Urea Cycle

- Role of Urea cycle: to prevent the accumulation of toxic NH$_4^+$. It incorporates nitrogen not used for biosynthetic purposes into urea, which serves as the waste nitrogen product in mammals.
- Urea is the major disposal form of amino groups derived from amino acids, and accounts for about 90% of the nitrogen-containing components of urine.
- Also urea cycle is responsible for de novo synthesis of arginine.
- The complete Urea Cycle is significantly only in liver.
- However some enzymes of the pathway are in other cells and tissues where they generate arginine & ornithine.
- E.g., Argininosuccinate Synthase, which catalyzes synthesis of the precursor to arginine, is in most tissues.
Reactions of the cycle

• **5-step** Urea Cycle (Small Krebs c., Krebs-Hensleit c., Ornithin c.)
• **function**: synthesis of non-toxic urea.
• The first two reactions leading to the synthesis of urea occur in the mitochondria, whereas the remaining cycle enzymes are located in the cytosol.
• One nitrogen of the urea molecule is supplied by free NH$_3$, and the other nitrogen by aspartate.
• So glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST).
• The carbon and oxygen of urea are derived from CO$_2$.
• Overall **urea cycle reaction**: $\text{Aspartate} + \text{NH}_3 + \text{CO}_2 + 3 \text{ATP} \rightarrow \text{urea} + \text{fumarate} + 2 \text{ADP} + \text{AMP} + 2 \text{P}_i + \text{PP}_i + 3 \text{H}_2\text{O}$
• Urea is produced by the liver, and then is transported in the blood to the kidneys for excretion in the urine.
Step 1: formation of Carbamoyl phosphate from ammonia, bicarbonate and ATP

- Enzyme Carbamoyl phosphate synthetase I.
- In liver cell mitochondrias.
- Ammonia released from the oxidative deamination of glutamate, by mitochondrial glutamate dehydrogenase, is incorporated in carbamoyl phosphate by using ATP and bicarbonate.
- Utilizes 2 ATP.
- Requires Mg\(^+\)
- Carbamoyl phosphate synthetase I requires N-acetylglutamate as a positive allosteric activator. The reaction is irreversible.
Carbamoyl Phosphate Synthase (Type I) catalyzes a 3-step reaction, with carbonyl phosphate and carbamate intermediates.

Carbamoyl phosphate synthetase (type) II participates in the biosynthesis of pyrimidines. It does not require N-acetylglutamate, and occurs in the cytosol.
Step 2: Formation of citrulline from ornithine and carbamoyl phosphate

- Enzyme **Ornithine transcarbamoylase**
- In liver mitochondria
- Citrulline is formed from transfer of the carbamoyl group to the γ-amino group of ornithine.
- The release of the high-energy phosphate of carbamoyl phosphate as inorganic phosphate drives the reaction in the forward direction.
- Citrulline passes from mitochondrial membrane to cytosol
- Ornithine is regenerated with each turn of the urea cycle.
Step 3: Formation of arginosuccinate from citrulline and aspartate

- This is the second nitrogen-acquiring reaction.
- Condensation of citrulline with aspartate to form arginosuccinate.
- The α-amino group of aspartate provides the second nitrogen that is ultimately incorporated into urea.
- Enzyme Argininosuccinate synthetase.
- Requires ATP and Mg\(^+\)

**Figure 18-11b**
*Lehninger Principles of Biochemistry, Fifth Edition*  
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Step 4: Formation of arginine and fumarate from arginosuccinate

- Enzyme Argininosuccinase
- Liver and kidney of mammals
- Cleaves arginosuccinate to form arginine and fumarate.
- The arginine formed by this reaction serves as the immediate precursor of urea.
- Fumarate produced in the urea cycle is hydrated to malate, providing a link with several metabolic pathways. For example, the malate can be transported into the mitochondria via the malate shuttle and reenter the tricarboxylic acid cycle. Alternatively, cytosolic malate can be oxidized to oxaloacetate, which can be converted to aspartate or glucose. (thus aspartate could be regenerated)
Step 5: Hydrolysis of arginine to form ornithine and urea

- **Enzyme Arginase**
- The arginine is hydrolyzed to produce the urea and to reform the ornithine.
- In liver cells cytosol.
- The ornithine reenters the mitochondrial matrix.
- Urea excreted (N rich, excellent solubility, harmless)
- Thus, whereas other tissues, such as the kidney, can synthesize arginine by these reactions, only the liver can cleave arginine and, thereby, synthesize urea.
Fate of urea

- Urea diffuses from the liver, and is transported in the blood to the kidneys, where it is filtered and excreted in the urine.
- A portion of the urea diffuses from the blood into the intestine, and is cleaved to $\text{CO}_2$ and $\text{NH}_3$ by bacterial urease.
- This ammonia is partly lost in the feces, and is partly reabsorbed into the blood.
- In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut.
- The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients.
Regulation of urea cycle

- **The Activity of the Urea Cycle Is Regulated at Two Levels**: the level of urea cycle enzyme synthesis and by allosteric regulation of the enzyme carbamoyl phosphate synthetase I.
- The flux of nitrogen through the urea cycle in an individual animal varies with diet. When the dietary intake is primarily protein, the carbon skeletons of amino acids are used for fuel, producing much urea from the excess amino groups.
- During prolonged starvation, when breakdown of muscle protein begins to supply much of the organism’s metabolic energy, urea production also increases substantially.
- These changes in demand for urea cycle activity are met over the long term by regulation of the rates of synthesis of the enzymes in the liver.
- All five enzymes are synthesized at higher rates in starving animals and in animals on very-high-protein diets than in well-fed animals eating primarily carbohydrates and fats.
- Animals on protein-free diets produce lower levels of urea cycle enzymes.
Regulation of urea cycle cont...

- The carbamoyl phosphate synthetase I, is allosterically activated by **N-acetylglutamate, which is synthesized from acetyl-CoA** and glutamate by **N-acetylglutamate synthase**.
- The steady-state levels of N-acetylglutamate are determined by the concentrations of glutamate and acetyl-CoA (the substrates for N-acetylglutamate synthase) and arginine (an activator of N-acetylglutamate synthase, and thus an activator of the urea cycle).
urea cycle
Aspartate –Arginosuccinate Shunt Links Urea Cycle and Citric Acid Cycle
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• Each cycle can operate independently and communication between them depends on the transport of key intermediates between the mitochondrion and cytosol.
• Several enzymes of the citric acid cycle, including fumarase (fumarate hydratase) and malate dehydrogenase, are also present as isozymes in the cytosol.
• The fumarate generated in cytosolic arginine synthesis can therefore be converted to malate in the cytosol, and these intermediates can be further metabolized in the cytosol or transported into mitochondria for use in the citric acid cycle.
• Aspartate formed in mitochondria by transamination between oxaloacetate and glutamate can be transported to the cytosol, where it serves as nitrogen donor in the urea cycle reaction catalyzed by argininosuccinate synthetase.
Hyperammonemia

- The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of serum ammonia are normally low (5–50 µmol/L).
- When liver function is decreased, due either to genetic defects of the urea cycle, or liver disease, blood levels can rise above 1,000 µmol/L.
- Ammonia has a direct neurotoxic effect on the CNS. For example, elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, sleepy, vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death.
- The two major types of hyperammonemia are: Acquired hyperammonemia Hereditary hyperammonemia
Acquired Hyperammonemia

- Liver disease is a common cause of hyperammonemia in adults.
- Liver diseases include viral hepatitis, ischemia, or hepatotoxins.
- In patients with liver disease, hepatic function is impaired and conversion ammonia to urea, therefore, severely impaired, leading to elevated levels of circulating ammonia.
- Cirrhosis of the liver caused by alcoholism, hepatitis or biliary obstruction may result in formation of collateral circulation around the liver.
- As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver.
Hereditary hyperammonemia
Urea Cycle Disorders

- Deficiency or absence of any one of urea cycle enzymes resulting in one urea cycle disorder.
- **Hyperammoniemia** is the characteristic and predominant cause of the symptoms.
- Complete deficiency of any enzyme of them leads to severe hyperammoniemia in the neonatal period and may result in coma and death.
- Partial enzyme deficiency leads to clinical picture correlated with the residual enzyme activity and the age of onset of the disease.
- In some cases the hyperammoniemia is **episodic** and this episodes can result from high protein diet or infections.
- Prevalence of disorders: 1/30,000 live births but may be more since some die undiagnosed.
Symptoms

Severe Illness: **First week**

- Usually normal first 24h
- Symptoms of hyperammonemia within 1-3 days

  Include: Feeding intolerance
  - Vomiting
  - Lethargy
  - Irritability
  - Respiratory Distress (hyperventilation)
  - Seizures
  - Coma
Hyperammoniemia Toxicity

- Hyperammoniemia cause **encephalopathy** (brain damage) mainly by these mechanisms:
- 1- Shift of glutamate dehydrogenase reaction toward the direction of glutamate formation.
- 2- The resulting depletion of \( \alpha \)-ketoglutarate, an essential Krebs Cycle intermediate, could impair energy metabolism in the brain.
- 3- Increased glutamine also cause brain damage. (Fig)
- 4- This would deplete glutamate – a neurotransmitter & precursor for synthesis of the neurotransmitter GABA.

\[
\begin{align*}
\alpha\text{-Ketoglutarate} + \text{NH}_4^+ & \rightarrow \text{Glutamate dehydrogenase} \rightarrow \text{NAD}^+ \rightarrow \text{NH}_3 + \text{ATP} \\
& \rightarrow \text{Glutamine synthetase} \rightarrow \text{ADP} + P_i
\end{align*}
\]
Diagrammatic representation of the pathophysiology responsible for hyperammonememic encephalopathy. The primary event is activation by ammonia of glutamine synthetase within the astrocyte, leading to the accumulation of glutamine within the astrocyte. This has a dual effect: (1) glutamine serves as an intracellular osmolyte, causing entry of water and astrocyte swelling and cerebral edema, and (2) the swollen astrocyte or the high glutamine concentration causes astrocyte dysfunction.
# Urea Cycle Disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Alternative names</th>
<th>Plasma amino acid concentrations</th>
<th>Urine orotic acid</th>
<th>Tissue for enzyme diagnosis</th>
<th>Genetics – gene (chromosome localisation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetylglutamate synthetase deficiency</td>
<td>NAGS deficiency</td>
<td>↑ glutamine ↑ alanine</td>
<td>N</td>
<td>Liver</td>
<td>AR – NAGS (chromosome 17q 21.31)</td>
</tr>
<tr>
<td>Carbamoyl phosphate synthetase deficiency</td>
<td>CPS deficiency</td>
<td>↑ glutamine ↑ alanine ↓ citrulline ↓ arginine</td>
<td>N</td>
<td>Liver</td>
<td>AR – CPS1 (chromosome 2p 35)</td>
</tr>
<tr>
<td>Ornithine transcarbamoylase deficiency</td>
<td>OTC deficiency</td>
<td>↑ glutamine ↑ alanine ↓ citrulline ↓ arginine</td>
<td>↑↑</td>
<td>Liver</td>
<td>X-linked – OTC (Xp21.1)</td>
</tr>
<tr>
<td>Argininosuccinic acid synthetase deficiency</td>
<td>Citrullinaemia</td>
<td>↑↑ citrulline ↓ arginine</td>
<td>↑</td>
<td>Liver/fibroblasts</td>
<td>AR – ASS (chromosome 9q 34)</td>
</tr>
<tr>
<td>Argininosuccinic acid lyase deficiency</td>
<td>Argininosuccinic aciduria (ASA)</td>
<td>↑ citrulline ↑ argininosuccinic acid ↓ arginine</td>
<td>↑</td>
<td>RBC/fibroblasts</td>
<td>AR – ASL (chromosome 7cen-q11.2)</td>
</tr>
<tr>
<td>Arginase deficiency</td>
<td>Hyperargininaemia</td>
<td>↑ arginine</td>
<td>↑</td>
<td>RBC/Liver</td>
<td>AR – ARG1 (chromosome 6q 23)</td>
</tr>
</tbody>
</table>

*AR*, autosomal recessive; *RBC*, red blood cells; *N*, normal
Treatment of Urea Cycle Disorders

1. **limiting protein intake** to the amount barely adequate to supply amino acids for growth, while adding to the diet the $\alpha$-keto acid analogs of essential amino acids.

2. **Feeding the patients with Benzoate or phenylacetate:** These compound react with glycine and glutamine respectively forming non-toxic compounds that are excreted in urine. Thus the body runs low in glycine and glutamine and starts synthesizing these AA using the ammonia available in system. Thus clearing the system of excess ammonia.

3. In the patients with N-acetylglutamate synthase deficiency, Carbamoyl glutamate (**NAG Analog**) can act as activator of carbamoyl phosphate synthase.

4. **Liver transplantation** has also been used, since liver is the organ that carries out Urea Cycle.