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:

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

\[ \rightarrow R_2\text{C} - \text{coo}^\text{(-)} + \text{NH}_2 \text{ amino acid (typically glutamate)} \]

Details of reaction mechanism:

pyridoxal phosphate
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 + $\alpha$-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):

\[
\text{Glutamate + oxaloacetate} \rightarrow \alpha\text{-ketoglutarate + aspartate}
\]
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

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

**Glutamine Synthetase:**

\[
\text{Glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{Glutamine} + \text{ADP} + \text{Pi}
\]
Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

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Glutamine is hydrolyzed to glutamate and ammonia:

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

Glutamate donates its amino group to form aspartate:

Glutamate-aspartate aminotransferase:

\[
\begin{align*}
\text{H} & \quad \text{O}_2\text{C} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{CO}_2^- \\
\text{H} & \quad \text{O}_2\text{C} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{CO}_2^- \\
\text{glutamate} & \quad \text{oxaloacetate} \\
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{O}_2\text{C} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{CO}_2^- \\
\text{H} & \quad \text{O}_2\text{C} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{CO}_2^- \\
\text{α-keto glutarate} & \quad \text{aspartate}
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate + ATP → carbonyl phosphate + ADP

carbonyl phosphate + NH₃ → carbamate + Pi

carbamate + ATP → carbamoyl phosphate + ADP
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

\[
\text{(-)OCC-CH}_2\text{CH}_2\text{CH}_2\text{NH-C}=\text{NH}_2 \rightarrow \text{(-)OOC-CH}_2\text{CH}_2\text{NH-C}=\text{NH}_2^+ + \text{AMP + PP}_i
\]

aspartate

Citrulline

Argininosuccinate
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Arginine + Fumarate
Arginase

Arginine $\rightarrow$ Ornithine

H$_2$O

Urea

$(-)\text{OOCC-CH}_2\text{CH}_2\text{NH}_3^+$
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Arginine
- Argininosuccinate
- Aspartate
- Oxaloacetate
- Malate
- Fumarate
- α-Ketoglutarate
- Citrate

The Urea Cycle and the TCA Cycle are connected through the exchange of metabolites.
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

bicarbonate → carbonyl phosphate → carbamate → carbamoyl phosphate
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

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

(repeating the figure from page 3 of your handout)

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

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

\[
\text{Asparagine} \rightarrow \text{aspartate} + \text{ammonia}
\]

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:

\[
\text{Glycine Synthase:} \quad (-)\text{OOC-C-NH}_3^+ \\
\text{Glycine} \quad \text{THF} \quad \text{N}^5-\text{N}^6-\text{methylene THF} \quad \text{CO}_2 + \text{NH}_4^+ \\
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine Hydroxymethyltransferase:} \quad (-)\text{OOC-CH-NH}_3^+ \\
\text{Serine} \quad \text{THF} \quad \text{N}^5-\text{N}^6-\text{methylene THF} \quad \text{Glycine} \\
\]

Serine Dehydratase:

\[
\text{Serine Dehydratase:} \quad (-)\text{OOC-CH-NH}_3^+ \\
\text{Serine} \quad \text{H}_2\text{O} \quad (-)\text{OOC-C-NH}_3^+ \\
\quad \text{H}_2\text{O} \quad (-)\text{OOC-C=NH}_2^+ \\
\quad \text{H}_2\text{O} \quad (-)\text{OOC-CH}_3 \\
\]

\[
\]

\[
\]

\[
\]

\[
\]

\[
\]

\[
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine → Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O

Enzyme: Phenylalanine hydroxylase

→ Tyrosine

→ Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

(You don’t need to know the rest)

Phenylalanine

CH₂

NH₃

+ (-)

O

CH₂

COO

(-)

Phenylpyruvate

(+)
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 $\xrightarrow{\text{Tyrosine hydroxylase}}$ Dihydroxyphenylalanine (L-DOPA)

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

Tryptophan $\rightarrow$ NAD$^+$

\[ 	ext{Tryptophan hydroxylase} \rightarrow 5\text{-hydroxytryptophan} \]

\[ \text{PLP-dependent decarboxylation} \rightarrow \text{Serotonin} \]
Synthesis of Bioactive Amines

Glutamate (PLP-dependent)

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

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

\[ \text{Glutamate decarboxylase} \]

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

Histidine (PLP-dependent)

\[ \text{N} \]

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

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

\[ \text{Histidine decarboxylase} \]

\[ \text{Histamine} \]
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