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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:

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

\( \text{NH}_2 \)
\( \text{NH}_2 \)
\( \alpha\text{-keto acid (typically alpha-ketoglutarate)} \)
\( \alpha\text{-keto acid} \)
\( \text{amino acid} \)
\( \text{amino acid} \)

Details of reaction mechanism:

\[ \text{amino acid} \]
\[ R-C-\text{coo}(-) \]
\[ \text{NH}_2 \]
\[ \text{O} \]
\[ \text{H} \]
\[ \text{CH} \]
\[ \text{OH} \]
\[ \text{BoCH} \]
\[ \text{BoCH} \]
\[ \text{BoCH} \]
\[ \text{BoCH} \]
\[ \text{pyridoxal phosphate} \]
\[ \text{H}_2\text{O} \]
\[ \text{H}^+ \]
\[ \text{N} \]
\[ \text{CH} \]
\[ \text{OH} \]
\[ \text{N} \]
\[ \text{CH} \]
\[ \text{CH} \]
\[ \text{pyridoxamine phosphate} \]
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 → 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:

\[ (\ce{\text{H}})_{\text{O}_2\text{C}\text{CH}_2\text{CH}_2\text{C}=\text{CO}_2}\text{NH}_2 \xrightarrow{\text{NAD(P)}} \text{glutamate} \xrightarrow{\text{mito}} (\ce{\text{H}})_{\text{O}_2\text{C}\text{CH}_2\text{CH}_2\text{C}=\text{CO}_2} + \text{NAD(P)H} \xrightarrow{\text{O}} \alpha\text{-ketoglutarate} + \text{ammonia} \]

Glutamine Synthetase:

\[ (\ce{\text{H}})_{\ce{\text{OOC-CH}_2\text{CH}_2\text{COO}^-}} \xrightarrow{\text{ATP+NH}_3} \text{glutamate} \xrightarrow{\text{ADP+P}} (\ce{\text{H}})_{\ce{\text{OOC-CH}_2\text{CH}_2\text{C}=\text{N}}} + \text{NH}_3 \]
Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{glutamine} & \quad \text{H}_2\text{O} \\
\text{H} & \quad \text{NH}_3 \\
\text{ammonia} & \quad \text{glutamate}
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase:} & \\
\text{Glutamate} & \quad \text{H} \\
\text{oxaloacetate} & \quad \text{H} \\
\text{aspartate} & \quad \text{H}
\end{align*}
\]
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

\[
\text{Citrulline} \xrightarrow{\text{ATP}} \text{Argininosuccinate} + \text{AMP} + \text{PP}_i
\]
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Fumarate $\rightarrow$ Arginine

$\text{H} \quad \text{(-)}_2\text{CCH}_2\text{C} - \text{CO}_2\text{(-)}$

$\text{H} \quad \text{(-)}_2\text{CCH}_2\text{CH}_2\text{NH} - \text{C} = \text{NH}_2\text{(+)}

$\text{H} \quad \text{NH}_3\text{(+)}

$\text{H} \quad \text{(-)}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{(-)}$

$\text{H} \quad \text{NH}_3\text{(+)}

$\text{H} \quad \text{NH}_2\text{(+)}}$
Arginase

{(-)OOCC-CH(CH₂)₂CH₂NH-C-NH₂}³

Arginine

H₂O

î

NH₂-C-NH₂

Urea

{(-)OOCC-CH(CH₂)₂CH₂NH₃⁺}³

Ornithine
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Citrulline
- Argininosuccinate
- Arginine

TCA Cycle:
- Oxaloacetate
- Malate
- Fumarate
- Citrate
- α-Ketoglutarate

Aspartate: $\text{H}^{+} \text{CCH}_2\text{C}=-\text{CO}_2\text{NH}_2$
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\text{glutamate} \xrightarrow{\text{NAD}(\text{P})} \text{NH}_2 \xrightarrow{\text{(mito)}} \text{NAD}(\text{P})\text{H} \xrightarrow{\alpha\text{-ketoglutarate}} \text{ammonia}
\]

**Glutamine Synthetase:**

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

\[
\text{NH}_3
\]
CPS I is Stimulated by NAG

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

(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>Enzyme</th>
<th>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

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

(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
We also already know how to degrade Glutamine:

\[
glutaminase \quad \text{Glutamine} \rightarrow \text{glutamate} + \text{ammonia}
\]

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

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

\[ \begin{align*}
\text{Glycine} & \quad \text{THF} \\
\text{NAD}^+ & \quad \text{NADH} \\
\text{N}^6-\text{N}^\alpha-\text{methylene} \text{ THF} & \quad \text{CO}_2 + \text{NH}_4^+
\end{align*} \]

Serine Hydroxymethyltransferase:

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

Serine Dehydratase:

\[ \begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{NH}_4^+ & \quad \text{CO}_2 + \text{CH}_3
\end{align*} \]
Methionine Cycle
And Biological Methyl Groups
Deficiency:
Alkaptonuria
“Ochronosis”

Phenylalanine and Tyrosine

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

Phenylalanine

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

Enzyme: Phenylalanine hydroxylase

Tyrosine

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

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

Isolucine

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

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

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

\[ \text{Glu} \]

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

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

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

\[ \text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{S-CoA} \]

Leucine

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

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

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

\[ \text{Glu} \]

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

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

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

\[ \text{CH}_3\text{CHCH}_2 - \text{C} - \text{S-CoA} \]

Valine

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

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

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

\[ \text{Glu} \]

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

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

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

\[ \text{CH}_3\text{CH} - \text{C} - \text{S-CoA} \]

(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 \[\xrightarrow{\text{Conversion}}\] Norepinephrine \[\xrightarrow{\text{Conversion}}\] Epinephrine
Synthesis of Bioactive Amines

Tryptophan \xrightarrow{\text{Tryptophan hydroxylase}} 5-hydroxytryptophan \xrightarrow{\text{PLP-dependent decarboxylation}} \text{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