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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:

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

$$\alpha\text{-keto acid (typically alpha-ketoglutarate)}$$

$$\alpha\text{-keto acid (typically glutamate)}$$

Details of reaction mechanism:

1. Amino acid
2. Pyridoxal phosphate
3. $H^+$
4. 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:

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

Glutamine Synthetase:

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

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{glutamine} & \quad \xrightarrow{\text{H$_2$O}} \quad \text{glutamate} \\
\xrightarrow{(-)\text{HNCOCCH}_2\text{CH}_2\text{COO}^-} & \quad \xrightarrow{(-)\text{HNCOCCH}_2\text{CH}_2\text{COO}^-} \\
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} & \quad + \quad \text{oxaloacetate} \quad \xrightarrow{\text{Glutamate-aspartate aminotransferase}} \quad \text{aspartate} \\
& \quad \xrightarrow{(-)\text{CH}_2\text{CH}_2\text{COO}^-} \quad \xrightarrow{(-)\text{CH}_2\text{CH}_2\text{COO}^-} \\
& \quad \xrightarrow{\text{NH}_3} \quad \xrightarrow{\text{NH}_3} \\
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate $\xrightleftharpoons{\text{ATP}}$ carbonyl phosphate $\xrightleftharpoons{\text{NH}_3} \rightarrow$ carbamate $\xrightleftharpoons{\text{ATP}}$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

\[
\begin{align*}
\text{aspartate} & \quad \text{Citrulline} \\
\text{ATP} & \quad \text{AMP + PP}_i
\end{align*}
\]
Argininosuccinate lyase

Argininosuccinate

H
\(-\)O\(_2\)C\(\text{CH}_2\text{CH}_2\text{NH}\)\(-\)C\(=\text{NH}_2^{(+)}\)
\(\text{NH}_3^{(+)}\)

\(-\)O\(_2\)C\(\text{CH}_2\text{CH}_2\text{NH}\)\(-\)C\(=\text{NH}_2^{(+)}\)
\(\text{NH}_3^{(+)}\)

\(-\)O\(_2\)C\(\text{CH}_2\text{CH}_2\text{NH}\)\(-\)C\(=\text{H}\)
\(\text{CO}_2^{(-)}\)

Fumarate

Arginine

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

\(-\)O\(_2\)C\(\text{CH}_2\text{CH}_2\text{NH}\)\(-\)C\(-\text{NH}_2^{(+)}\)
\(\text{NH}_3^{(+)}\)
Arginase

\[
\begin{align*}
\text{Arginine} & \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{Urea} \\
\text{Ornithine} &
\end{align*}
\]
Urea Cycle Connects to TCA Cycle

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

**TCA Cycle**
- Oxaloacetate → Malate → Fumarate → α-Ketoglutarate → Citrate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

Glutamine Synthetase:
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

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

<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><strong>Elevated CP causes synthesis of Orotate</strong></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

(repeating the figure from page 3 of your handout)

bicarbonate $\rightarrow$ carbamoyl phosphate $\rightarrow$ carbamate $\rightarrow$ 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:

- Glutamine $\rightarrow$ glutamate + ammonia
- Asparagine $\rightarrow$ aspartate + ammonia

We also already know how to degrade Glutamine:

$$\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}$$

...and by analogy, how to degrade Asparaginase:

$$\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}$$
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:

\[
\text{NAD}^+ \quad \text{NADH} \quad \text{THF} \quad N^6-N^4\text{-methylene THF} \quad \text{CO}_2 \quad \text{NH}_4^+ \\
\text{Glycine} \quad \text{THF} \quad \text{Serine} \quad \text{Glycine}
\]

Serine Hydroxymethyltransferase:

\[
\text{NAD}^+ \quad \text{NADH} \quad \text{THF} \quad N^6-N^4\text{-methylene THF} \quad \text{CO}_2 \quad \text{NH}_4^+ \\
\text{Glycine} \quad \text{THF} \quad \text{Serine} \quad \text{Glycine}
\]

Serine Dehydratase:

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

(Deficiency: Alkaptonuria, "Ochronosis")
Branched Chain Amino Acids

Isoleucine

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

Leucine

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

Valine

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

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

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

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

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

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

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

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

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

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

(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 $\xrightarrow{\text{}}$ Norepinephrine $\xrightarrow{\text{}}$ Epinephrine
Synthesis of Bioactive Amines

Tryptophan (NH₃) → 5-hydroxytryptophan (NH₃) → PLP-dependent decarboxylation → CO₂ → Serotonin

CH₂ ─ CH ─ COO⁻

Tryptophan hydroxylase
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