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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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Supplementary study material on the Web:
http://seqcore.brcf.med.umich.edu/mcb500

There are also PDF’s of class handouts with supplemental information available in the table of contents for this course.
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:

\[
R_1\text{-C-}\text{coo}^{(-)} + \text{NH}_2\text{ amino acid} \quad \xrightarrow{\alpha\text{-keto acid (typically alpha-ketoglutarate)}} \quad \text{R}_1\text{-C-}\text{coo}^{(-)} + \text{R}_2\text{-C-}\text{coo}^{(-)} \quad \text{NH}_2\text{ amino acid (typically glutamate)}
\]

Details of reaction mechanism:

1. **Amino Acid**
   \[
   R\text{-C-}\text{coo}^{(-)} + \text{NH}_2 + \text{O} + \text{CH}_3\text{pyridoxal phosphate} \rightarrow \text{H}_2\text{O}
   \]

2. **Pyridoxal Phosphate**
   \[
   \text{Pyridoxal phosphate} \rightarrow \text{H}_2\text{O}
   \]

3. **Pyridoxamine Phosphate**
   \[
   \text{Pyridoxamine phosphate} \rightarrow \text{H}_2\text{O}
   \]
Transfer the amine back to an acceptor α-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):

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

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} & \quad \text{ATP} + \text{NH}_3 \\
\text{ADP} + P_i & \quad \text{glutamine}
\end{align*}
\]
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 $\rightarrow$ carbonyl phosphate $\rightarrow$ carbamate $\rightarrow$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline

Argininosuccinate

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

Argininosuccinate → Fumarate → Arginine

Chemical structures are shown, illustrating the enzymatic reaction process.
Arginase

Arginine $\rightarrow$ Urea $\rightarrow$ Ornithine

H$_2$O
Urea Cycle Connects to TCA Cycle
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\text{Glutamate} \quad \xrightarrow{\text{NAD}(P)} \quad \text{NAD}(P)H
\]

\[
\text{NH}_2
\]

\[
\text{H}
\]

\[
\text{(-)O}_2\text{CCH}_2\text{CH}_2\text{C}-\text{CO}_2\text{H}
\]

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

\[
\alpha\text{-Ketoglutarate} \quad \text{ammonia}
\]

Glutamine Synthetase:

\[
\text{Glutamine} \quad \xrightarrow{\text{ATP}+\text{NH}_3} \quad \text{Glutamate}
\]

\[
\text{H}
\]

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

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

\[
\text{ATP} \quad \text{ADP}+\text{P}_i
\]

\[
\text{NH}_3
\]

\[
\text{(-)OOC-C-CH}_2\text{NH}_2\text{(+)}
\]
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 + NH₃ → carbamate + P₃

carbamate + ATP → carbamoyl phosphate + ADP
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>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

\[
\text{(-) ooc} \quad \text{CH}_2\text{CH}_2\text{C} \quad \text{OO} + \quad \text{CoA-C} = \text{O} \quad \xrightarrow{\text{N-acetyl glutamate synthetase}} \quad \text{NH}_2 \quad \text{CH}_2 \quad \text{CH}_3 \quad \text{OO} \quad \text{NH}
\]

glutamate \hspace{1cm} \text{acetyl CoA} \hspace{1cm} \text{N-acetyl glutamate (NAG)}

(repeating the figure from page 3 of your handout)

\[
\text{HO-C-0} \quad \xrightarrow{\text{ATP}} \quad \text{HO-C-O(P)} \quad \xrightarrow{\text{NH}_3} \quad \text{HO-C-NH}_2 \quad \xrightarrow{\text{ATP}} \quad \text{P}_i \quad \text{HO-C-NH}_2 \quad \xrightarrow{\text{ADP}} \quad \text{carbamoyl phosphate}
\]

bicarbonate \hspace{1cm} \text{carbonyl phosphate} \hspace{1cm} \text{carbamate} \hspace{1cm} \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:

$$\text{H} \quad (\text{CH}_2\text{C}_2\text{H}_2\text{C}_2\text{C}_2\text{CO}_2^-)$$

$$\text{H} \quad (\text{CH}_2\text{C}_2\text{H}_2\text{C}_2\text{C}_2\text{CO}_2^-)$$

$$\text{NH}_3 \quad \text{oxaloacetate}$$

$$\text{aspartate}$$

$$\text{H} \quad (\text{CH}_2\text{C}_2\text{H}_2\text{C}_2\text{CO}_2^-)$$

$$\text{H} \quad (\text{CH}_2\text{C}_2\text{H}_2\text{C}_2\text{CO}_2^-)$$

$$\text{NH}_3 \quad \alpha\text{-ketoglutarate}$$

$$\text{glutamate}$$

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparagine:

Asparagine $\xrightarrow{\text{asparaginase}}$ 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 \( \alpha \)-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

**Glycine Synthase:**

\[ (-)\text{OOC}_-\text{C}_-\text{NH}_3^{(+)} \]

\[ \text{Glycine} \]

\[ \text{NAD}^{(+)} \rightarrow \text{NADH} \]

\[ \text{THF} \rightarrow \text{N}^5-\text{N}^\circ - \text{methylene THF} \]

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

**Serine Hydroxymethyltransferase:**

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

\[ \text{CH}_2\text{OH} \]

\[ \text{Serine} \]

\[ \text{THF} \rightarrow \text{N}^5-\text{N}^\circ - \text{methylene THF} \]

\[ \text{Glycine} \]

**Serine Dehydratase:**

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

\[ \text{CH}_2\text{OH} \]

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

\[ \text{Serine} \]

\[ \rightarrow (-)\text{OOC}_-\text{C}_-\text{NH}_3^{(+)} \]

\[ \text{Serine} \]

\[ \rightarrow (-)\text{OOC}_-\text{C}_-\text{NH}_2^{(+)} \]

\[ \text{Serine} \]

\[ \rightarrow (-)\text{OOC}_-\text{C}_-\text{NH}_2^{(+)} \]

\[ \text{Serine} \]

\[ \rightarrow (-)\text{OOC}_-\text{C}_-\text{NH}_2^{(+)} \]

\[ \text{Serine} \]

\[ \rightarrow (-)\text{OOC}_-\text{C}_-\text{NH}_2^{(+)} \]

\[ \text{Serine} \]

\[ \rightarrow (-)\text{OOC}_-\text{C}_-\text{NH}_2^{(+)} \]

\[ \text{Serine} \]

\[ \rightarrow (-)\text{OOC}_-\text{C}_-\text{NH}_2^{(+)} \]
Methionine Cycle
And Biological Methyl Groups
Deficiency:
Alkaptonuria
"Ochronosis"

Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine

\[
\begin{array}{c}
\text{NH}_3 \\
\text{CH}_2-\text{CH}-\text{COO} \quad (-)
\end{array}
\]

Enzyme: Phenylalanine hydroxylase

Tetrahydrobiopterin + O\textsubscript{2}
Dihydrobiopterin + H\textsubscript{2}O

Phenylketonuria
(no phenylalanine hydroxylase)

Phenylpyruvate

Tyrosine

\[
\begin{array}{c}
\text{NH}_3 \\
\text{CH}_2-\text{CH}-\text{COO} \quad (-)
\end{array}
\]

Homogentisate

Deficiency:
Alkaptonuria
"Ochronosis"

Enzyme: homogentisate dioxygenase

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

Isoleucine

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

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

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

\[
\rightarrow \text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

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

--- Transamination ---

\[
\rightarrow \text{Glu}
\]

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

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

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

\[
\rightarrow \text{Glu}
\]

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

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

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

\[
\rightarrow \text{Glu}
\]

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

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

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

\[
\rightarrow \text{Glu}
\]

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

Tyrosine

Tyrosine hydroxylase

Dihydroxyphenylalanine (L-DOPA)

Dopamine

Norepinephrine

Epinephrine
Synthesis of Bioactive Amines

Tryptophan → 5-hydroxytryptophan → Serotonin

- Tryptophan hydroxylase
- PLP-dependent decarboxylation
- NAD+
Synthesis of Bioactive Amines

Glutamate

\[ (-)\text{COO}CH_2CH_2CHCH_2COO\overset{\text{Glutamate decarboxylase (PLP-dependent)}}{\rightarrow}(-)\text{COO}CH_2CH_2CHCH_2-NH_3 \]
\[ \text{γ-aminobutyric acid (GABA)} \]

Histidine

\[ (-)\text{CH_2CHCHCOO}\overset{\text{Histidine decarboxylase (PLP-dependent)}}{\rightarrow}(-)\text{CH_2CHCH-NH_3} \]
\[ \text{Histamine} \]
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