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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:

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

α-keto acid (typically α-ketoglutarate)

Details of reaction mechanism:

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

Some amino acid + α-ketoglutarate → 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):

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

**Glutamate Dehydrogenase:**

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

**Glutamine Synthetase:**

\[
\text{H} \quad \xrightarrow{\text{ATP} + \text{NH}_3} \quad \text{H} \\
\text{glutamate} \quad \xrightarrow{\text{ADP} + \text{P}_i} \quad \text{glutamine}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{Glutamine} \rightarrow \text{Glutamate} + \text{NH}_3
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:}
\]
Carbamoyl phosphate synthetase I

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

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

\[
\text{aspartate} \quad \xrightarrow{\text{ATP}} \quad \text{argininosuccinate} \quad \xrightarrow{\text{AMP + PP}_i} \quad \text{Citrulline}
\]
Argininosuccinate lyase

\[-\text{H}((-)\text{O}_2\text{CCH}_2\text{C}(-\text{CO}_2)^{-}\text{H})\text{NH}_2\text{NH}_3^{(+)}\]

\[-\text{CH}_2\text{CH}_2\text{NH}-\text{c}=\text{NH}_2^{(+)}\]

Argininosuccinate

\[-\text{H}((-)\text{O}_2\text{C}-\text{c}=\text{c}=\text{CO}_2)^{-}\text{H}\]

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

Fumarate

\[-\text{H}((-)\text{O}_2\text{CCH}_2\text{CH}_2\text{NH}-\text{c}=-\text{NH}_2\text{NH}_2^{(+)}\]

Arginine
Arginase

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

Arginine → Urea → Ornithine
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Citrulline
- Arginine
- Argininosuccinate

TCA Cycle:
- Oxaloacetate
- Malate
- Fumarate
- α-Ketoglutarate
- Citrate

Urea:
- H \((\text{C}\text{H}_2\text{C=CO}_2\text{H})\)
- Aspartate

H \((\text{C}_\text{H}_2\text{C=CO}_2\text{H})\)
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\text{Glutamate} \xrightarrow{\text{NAD}(P)} \text{NH}_2 \xrightarrow{\text{mito}} \text{NAD}(P)H \xrightarrow{\alpha\text{-ketoglutarate}} \text{ammonia}
\]

Glutamine Synthetase:

\[
\text{Glutamine Synthetase:} \quad \text{Glutamate} \xrightarrow{\text{ATP}+\text{NH}_3} \text{NH}_3 \xrightarrow{\text{ADP}+\text{P}_i} \text{Glutamine}
\]
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
Complicating the picture: Other tissues may be involved

Muscle:
- Amino acids: Transamination, Deamination
- NH₄⁺
- Alamine
- Glutamate
- Glutamine

Intestine:
- Glutamine
- Alanine
- NH₄⁺
- Citrulline

Kidney:
- Glutamine
- NH₃
- NH₄⁺
- Arginine
- Citrulline

Liver:
- Glutamine
- Alamine
- Glu
- Aspartate
- NH₄⁺
- Arginine
- Urea
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

Glutamate + Acetyl CoA → N-acetyl glutamate

(repeating the figure from page 3 of your handout)

bicarbonate → carbonyl phosphate → carbamate → 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:

\[
\begin{align*}
&(-\text{O}_2\text{CCH}_2\text{C} - \text{CO}_2^{(-}) \\
&\text{NH}_3 \\
&\text{aspartate} \\
\nonumber
\end{align*}
\]

\[
\begin{align*}
&(-\text{O}_2\text{CCH}_2\text{H}_2\text{C} - \text{CO}_2^{(-}) \\
&\text{NH}_3 \\
&\text{glutamate} \\
\nonumber
\end{align*}
\]

\[
\begin{align*}
&(-\text{O}_2\text{CCH}_2\text{H}_2\text{C} - \text{CO}_2^{(-}) \\
&\text{NH}_3 \\
&\text{alanine} \\
\nonumber
\end{align*}
\]

\[
\begin{align*}
&(-\text{O}_2\text{CCH}_2\text{C} - \text{CO}_2^{(-}) \\
&\text{Oxaloacetate} \\
\nonumber
\end{align*}
\]

\[
\begin{align*}
&(-\text{O}_2\text{CCH}_2\text{H}_2\text{C} - \text{CO}_2^{(-}) \\
&\alpha\text{-ketoglutarate} \\
\nonumber
\end{align*}
\]

\[
\begin{align*}
&(-\text{O}_2\text{CCH}_2\text{H}_2\text{C} - \text{CO}_2^{(-}) \\
&\text{Pyruvate} \\
\nonumber
\end{align*}
\]

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparagine:

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate + ammonia}
\]
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:

\[
\begin{align*}
\text{Glycine} & \quad \text{NAD}^+ \quad \text{NADH} \\
\text{THF} & \quad \text{N}^5 - \text{N}^\text{H}_2 \text{methylene THF} \\
\text{CO}_2 & \quad + \quad \text{NH}_4^+ 
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Serine} & \quad \text{THF} \quad \text{N}^5 - \text{N}^\text{H}_2 \text{methylene THF} \\
\text{Glycine} & \quad \text{NAD}^+ \quad \text{NADH} \\
\text{H}_2\text{O} & \quad \text{NH}_4^+ 
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{H}_2\text{O} & \quad \text{NH}_4^+ \\
\text{H}_2\text{O} & \quad \text{NH}_4^+ \\
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine: $\text{NH}_3$ \hspace{1cm} Dihydrobiopterin + H$_2$O

\[ (+) \]

Homogentisate: Enzyme: homogentisate dioxygenase

Deficiency: Alkaptonuria “Ochronosis”

Phenylpyruvate: Phenylketonuria (no phenylalanine hydroxylase)

Tyrosine: $\text{NH}_3$
Branched Chain Amino Acids

Isoleucine

\[
\text{CH}_3\text{CH}_2\text{CH}(-)\text{CH}(-)\text{COOH}
\]

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

\[
\alpha-\text{KG}
\]

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

\[
\text{CH}_3
\]

\[
\text{(continues on to degradation path similar to } \beta\text{-oxidation of fatty acids)}
\]

Leucine

\[
\text{CH}_3\text{CHCH}_2\text{CH}(-)\text{COOH}
\]

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

\[
\alpha-\text{KG}
\]

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

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

Valine

\[
\text{CH}_3\text{CH}(-)\text{COOH}
\]

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

\[
\alpha-\text{KG}
\]

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

\[
\text{CH}_3
\]
Synthesis of Bioactive Amines

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

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

Tryptophan to 5-hydroxytryptophan:

- Tryptophan hydroxylase
- PLP-dependent decarboxylation
- CO₂

NAD⁺ to Serotonin:

- NH₃
Synthesis of Bioactive Amines

**Glutamate**

\[\text{COO}^\text{(-)} - \text{CH}_2\text{CH}_2\text{CH} - \text{COO}^\text{(-)} \quad \xrightarrow{\text{Glutamate decarboxylase (PLP-dependent)}} \quad \text{COO}^\text{(-)} - \text{CH}_2\text{CH}_2\text{CH}_2\text{--NH}_3^\text{(+)}}\]

\[\text{Glutamate} \quad \underrightarrow{\text{Ammonia}} \quad \gamma\text{-aminobutyric acid (GABA)}\]

**Histidine**

\[\text{H}_{\text{N}}\text{-CH}_2\text{-CH} - \text{COO}^\text{(-)} \quad \xrightarrow{\text{Histidine decarboxylase (PLP-dependent)}} \quad \text{H}_{\text{N}}\text{-CH}_2\text{-CH}_2\text{--NH}_3^\text{(+)}}\]

\[\text{Histidine} \quad \underrightarrow{\text{Ammonia}} \quad \text{Histamine}\]
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