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:

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

\( \alpha\)-keto acid (typically \( \alpha\)-ketoglutarate) \\
\( \alpha\)-keto acid (typically glutamate)

Details of reaction mechanism:

\[ R\text{-C-}\text{coo}^{(-)} + \text{amino acid} + \text{pyridoxal phosphate} \rightarrow \text{H^+} \]

\[ \text{amino acid} \rightarrow \text{pyridoxamine phosphate} \]
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:

\[
\begin{align*}
\text{Glutamate} & \quad \xrightarrow{\text{NAD}(P)} \quad \text{NH}_2 \\
& \quad \xrightarrow{\text{mito}} \quad \text{Glutamate} \\
& \quad \xrightarrow{\text{NAD}(P)H} \quad \text{\(\alpha\)-Ketoglutarate} + \text{NH}_3
\end{align*}
\]

Glutamine Synthetase:

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

Glutamine is hydrolyzed to glutamate and ammonia:

[Chemical reaction diagram]

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

Glutamate donates its amino group to form aspartate:

[Chemical reaction diagram]
Carbamoyl phosphate synthetase I
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[ \text{NH}_2C\text{OP}_4\text{(O)}^{-} \]

Ornithine

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

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

Citrulline

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

\[ \text{Citrulline} \xrightarrow{\text{ATP}} \text{Argininosuccinate} \]

\[ \text{aspartate} \]
Argininosuccinate lyase

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

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

\[ + \text{H}_2\text{O} \rightarrow \text{Urea} \]
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Argininosuccinate
- Arginine
- Aspartate
- Oxaloacetate
- Malate
- Fumarate
- TCA Cycle
- Citrate
- α-Ketoglutarate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \rightarrow \text{carbonyl phosphate} & \rightarrow \text{carbamate} & \rightarrow \text{carbamoyl phosphate} \\
\text{ATP} & \rightarrow & \text{ATP} & \rightarrow & \text{ATP} \\
\text{ADP} & \rightarrow & \text{ADP} & \rightarrow & \text{ADP} \\
\end{align*}
\]
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>Abbreviation</th>
<th>Description</th>
</tr>
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<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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} & \quad \text{carbonyl phosphate} & \quad \xrightarrow{\text{ADP}} & \quad \text{carbamoyl phosphate} \\
\end{align*}
\]
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:

\[
\begin{align*}
\text{Glutamine} & \overset{\text{glutaminase}}{\rightarrow} \text{glutamate} + \text{ammonia} \\
\text{Asparagine} & \overset{\text{asparaginase}}{\rightarrow} \text{aspartate} + \text{ammonia}
\end{align*}
\]

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparagine:

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

\[
\text{Glycine} \quad \xrightarrow{NAD^+ / NADH} \quad \text{CO}_2 + \text{NH}_4^+ \\
\text{THF} \quad \xrightarrow{N^5-N^0- \text{methylene THF}}
\]

**Serine Hydroxymethyltransferase:**

\[
\text{Serine} \quad \xrightarrow{\text{THF} / N^5-N^0- \text{methylene THF}} \quad \text{Glycine}
\]

**Serine Dehydratase:**

\[
\text{Serine} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{Glycine} + \text{NH}_4^+
\]
Methionine Cycle
And Biological Methyl Groups
Deficiency:

Alkaptonuria
“Ochronosis”

Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine → Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O → Tyrosine

Phenylalanine hydroxylase

Phenylketonuria (no phenylalanine hydroxylase)

Phenylketonuria

Phenylpyruvate

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

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

Isoleucine

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

Leucine

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

Valine

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

\[\text{--------- Transamination ---------}\]

\[\text{--------- Branched-chain } \alpha\text{-keto acid dehydrogenase ---------}\]

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

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

(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 → 5-hydroxytryptophan → Serotonin

- Tryptophan hydroxylase
- PLP-dependent decarboxylation
- NAD+ → CO₂
Synthesis of Bioactive Amines

Glutamate

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

Glutamate decarboxylase (PLP-dependent)

\[ \text{NH}_3 \]

\[ \gamma\text{-aminobutyric acid (GABA)} \]

Histidine

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

Histidine decarboxylase (PLP-dependent)

\[ \text{NH}_3 \]

\[ \text{CH}_2\text{CH}_2\text{CH} - \text{NH}_3 \]

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