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:

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

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

\[\text{NH}_2\]

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

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

\[\text{NH}_2\]

Details of reaction mechanism:

\[\text{amino acid} \rightarrow \text{pyridoxal phosphate} \rightarrow \text{amino acid} \rightarrow \text{pyridoxamine phosphate}\]
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):

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

Glutamate Dehydrogenase:

\[
\begin{align*}
\text{Glutamate:} & \quad \overset{\text{(-)}\text{O}_2\text{C} \text{CH}_2\text{CH}_2\text{C} - \text{CO}_2\text{(-)} \text{NH}_2}{\text{mito}} & \rightarrow & \text{NAD(P)} & \rightarrow & \text{NAD(P)H} & \rightarrow & \overset{\text{(-)}\text{O}_2\text{C} \text{CH}_2\text{CH}_2\text{C} - \text{CO}_2\text{(-)} \text{NH}_3}{\text{mito}} \\
\text{glutamate} & & & & & & & \alpha\text{-ketoglutarate} & \text{ammonia}
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{Glutamine:} & \quad \overset{\text{(-)}\text{OOC} \text{CH}_2\text{CH}_2\text{COO}(-)}{\text{(+)}\text{H}} & \rightarrow & \text{ATP} + \text{NH}_3 & \rightarrow & \text{ADP} + \text{P}_i & \rightarrow & \overset{\text{(-)}\text{OOC} \text{CH}_2\text{CH}_2\text{COO}(-)}{\text{(+)}\text{NH}_3} \\
\text{glutamate} & & & & & & & \text{glutamine}
\end{align*}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{H}_2\text{O} + \text{NH}_3 + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{NH}_3 + \text{H}^+ 
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} + \text{oxaloacetate} \rightarrow \text{aspartate} + \text{o-keto glutarate} 
\]
Carbamoyl phosphate synthetase I

bicarbonate → ATP → carbonyl phosphate → NH₃ → carbamate → ATP → carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline $\rightarrow$ Argininosuccinate

aspartate + ATP $\rightarrow$ AMP + PP$_i$
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Arginine $\rightarrow$ Argininosuccinate

Fumarate
Arginase

\[
\overset{(-)\text{HOC}}{\text{C}} - \overset{+}{\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}-\overset{+}{\text{NH}_2}}{\text{C-NH}_2} + \overset{-}{\text{H}_2\text{O}} \rightarrow \overset{(-)\text{HOC}}{\text{C}} - \overset{+}{\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_3^{+}}
\]

Arginine $\rightarrow$ Ornithine

Urea
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \rightarrow \quad \text{carbonyl phosphate} \\
\text{carbamoyl phosphate}
\end{align*}
\]
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>Condition</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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
\end{align*}
\]
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:

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparagine:

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
Amino Acids are categorized as ‘Glucogenic’ or ‘ketogenic’ or both.

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:

\[
\text{Glycine} \rightarrow \text{THF} \oplus \text{N}^5\text{N}^\text{0}\text{-methylene THF} \rightarrow \text{NAD}^+ \rightarrow \text{NADH} \rightarrow \text{CO}_2 \oplus \text{NH}_4^+
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine} \rightarrow \text{THF} \oplus \text{N}^5\text{N}^\text{0}\text{-methylene THF} \rightarrow \text{Glycine}
\]

Serine Dehydratase:

\[
\text{Serine} \rightarrow \text{H}_2\text{O} \rightarrow \text{Serine} \rightarrow \text{H}_2\text{O} \rightarrow \text{NH}_4^+
\]

\[
\text{Serine} \rightarrow \text{H}_2\text{O} \rightarrow \text{Serine} \rightarrow \text{H}_2\text{O} \rightarrow \text{NH}_4^+
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine $\rightarrow$ Tyrosine

\[ \text{Enzyme: Phenylalanine hydroxylase} \]

\[ \text{Homogentisate} \]

Deficiency: Alkaptonuria “Ochronosis”

\[ \text{Enzyme: homogentisate dioxygenase} \]

Phenylpyruvate

\[ \text{Phenylketonuria} \ (\text{no phenylalanine hydroxylase}) \]
Branched Chain Amino Acids

Isoleucine  Leucine  Valine

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

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

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

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

(continues on to degradation path similar to \( \beta \)-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 → 5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase

PLP-dependent decarboxylation
Synthesis of Bioactive Amines

Glutamate

COO\(^{(-)}\)CH\(_2\)CH\(_2\)CH\(\_\)COO\(^{(-)}\)

Glutamate decarboxylase (PLP-dependent)

\(\gamma\)-aminobutyric acid (GABA)

\(\gamma\)-aminobutyric acid

Histidine

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

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