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:

\[ \text{R}_1\text{C}-\text{coo}^(-) + \text{R}_2\text{C}-\text{coo}^(-) \rightarrow \text{R}_1\text{C}-\text{coo}^(-) + \text{R}_2\text{C}-\text{coo}^(-) \]

\[ \alpha\text{-keto acid (typically \alpha\text{-ketoglutarate})} \]

Details of reaction mechanism:

\[ \text{amino acid} \]

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

\[ \text{NH}_2 \]

\[ + \text{O} \]

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

\[ \text{pyridoxal phosphate} \]

\[ \text{H}^+ \]

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

\[ \text{N} \]

\[ \text{CH}_3 \]

\[ \text{H} \]

\[ \text{H} \]

\[ \text{pyridoxamine phosphate} \]

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

\[ \text{NH}_2 \]

\[ + \text{NH}_2 \]

\[ \text{HCH} \]

\[ \text{CH}_3 \]

\[ \text{H} \]

\[ \text{pyridoxamine phosphate} \]
Transfer the amine back to an acceptor α-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 + 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:**

\[
\text{glutamate} + \text{NAD}(P) \rightarrow \text{NAD}(P)\text{H} + \alpha\text{-ketoglutarate} + \text{NH}_3
\]

**Glutamine Synthetase:**

\[
\text{glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamine} + \text{ADP} + \text{P}_i
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{Glutamine} & \rightarrow \text{Glutamate} + \text{NH}_3 \\
\text{H}_2\text{O} & \\
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase:} & \\
\text{Glutamate} + \text{oxaloacetate} & \rightarrow \text{aspartate} + \text{α-keto glutarate} \\
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{NH}_3}$ carbamate $\xrightarrow{\text{ATP}}$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

\[
\begin{align*}
\text{Citrulline} & \quad \text{aspartate} \\
\text{Argininosuccinate} & \quad \text{ATP} \\
\text{AMP} + \text{PP}_i &
\end{align*}
\]
Argininosuccinate lyase

Argininosuccinate $\xrightarrow{\text{Argininosuccinate lyase}}$ Arginine $+$ Fumarate
Arginase

\[
\text{Arginine} \xrightarrow{\text{H}_{2}O} \text{Urea} \xrightarrow{\text{H}_{2}O} \text{Ornithine}
\]

\[
\text{(-)}\text{oOC-C-CH}_{2}\text{CH}_{2}\text{CH}_{2}\text{NH-C-NH}_{2}^{(+3)} \xrightarrow{\text{H}_{2}O} \text{NH}_{2}\text{C-C-NH}_{2} \xrightarrow{\text{H}_{2}O} \text{NH}_{3}{\text{C-CH}_{2}\text{CH}_{2}\text{NH}_{3}^{(+3)}}
\]
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine → Citrulline → Argininosuccinate → Arginine → Urea

TCA Cycle:
- Oxaloacetate → Malate → Fumarate → α-Ketoglutarate → Citrate
- Aspartate (H\(^{+}\) \(-\text{C}_{2}\text{H}_{2}\text{CH}_{2}\text{C}=\text{CO}_{2}^{(-)}\) \(\text{NH}_{2}\))
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbonyl phosphate} \\
& \xrightarrow{\text{ADP}} \quad \text{carbamate} \\
& \xrightarrow{\text{P_i}} \quad \text{carbamoyl phosphate}
\end{align*}
\]
Complicating the picture: Other tissues may be involved

Muscle:
- Amino acids: Transamination, Deamination
- Alanine → Glutamate → Glutamine → NH₄⁺
- Purine deamination: NH₄⁺

Intestine:
- Glutamine
- Alanine → NH₄⁺ → Citrulline

Kidney:
- Glutamine → NH₃ → NH₄⁺
- Citrulline
- Arginine

Liver:
- Glutamine
- Alanine → Glu → Aspartate → NH₄⁺
- Arginine → Urea
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>Enzyme</th>
<th>Metabolite Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSD</td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
</tr>
<tr>
<td>OTCD</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

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

(repeating the figure from page 3 of your handout)

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

\[
\begin{align*}
\text{H} & \quad \text{(-O}_{2}\text{CCH}_{2}\text{C}-\text{CO}_{2}(-)} \\
\text{NH}_{3} & \quad \text{aspartate} \\
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{(-O}_{2}\text{CCH}_{2}\text{CH}_{2}\text{C}-\text{CO}_{2}(-)} \\
\text{NH}_{3} & \quad \text{glutamate} \\
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{(-O}_{2}\text{CCH}_{2}\text{CH}_{2}\text{C}-\text{CO}_{2}(-)} \\
\text{NH}_{3} & \quad \text{oxaloacetate} \\
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{(-O}_{2}\text{CCH}_{2}\text{CH}_{2}\text{CH}_{2}\text{C}-\text{CO}_{2}(-)} \\
\text{NH}_{3} & \quad \text{\alpha-ketoglutarate} \\
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{(-O}_{2}\text{CCH}_{2}\text{CH}_{2}\text{CH}_{2}\text{C}-\text{CO}_{2}(-)} \\
\text{NH}_{3} & \quad \text{pyruvate} \\
\end{align*}
\]

We also already know how to degrade Glutamine:

Glutamine \text{ glutaminase} \rightarrow \text{ glutamate + ammonia}

…and by analogy, how to degrade Asparagine:

Asparagine \text{ asparaginase} \rightarrow \text{ aspartate + 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:

\[
\begin{align*}
\text{Glycine} & \quad \text{NAD}^+ \\
\text{THF} & \quad N^6\text{-N}^\text{10- methylene THF}
\end{align*}
\]

\[
\begin{align*}
\text{Glycine} & \quad \text{CO}_2 + \text{NH}_4^+
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Serine} & \quad \text{THF} \\
\text{N}^6\text{-N}^\text{10- methylene THF} & \quad \text{Glycine}
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O}
\end{align*}
\]

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O}
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

\[
\begin{align*}
\text{Phenylalanine} & \xrightarrow{\text{Enzyme: Phenylalanine hydroxylase}} \text{Tyrosine} \\
\text{Phenylpyruvate} & \end{align*}
\]

Deficiency: Alkaptonuria (“Ochronosis”)

Enzyme: homogentisate dioxygenase

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

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

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

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

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

------------- Branched-chain \(\alpha\text{-keto acid dehydrogenase} \) -------------

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

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

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

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

Tyrosine $\xrightarrow{\text{Tyrosine hydroxylase}}$ Dihydroxyphenylalanine (L-DOPA)

Dopamine $\xrightarrow{\text{}}$ Norepinephrine $\xrightarrow{\text{}}$ Epinephrine
Synthesis of Bioactive Amines

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

5-hydroxytryptophan \[\xrightarrow{\text{PLP-dependent decarboxylation}}\] Serotonin
Synthesis of Bioactive Amines

\[ \text{Glutamate} \xrightarrow{\text{Glutamate decarboxylase (PLP-dependent)}} \gamma\text{-aminobutyric acid (GABA)} \]

\[ \text{Histidine} \xrightarrow{\text{Histidine decarboxylase (PLP-dependent)}} \text{Histamine} \]
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