M1 - Renal, Fall 2007

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Nitrogen Metabolism (and Related Topics)

- Amino Acid Metabolism (Nitrogen metabolism)
- Folate Metabolism (“One-Carbon pathways”)
- Nucleotide Metabolism

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There are also PDF’s of class handouts with supplemental information available in the table of contents for this course.

Supplementary study material on the Web:
http://seqcore.brcf.med.umich.edu/mcb500
Protein Degradation:

• Endogenous proteins degrade continuously
  - Damaged
  - Mis-folded
  - Un-needed
• Dietary protein intake - mostly degraded

Nitrogen Balance - expresses the patient’s current status - are they *gaining* or *losing* net Nitrogen?
Transaminases Collect Amines

General reaction overview:

\[
R_1\text{-}C\text{-}\text{coo}^{(-)} + R_2\text{-}C\text{-}\text{coo}^{(-)} \rightarrow R_1\text{-}C\text{-}\text{coo}^{(-)} + R_2\text{-}C\text{-}\text{coo}^{(-)}
\]

\(\alpha\)-keto acid (typically \(\alpha\)-ketoglutarate) and amino acid (typically glutamate).

Details of reaction mechanism:

Amino acid

\[
\begin{align*}
& \quad \text{H} \\
& R-\text{C-} \text{coo}^{(-)} \\
& \quad \text{NH}_2 \\
& \quad + O \\
& \quad \text{CH}_3 \\
& \quad \text{pyridoxal phosphate}
\end{align*}
\]

\[
\text{H} + R-\text{C-} \text{coo}^{(-)} \rightarrow R-\text{C-} \text{coo}^{(-)}
\]

\[
\text{H} + R-\text{C-} \text{coo}^{(-)} \rightarrow R-\text{C-} \text{coo}^{(-)}
\]

\[
\text{H} + R-\text{C-} \text{coo}^{(-)} \rightarrow R-\text{C-} \text{coo}^{(-)}
\]

\[
\text{H}_2\text{O}
\]

\[
\alpha\text{-ketoo acid}
\]

\[
\begin{align*}
& \quad \text{R-} \text{C-} \text{coo}^{(-)} \\
& \quad + \text{NH}_2 \\
& \quad \text{HCH}_3 \\
& \quad \text{pyridoxamine phosphate}
\end{align*}
\]
Transfer the amine back to an acceptor α-keto acid

\[
\text{pyridoxamine phosphate} + \text{α-keto acid} \rightarrow \text{pyridoxal phosphate} + \text{amino acid}
\]
In peripheral tissues, transaminases *tend* to form Glutamate when they catabolize amino acids.

In other words, alpha-ketoglutarate is the preferred acceptor, and Glutamate is the resulting amino acid:

Some amino acid + α-ketoglutarate \(\rightarrow\) some alpha keto acid + Glutamate
Glutamate can donate its amines to form other amino acids as needed

A specific example - production of Aspartate in liver (described a few slides from now):

Glutamate + oxaloacetate $\rightarrow$ $\alpha$-ketoglutarate + aspartate
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[ \text{glutamate} \xrightarrow{\text{NAD}(P)} \text{NAD}(P)H \]

\[ \text{NAD}(P)H \xrightarrow{\text{mito}} \text{\(\alpha\)-ketoglutarate} + \text{ammonia} \]

**Glutamine Synthetase:**

\[ \text{glutamate} \xrightarrow{\text{ATP} + \text{NH}_3} \text{glutamine} \]

\[ \text{ADP} + \text{P}_i \]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

Glutamate donates its amino group to form aspartate:

Glutamate-aspartate aminotransferase:

Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.
Carbamoyl phosphate synthetase I

bicarbonate $\rightleftharpoons$ carbonyl phosphate $\rightleftharpoons$ carbamate $\rightleftharpoons$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline → Argininosuccinate

\(-\text{boC} - \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} - \text{NH}_2\)

aspartate

\(-\text{oC} - \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} = \text{NH}_2^{(+)}\)

ATP → AMP + PP_1
Arginase

\[
\text{Arginine} \xrightarrow{\text{H}_2\text{O}} \text{Urea} \rightarrow \text{Ornithine}
\]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\begin{align*}
\text{glutamate} & \xrightarrow{\text{NAD}(P)} \text{α-ketoglutarate} + \text{NH}_3 \\
\text{NAD}(P)H & \xrightarrow{\text{mito}} \text{NAD}(P)
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamine} & \xrightarrow{\text{ATP} + \text{NH}_3} \text{glutamate} \\
\text{ADP} + P_i & \xrightarrow{\text{NH}_3} \text{glutamine}
\end{align*}
\]
The diagram illustrates the urea cycle and the synthesis of arginine and fumarate in liver mitochondria and cytoplasm. Here is a step-by-step explanation:

1. **2ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate**
   - Carbamoyl phosphate is formed from ATP, HCO₃⁻, and NH₃.

2. **2ADP + P₃ → NH₃**
   - NH₃ is released from the reaction.

3. **NH₃ + Carbamoyl phosphate → Ornithine**
   - Ornithine is produced from the reaction of NH₃ and carbamoyl phosphate.

   Ornithine is then converted to citrulline in the cytoplasm.

4. **Citrulline + ATP → Argininosuccinate**
   - Argininosuccinate is synthesized from citrulline and ATP.

5. **Argininosuccinate + H₂O → Arginine**
   - Arginine is produced from argininosuccinate.

6. **Arginine + ATP → Citrulline**
   - Citrulline is produced from arginine and ATP.

7. **Citrulline + Aspartate → Fumarate**
   - Fumarate is produced from citrulline and aspartate.

8. **Fumarate + H₂O → Pyruvate**
   - Pyruvate is produced from fumarate and water.

This cycle is crucial for the detoxification of ammonia and the production of urea and arginine.
CPS I is Stimulated by NAG

\[
\begin{align*}
\text{glutamate} & \quad + \quad \text{acetyl CoA} \\
\quad \text{N-acetyl glutamate (NAG)}
\end{align*}
\]

(repeating the figure from page 3 of your handout)
Complicating the picture: Other tissues may be involved
Why is Ammonia Toxic?
Why is Ammonia Toxic?

• Possible neurotoxic effects on glutamate levels (and also GABA) (due to shifting equilibria of reactions involving these compounds)
Why is Ammonia Toxic?

• Possible neurotoxic effects on glutamate levels (and also GABA)
  (due to shifting equilibria of reactions involving these compounds)

• Possible metabolic/energetics effects:
  - alpha-ketoglutarate levels
  - glutamate levels
  - glutamine
Inherited Defects of Urea Cycle Enzymes: Diagnosis

Defects are diagnosed based on the metabolites seen in the blood and/or urine.

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSD</td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
</tr>
<tr>
<td>OTC</td>
<td>Elevated CP causes synthesis of Orotate</td>
</tr>
<tr>
<td>ASD</td>
<td>Elevated citrulline</td>
</tr>
<tr>
<td>ALD</td>
<td>Elevated argininosuccinate</td>
</tr>
<tr>
<td>AD</td>
<td>Elevated arginine</td>
</tr>
</tbody>
</table>
CPS I is Stimulated by NAG

(repeating the figure from page 3 of your handout)
Clinical Management of Urea Cycle Defects

• Dialysis to remove ammonia
• Provide the patient with alternative ways to excrete nitrogenous compounds:
  * Intravenous sodium benzoate or phenylacetate
  * Supplemental arginine

• Levulose - acidifies the gut
• Low protein diet
Degrading the Amino Acid Carbon Backbone
Easily-degraded products after transamination:

Glutaminase

Glutamine $\rightarrow$ glutamate + ammonia

Asparaginase

Asparagine $\rightarrow$ aspartate + ammonia
Many amino acids are purely glucogenic: Glutamate, aspartate, alanine, glutamine, asparagine,…

Some amino acids are both gluco- and ketogenic: Threonine, isoleucine, phenylalanine, tyrosine, tryptophan

The only PURELY ketogenic Amino Acids: leucine, lysine
Amino acids with 5-carbon backbones tend to form \( \alpha \)-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

**Glycine Synthase:**

Glycine

\[ \text{H} \quad \text{C} \quad \text{NH}_3^{(+)} \]

\[ \text{THF} \]

\[ \text{N}^5\text{N}^0\text{-methylene THF} \]

\[ \text{CO}_2 \quad + \quad \text{NH}_4^{(+)} \]

**Serine Hydroxymethyltransferase:**

Serine

\[ \text{H}_2\text{O} \quad \text{S} \quad \text{O} \quad \text{C} \quad \text{C} \quad \text{H} \quad \text{N} \quad \text{H}_2^{(+)} \]

\[ \text{THF} \]

\[ \text{N}^5\text{N}^0\text{-methylene THF} \]

**Serine Dehydratase:**

Serine

\[ \text{H}_2\text{O} \quad \text{S} \quad \text{O} \quad \text{C} \quad \text{C} \quad \text{H} \quad \text{N} \quad \text{H}_2^{(+)} \]

\[ \text{H}_2\text{O} \quad \text{S} \quad \text{O} \quad \text{C} \quad \text{C} \quad \text{H}_2 \quad \text{N} \quad \text{H}_2^{(+)} \]
Methionine Cycle
And Biological Methyl Groups
Deficiency:
Alkaptonuria
"Ochronosis"

Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine
\[ \text{H} \text{C} \text{H} \text{C} \text{CO} \]
\[ \text{NH}_3 \]
\[ (+) \]

Tetrahydrobiopterin + O\(_2\)
\[ \text{Dihydrobiopterin} + \text{H}_2\text{O} \]

Enzyme: Phenylalanine hydroxylase

Tyrosine
\[ \text{HO} \text{H} \text{C} \text{H} \text{C} \text{CO} \]
\[ \text{NH}_3 \]
\[ (+) \]

Homogentisate

Phenylketonuria
(no phenylalanine hydroxylase)

Phenylpyruvate
\[ \text{H} \text{C} \text{H} \text{C} \text{CO} \]
\[ \text{O} \]

Deficiency:
Alkaptonuria
"Ochronosis"

Enzyme: homogentisate dioxygenase
(You don’t need to know the rest)
Branched Chain Amino Acids

Isoleucine

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{CH} \quad \text{COO} \\
\text{CH}_3 & \quad \text{NH}_3
\end{align*}
\]

\[\alpha-KG\]

\[\text{Glu}\]

\[\text{NAD}^+\text{CoASH}\]

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{C} \quad \text{COO} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

Leucine

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{CH} \quad \text{COO} \\
\text{CH}_3 & \quad \text{NH}_3
\end{align*}
\]

\[\alpha-KG\]

\[\text{Glu}\]

\[\text{NAD}^+\text{CoASH}\]

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{C} \quad \text{COO} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

Valine

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{CH} \quad \text{COO} \\
\text{CH}_3 & \quad \text{NH}_3
\end{align*}
\]

\[\alpha-KG\]

\[\text{Glu}\]

\[\text{NAD}^+\text{CoASH}\]

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{C} \quad \text{COO} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

------------------ Transamination ---------------

--- Branched-chain \(\alpha\)-keto acid dehydrogenase ---

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

\[\text{NADH} + \text{CO}_2\]

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

\[\text{NADH} + \text{CO}_2\]

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \text{O}
\end{align*}
\]

\[\text{NADH} + \text{CO}_2\]

(continues on to degradation path similar to \(\beta\)-oxidation of fatty acids)
Synthesis of Bioactive Amines

Tyrosine

Tyrosine hydroxylase

Dihydroxyphenylalanine (L-DOPA)

Dopamine

Norepinephrine

Epinephrine
Synthesis of Bioactive Amines

Tryptophan + NAD+ → 5-hydroxytryptophan + CO₂

5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase

PLP-dependent decarboxylation
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

Histidine decarboxylase (PLP-dependent)

Histamine
NON-Essential Amino Acids:

Glutamate, aspartate, alanine, glutamine, asparagine, (proline), glycine, serine (cysteine, tyrosine)

Essential Amino Acids:

Arginine (!), phenylalanine, methionine, histidine, Isoleucine, leucine, valine, threonine, tryptophan, lysine