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:

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

$R_1\text{C}$ and $R_2\text{C}$ represent amino acids, typically $\alpha$-ketoglutarate.

Details of reaction mechanism:

1. Amino acid
2. $R\text{C}-\text{coo}^\text{(-)}$ + pyridoxal phosphate
3. $\text{H}_2\text{O}$
4. $R\text{C}-\text{coo}^\text{(-)}$ + $H^+$
5. $R\text{C}-\text{coo}^\text{(-)}$ + $\text{NH}_2$
6. 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 + α-ketoglutarate → 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:

\[
\text{Glutamate Dehydrogenase:} \quad (-)^\text{O}_2\text{C}_\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-) \xrightarrow{\text{NAD}(P)} \text{NAD}(P)\text{H} \xrightarrow{\text{mito}} (-)^\text{O}_2\text{C}_\text{CH}_2\text{CH}_2\text{C}-\text{CO}_2(-) + \text{NH}_3
\]

Glutamine Synthetase:

\[
\text{Glutamine Synthetase:} \quad (-)^\text{OOC}_\text{C}-\text{CH}_2\text{CH}_2\text{COO}(-) \xrightarrow{\text{ATP}+\text{NH}_3} \text{ATP} \xrightarrow{\text{ADP}+\text{P}_i} (-)^\text{OOC}_\text{C}-\text{CH}_2\text{CH}_2\text{C}^\text{NH}_3_3
\]
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:

\[
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} + \text{Oxaloacetate} \rightarrow \text{Aspartate} + \text{α-keto glutarate}
\]
Carbamoyl phosphate synthetase I

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

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

\[
\begin{align*}
\text{Argininosuccinate} & \rightarrow \text{Arginine} + \text{Fumarate} \\
\text{(-OOOC-CH}_2\text{CH}_2\text{CH}_2\text{NH-CH=NH}_2} & \rightarrow \text{(-OOOC-CH}_2\text{CH}_2\text{CH}_2\text{NH-CH=NH}_2 \\
\text{(-O}_2\text{C-CH}_2\text{C-CH}=\text{CO}_2} & \rightarrow \text{(-O}_2\text{C-CH}_2\text{CH}_2\text{NH-CH=NH}_2}
\end{align*}
\]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\begin{array}{c}
-\text{H}O\text{C}CH_2CH\text{C}-\text{CO}_2^- \\
\text{NH}_2
\end{array}
\]

\[\text{glutamate} \xrightarrow{\text{NAD}(P)} \text{NAD}(P)\text{H} \xrightarrow{\text{mito}} \begin{array}{c}
-\text{H}O\text{C}CH_2CH\text{C}-\text{CO}_2^- \\
\text{NH}_3
\end{array}
\]

\[\alpha\text{-ketoglutarate} + \text{ammonia}\]

Glutamine Synthetase:

\[
\begin{array}{c}
-\text{OOC}C\text{CH}_2\text{CH}_2\text{COO}^- \\
\text{NH}_3^-\text{H}^3
\end{array}
\]

\[\text{glutamate} \xrightarrow{\text{ATP}+\text{NH}_3} \text{ADP}+\text{P}_i \xrightarrow{\text{glutamine}} \begin{array}{c}
-\text{OOC}C\text{CH}_2\text{CH}_2\text{C}^- \\
\text{NH}_3^-\text{NH}_2^\text{+}
\end{array}
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

bicarbonate + ATP → carbamoyl phosphate

Carbamoyl phosphate + NH₃ → carbamate

Carbamoyl phosphate + ATP → 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.

<table>
<thead>
<tr>
<th>Cpsd</th>
<th>No elevation except ammonia; diagnosed by elimination.</th>
</tr>
</thead>
<tbody>
<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

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

(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:

\[
\begin{align*}
\text{Glutamine} & \rightarrow \text{glutamate} + \text{ammonia} \\
\text{Asparagine} & \rightarrow \text{aspartate} + \text{ammonia}
\end{align*}
\]

We also already know how to degrade Glutamine:

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

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

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
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:

\[
\text{Glycine} \quad \text{(+)NH}_3 \quad \text{THF} \quad \text{N}^5-\text{N}^0-\text{methylene THF} \quad \text{CO}_2 + \text{NH}_4^{(+)}
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine} \quad \text{(+)NH}_3 \quad \text{THF} \quad \text{N}^5-\text{N}^0-\text{methylene THF} \quad \text{Glycine}
\]

Serine Dehydratase:

\[
\text{Serine} \quad \text{H}_2\text{O} \quad \text{(-)OOC} - \text{C} - \text{NH}_3 \quad \text{(+)NH}_3 \quad \text{H}_2\text{O} \quad \text{(+)OOC} - \text{C} - \text{O} \quad \text{CH}_3
\]
Methionine Cycle
And Biological Methyl Groups
Deficiency:
Alkaptonuria
"Ochronosis"

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

Phenylalanine → Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O → Tyrosine

Enzyme: Phenylalanine hydroxylase

Phenylketonuria (no phenylalanine hydroxylase) → Phenylpyruvate

Tyrosine → Homogentisate

Deficiency: Alkaptonuria "Ochronosis"

Enzyme: homogentisate dioxygenase

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

Isoleucine

Leucine

Valine

--- Branched-chain α-keto acid dehydrogenase ---

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

Tyrosine $\rightarrow$ Dihydroxyphenylalanine (L-DOPA) $\rightarrow$ Dopamine $\rightarrow$ Norepinephrine $\rightarrow$ Epinephrine

Tyrosine hydroxylase
Synthesis of Bioactive Amines

Tryptophan → NAD+ → Tryptophan hydroxylase → 5-hydroxytryptophan → PLP-dependent decarboxylation → CO₂ → Serotonin
Synthesis of Bioactive Amines

Glutamate

\[ \text{COO}^{(-)} - \text{CH}_2\text{CH}_2\text{CH} - \text{COO} \]

\[ \text{NH}_3 \]

(+)

Glutamate decarboxylase (PLP-dependent)

\[ \text{COO}^{(-)} - \text{CH}_2\text{CH}_2\text{CH}_2 - \text{NH}_3 \]

(+)

γ-aminobutyric acid (GABA)

Histidine

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

\[ \text{NH}_3 \]

(+)

Histidine decarboxylase (PLP-dependent)

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

(+)

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