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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:

$$R_1\text{C-} COO^- + R_2\text{C-} COO^- \rightarrow R_1\text{C-} COO^- + R_2\text{C-} COO^-$$

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

Details of reaction mechanism:

$$\text{amino acid} \quad \text{H} \quad \text{R-C-} COO^- \quad \text{H} \quad \text{R-C-} COO^- \quad \text{H} \quad \text{R-C-} COO^-$$

$$\text{H}_{2}\text{O} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N}$$

$$\text{pyridoxal phosphate} \quad \text{pyridoxamine phosphate}$$
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:**

\[ \text{H} \quad \text{NAD}(P) \quad \text{NAD}(P)H \quad \text{mito} \quad \text{NH}_3 \]

\[ \text{(-)}\text{O}_2\text{CCH}_2\text{CH}_2\text{C-}\text{CO}_2\text{(-)} \quad \text{glutamate} \]

\[ \text{(-)}\text{O}_2\text{CCH}_2\text{CH}_2\text{C-}\text{CO}_2\text{(-)} + \text{NH}_3 \quad \text{a-ketoglutarate} \quad \text{ammonia} \]

**Glutamine Synthetase:**

\[ \text{(-)}\text{OOC-} \quad \text{H} \quad \text{NH}_3 \quad \text{(+)} \quad \text{glutamate} \]

\[ \text{(-)}\text{OOC-} \quad \text{H} \quad \text{NH}_3 \quad \text{(+)} \quad \text{glutamine} \]

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

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{glutamine} & \quad \text{glutamate} \\
\text{H} & \quad \text{H} \\
\text{NH}_3 & \quad \text{NH}_3 \\
\text{CH}_2\text{CH}_2\text{C}=\text{O} & \quad \text{CH}_2\text{CH}_2\text{C}=\text{O} \\
\text{O} & \quad \text{OH} \\
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} & \quad \text{oxaloacetate} \\
\text{H} & \quad \text{H} \\
\text{NH}_3 & \quad \text{NH}_3 \\
\text{CO}_{2} & \quad \text{CO}_{2} \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{O} \\
\text{C} & \quad \text{C} \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate \[ \rightarrow \text{ATP} \rightarrow \text{carbonyl phosphate} \rightarrow \text{NH}_3 \rightarrow \text{carbamate} \rightarrow \text{ATP} \rightarrow \text{carbamoyl phosphate} \]
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[ \text{(-OOCC} \quad \text{H}_2\text{C}_2\text{H}_2\text{C}_2\text{NH}_3^{(+)} \quad \text{NH}_3 \quad \text{(-O)} \quad \text{H}_2\text{C}_2\text{H}_2\text{C}_2\text{NH}_2 \text{(-O)} \quad \text{C} \quad \text{NH}_2 \]

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline → Aspartate → Argininosuccinate

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

\[
\text{Argininosuccinate} \rightarrow \text{Arginine} + \text{Fumarate}
\]
Arginase

\[
\begin{align*}
\text{Arginine} & \quad \xrightarrow{H_2O} \quad \text{Ornithine} \\
(-)\text{ooC} & \quad \text{CH}_2\text{CH}_2\text{CH}_2\text{NH} - \text{C} - \text{NH}_2 \\
\text{NH} & \quad \text{NH}_2 \\
(+)^3 & \\
\text{Urea} & \quad \text{H} \\
\text{NH}_2 \quad \text{C} - \text{NH}_2 \\
\text{NH}_3 & \quad \text{NH}_2 \\
(+)^3 & 
\end{align*}
\]
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Citrulline
- Arginine
- Argininosuccinate

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

Aspartate:
\[ \text{Aspartate} = \text{H} + \left( \text{H}_2\text{CCH}_2\text{C}=\text{CO}_2\text{H} \right) \]
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\begin{align*}
\text{Glutamate} & \quad \text{NAD}(P) \\
\text{H} & \quad \rightarrow \\
\text{NH}_2 & \quad \text{(mito)} \\
\text{glutamate} & \quad \text{NAD}(P)H & \quad \text{α-ketoglutarate} & \quad \text{ammonia}
\end{align*}
\]

**Glutamine Synthetase:**

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

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

(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>Acronym</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

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

(repeating the figure from page 3 of your handout)

bicarbonate + ATP → ADP + carbonyl phosphate

carbonyl phosphate + NH₃ → carbamoyl phosphate
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:

\[
\begin{align*}
\text{Glutamine} & \overset{\text{glutaminase}}{\longrightarrow} \text{glutamate} + \text{ammonia} \\
\text{Asparagine} & \overset{\text{asparaginase}}{\longrightarrow} \text{aspartate} + \text{ammonia}
\end{align*}
\]
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
Methionine Cycle
And Biological Methyl Groups
Deficiency: Alkaptonuria

"Ochronosis"

Phenylalanine and Tyrosine

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

Phenylalanine

\[ \text{NH}_3 \]

\[ (+) \text{Tetrahydrobiopterin + O}_2 \]

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

Enzyme: Phenylalanine hydroxylase

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

Tyrosine

\[ \text{NH}_3 \]

\[ (+) \]

Homogentisate

Deficiency: Alkaptonuria

"Ochronosis"

Phenylalanine

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

Phenylpyruvate

Enzyme: homogentisate dioxygenase

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

Isoleucine

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

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

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

\[\rightarrow \text{Glu}\]

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

\[\rightarrow \text{NAD}^\cdot\text{CoASH}\]  

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

Leucine

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

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

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

\[\rightarrow \text{Glu}\]

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

\[\rightarrow \text{NAD}^\cdot\text{CoASH}\]  

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

Valine

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

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

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

\[\rightarrow \text{Glu}\]

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

\[\rightarrow \text{NAD}^\cdot\text{CoASH}\]  

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

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

--- Branched-chain \(\alpha\)-keto acid dehydrogenase ---

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

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

\[\rightarrow \text{NAD}^\cdot\text{CoASH}\]  

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

(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 $\xrightarrow{}$ Norepinephrine $\xrightarrow{}$ Epinephrine
Synthesis of Bioactive Amines

Tryptophan → Tryptophan hydroxylase → 5-hydroxytryptophan → PLP-dependent decarboxylation → CO₂ → Serotonin

\[ \text{Tryptophan} \rightarrow \text{Tryptophan hydroxylase} \rightarrow \text{5-hydroxytryptophan} \rightarrow \text{PLP-dependent decarboxylation} \rightarrow \text{CO}_2 \rightarrow \text{Serotonin} \]
Synthesis of Bioactive Amines

- Glutamate
  - COO⁻CH₂CH₂CH−COO⁻
  - Glutamate decarboxylase (PLP-dependent)
  - \(\gamma\)-aminobutyric acid (GABA)

- Histidine
  - \(\text{Histidine}\) \(\text{CH}_2\text{CH}^{-}\text{COO}^-\)
  - Histidine decarboxylase (PLP-dependent)
  - 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