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:**

\[ \text{R}_1\text{C}^{-}\text{coo}^{(-)} + \text{R}_2\text{C}^{-}\text{coo}^{(-)} \rightarrow \text{R}_1\text{C}^{-}\text{coo}^{(-)} + \text{R}_2\text{C}^{-}\text{coo}^{(-)} \]

\[ \text{NH}_2 \quad \text{NH}_2 \]

α-keto acid (typically α-ketoglutarate)

α-keto acid (typically glutamate)

**Details of reaction mechanism:**

![Chemical reaction mechanism diagram]
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 $\rightarrow$ 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:

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

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:

\[
\text{Glutamine} \xrightarrow{\text{H}^+} \text{Glutamate} + \text{NH}_3
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} + \text{Oxaloacetate} \rightarrow \text{α-Keto glutarate} + \text{Aspartate}
\]
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{NH}_3} \xrightarrow{\text{ATP}}$ carbamate $\xrightarrow{\text{ADP}}$ carbamoyl phosphate
Argininosuccinate synthetase

\[
\text{Aspartate} \quad \xrightarrow{\text{ATP}} \quad \text{Citrulline} \quad \xrightarrow{\text{AMP + PP}_i} \quad \text{Argininosuccinate}
\]
Argininosuccinate lyase

\[
\begin{align*}
\text{Argininosuccinate} & \rightarrow \text{Arginine} \\
\text{Fumarate} & \rightarrow 
\end{align*}
\]
Arginase

Arginine $\xrightarrow{\text{H}_2\text{O}}$ Ornithine

Urea
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

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

**Glutamine Synthetase:**

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

N-acetyl glutamate (NAG)

(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>Enzyme</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

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

(repeating the figure from page 3 of your handout)

\[
\text{bicarbonate} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
\]
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:

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:

\[
\begin{align*}
\text{Glycine} & \quad \text{NAD}^+ \quad \text{NADH} \quad \text{THF} \quad \text{N}^6-\text{N}^\theta \text{ methylene THF} \\
\text{Glycine} & \quad \text{CO}_2 \quad + \quad \text{NH}_4^+ \\
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Serine} & \quad \text{THF} \quad \text{N}^6-\text{N}^\theta \text{ methylene THF} \quad \text{Glycine} \\
\text{Serine} & \quad \text{THF} \quad \text{N}^6-\text{N}^\theta \text{ methylene THF} \\
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{H}_2\text{O} \\
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\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups

Methionine

S-Adenosyl Methionine

S-Adenosyl Homocysteine

Homocysteine

Serine

Cysteine

(remainder of homocysteine degraded for energy)
Deficiency: 

*Alkaptonuria*  

"Ochronosis"

---

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

- **Phenylalanine**
  - $\text{NH}_3$
  - $\text{CH}_2 - \text{CH} - \text{COO}^{(-)}$

  **Tetrahydrobiopterin** $+ \text{O}_2$

  **Dihydrobiopterin** $+ \text{H}_2\text{O}$

  **Enzyme:** Phenylalanine hydroxylase

  **Tyrosine**
  - $\text{NH}_3$
  - $\text{CH}_2 - \text{CH} - \text{COO}^{(-)}$

  **Homogentisate**

- **Phenylketonuria** (no phenylalanine hydroxylase)

- **Phenylpyruvate**

  

- Deficiency: Alkaptonuria  
  "Ochronosis"

- Enzyme: homogentisate dioxygenase

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

Isoleucine

Leucine

Valine

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

--------- 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}} \text{Dihydroxyphenylalanine (L-DOPA)}

Dopamine \xrightarrow{} \text{Norepinephrine} \xrightarrow{} \text{Epinephrine}
Synthesis of Bioactive Amines

Tryptophan \[\xrightarrow{\text{ TRYPTOPHAN HYDROXYLASE }}\] 5-Hydroxytryptophan

PLP-dependent decarboxylation

CO₂

Serotonin
Synthesis of Bioactive Amines

Glutamate (PLP-dependent) → γ-aminobutyric acid (GABA)

Histidine (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