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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:

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

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

Details of reaction mechanism:

1. **Amino acid**
   \[
   \text{H} - R - \text{C-}^{\text{coo}}(-)
   \]
   \[
   + \text{NH}_2\]
   \[
   \text{OCH}_2\text{OH}
   \]
   \[
   \overset{\text{pyridoxal phosphate}}{\text{H_2O}}
   \]

2. **Pyridoxal phosphate**
   \[
   \overset{\text{H^+}}{\text{HCH}}
   \]
   \[
   \overset{\text{N}}{\text{CH}_3}
   \]

3. **Pyridoxamine phosphate**
   \[
   \overset{\text{HCH}}{\text{HCH}}
   \]
   \[
   \overset{\text{NH}_2}{\text{H}}
   \]

4. **α-keto acid**
   \[
   \overset{\text{R-C-}^{\text{coo}}(-)}{\text{H}}
   \]
   \[
   + \text{NH}_2\]
   \[
   \text{OCH}_2\text{OH}
   \]

where \(R_1\) and \(R_2\) represent different amino acids.
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:**

\[
\begin{align*}
\text{glutamate} & \quad \rightarrow \quad \text{NAD}(P) \\
\text{NAD}(P)H & \quad \rightarrow \quad \alpha\text{-ketoglutarate} \quad \text{ammonia}
\end{align*}
\]

**Glutamine Synthetase:**

\[
\begin{align*}
\text{glutamate} & \quad \rightarrow \quad \text{ATP} + \text{NH}_3 \\
\text{ATP} + \text{NH}_3 & \quad \rightarrow \quad \text{ADP} + \text{P}_i \\
\end{align*}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{H} \quad \text{NH}_3 \\
\text{H} \quad \text{NH}_3
\]

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:}
\]

\[
(\overset{-}{\text{H}} \quad \overset{-}{\text{H}} \quad \overset{-}{\text{H}} \quad \text{CO}_2^- + \quad \overset{-}{\text{H}} \quad \overset{-}{\text{H}} \quad \overset{-}{\text{H}} \quad \text{CO}_2^-) \\
\text{Glutamate} \quad \text{oxaloacetate} \quad \alpha\text{-keto glutarate} \quad \text{aspartate}
\]
Carbamoyl phosphate synthetase I

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

Carbamoyl phosphate

\[
\text{NH}_2 - C - \text{O} \quad \text{Pi}
\]

Ornithine

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

Citrulline

\[
(-)\text{OOC} - C - \text{CH}_2 \text{CH}_2 \text{CH}_2 \text{NH} - \text{C} - \text{NH}_2
\]
Argininosuccinate synthetase

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

Argininosuccinate $\rightarrow$ Fumarate $\rightarrow$ Arginine
Arginase

\[
\text{Arginine} \xrightarrow{H_2O} \text{Ornithine}
\]

\[
(-)\text{C}(-\text{NH}_2)\text{C}(\text{CH}_2\text{CH}_2\text{NH}_3^+)\xrightarrow{H_2O}\text{C}(\text{CH}_2\text{CH}_2\text{NH}_3^+)
\]
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Argininosuccinate
- Arginine
- Urea

TCA Cycle
- Oxaloacetate
- Malate
- Fumarate
- α-Ketoglutarate
- Citrate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\begin{align*}
&\text{glutamate} & & \text{NAD}(P) \\
&\xrightarrow{\text{mite}} & & \text{NAD}(P)H \\
& & & \alpha\text{-ketoglutarate} \\
& & & \text{ammonia}
\end{align*}
\]

Glutamine Synthetase:

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

\[
\text{glutamate} + \text{acetyl CoA} \xrightarrow{\text{N-acetyl glutamate synthetase}} \text{N-acetyl glutamate (NAG)}
\]

(repeating the figure from page 3 of your handout)

\[
\text{bicarbonate} \xrightarrow{\text{ATP}} \text{carbonyl phosphate} \xrightarrow{\text{NH}_3} \text{carbamate} \xrightarrow{\text{ATP}} \text{carbamoyl phosphate}
\]
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>Condition</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

\[
\begin{align*}
\text{glutamate} & \quad \text{acetyl CoA} \\
\overset{(-)}{\text{OOC}} & \quad \overset{(-)}{\text{CoA-C-OH}} \\
\text{NH}_2 & \quad \text{C=O} \\
\overset{(+)}{\text{CH}_3} & \quad \overset{(+)}{\text{CH}_3}
\end{align*}
\]

\[
\overset{\text{N-acetyl glutamate (NAG)}}{\text{N-acetyl glutamate synthetase}}
\]

(repeating the figure from page 3 of your handout)

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

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

\[
\begin{align*}
\text{Glycine} & \quad \text{NAD}^+ \quad \text{NADH} \\
& \quad \text{THF \quad N}^5\text{N}^\text{O}^- \text{methylene THF} \\
& \quad \text{CO}_2 \quad + \quad \text{NH}_4^+
\end{align*}
\]

**Serine Hydroxymethyltransferase:**

\[
\begin{align*}
\text{Serine} & \quad \text{NAD}^+ \quad \text{NADH} \\
& \quad \text{THF \quad N}^5\text{N}^\text{O}^- \text{methylene THF} \\
& \quad \text{Glycine}
\end{align*}
\]

**Serine Dehydratase:**

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
& \quad \text{NH}_4^+
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine + Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O

Enzyme: Phenylalanine hydroxylase

Phenylpyruvate

Tyrosine → Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

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

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

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

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

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

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

\[ \text{CH}_2\text{CHCOO}^\text{(-)} \quad \text{NH}_3\text{(+)} \quad \text{CH}_2\text{CHCOO}^\text{(-)} \quad \text{NH}_3\text{(+)} \]

\[ \text{NAD}^+ \]

\[ \text{CO}_2 \]
Synthesis of Bioactive Amines

- Glutamate decarboxylase (PLP-dependent)
- Histidine decarboxylase (PLP-dependent)

Glutamate $\rightarrow$ GABA

Histidine $\rightarrow$ 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