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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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Director, DNA Sequencing Core
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:

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

Details of reaction mechanism:

\[
\text{Amino acid} + \text{pyridoxal phosphate} \rightarrow \text{alpha-keto acid} + \text{pyridoxamine phosphate}
\]
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:

\[ \text{Some amino acid} + \alpha \text{-ketoglutarate} \rightarrow \text{some alpha keto acid} + \text{Glutamate} \]
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} & \rightarrow \text{NAD(P)} \quad \text{(mito)} \\
& \rightarrow \text{NAD(P)H} \\
& \rightarrow \text{\(\alpha\)-ketoglutarate} + \text{ammonia}
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{ATP} + \text{NH}_3 & \rightarrow \text{ADP} + \text{P}_i \\
\text{glutamate} & \rightarrow \text{glutamine}
\end{align*}
\]
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:}
\[
\text{Glutamate} + \text{oxaloacetate} \rightarrow \text{aspartate} + \text{\alpha-keto glutarate}
\]
```
Carbamoyl phosphate synthetase I

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

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline

Argininosuccinate
Argininosuccinate lyase

Argininosuccinate → Arginine

\(-\)\(\text{O}_2\text{C}-\text{C}(-)\text{H}_2\text{CH}_2\text{NH}(-)\text{C}=\text{NH}^{(+)}\) → \(-\)\(\text{O}_2\text{C}-\text{C}(-)\text{H}(-)\text{H}_2\text{CH}_2\text{NH}(-)\text{C}(-)\text{NH}_2\)
Arginase

\[
\text{Arginine} \xrightarrow{\text{H}_2\text{O}} \text{Ornithine} + \text{Urea}
\]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[
\text{glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamine} \rightarrow \text{ADP} + \text{P}_i
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \rightarrow \text{carbonyl phosphate} \\
\xrightarrow{\text{ATP}} & \text{carbamide} \\
\xrightarrow{\text{ATP}} & \text{carbamoyl phosphate}
\end{align*}
\]
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
2 ATP + HCO₃⁻ + NH₃ → Carboxamoyl phosphate
2 ADP + P₃ → Ornithine
Liver mitochondrion
Liver cytoplasm

Ornithine → Citrulline

Citrulline → Argininosuccinate

Argininosuccinate → Fumarate

NH₃ + H₂O → Urea
NH₃ + H₂O → Aspartate

(-boc-C-(CH₂)₃CH₂CH₂NH₃⁺)
(-boc-C-(CH₂)₃CH₂CH₂NH₂)
(-boc-C-(CH₂)₃CH₂CH₂NH⁻)
(-boc-C-(CH₂)₃CH₂CH₂NH₃⁺)
(-boc-C-(CH₂)₃CH₂CH₂NH₃⁺)
(-boc-C-(CH₂)₃CH₂CH₂NH₂)
(-boc-C-(CH₂)₃CH₂CH₂NH⁻)
(-boc-C-(CH₂)₃CH₂CH₂NH₃⁺)
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>Condition</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

\[
\text{glutamate} \quad \text{acetyl CoA} \quad \text{N-acetyl glutamate (NAG)}
\]

(repeating the figure from page 3 of your handout)
1. $2 \text{ATP} + \text{HCO}_3^- + \text{NH}_3 \rightarrow \text{Carbamoyl phosphate}$
   \[ \text{NH}_2 \overset{\text{H}}{\overset{\text{O}}{\overset{\text{P}}{\text{C}}} \overset{\text{NH}_3}{\text{NH}_3}}^{\text{(-)}} \]

2. $2 \text{ADP} + \text{P}_i \rightarrow \text{ATP}$
   \[ \overset{\text{H}}{\overset{\text{O}}{\overset{\text{P}}{\text{C}}} \overset{\text{NH}_3}{\text{NH}_3}}^{\text{(-)}} \]

3. Liver mitochondrion
   - Ornithine
   - Citrulline

4. Liver cytoplasm
   - Ornithine
   - Urea
   - Arginine
   - Argininosuccinate
   - Fumarate

5. $\text{NH}_2 \overset{\text{H}}{\overset{\text{O}}{\overset{\text{P}}{\text{C}}} \overset{\text{NH}_3}{\text{NH}_3}}^{\text{(-)}}$
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:

\[
\begin{align*}
\text{H} & \quad \text{transamination} \quad \text{H} \\
\text{H} & \quad \text{transamination} \\
\text{H} & \quad \text{transamination}
\end{align*}
\]

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparagine:

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
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 $\alpha$-ketoglutarate.
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

\[
\begin{align*}
\text{Phenylalanine} & \xrightarrow{\text{Phenylalanine hydroxylase}} \text{Tyrosine} \\
\text{Phenylpyruvate} & \xrightarrow{\text{Enzyme: homogentisate dioxygenase}} \text{Homogentisate}
\end{align*}
\]
Branched Chain Amino Acids

Isoleucine

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

\[\text{CH}_3\text{NH}^+ \]

\[\text{α-KG}\]

\[\text{Glu}\]

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

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

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

Leucine

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

\[\text{CH}_3\text{NH}^+ \]

\[\text{α-KG}\]

\[\text{Glu}\]

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

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

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

Valine

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

\[\text{CH}_3\text{NH}^+ \]

\[\text{α-KG}\]

\[\text{Glu}\]

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

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

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

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

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

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

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

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

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

Tyrosine $\rightarrow$ Dihydroxyphenylalanine (L-DOPA) via Tyrosine hydroxylase

Dopamine $\rightarrow$ Norepinephrine $\rightarrow$ Epinephrine
Synthesis of Bioactive Amines

Tryptophan

\[ \text{Tryptophan hydroxylase} \]

5-hydroxytryptophan

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

\[ \text{CO}_2 \]

Serotonin

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

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

\[
\text{Histidine} \quad \xrightarrow{\text{Histidine decarboxylase (PLP-dependent)}} \quad \text{Histamine}
\]
NON-Essential Amino Acids:

- Glutamate, aspartate, alanine, glutamine, asparagin e, (proline), glycine, serine (cysteine, tyrosine)

Essential Amino Acids:

- Arginine (!), phenylalanine, methionine, histidine, Isoleucine, leucine, valine, threonine, tryptophan, lysine