M1 - Renal, Fall 2007

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Viewer discretion advised: Material may contain medical images that may be disturbing to some viewers.
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:

\[
R_1-C-\text{coo}^{(-)} + R_2-C-\text{coo}^{(-)} \rightarrow R_1-C-\text{coo}^{(-)} + R_2-C-\text{coo}^{(-)}
\]
\[
\text{NH}_2 \quad \text{NH}_2
\]
\[\alpha\text{-keto acid} \quad (\text{typically alpha-ketoglutarate}) \quad \alpha\text{-keto acid} \quad \text{(typically glutamate)}
\]

Details of reaction mechanism:

\[
\begin{align*}
\text{amino acid} & \\
\text{H} & \\
R-C-\text{coo}^{(-)} & \\
\text{NH}_2 & \\
\text{O} & \\
\text{H}_2\text{O} & \\
\text{pyridoxal phosphate} & \\
\end{align*}
\]
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:

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

Glutamate + oxaloacetate $\rightarrow$ $\alpha$-ketoglutarate + aspartate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[
\text{glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamine} + \text{ADP} + \text{Pi}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

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

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

Glutamate donates its amino group to form aspartate:

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

bicarbonate $\xrightarrow{ATP} \text{carbonyl phosphate} \xrightarrow{NH_3} \text{carbamate} \xrightarrow{ATP} \text{carbamoyl phosphate}$
Ornithine Transcarbamoylase

Carbamoyl phosphate

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

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

Ornithine

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

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

Citrulline
Argininosuccinate synthetase

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

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

\[ \text{Arginine} \xrightarrow{H_2O} \text{Ornithine} \]
Urea Cycle Connects to TCA Cycle

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

TCA Cycle

Red and blue pathways indicate the connection between the Urea and TCA cycles.
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

![Chemical reaction diagram]

(repeating the figure from page 3 of your handout)

![Chemical reaction diagram]
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

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

(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:

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

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade 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:**

\[
\begin{array}{c}
\text{Glycine} \\
\text{NAD}^{+} \\
\text{THF} \quad \text{N}^6-\text{N}^2- \text{methylene THF} \\
\text{CO}_2 \quad + \quad \text{NH}_4^{+}
\end{array}
\]

**Serine Hydroxymethyltransferase:**

\[
\begin{array}{c}
\text{Serine} \\
\text{THF} \quad \text{N}^6-\text{N}^2- \text{methylene THF} \\
\text{Glycine}
\end{array}
\]

**Serine Dehydratase:**

\[
\begin{array}{c}
\text{Serine} \\
\text{H}_2\text{O} \\
\text{H}_2\text{O} \\
\text{NH}_4^{+} \\
\text{CH}_3
\end{array}
\]
Methionine Cycle And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine \( \rightarrow \) Dihydrobiopterin + \( \text{O}_2 \) \( \rightarrow \) Tetrahydrobiopterin + \( \text{H}_2\text{O} \)

Enzyme: Phenylalanine hydroxylase

\( \rightarrow \) Tyrosine

\( \rightarrow \) Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

Phenylpyruvate

Phenylketonuria (no phenylalanine hydroxylase)
Branched Chain Amino Acids

Isoleucine  Leucine  Valine

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} - & \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{NH}_3^{(+)} \\
\text{α-KG} & \\
\text{Glu} & \\
\text{CH}_3\text{CH}_2\text{CH} - & \text{C} - \text{COO}^{(-)} \\
\text{CH}_3 & \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 & \quad 2
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 - & \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{NH}_3^{(+)} \\
\text{α-KG} & \\
\text{Glu} & \\
\text{CH}_3\text{CHCH}_2 - & \text{C} - \text{COO}^{(-)} \\
\text{CH}_3 & \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 & \\
\text{CH}_3\text{CH} - & \text{C} - \text{COO}^{(-)} \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 &
\end{align*}
\]

--- Branched-chain α-keto acid dehydrogenase ---

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} - & \text{C} - \text{S-CoA} \\
\text{CH}_3 & \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 & \quad 2
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 - & \text{C} - \text{S-CoA} \\
\text{CH}_3 & \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 & \\
\text{CH}_3\text{CH} - & \text{C} - \text{S-CoA} \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 &
\end{align*}
\]

(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 $\xrightarrow{\text{Monoamine oxidase}}$ Norepinephrine $\xrightarrow{\text{Epinephrine synthase}}$ 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 (PLP-dependent)}}\;\text{\(\gamma\)-aminobutyric acid (GABA)}
\]

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