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M1 - Renal, Fall 2007

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<http://hdl.handle.net/2027.42/64946>
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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
Amino Acid metabolism

- Amino acids
- Glu, Gln, Asp, NH₃
- Urea

Folate metabolism

- Methylene THF
- Met Cycle

TCA Cycle

oxaloacetate

- fumarate

Nucleic Acid metabolism

- Purines
- DNA
- RNA
- Pyrimidines
- Uric Acid
- (energy)
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:

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

\( R_1 \) and \( R_2 \) are amino acids, typically \( \alpha \)-keto acid (glutamate).

Details of reaction mechanism:

\[ \text{pyridoxal phosphate} + \text{amino acid} \rightarrow \text{cofactor} \rightarrow \text{product} \]
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 + $\alpha$-ketoglutarate $\rightarrow$ 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 → α-ketoglutarate + aspartate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\text{glutamate} \rightarrow \text{NAD(P)} \rightarrow \text{α-ketoglutarate} + \text{ammonia}
\]

Glutamine Synthetase:

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

Glutamine is hydrolyzed to glutamate and ammonia:

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

Glutamate donates its amino group to form aspartate:

Glutamate-aspartate aminotransferase:
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{ATP} \text{carbonyl phosphate} \xrightarrow{NH_3, Pi} \text{carbamate} \xrightarrow{ATP} \text{carbamoyl phosphate}$
Ornithine Transcarbamoylase
Argininosuccinate lyase
Arginase

- Arginine $\rightarrow$ Urea $\rightarrow$ Ornithine

\[ (-)^{\text{OOOC}} \text{C} \bigg| \text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \bigg| \text{C} \text{-NH}_3 \]

\[ \text{H} \bigg\Downarrow \text{NH}_2 \bigg| \text{CH}_2\text{NH}_2 \]

\[ \text{H}_2\text{O} \bigg\Downarrow \text{NH}_2 \bigg| \text{C} \bigg| \text{O} \bigg| \text{NH}_2 \bigg| \text{C} \text{-NH}_3 \]

\[ \text{H} \bigg\Downarrow \text{NH}_3 \bigg| \text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_3 \]
The image depicts the urea cycle, a metabolic pathway in which the liver converts ammonia to urea. The cycle involves the following reactions:

1. Carbamoyl phosphate is synthesized from 2 ATP + HCO₃⁻ + NH₃.
2. Carbamoyl phosphate reacts with ornithine to form citrulline.
3. Citrulline is converted to arginine in the cytoplasm.
4. Arginine is then converted to argininosuccinate in the mitochondrion.
5. Argininosuccinate is hydrolyzed to fumarate and ornithine.

The cycle is crucial for the detoxification of ammonia produced by the deamination of amino acids.
Urea Cycle Connects to TCA Cycle

- **Urea Cycle**
  - Ornithine → Citrulline → Argininosuccinate → Arginine → Urea

- **TCA Cycle**
  - Oxaloacetate → Malate → α-Ketoglutarate → Citrate

- **Aspartate**
  - \(-\text{b}_2\text{c}\text{H}_1\text{C}=\text{co}_2\text{^-}\text{NH}_2\)

- **Fumarate**
  - \(-\text{b}_2\text{c}=\text{H}_1\text{C}=\text{co}_2\text{^-}\text{H}\)
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \rightarrow \text{carbonyl phosphate} \\
\text{carbamoyl phosphate} & \\
\end{align*}
\]
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>Enzyme</th>
<th>Characteristic</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

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

(repeating the figure from page 3 of your handout)

bicarbonate \[ \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate} \]
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:

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:

\[ \text{Glycine Synthase:} \quad \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{H}}{\text{H}} \]

\[ \text{Glycine} \quad \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{C}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{H}}{\text{H}} \]

\[ \text{Glycine} \quad \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{C}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{H}}{\text{H}} \]

\[ \text{THF} \quad \overset{(-)}{\text{N}}^5 - \overset{(-)}{\text{N}}^0 - \text{methylene THF} \]

\[ \text{CO}_2 \quad + \quad \text{NH}_4^{(+)} \]

Serine Hydroxymethyltransferase:

\[ \text{Serine Hydroxymethyltransferase:} \quad \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{CH}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{CH}_2\text{OH}}{\text{CH}_2\text{OH}} \]

\[ \text{Serine} \quad \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{C}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{CH}_2\text{OH}}{\text{CH}_2\text{OH}} \]

\[ \text{THF} \quad \overset{(-)}{\text{N}}^5 - \overset{(-)}{\text{N}}^0 - \text{methylene THF} \]

\[ \text{Glycine} \quad \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{C}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{CH}_2\text{OH}}{\text{CH}_2\text{OH}} \]

Serine Dehydratase:

\[ \text{Serine Dehydratase:} \quad \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{CH}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{CH}_2\text{OH}}{\text{CH}_2\text{OH}} \]

\[ \text{Serine} \quad \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{C}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{CH}_2\text{OH}}{\text{CH}_2\text{OH}} \]

\[ \text{H}_2\text{O} \]

\[ \text{H}_2\text{O} \]

\[ \text{H}_2\text{O} \]

\[ \text{H}_2\text{O} \]

\[ \text{NH}_4^{(+)} \]

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

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

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\[ \overset{(-)}{\text{OOC}} - \overset{(-)}{\text{C}} - \overset{(-)}{\text{NH}}_3 \overset{(+)\text{CH}_2\text{OH}}{\text{CH}_2\text{OH}} \]
Methionine Cycle
And Biological Methyl Groups
Deficiency: Alkaptonuria

Tetrahydrobiopterin + O_2 → Dihydrobiopterin + H_2O

Phenylalanine → Tyrosine

Enzyme: Phenylalanine hydroxylase

Phenylalanine

(+)
NH_3

Dihydrobiopterin

Phenylpyruvate

(-)

O

Tyrosine

(+)
NH_3

Homogentisate

Enzyme: homogentisate dioxygenase

Deficiency: Alkaptonuria

“Ochronosis”

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

Isoleucine

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

Leucine

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

Valine

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

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

Glu

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

\[
\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{S-CoA} \\
\text{CH}_3\text{NH}_3^{(+)} \\
\text{NAD}^+, \text{CoASH} \\
\text{NADH} + \text{CO}_2
\]

\[
\text{CH}_3\text{CHCH}_2 - \text{C} - \text{S-CoA} \\
\text{CH}_3\text{NH}_3^{(+)} \\
\text{NAD}^+, \text{CoASH} \\
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\text{CH}_3\text{CH} - \text{C} - \text{S-CoA} \\
\text{CH}_3\text{NH}_3^{(+)} \\
\text{NAD}^+, \text{CoASH} \\
\text{NADH} + \text{CO}_2
\]

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

Tyrosine + Tyrosine hydroxylase \rightarrow \text{Dihydroxyphenylalanine (L-DOPA)}

Dihydroxyphenylalanine \rightarrow \text{Dopamine} \rightarrow \text{Norepinephrine} \rightarrow \text{Epinephrine}
Synthesis of Bioactive Amines

Tryptophan

\[ \text{Tryptophan hydroxylase} \]

\[ \text{5-hydroxytryptophan} \]

\[ \text{PLP-dependent decarboxylation} \]

\[ \text{CO}_2 \]

Sero tonin

\[ \text{NAD}^+ \]
Synthesis of Bioactive Amines

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