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)

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

Details of reaction mechanism:

amino acid

\[
\text{R}_1\text{C} - \text{coo}(-) + \text{H} + \text{O} \rightarrow \text{R}_1\text{C} - \text{coo}(-) + \text{H}^+ \rightarrow \text{R}_1\text{N} - \text{COO}(-) + \text{CH}_3 \rightarrow \text{R}_1\text{C} - \text{COO}(-) + \text{H}_2\text{O}
\]

\text{pyridoxal phosphate}

\[
\text{R}_1\text{C} - \text{COO}(-) + \text{NH}_2 \rightarrow \text{R}_1\text{N} - \text{COO}(-) + \text{N}_2 \rightarrow \text{R}_1\text{C} - \text{COO}(-) + \text{NH}_2 \rightarrow \text{R}_1\text{C} - \text{COO}(-) + \text{N}_2
\]

\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:

\[
\text{Some amino acid} + \alpha-\text{ketoglutarate} \rightarrow \text{some alpha keto acid} + \text{Glutamate}
\]
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:**

\[
\begin{align*}
\text{glutamate} & \xrightarrow{\text{NAD}(P)^{+}} \text{NAD}(P)H \\
& \xrightarrow{\text{mito}} \text{α-ketoglutarate} + \text{NH}_3
\end{align*}
\]

**Glutamine Synthetase:**

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

Glutamine is hydrolyzed to glutamate and ammonia:

Glutamate donates its amino group to form aspartate:

Glutamate-aspartate aminotransferase:
Carbamoyl phosphate synthetase I

bicarbonate $\rightarrow$ carbonyl phosphate $\rightarrow$ carbamate $\rightarrow$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

\[ \text{Argininosuccinate} \rightarrow \text{Arginine} + \text{Fumarate} \]
Arginase

Arginine $\rightarrow$ Urea $\rightarrow$ Ornithine

$\text{(-)}\text{OOO}-\text{C} \equiv \text{CH}_2\text{CH}_2\text{CH}_2\text{NH}^-\text{C} \equiv \text{NH}_2$ $\rightarrow$ Urea $\rightarrow$ Ornithine

Arginine

Ornithine

$\text{H}_2\text{O}$
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\text{glutamate} \xrightarrow{\text{NAD}(P)} \text{α-ketoglutarate} + \text{NH}_3
\]

**Glutamine Synthetase:**

\[
\text{ATP} + \text{NH}_3 \rightarrow \text{ADP} + P_i + \text{glutamine}
\]
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)

bicarbonate $\rightarrow$ carbonyl phosphate $\rightarrow$ carbamate $\rightarrow$ carbamoyl phosphate

ATP

ADP

NH$_3$

P$_i$
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.

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>CPSD</strong></td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
</tr>
<tr>
<td><strong>OTCD</strong></td>
<td>Elevated CP causes synthesis of Orotate</td>
</tr>
<tr>
<td><strong>ASD</strong></td>
<td>Elevated citrulline</td>
</tr>
<tr>
<td><strong>ALD</strong></td>
<td>Elevated argininosuccinate</td>
</tr>
<tr>
<td><strong>AD</strong></td>
<td>Elevated arginine</td>
</tr>
</tbody>
</table>
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)
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:

\[
\text{H} \\
(-\text{O}_2\text{C}\text{CH}_2\text{C} \text{–} \text{CO}_2(-) \\
\text{NH}_3 \quad (+)
\] aspartate
\[
\text{H} \\
(-\text{O}_2\text{C}\text{CH}_2\text{CH}_2\text{C} \text{–} \text{CO}_2(-) \\
\text{NH}_3 \quad (+)
\] glutamate
\[
\text{H} \\
\text{CH}_3 \text{–} \text{CO}_2(-) \\
\text{NH}_3 \quad (+)
\] alanine

\[
\text{H} \\
(-\text{O}_2\text{C}\text{CH}_2\text{C} \text{–} \text{CO}_2(-) \\
\] oxaloacetate
\[
(-\text{O}_2\text{C}\text{CH}_2\text{CH}_2\text{C} \text{–} \text{CO}_2(-) \\
\] α-ketoglutarate
\[
\text{CH}_3 \text{–} \text{CO}_2(-) \\
\] pyruvate

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 $\alpha$-ketoglutarate.
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

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

\[
\begin{align*}
\text{Glycine} & \quad \text{CO}_2 \quad + \quad \text{NH}_4^+ \\
\end{align*}
\]

Serine Hydroxymethyltransferase:

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

\[
\begin{align*}
\text{Serine} & \quad \text{Glycine} \\
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{Serine} & \quad \text{NH}_4^+ \\
\end{align*}
\]
Methionine Cycle And Biological Methyl Groups

Methionine

S-Adenosyl Methionine

Homocysteine

S-Adenosyl Homocysteine

Serine

Cysteine

(remainder of homocysteine degraded for energy)
Phenylalanine and Tyrosine

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

Phenylalanine → Phenylalanine hydroxylase → Tyrosine

Enzyme: Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O

Phenylpyruvate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

Homogentisate

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

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

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

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

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

\[
\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{COO}^{(-)} \\
\text{CH}_3
\]

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

\[
\text{CH}_3\text{CH} - \text{C} - \text{COO}^{(-)} \\
\text{CH}_3
\]

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

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

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

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

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

\[
\text{NADH} + \text{CO}_2 \\
\text{NAD}^+ \text{ CoASH}
\]

\[
\text{NADH} + \text{CO}_2 \\
\text{NAD}^+ \text{ CoASH}
\]

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

Tyrosine $\rightarrow$ Dihydroxyphenylalanine (L-DOPA) via Tyrosine hydroxylase

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

Tryptophan $\rightarrow$ NAD$^+$

Tryptophan $\rightarrow$ 5-hydroxytryptophan

5-hydroxytryptophan $\rightarrow$ Serotonin

PLP-dependent decarboxylation

Tryptophan hydroxylase
Synthesis of Bioactive Amines

Glutamate

\[
\text{Glutamate decarboxylase (PLP-dependent)}
\]

\[\text{(-)}\ \text{COO} - \text{CH}_2 \text{CH}_2 \text{CH} - \text{COO} (\text{(+)}\ \text{NH}_3) \]

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

Histidine

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

\[
\begin{align*}
\text{Histidine} & \rightarrow \text{Histamine} \\
\text{(-)}\ \text{CH}_2 \text{CH} - \text{COO} (\text{(+)}\ \text{NH}_3) & \rightarrow \text{(-)}\ \text{CH}_2 \text{CH}_2 \text{CH} - \text{NH}_3 (\text{(+)}
\end{align*}
\]
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