A Case Study of the Urea Cycle

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Introduction

Dietary amino acids are not stored in the body as a fuel source. The carbon skeletons eventually enter the Tricarboxylic acid (Krebs) cycle as either TCA cycle intermediates or as acetyl CoA. The nitrogen of the amino acids must also be disposed of. This nitrogen eventually becomes free ammonium ion in the blood stream. Ammonia increases the pH of its environment and is neurotoxic. It is not clear how or why ammonia is toxic. It is clear that it can freely permeate the blood-brain barrier. Possibilities of its neurotoxicity include a conversion of α-ketoglutarate to glutamate by mass action via glutamate dehydrogenase (see figure 1). This reaction removes free ammonium from the blood but it also depletes the stores of α-ketoglutarate, a TCA cycle intermediate. As the TCA cycle slows, aerobic respiration slows and cell death can occur. If ammonia is not disposed of properly, lethargy, coma and death can occur. The excess glutamine produced is also a precursor the brain inhibitory neurotransmitter GABA (γ-aminobutyric acid). GABA inhibits neural activity and leads to a feeling of calmness and overall muscle relaxation. Ammonium ion can also combine with bicarbonate to form the amino acid glycine.

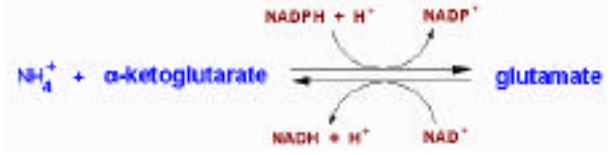


Figure 1. Glutamate dehydrogenase reaction.

The urea cycle, first introduced to us by Hans Krebs, is the principle outlet for nitrogen disposal. Transaminations of dietary amino acids are catalyzed by aminotranferases and use α-ketoglutarate to form an α-ketoacid and glutamate from the original amino acid (see Figure 2). Glutamate dehydrogenase then deaminates the glutamate to α-ketoglutarate and free ammonium ion (NH_4^+) (see figure 1). This toxic ammonium ion then enters the urea cycle in the form of carbamoyl phosphate and is eventually excreted in the urine as urea.

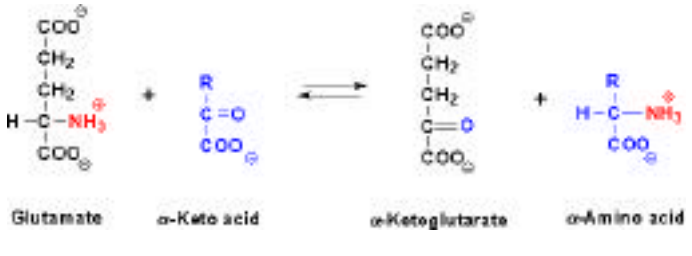


Figure 2. Transamination reaction.

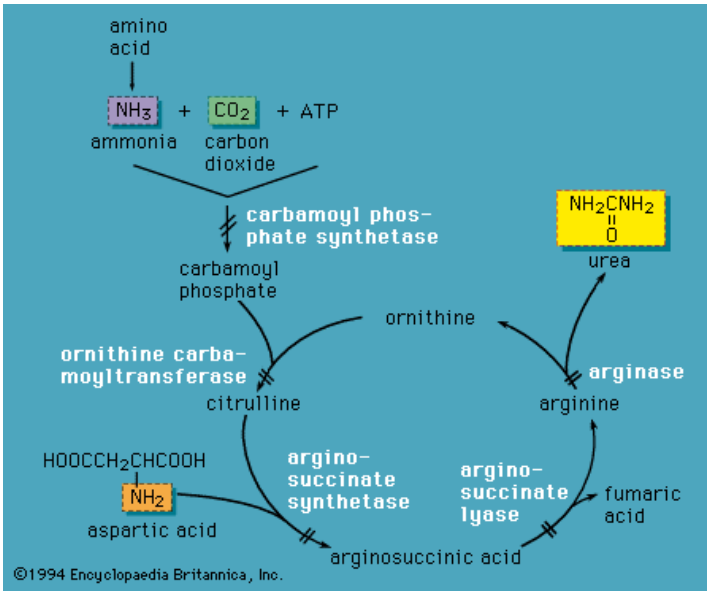


Figure 3. Urea Cycle

Case 1

A mother brings her 3-month-old girl in to the emergency room after a night of severe vomiting. Doctors observe lethargy, irritability, ataxia, and a general failure to thrive in the infant. Blood work is done (see table 1). Glycine levels are also noted to be high. The doctors treat her with arginine, sodium benzoate, and phenylacetate.

Table 1. Blood Test Results for Case 1.

	Patient	Normal controls
Ammonia	540 µM	90 – 150 µM
Urea	5 mg/dL	15 mg/dL
Citrulline	25 µM	2000 µM

Case 2

A 34-year-old male comes in to the emergency room complaining of recurrent nighttime vomiting, insomnia, and diarrhea. His wife tells doctors that she has noticed that he seems to be confused after meals and is very lethargic overall. Blood work is done (see table 2) and the doctors also treat him with arginine, sodium benzoate and phenylacetate.

Table 2. Blood Test Results for Case 2.

	Patient	Normal controls
Ammonia	485 µM	10 – 40 µM
Urea	10 mg/dL	15 mg/dL
Citrulline	25 µM	1,665 µM

Questions

1. In both cases, liver samples of the patients were taken and the doctors determine that both the patients have an enzymatic, metabolic defect of the urea cycle. Using table 1, basic metabolic logic, and/or the OMIM database, determine the enzyme deficiency of these two cases.

2. Knowing that the above enzyme uses an ATP and converts it to an AMP and pyrophosphate, propose a mechanism for the enzyme reaction.

3. Using the OMIM database, determine the location of the defective gene.

4. What is the purpose of the arginine in the treatment procedure? The sodium benzoate and phenylacetate?

Case Summary

The little girl in this case has classical citrullinemia, a rare metabolic defect caused by the deficiency of arginosuccinate synthase (ASS). ASS is an essential enzyme in the urea cycle that is found on chromosome 9q34. It combines citrulline and aspartate using ATP to form arginosuccinate. Because AMP is a product also, we know that either a pyrophosphate or an adenylate intermediate is formed. Symptoms of citrullinemia included severe vomiting, hyperammonemia, lethargy, ataxia (irregular muscular coordination), seizures, irritability, cerebral edema, developmental delay, and eventual mental retardation and coma. The man in Case 2 also has citrullinemia. It is citrullemia type II, adult-onset citrullemia. In type II citrullinemia behavioral symptoms can present themselves like protein avoidance and a peculiar fondness for beans, peas and peanuts since childhood. Beans, peas and peanuts are all rich in arginine and patients tend to want them in a self-treatment they don't even realize. Both forms of citrullinemia are autosomal recessive and map to the same gene. It is unclear why type II can remain semi-dormant for so long. Treatment for both classical and type II citrullinemia consist of arginine replacement therapy and sodium benzoate and phenylacetate. Because the cycle is truncated, arginine becomes an essential amino acid (the cycle *is* its synthesis pathway) and it needs to be supplemented in the diet. Glycine is a natural product of hyperammonemia. Glycine will combine with the benzoate to produce hippurate. Phenylacetate will combine with the excess glutamine formed to make phenylacetylglutamine. Both hippurate and phenylacetylglutamine are safely excreted in the urine, thus removing the toxic ammonium from the body.

Resources:

Citrullinemia on OMIM http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?215700 and http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?603471

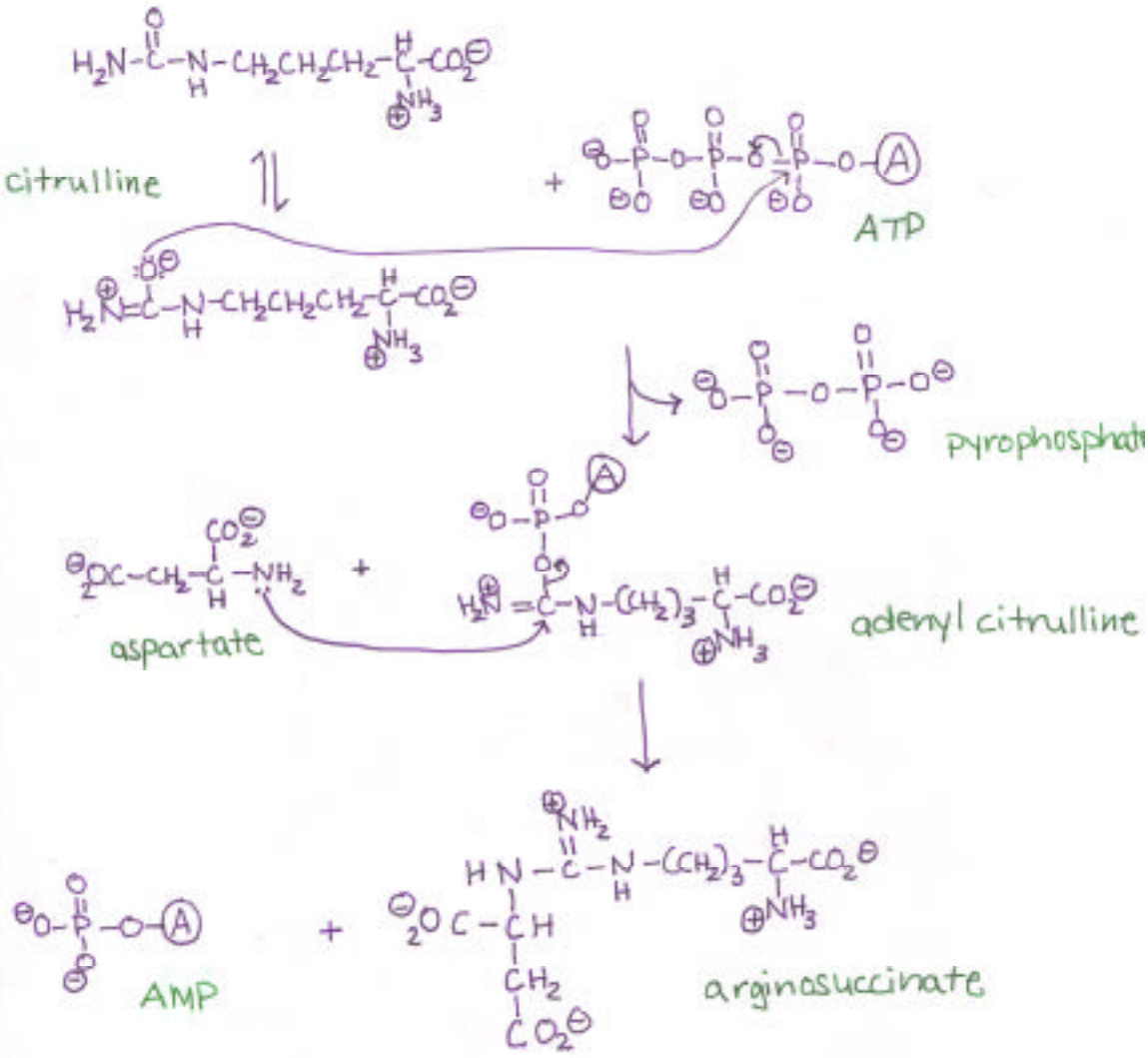
Nitrogen metabolism http://web.indstate.edu/thcme/mwking/nitrogen-metabolism.html

Table Data: http://www.laurushealth.com/library/healthguide/MedicalTests/_showTopic.asp?topic_id=13055&sequence=7

http://www.icondata.com/health/pedbase/files/CITRULLI.HTM

Sass, JO and Skladal, D. Plasma concentrations and renal clearance of orotic acid in arginosuccinic acid synthetase deficiency. *Pediatric Nephrology*, (1999) 13:912-916.

Mechanism of Arginosuccinate Synthase



Alternative Nitrogen Disposal Pathways