J. Inher. Metab. Dis. 21 (Suppl 1) (1998) 101–111

Alternative pathway therapy for urea cycle disorders

F. Feillet and J. V. Leonard*
Biochemistry, Endocrine and Metabolic Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

* *Correspondence*

Summary: In man the major pathway for the disposal of waste nitrogen is the urea cycle; in inborn errors of this pathway, nitrogen flux is reduced. As a result there is accumulation of ammonia and glutamine with disordered metabolism of other amino acids. Nitrogen homeostasis can be restored in these patients with a low-protein diet combined with compounds that create alternative pathways for nitrogen excretion. The introduction of these compounds has been a major advance in the management of these inborn errors and as a result the outcome, particularly for those treated early, has improved.

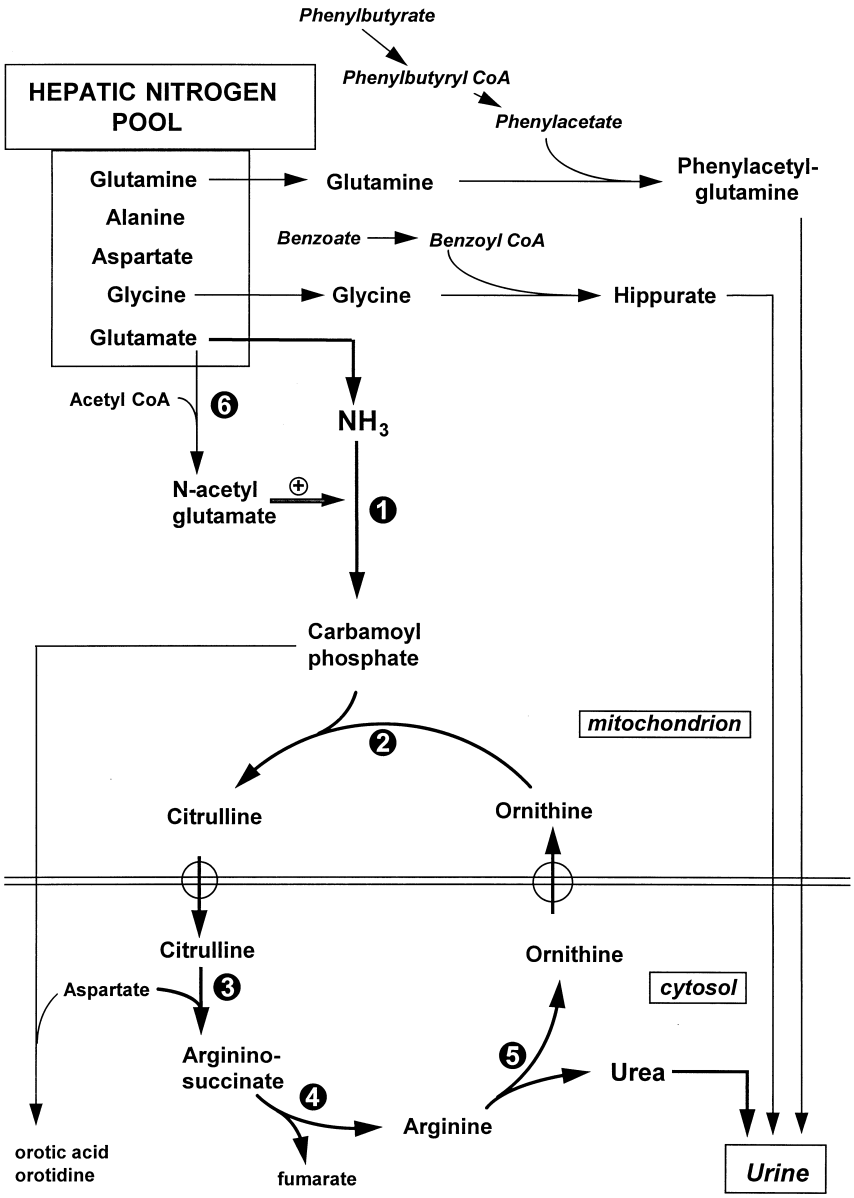
THE UREA CYCLE

Surplus nitrogen cannot be stored and has to be excreted. In mammals the major pathway for the metabolism of waste nitrogen is the urea cycle. In children on a protein intake of 1.25 g/kg, about 50% of the urinary nitrogen is excreted as urea (Brusilow and Maestri 1996). Quantitatively, 1 g of protein contains approximately 0.16 g of nitrogen which, if catabolized completely, will be converted to 5.7 mmol of urea.

The net effect of the urea cycle is to convert two nitrogen atoms derived from ammonia and aspartate to urea. The biochemical steps in the cycle are shown in Figure 1. Ammonia is probably derived from several sources (Brusilow and Horwich 1995) and is converted to carbamoyl phosphate by carbamoyl-phosphate synthase. This enzyme requires an allosteric activator *N*-acetylglutamate for full activity. The carbamoyl phosphate condenses with ornithine to form citrulline which then reacts with aspartate to form argininosuccinate. This compound is then hydrolysed to arginine and fumarate. The arginine is cleaved by arginase, releasing urea with ornithine being reformed. Within the urea cycle, ornithine acts as a carrier, being neither formed nor lost.

INBORN ERRORS OF THE UREA CYCLE

Defects of each step have now been described and are listed in Figure 1. The presentation is highly variable: those presenting in the newborn period usually have an



overwhelming illness but it may be subtle in those who present later in childhood or adult life (Brusilow and Horwich 1995; Leonard 1995). As a result of the inborn error, the flux in the pathway is reduced and all the defects are associated with hyperammonaemia and increased concentrations of glutamine in plasma (Brusilow and Horwich 1995). The metabolism of other amino acids is disordered depending on the site of the metabolic block. The concentrations of amino acids in the pathway immediately proximal to the enzyme defect will be increased and of those beyond the block decreased (Figure 1). For many years the mainstay of treatment was a low-protein diet but the metabolic control was frequently not satisfactory. The development of compounds that increase the excretion of nitrogen by alternative pathways has been an important breakthrough in the management of these disorders (Brusilow et al 1979).

NEUROTOXICITY OF UREA CYCLE INTERMEDIATES

Ammonia increases the transport of tryptophan across the blood–brain barrier with consequent increase in the production and release of serotonin (Bachmann and Colombo 1983). Some of the symptoms of hyperammonaemia can be explained on this basis and restriction of dietary tryptophan reverses some symptoms, particularly anorexia, in patients with these disorders (Hyman et al 1987). Ammonia induces many other electrophysiological, vascular and biochemical changes in experimental models, but it is not known to what extent these are relevant to the problems of hyperammonaemia in man (Surtees and Leonard 1989).

Glutamine can also be shown to accumulate at high concentrations both in experimental models (Brusilow and Horwich 1995) and also in man *in vivo* using proton nuclear magnetic resonance spectroscopy (Connelly et al 1993). The concentrations are such that the increase in osmolality could be responsible for changes in the intracellular water content and cerebral oedema.

Figure 1 (*opposite*) Pathways for the disposal of waste nitrogen: The urea cycle and alternative pathways of nitrogen excretion

Enzymes	*Inborn error of metabolism*
1. Carbamoyl-phosphate synthase	Carbamoyl-phosphate synthase deficiency
2. Ornithine transcarbamoylase	Ornithine transcarbamoylase deficiency
3. Argininosuccinate synthetase	Citrullinaemia
4. Argininosuccinate lyase	Argininosuccinic aciduria
5. Arginase	Arginase deficiency
6. *N*-Acetylglutamate synthase	*N*-Acetylglutamate synthase deficiency

Benzoate and phenylbutyrate are activated, conjugated to glycine and glutamine respectively and excreted, thereby creating an alternative pathway for nitrogen excretion

The clinical problems of arginase deficiency are distinct from other urea cycle disorders and it seems probable that the high arginine concentrations are neurotoxic, although the mechanism is unknown. Arginine deficiency may also contribute to the same symptoms in other disorders (Kline et al 1981).

TREATMENT

The aim of treatment is to correct the biochemical abnormalities and yet at the same time ensure that all the nutritional needs are met. The strategies used are to reduce protein intake to reduce the nitrogen flux through the urea cycle; secondly to utilize alternative pathways of nitrogen excretion; and thirdly to replace nutrients that are deficient.

Low-protein diet

Most patients with urea cycle disorders require a low-protein diet. The exact quantity of protein will depend on the inborn error, the age of the patient and the severity of the disorder. For some patients, particularly those with severe disorders and those with marked protein aversion, the diet may need to be supplemented with essential amino acids (Brusilow and Horwich 1995; Leonard 1995).

Alternative pathways for nitrogen excretion

In many patients diet alone is not sufficient to control the metabolic derangement, so that additional therapy is necessary. A major advance in this field has been the development of compounds that increase the removal of waste nitrogen (Brusilow et al 1979). By giving these substances, nitrogen is converted to compounds other than urea and is excreted. Hence the load on the urea cycle is reduced (Figure 1). The first compounds introduced were arginine and sodium benzoate. Later phenylacetate was used but this has now been superseded by phenylbutyrate.

Sodium benzoate: Benzoate is conjugated with glycine to form hippurate (Tremblay and Qureshi 1993) which is rapidly excreted in the urine (Figure 1). The possibility that sodium benzoate could be used to increase waste nitrogen excretion was first recognized by Lewis early this century (Lewis 1914) and it is well suited for this because its renal clearance is five times the glomerular filtration rate (Brusilow et al 1979). For each mole of benzoate given, 1 mole of nitrogen is removed. In practical terms 1 g of benzoate would, if completely converted to hippurate and excreted, result in the removal of the equivalent of 0.6 g protein.

Sodium benzoate is usually given in doses up to 250 mg/kg per day, but in acute emergencies this can be increased to 500 mg/kg per day. Following a dose of 250 mg/kg, the nitrogen removed by sodium benzoate, if conjugation is complete,

will be equivalent 0.15 g/kg of protein. Plasma ammonia and glutamine concentrations decrease (Brusilow and Maestri 1996). This, combined with a low-protein diet, may be sufficient in those with mild defects and it has been used widely with beneficial effects (Batshaw 1983; Letarte et al 1985; Takeda et al 1983).

Pharmacokinetics: Studies of the pharmacokinetics have shown that benzoate is rapidly converted to hippurate which is then cleared more slowly (Kubota and Ishizaki 1991). In this study, the mean maximum rate of clearance was 23 mg/h per kg, which is close to the maximum dose used clinically. However, there is wide variation in the recovery of the benzoate reported, which may be as low as 41% (Barshop et al 1989). The reasons for this have not been investigated, although a small quantity may be excreted as the glucuronide, which will reduce its efficacy. Studies of patients on their regular medication confirm that sodium benzoate is rapidly converted to hippurate but cleared more slowly (Figure 2). It has been recommended that plasma concentrations should not exceed 4.5 mmol/L (Simell et al 1986). In the neonatal period, induction of hippurate synthesis may be delayed, so that plasma benzoate concentrations may reach potentially toxic concentrations and should therefore be monitored (C. Bachmann, personal communication).

Adverse effects: In animal studies sodium benzoate induces a rise in plasma ammonia concentrations coupled to a decrease in ATP and acetyl-CoA (Palekar and Kalbag 1991). This was thought to be caused by competition for free CoA (Griffith et al 1989) and the effect could be reversed with *N*-carbamoyl glutamate or carnitine (O'Connor et al 1987, 1989). In sparse-fur mouse benzoate impairs several mitochondrial pathways including the urea cycle, fatty acid oxidation and the citric acid

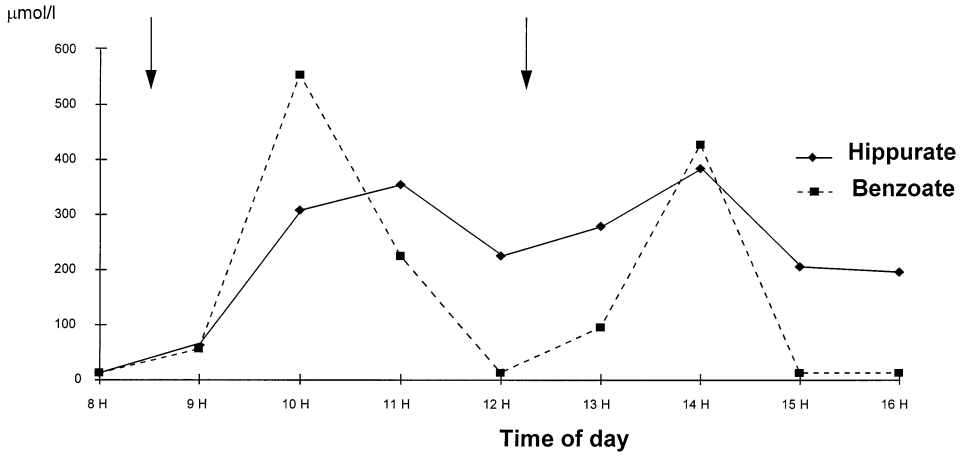


Figure 2 Late-onset ornithine transcarbamylase deficiency in a boy (S.H.) treated with sodium benzoate (375 mg/kg per day). Profile of plasma benzoate and hippurate during routine therapy. Arrows indicate each dose of 125 mg/kg

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.