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
Amino Acid metabolism

Amino acids

Glu, Gln, Asp, NH₃

Urea

Folate metabolism

Methylen THF

Met Cycle

Nucleic Acid metabolism

Purines

DNA

RNA

Pyrimidines

Uric Acid

(energy)
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:

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

Details of reaction mechanism:

\[ \text{pyridoxal phosphate} \rightarrow \text{pyridoxamine phosphate} \]
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:

Some amino acid + α-ketoglutarate $\rightarrow$ 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:

\[
\text{Glutamate} \rightarrow \text{NAD}(P) \xrightarrow{\text{mito}} \text{NAD}(P)H \xrightarrow{\text{mito}} \text{\alpha-ketoglutarate} + \text{ammonia}
\]

Glutamine Synthetase:

\[
\text{ATP} + \text{NH}_3 \xrightarrow{\text{ATP}} \text{ADP} + \text{P}_i \xrightarrow{\text{ADP}} \text{glutamine}
\]
Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{glutamine} \quad \rightarrow \quad \text{glutamate} + \text{NH}_3
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:}
\text{Glutamate} + \text{oxaloacetate} \rightarrow \text{aspartate} + \alpha\text{-keto glutarate}
\]
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

Citrulline

Argininosuccinate
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Arginine $\rightarrow$ Fumarate

$\text{Chemical Structures}$
Urea Cycle Connects to TCA Cycle

- Ornithine → Citrulline
- Argininosuccinate → Fumarate
- Oxaloacetate
- α-Ketoglutarate
- Citrate
- Aspartate

Urea Cycle: Ornithine → Citrulline → Argininosuccinate → Arginine → Urea
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\text{Glutamate} \quad \xrightarrow{\text{NAD}(P)} \quad \text{NAD}(P)H
\]

\[
\text{H} \quad \xrightarrow{\text{(mito)}} \quad \text{NH}_2
\]

\[
(-)\text{O}_2\text{CCH}_2\text{CHC}-\text{CO}_2\text{NH}_2
\]

\[
\text{glutamate} \quad \xrightarrow{\text{NAD}(P)H} \quad \alpha\text{-ketoglutarate} + \text{ammonia}
\]

Glutamine Synthetase:

\[
(-)\text{OOC}\text{-C}\text{-CH}_2\text{COO}^- \quad \text{ATP} + \text{NH}_3 \quad \text{ADP} + P_i
\]

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

\[
\text{glutamate} \quad \xrightarrow{\text{ATP} + \text{NH}_3} \quad \text{glutamine}
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \text{ATP} \\
\text{carbonyl phosphate} & \quad \text{NH}_3 \\
\text{carbamate} & \quad \text{ATP}
\end{align*}
\]

\[
\begin{align*}
\text{ADP} & \quad \text{ADP}
\end{align*}
\]
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
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>OTCO</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{H} & \quad \text{OOC} \quad \text{C} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{NH}_3 \\
(+^2) & \quad \text{H} \quad \text{OOC} \quad \text{C} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{NH}_3 \\
\text{glutamate} & \quad \text{acetyl CoA} \\
& \quad \text{N-acetyl glutamate (NAG)}
\end{align*}

(repeating the figure from page 3 of your handout)

\begin{align*}
\text{HO-C-O} & \quad \text{ATP} \\
\text{bicarbonate} & \quad \text{ADP} \\
\text{HO-C-O} & \quad \text{carbonyl phosphate} \\
\text{HO-C-NH} & \quad \text{Pi} \\
\text{carbamate} & \quad \text{ADP} \\
\text{HO-C-NH} & \quad \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:

\[
\begin{align*}
\text{aspartate} & \quad \text{transamination} \quad \text{oxaloacetate} \\
\text{glutamate} & \quad \text{transamination} \quad \alpha\text{-ketoglutarate} \\
\text{alanine} & \quad \text{transamination} \quad \text{pyruvate}
\end{align*}
\]

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:**

\[
\text{H} \quad \overset{(-)\text{OOC-CH-NH}_3}{\text{NH}_3(+) \quad \text{H}} \quad \text{CO}_2 + \text{NH}_4(+) \quad \text{NAD}^+(+) \quad \text{NADH} \quad \text{THF} \quad \text{N}^6-\text{N}^0-\text{methylene THF}
\]

**Serine Hydroxymethyltransferase:**

\[
\text{(-)OOC-CH-NH}_3 \quad \overset{\text{CH}_2\text{OH}}{\text{(-)OOC-C=CH-NH}_3 +} \quad \text{THF} \quad \text{N}^6-\text{N}^0-\text{methylene THF} \quad \text{Glycine}
\]

**Serine Dehydratase:**

\[
\text{(-)OOC-CH-NH}_3 \quad \overset{\text{CH}_2\text{OH}}{\text{(-)OOC-C=NH}_3(+) \quad \text{H}_2\text{O}} \quad \text{(-)OOC-C=NH}_2(+) \quad \text{H}_2\text{O} \quad \text{(-)OOC-C=O} \quad \text{H}_2\text{O}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine: 

\[
\begin{align*}
\text{+} & \quad \text{NH}_3 \\
\text{CH}_2 & \quad \text{CH} & \quad \text{COO} \\
\text{[Phenylalanine]} \\
\end{align*}
\]

\[
\begin{align*}
\text{Enzyme: Phenylalanine hydroxylase} \\
\end{align*}
\]

Tyrosine: 

\[
\begin{align*}
\text{+} & \quad \text{NH}_3 \\
\text{CH}_2 & \quad \text{CH} & \quad \text{COO} \\
\text{[Tyrosine]} \\
\end{align*}
\]

Phenylpyruvate: 

\[
\begin{align*}
\text{CH}_2 & \quad \text{C} & \quad \text{COO} \\
\text{[Phenylpyruvate]} \\
\end{align*}
\]

Deficiency: 

- Alkaptonuria
- Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

Homogentisate

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

Isoleucine

\[
\text{CH}_3\text{CH}_2\text{CH} - \text{CH} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \quad \text{NH}_3^{(+)}
\]
\[
\alpha - \text{KG}
\]
\[
\text{Glu}
\]
\[
\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{COO}^{(-)}
\]
\[
\text{CH}_3
\]

Leucine

\[
\text{CH}_3\text{CHCH}_2 - \text{CH} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \quad \text{NH}_3^{(+)}
\]
\[
\alpha - \text{KG}
\]
\[
\text{Glu}
\]
\[
\text{CH}_3\text{CHCH}_2 - \text{C} - \text{COO}^{(-)}
\]
\[
\text{CH}_3
\]

Valine

\[
\text{CH}_3\text{CH} - \text{CH} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \quad \text{NH}_3^{(+)}
\]
\[
\alpha - \text{KG}
\]
\[
\text{CH}_3\text{CH} - \text{C} - \text{COO}^{(-)}
\]
\[
\text{CH}_3
\]

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

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

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

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

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

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

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

Tyrosine $\rightarrow$ Dihydroxyphenylalanine (L-DOPA)

Tyrosine hydroxylase

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

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

$\text{NH}_3$ $\text{NAD}^+$ $\text{CO}_2$
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

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