Clinical biochemistry second stage lecture 3 Amino acid metabolism

**FORMATION OF AMMONIA and UREA CYCLE**

The sources and fate of ammonia are shown in Figure 1. The first step in the catabolism of amino acids is to remove the amino group as **ammonia**. This is the major source of ammonia. However ,small quantities of ammonia may also be formed from catabolism of purine and pyrimidine bases Ammonia is **highly toxic** especially to the nervous system. Detoxification of ammonia is by conversion to urea and excretion through urine.



Figure 1. Sources and fate of ammonia

**DISPOSAL/DETOXIFICATION OF AMMONIA**

**1. First line of Defense (Trapping of ammonia)**

Being highly toxic, ammonia should be eliminated or detoxified, as and when it is formed. Even very minute quantity of ammonia may produce toxicity in central nervous system. But, ammonia is always produced by almost all cells, including neurons. The intracellular ammonia is immediately trapped by glutamic acid to form **glutamine**, especially in brain cells (Fig. 2). The glutamine is then transported to liver, where the reaction is reversed by the enzyme **glutaminase** (Fig. 2). The ammonia thus generated is immediately detoxified into urea .Aspartic acid may also undergo similar reaction to form **asparagine**.



Figure 2.Ammonia trapping as glutamine

**2. Transportation of Ammonia**

Inside the cells of almost all tissues, the transamination of amino acids produce glutamic acid. However, glutamate dehydrogenase is available only in the liver. Therefore, the final deamination and production of ammonia is taking place in the liver (Fig. 3). Thus, **glutamic acid** acts as the link between amino groups of amino acids and ammonia. The concentration of glutamic acid in blood is 10 times more than other amino acids.**Glutamine** is the transport forms of ammonia from brain and intestine to liver; while alanine is the transport form from muscle

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Fig. 3 **oxidative deamination**

**3. Final disposal**

The ammonia from all over the body thus reaches liver. It is then **detoxified to urea by liver** cells,and then excreted through kidneys. **Urea is the endproduct of protein metabolism.**

Although Ammonia is toxic and has to be immediately detoxified, in kidney cells, ammonia is purposely generated from glutamine with the helpof glutaminase. This is for buffering the acids, and maintaining acid-base balance (see Fig. 4).



Figure 4. Ammonia as buffer

**UREA CYCLE**

The two nitrogen atoms of urea are derived from two different sources, one from ammonia and the other directly from the alpha amino group of aspartic acid. Steps of urea cycle are the following

**Step 1. Formation of Carbamoyl Phosphate**

One molecule of ammonia condenses with CO2 in the presence of **two molecules of ATP** to form carbamoyl phosphate. The reaction is catalysed by the mitochondrial enzyme **carbamoyl phosphate synthetase-I (CPS-I).**. CPS-I reaction is the **rate-limiting step** in urea formation. It is irreversible and allosterically regulated..

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Figure 5 .Urea cycle, summary. Note that aspartate enters and fumarate leaves at different

steps.

**Step 2. Formation of Citrulline**

The second reaction is also **mitochondrial.** The carbamoyl group is transferred to the NH2 group of ornithine by **ornithin transcarbamoylase** (OTC) and produce **citrulline** The citrulline leaves the mitochondria and further reactions are taking place in **cytoplasm**. Citrulline is neither present in tissue proteins nor in blood; but it is **present in milk.**

**Step 3. Formation of Argininosuccinate**

One molecule of aspartic acid adds to citrulline forming a carbon to nitrogen bond which provides the 2nd nitrogen atom of urea. **Argininosuccinate synthetase** catalyses the reaction (Figs 6 step 3). This needs hydrolysis of ATP to AMP level, so **two high energy phosphate bonds** are utilized. The PPi is an inhibitor of this step.

**Step 4. Formation of Arginine**

Argininosuccinate is cleaved by **argininosuccinate lyase** (argininosuccinase) to arginine and fumarate(Figs 6, step 4). The enzyme is inhibited by fumarate. But this is avoided by the cytoplasmic localization of the enzyme. The fumarate formed may be funnelled into TCA cycle to be converted to malate and then to oxaloacetateto be transaminated to aspartate (Fig. 6). Thus the urea cycle is linked to TCA cycle through fumarate. The 3rd and 4th steps taken together maybe summarized as:

Citrulline + aspartate → Arginine + fumarate



Figure 6 .Urea cycle and its relation with citric acid cycle

**Step 5. Formation of Urea**

The final reaction of the cycle is the hydrolysis of arginine to urea and ornithine by arginase (Fig. 6 .step 5). The ornithine returns to the mitochondria to react with another molecule of carbamoyl phosphate so that the cycle will proceed.Thus, ornithine may be considered as a catalyst which enters the reaction and is regenerated.

**Energetics of Urea Cycle**

The overall reaction may be summarized as:

NH3 + CO2 + Aspartate →Urea + fumarate

During these reactions, 2 ATPs are used in the1st reaction. Another ATP is converted to AMP +PPi in the 3rd step, which is equivalent to 2 ATPs.The urea cycle consumes **4 high energy phosphatebonds.** However, fumarate formed in the4th step may be converted to malate. Malate when oxidised to oxaloacetate produces 1 NADH equivalent to 2.5 ATP. So **net energy expenditureis only 1.5 high energy phosphates**. The ureacycle and TCA cycle are interlinked, and so, it is called as "**urea bicycle**"

. **Regulation of the Urea Cycle**

**1. Coarse Regulation**

The enzyme levels change with the protein content of diet. During starvation, the activity of urea cycle enzymes is elevated to meet the increased rate of protein catabolism.

**2. Fine Regulation**

The major regulatory step is catalyzed by CPS-I where the positive effector is **N-acetyl glutamate**(NAG). It is formed from glutamate and acetyl CoA(Fig. 7). Arginine is an activator of NAG synthase.



Fig. 7 NAG synthesis and breakdown

**3. Compartmentalization**

The urea cycle enzymes are located in such a way that the first two enzymes are in the mitochondria matrix. The inhibitory effect of fumarate on its own formation is minimized because argininosuccinatelyase is in the cytoplasm, while fumarase is mitochondria .

**Disorders of Urea Cycle**

A urea cycle disorder is a genetic disorder caused by a mutation that results in a deficiency of one of the enzymes in the urea cycle. These enzymes are responsible for removing ammonia from the blood stream.

Severe deficiency or total absence of activity of any of the first four enzymes (CPS1, OTC, ASS, ASL) in the urea cycle or the cofactor producer (NAGS) results in the accumulation of ammonia (hyperammonemia) and other precursor metabolites during the first few days of life. When the block is in one of the earlier steps, the condition is more severe ,since ammonia itself accumulates. Deficiencies of later enzymes result in the accumulation of other intermediates which are less toxic and hence symptoms are less. As a general description,disorders of urea cycle are characterized by hyperammonemia, encephalopathy and respiratory alkalosis. Clinical symptoms include vomiting, irritability, lethargy and severe mental retardation. Infants appear normal at birth, but within days progressive lethargy sets in. Metabolic stress ors -- viruses, high protein intake, excessive exercise or dieting, surgery, or a drug as prednisone or other corticosteroid -- can create excessive ammonia in the body resulting in severe neurological symptoms.  Treatment is more or less similar in the different types of disorders. Low protein diet with sufficient arginine and energy by frequent feeding can minimize brain damage since ammonia levels do not increase very high results in toxic symptoms.

Brain is very sensitive to ammonia. glutamate and γ-aminobutyrateGABA play a role in ammonia-induced toxicity .ammonia entering the brain by diffusion across the blood-brain barrier and to incorporate this ammonia into glutamine. production of glutamine producing an osmotic stress in brain cell and also decrease the availability ofα ketoglutaratrease for citric acid cycle so decrease the level of ATP as source of energy in brain. Different urea cycle disorders are shown in following Table

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Urea Cycle Disorders:

| **Disease Name** |  | **Enzyme defect** |
| --- | --- | --- |
| Carbamoylphosphate synthetase I deficiency | CPS1 1 | Carbamoyl-phosphate synthase |
| [Ornithine transcarbamylase deficiency](http://www.ncbi.nlm.nih.gov/books/n/gene/otc-def/) | OTC | Ornithine carbamoyltransferase |
| ASS deficiency ([Citrullinemia type I](http://www.ncbi.nlm.nih.gov/books/n/gene/ctlm/)) | ASS1 | Argininosuccinate synthase |
| [ASL deficiency](http://www.ncbi.nlm.nih.gov/books/n/gene/args-aciduria/) (argininosuccinic aciduria) | ASL | Argininosuccinate lyase |
| [Arginase deficiency](http://www.ncbi.nlm.nih.gov/books/n/gene/arg1/) | ARG1 | Arginase-1 |
| NAGS deficiency | NAGS | N-acetylglutamate synthase |

Since **Citrulline** is present in significant quantities in milk, breast milk is to be avoided in citrullinemia

**Hepatic Coma (Acquired Hyperammonemia)**

In diseases of the liver, hepatic failure can finally lead to hepatic coma and death. Hyperammonemia is the characteristic feature of liver failure. The condition is also known as portal systemic encephalopathy. Normally the ammonia and other toxic compounds produced by intestinal bacterial metabolism are transported to liver by portal circulation and detoxified by the liver. But when there is portal systemic shunting of blood, the toxins bypass the liver and their concentration in systemic circulation rises.The signs and symptoms are mainly pertaining to CNS dysfunction (altered sensorium, convulsions), withholding hepatotoxic drugs and maintenance of electrolyte and acid base balance are the main lines of management.