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

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

\[ R_1-C-COO^(-) + R_2-C-COO^(-) \rightarrow \alpha\text{-keto acid (typically } \alpha\text{-ketoglutarate)} + \alpha\text{-keto acid (typically glutamate)} \]

**Details of reaction mechanism:**

[Diagram showing the reaction mechanism involving amino acids, pyridoxal phosphate, and pyridoxamine phosphate.]
Transfer the amine back to an acceptor $\alpha$-keto 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 + $\alpha$-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:**

\[
\text{glutamate} \xrightarrow{\text{NAD}(P)} \text{NAD}(P)H \xrightarrow{\text{mito}} \text{\text{\'\text{O}_2C\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-)}} + \text{NH}_3
\]

\[
\text{\text{\'\text{O}_2C\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-)}} + \text{NH}_3
\]

**Glutamine Synthetase:**

\[
\text{\text{\'\text{O}_2C\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-)}} \xrightarrow{\text{ATP+NH}_3} \text{\text{\'\text{O}_2C\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-)}} + \text{ADP} + \text{P}_i
\]

\[
\text{\text{\'\text{O}_2C\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-)}} + \text{NH}_3
\]

\[
\text{\text{\'\text{O}_2C\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-)}} + \text{NH}_3
\]

\[
\text{\text{\'\text{O}_2C\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-)}} + \text{NH}_3
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{Glutamine} \rightarrow \text{Glutamate} + \text{NH}_3
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:} \quad \text{Glutamate} + \text{oxaloacetate} \rightarrow \text{aspartate} + \alpha\text{-keto glutarate}
\]
1. ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate

2. 2ADP + P₃ → Ornithine + Citrulline

3. Ornithine + α-Ketoglutarate + H₂O → Urea + NH₃

4. Arginine + ATP → Argininosuccinate + Aspartate

5. Argininosuccinate + AMP + P₃ → Fumarate + NH₃
Carbamoyl phosphate synthetase I

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

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline

Aspartate

Argininosuccinate
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Arginine + Fumarate
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Arginine
- Argininosuccinate
- Aspartate
- Oxaloacetate
- Malate
- Fumarate
- Citrate
- α-Ketoglutarate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} & \quad \xrightarrow{\text{ATP} + \text{NH}_3} \quad \text{glutamine} \\
\text{ADP} + P_i
\end{align*}
\]
CPS I is Stimulated by NAG

-glutamate + acetyl CoA → N-acetyl glutamate (NAG)

(repeating the figure from page 3 of your handout)

bicarbonate → carbonyl phosphate → carbamate → carbamoyl phosphate
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.

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \text{ATP} \quad \text{ADP} \\
\text{carbonyl phosphate} & \quad \text{NH}_3 \\
\text{carbamate} & \quad \text{ATP} \quad \text{ADP} \\
\text{carbamoyl phosphate} &
\end{align*}
\]
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:

We also already know how to degrade Glutamine:

\[
\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}
\]

...and by analogy, how to degrade Asparagine:

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
Amino Acids are categorized as ‘Glucogenic’ or ‘ketogenic’ or both.

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 α-ketoglutarate.
Degradation and Biosynthesis of Serine and Glycine

**Glycine Synthase:**

\[
\begin{array}{c}
\text{Glycine} \\
(-)\text{OOC} - \text{C} - \text{NH}_3^+ \\
\text{THF} \\
N^6 - N^0 - \text{methylene THF}
\end{array}
\]

\[
\begin{array}{c}
\text{CO}_2 + \text{NH}_4^+ \\
\text{NAD}^+ \\
\text{NADH}
\end{array}
\]

**Serine Hydroxymethyltransferase:**

\[
\begin{array}{c}
\text{Serine} \\
(-)\text{OOC} - \text{CH} - \text{NH}_3^+ \\
\text{CH}_2\text{OH} \\
\text{THF} \\
N^6 - N^0 - \text{methylene THF}
\end{array}
\]

**Serine Dehydratase:**

\[
\begin{array}{c}
\text{Serine} \\
(-)\text{OOC} - \text{CH} - \text{NH}_3^+ \\
\text{CH}_2\text{OH} \\
\text{H}_2\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{(-)OOC} - \text{C} - \text{NH}_3^+ \\
\text{CH}_2\text{OH}
\end{array}
\]

\[
\begin{array}{c}
\text{(-)OOC} - \text{C} - \text{NH}_2^+ \\
\text{CH}_3
\end{array}
\]

\[
\begin{array}{c}
\text{(-)OOC} - \text{C} - \text{O} \\
\text{CH}_3
\end{array}
\]

\[
\begin{array}{c}
\text{NH}_4^+ \\
\text{H}_2\text{O}
\end{array}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

(Normal path shown in black, pathological reaction shown in red)

Phenylalanine $\rightarrow$ Tyrosine

Phenylalanine hydroxylase

Tetrahydrobiopterin + $O_2$ \rightarrow Dihydrobiopterin + $H_2O$

(+) NH$_3$

Phenylalanine $\rightarrow$ Phenylpyruvate

Phenylketonuria (no phenylalanine hydroxylase)

Deficiency: Alkaptonuria "Ochronosis"

Enzyme: homogentisate dioxygenase

Homogentisate

(You don’t need to know the rest)
Branched Chain Amino Acids

Isoleucine  Leucine  Valine

\[
\begin{align*}
R^3 
\text{CH}_3 \text{CH}_2 \text{CH} & \quad \text{CH} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{NH}_3^{(+)}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 \text{CHCH}_2 & \quad \text{CH} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{NH}_3^{(+)}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 \text{CH} & \quad \text{CH} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{NH}_3^{(+)}
\end{align*}
\]

\[\alpha\text{-}\text{KG}\]

\[\text{Glu}\]

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

\[\text{NADH} + \text{CoASH}\]

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

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

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

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

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

--- Transamination ---

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

Tyrosine → Dihydroxyphenylalanine (L-DOPA)

Tyrosine hydroxylase

Dopamine → Norepinephrine → Epinephrine
Synthesis of Bioactive Amines

Tryptophan

\[ \text{Tryptophan hydroxylase} \rightarrow 5\text{-hydroxytryptophan} \]

\[ \text{PLP-dependent decarboxylation} \rightarrow \text{Serotonin} \]

\[ \text{NAD}^+ \]
Synthesis of Bioactive Amines

Glutamate

\[ \text{COO} \text{CH}_2 \text{CH}_2 \text{CH} \equiv \text{COO} \]

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

Glutamate decarboxylase (PLP-dependent)

\[ \text{COO} \text{CH}_2 \text{CH}_2 \text{CH}_2 \equiv \text{NH}_3 \]

\[ (-) \quad (+) \]

\[ \gamma \text{-aminobutyric acid (GABA)} \]

Histidine

\[ \text{CH}_2 \equiv \text{CH} \equiv \text{COO} \]

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

Histidine decarboxylase (PLP-dependent)

\[ \text{CH}_2 \equiv \text{CH}_2 \equiv \text{NH}_3 \]

\[ (-) \quad (+) \]

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