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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:

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

\(\alpha\)-keto acid (typically alpha-ketoglutarate) + amino acid

Details of reaction mechanism:

\[
\text{amino acid} + \text{pyridoxal phosphate} \rightarrow \text{H}^+ \text{pyridoxamine phosphate} + \text{H}_2\text{O}
\]
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):

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

**Glutamate Dehydrogenase:**

\[
\begin{align*}
\text{H} & & \text{NAD(P)} \\
\text{NH}_2 & & \text{(mito)} \\
\text{glutamate} & & \text{NAD(P)H} \\
& & \alpha\text{-ketoglutarate} + \text{ammonia}
\end{align*}
\]

**Glutamine Synthetase:**

\[
\begin{align*}
\text{ATP} + \text{NH}_3 & & \text{ADP} + P_i \\
\text{glutamate} & & \text{glutamine}
\end{align*}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{glutamine} & \quad \xrightarrow{\text{hydrolysis}} \quad \text{glutamate} + \text{NH}_3 \\
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} + \text{oxaloacetate} & \quad \xrightarrow{\text{reaction}} \quad \text{α-keto glutarate} + \text{aspartate} \\
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{NH}_3}$. carbamate $\xrightarrow{\text{ATP}}$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[ \text{Ornithine} \rightarrow \text{Citrulline} \]

\[ \text{Ornithine} \rightarrow \text{Citrulline} \]
Argininosuccinate synthetase

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

Argininosuccinate → Arginine

\[-\text{O}_{2}\text{C}\text{CH}_{2}\text{CH}_{2}\text{NH}-\text{C}=\text{NH}_{2}^{(+)}\] → \[-\text{O}_{2}\text{C}\text{CH}_{2}\text{CH}_{2}\text{NH}-\text{C}=\text{NH}_{2}\]

Fumarate
Arginase

\[
\text{Arginine} \xrightarrow{\text{H}_2\text{O}} \text{Ornithine} \xrightarrow{\text{Urea}}
\]

\[
\text{(-)OO} - \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} - \text{NH}_2 \\
\text{NH}_2 \\
\text{NH}_2 \\
\text{(+)}^3 \\
\text{H} \\
\text{(+)}^3
\]

\[
\text{(-)OO} - \text{C} - \text{CH}_2\text{CH}_2\text{NH}_3^+ \\
\text{NH}_2 \\
\text{(+)}^3
\]

\[
\text{NH}_2 \\
\text{C} - \text{NH}_2 \\
\text{H}_2\text{O}
\]
Urea Cycle Connects to TCA Cycle

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

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

\[
\text{(-)} \quad \text{OOC} \quad \text{C} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{OH} \\
\text{(-)} \quad \text{OOC} \quad \text{C} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{OH}
\]

\[
\text{glutamate} \quad + \quad \text{acetyl CoA} \quad \xrightarrow{\text{N-acetyl glutamate synthetase}} \quad \text{N-acetyl glutamate (NAG)}
\]

(repeating the figure from page 3 of your handout)

\[
\xrightarrow{\text{ATP}} \quad \xrightarrow{\text{ADP}} \quad \text{bicarbonate} \quad \xrightarrow{\text{carbonyl phosphate}} \quad \xrightarrow{\text{ADP}} \quad \text{carbamyl phosphate}
\]
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>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 → carbamoyl phosphate + ADP

 carbamoyl phosphate + ATP → carbamate + ADP

 carbamate + 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:

\[
\begin{align*}
\text{NH}_3 & \quad \text{aspartate} \\
\text{H} & \quad \text{glutamate} \\
\text{CH}_3 & \quad \text{alanine} \\
\end{align*}
\]

We also already know how to degrade Glutamine:

\[
\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}
\]

…and by analogy, how to degrade Asparagaine:

\[
\text{Asparagaine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
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 \(\alpha\)-ketoglutarate.
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

\[
\begin{array}{c}
\text{Glycine Synthase:} \\
\text{Glycine} \\
\text{(\(-\text{OOC-C-\text{NH}_3}\))} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Glycine Synthase:} \\
\text{Glycine} \\
\text{(\(-\text{OOC-C-\text{NH}_3}\))} \\
\end{array}
\]

Serine Hydroxymethyltransferase:

\[
\begin{array}{c}
\text{Serine Hydroxymethyltransferase:} \\
\text{Serine} \\
\text{(\(-\text{OOC-C-\text{CH-\text{NH}_3}\))} \\
\text{CH}_2\text{OH} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Serine Hydroxymethyltransferase:} \\
\text{Serine} \\
\text{(\(-\text{OOC-C-\text{CH-\text{NH}_3}\))} \\
\text{CH}_2\text{OH} \\
\end{array}
\]

Serine Dehydratase:

\[
\begin{array}{c}
\text{Serine Dehydratase:} \\
\text{Serine} \\
\text{(\(-\text{OOC-C-\text{NH}_3}\))} \\
\text{CH}_2\text{OH} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Serine Dehydratase:} \\
\text{Serine} \\
\text{(\(-\text{OOC-C-\text{NH}_3}\))} \\
\text{CH}_2\text{OH} \\
\end{array}
\]
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

Enzyme: Phenylalanine hydroxylase

Tyrosine

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

Phenylpyruvate

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

Isoleucine  
\[
\begin{array}{c}
\text{CH}_3\text{CH}_2\text{CH} - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \text{NH}_3^{(+)}
\end{array}
\]

\[\alpha-KG\]

\[\text{Glu}\]

\[
\begin{array}{c}
\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{COO}^{(-)} \\
\text{CH}_3
\end{array}
\]

Leucine  
\[
\begin{array}{c}
\text{CH}_3\text{CHCH}_2 - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \text{NH}_3^{(+)}
\end{array}
\]

\[\alpha-KG\]

\[\text{Glu}\]

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

Valine  
\[
\begin{array}{c}
\text{CH}_3\text{CH} - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \text{NH}_3^{(+)}
\end{array}
\]

\[\alpha-KG\]

\[\text{Glu}\]

\[
\begin{array}{c}
\text{CH}_3\text{CH} - \text{C} - \text{COO}^{(-)} \\
\text{CH}_3
\end{array}
\]

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

\[
\begin{array}{c}
\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{S-CoA} \\
\text{CH}_3
\end{array}
\]

\[
\begin{array}{c}
\text{CH}_3\text{CHCH}_2 - \text{C} - \text{S-CoA} \\
\text{CH}_3
\end{array}
\]

\[
\begin{array}{c}
\text{CH}_3\text{CH} - \text{C} - \text{S-CoA} \\
\text{CH}_3
\end{array}
\]

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

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

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

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

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

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

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

Tyrosine → Tyrosine hydroxylase → Dihydroxyphenylalanine (L-DOPA)

Dopamine → Norepinephrine → Epinephrine
Synthesis of Bioactive Amines

Tryptophan → 5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase

PLP-dependent decarboxylation

NAD⁺
Synthesis of Bioactive Amines

\[ \text{Glutamate} \xrightarrow{\text{Glutamate decarboxylase} \ (\text{PLP-dependent})} \gamma\text{-aminobutyric acid (GABA)}} \]

\[ \text{Histidine} \xrightarrow{\text{Histidine decarboxylase} \ (\text{PLP-dependent})} \text{Histamine} \]
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