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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
Amino Acid metabolism

- Amino acids
  - Glu, Gln, Asp, NH$_3$
  - 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:

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

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

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

Details of reaction mechanism:

\[
\text{amino acid} + \text{H}^+ + \text{H}_2\text{O} \rightarrow \text{pyridoxal phosphate} \rightarrow \text{pyridoxamine phosphate}
\]
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:

Some amino acid + α-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):

\[ \text{Glutamate} + \text{oxaloacetate} \rightarrow \alpha\text{-ketoglutarate} + \text{aspartate} \]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[ \text{glutamate} \xrightarrow{\text{ATP} + \text{NH}_3} \text{glutamine} \xrightarrow{\text{ADP} + P_i} \text{glutamine} \]
Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{glutamine} & \rightarrow \text{glutamate} + \text{NH}_3
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase:} & \\
\text{Glutamate} + \text{oxaloacetate} & \rightarrow \text{α-keto glutarate} + \text{aspartate}
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate → carbonyl phosphate → carbamate → carbamoyl phosphate

ATP
ADP
NH₃
Pi

ATP
ADP
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

Arginine $\xrightarrow{\text{H}_2\text{O}}$ Urea $\xrightarrow{\text{Ornithine}}$
Urea Cycle Connects to TCA Cycle

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

Urea Cycle

TCA Cycle
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbonyl phosphate} \\
\text{carbamic acid} & \quad \xrightarrow{\text{ATP}} \quad \text{carbamoyl phosphate} \\
\end{align*}
\]
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><strong>CPSD</strong></th>
<th>No elevation except ammonia; diagnosed by elimination.</th>
</tr>
</thead>
<tbody>
<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

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\begin{align*}
\text{glutamate} & \quad + \quad \text{acetyl CoA} \\
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(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbonyl phosphate} \\
\text{carbamate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbamoyl phosphate}
\end{align*}
\]
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:

\[ \text{Glutamine} \rightarrow \text{glutamate} + \text{ammonia} \]

\[ \text{Asparagine} \rightarrow \text{aspartate} + \text{ammonia} \]

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:

Glycine

Serine Hydroxymethyltransferase:

Serine

Serine Dehydratase:

Serine
Methionine Cycle
And Biological Methyl Groups

\[
\text{CH}_3-\text{S-CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^{-} \\
\text{Methionine}
\]

\[
\text{CH}_3-\text{S-CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^{-} \\
\text{S-Adenosyl Methionine}
\]

\[
\text{CH}_3-\text{S-CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^{-} \\
\text{S-Adenosyl Homocysteine}
\]

\[
\text{CH}_3-\text{S-CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^{-} \\
\text{Homocysteine}
\]

\[
\text{CH}_3-\text{S-CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^{-} \\
\text{Serine}
\]

\[
\text{CH}_3-\text{S-CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^{-} \\
\text{Cysteine}
\]

(remainder of homocysteine degraded for energy)
Phenylalanine and Tyrosine

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

**Deficiency:**

- Alkaptonuria
- "Ochronosis"

Phenylalanine

\[
\text{Phenylalanine} \rightarrow \text{Tetrahydrobiopterin} + \text{O}_2 \rightarrow \text{Dihydrobiopterin} + \text{H}_2\text{O}
\]

Enzyme: Phenylalanine hydroxylase

Tyrosine

\[
\text{Tyrosine} \rightarrow \text{Homogentisate}
\]

Enzyme: Homogentisate dioxygenase

Phenylalanine hydroxylase (no phenylalanine hydroxylase)

Phenylpyruvate

Deficiency: Alkaptonuria "Ochronosis"

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

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

\[\alpha-KG\]

\[\text{Glu}\]

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

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

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

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

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

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

\[\alpha-KG\]

\[\text{Glu}\]

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

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

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

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

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

\[\alpha-KG\]

\[\text{Glu}\]

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

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

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

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

(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 \[\text{NH}_3^{(+)}\] \[\text{Tryptophan hydroxylase}\] 5-hydroxytryptophan \[\text{NH}_3^{(+)}\] \[\text{PLP-dependent decarboxylation}\] \[\text{CO}_2\] Serotonin

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

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

Histidine decarboxylase (PLP-dependent)

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