Many private mutations	Amniocytes/chorionic villi	Not informative	
AL (ASA-uria)	Many private mutations	Amniocytes/chorionic villi	ASA (AF)	Enzyme activity and ASA concentration should both be assayed
Argininemia I		Fetal red cells		
LPI		Amniocytes/chorionic villi		
HHH		Amniocytes		Assay in cultured, not frozen sample

<sup>a</sup> Varies with gestational age. Intrauterine liver biopsies (week 16–17) have been performed, but are not without risk of fetal liver hemorrhage. A simultaneous control sample is usually required (instability of enzyme and transport conditions!).

### 11.11 DNA Analysis

Can be performed in some CPS deficiency (microsatellite analysis) most OTC deficiencies (RFLP rarely mutation analysis as first step), AS, AL, arginase deficiency, LPI, HHH and HIHA syndrome, not in NAGS deficiency.

The performing laboratory should be contacted before sample collection if possible; otherwise EDTA blood or tissue samples should be collected for DNA extraction.

### 11.12 Initial Treatment (Management while awaiting results)

As stated in the introduction, a rapid diagnosis is required in all instances, with collections of blood and simultaneous spot urines as outlined above. Before initiating any emergency treatment, one must ask the question whether treatment is desirable, if at all, especially in newborns where the prognosis is still reserved (e.g. in known male OTC deficiencies, except for the milder variants, which in a few instances can present with hyperammonemia at a few days of life).

The emergency treatment aims at stopping the endogenous and exogenous protein supply, at supplementing the missing arginine and at giving excess carnitine in order to replenish its free stores and trigger the urinary excretion of pathologic acylcarnitines in organic acidurias.

- Stop per oral protein supply or i.v. amino acid preparations.
- Glucose 8–10 mg/kg per minute i.v. (with insulin if needed); check plasma lactate 2 hours after start!
- Arginine HCl i.v. 2 mmol/kg b.w. as priming dose in 2 hours and then 2 mmol/kg per 24 h.
- Carnitine i.v. 50 mmol/kg b.w. as priming dose in hours and then 300 mg/kg b.w. per 24 h. Stop when organic aciduria has been excluded.

### 11.13 Summary/Comments

For improving the prognosis of inherited hyperammonemias, a major precondition is a timely and rapid accurate diagnosis in order to avoid irreversible damage to the patients brain. This is a motivating challenge to the technicians of well trained and experienced centers in close collaboration with clinical dieticians and other personnel which give guidance and support to the parents. A well equipped laboratory with validated methods and quality assurance is needed and must be prepared to work in emergency situations. The burden continues after a diagnosis is made according to the algorithm presented (without short-cuts) because long term therapy must be adapted to the individual patient with his individual ammonia detoxifying capacity and nitrogen load [13]. Overtreatment with excessively restricted essential amino acids (especially plasma isoleucine <25 µmol/L) is a major problem with inexperienced teams, who focus primarily on the ammonia levels. An understanding of the biochemical pathways and their

complexity is needed for adequate interpretation, for which the professionals in the laboratory can be of great help to the clinician.

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