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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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Director, DNA Sequencing Core
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:**

\[
\begin{align*}
R_1\text{-C-} & \text{coo}^(-) + R_2\text{-C-} \text{coo}^(-) \\
\text{amino} & \text{acid} \quad \alpha\text{-keto} \\
\text{alpha-ketoglutarate} & \text{acid} \\
\end{align*}
\]

\[
\begin{align*}
R_1\text{-C-} \text{coo}^(-) + R_2\text{-C-} \text{coo}^(-) \\
\text{amino} & \text{acid} \\
\text{typically glutamate} & \text{acid} \\
\end{align*}
\]

**Details of reaction mechanism:**

\[
\begin{align*}
\text{amino} & \text{acid} \\
H & \text{acid} \\
R\text{-C-} \text{coo}^(-) & \text{R-C-} \text{coo}^(-) \\
\text{pyridoxal} & \text{pyridoxal} \\
\text{phosphate} & \text{phosphate} \\
\end{align*}
\]

\[
\begin{align*}
\text{H} & \text{H} \\
N & N \\
\text{CH} & \text{CH} \\
\text{water} & \text{H}^+ \\
\text{NH}_2 & \text{R-C-} \text{coo}^(-) \\
\text{HCH} & \text{NH}_2 \\
\text{pyridoxamine} & \text{pyridoxamine} \\
\text{phosphate} & \text{phosphate} \\
\end{align*}
\]
Transfer the amine back to an acceptor $\alpha$-keto acid

\[
\begin{align*}
\text{pyridoxamine phosphate} + R-C-\text{COO}^{(-)} & \rightarrow \text{pyridoxal phosphate} + R-C-\text{COO} \\
\text{pyridoxamine phosphate} & \quad \text{pyridoxal phosphate} \\
\alpha\text{-keto acid} & \quad \text{amino acid}
\end{align*}
\]
Some amino acid $+$ $\alpha$-ketoglutarate $\rightarrow$ some alpha keto acid $+$ Glutamate

In other words, alpha-ketoglutarate is the preferred acceptor, and Glutamate is the resulting amino acid:

In peripheral tissues, transaminases tend to form Glutamate when they catabolize amino acids.
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:

\[
\begin{align*}
\text{glutamate} & \rightarrow \text{NAD(P)} \\
\text{α-ketoglutarate} + \text{ammonia} & \left(\text{mito}\right)
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} & \rightarrow \text{glutamine} \\
\text{ATP} + \text{NH}_3 & \rightarrow \text{ADP} + \text{P}_i
\end{align*}
\]
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:

\[ \text{Glutamate-aspartate aminotransferase:} \]

\[ \text{Glutamate} + \text{Oxaloacetate} \rightarrow \text{α-Keto glutarate} + \text{Aspartate} \]
Carbamoyl phosphate synthetase I

bicarbonate → carbonyl phosphate → carbamyl phosphate → carbamate → carboxamidyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline

Argininosuccinate

Aspartate

ATP → AMP + PPI
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Arginine $\rightarrow$ Fumarate
Arginase

Arginine $\rightarrow$ Ornithine

Arginine $\rightarrow$ Urea $\rightarrow$ Ornithine
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Citrulline
- Arginine
- Argininosuccinate

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

Urea:
- Aspartate
- \(-b\text{CCH}_{2}\text{C}-\text{co}_{2}^{-}\)
- \(\text{NH}_{2}\)
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\begin{align*}
\text{glutamate} & \xrightarrow{\text{NAD}(P)} \text{α-ketoglutarate} + \text{NH}_3 \\
\text{NAD}(P)H & \text{(mito)}
\end{align*}
\]

**Glutamine Synthetase:**

\[
\begin{align*}
\text{glutamate} & \xrightarrow{\text{ATP}+\text{NH}_3} \text{glutamine} \\
\text{ATP} & \rightarrow \text{ADP} + P_i
\end{align*}
\]
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)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbonyl phosphate} \\
\text{carbamate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbamoyl phosphate}
\end{align*}
\]
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>Enzyme</th>
<th>Diagnosis</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

\[
\text{(-)}\text{HOOOC-C-CH}_2\text{CH}_2\text{C}=\text{O} + \text{CoA-C}=\text{O} \xrightarrow{\text{N-acetyl glutamate synthetase}} \text{(-)}\text{HOOOC-C-CH}_2\text{CH}_2\text{C}=\text{O} \text{N-acetyl glutamate (NAG)}
\]

(repeating the figure from page 3 of your handout)

\[
\text{HO-C-O} \xrightarrow{\text{ATP}} \text{HO-C-OP} \xrightarrow{\text{NH}_3} \text{HO-C-NH}_2 \xrightarrow{\text{ATP}} \text{PO-C-NH}_2
\]
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: \( \text{NH}_3 \text{H} \overset{\text{transamination}}{\rightarrow} \overset{-1}{\overset{\text{transamination}}{\text{CCH}_2C^{-}}}{\text{NH}_3} + \overset{-1}{\overset{\text{transamination}}{\text{CH}_2^{-}}}{\text{CO}_2} \)
- Asparagine: \( \overset{-1}{\overset{\text{transamination}}{\text{CCH}_2C^{-}}}{\text{NH}_3} \overset{\text{transamination}}{\rightarrow} \overset{-1}{\overset{\text{transamination}}{\text{CCH}_2CH_2^{-}}}{\text{NH}_3} \)

We also already know how to degrade Glutamine:

Glutamine \( \overset{\text{glutaminase}}{\rightarrow} \) glutamate + ammonia

…and by analogy, how to degrade Asparagine:

Asparagine \( \overset{\text{asparaginase}}{\rightarrow} \) 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:

Glycine

Serine Hydroxymethyltransferase:

Serine

Serine Dehydratase:

Serine
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(normal path shown in black, pathological reaction shown in red)

Phenylalanine

(-)\[\text{NH}_3\]

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

Enzyme: Phenylalanine hydroxylase

Tetrahydrobiopterin + O\(_2\)

Dihydrobiopterin + H\(_2\)O

(+)

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

Tyrosine

(+)

\[\text{NH}_3\]

Phenylpyruvate

Deficiency: Alkaptonuria “Ochronosis”

Homogentisate

Enzyme: homogentisate dioxygenase

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

Isoleucine
\[
\text{CH}_3\text{CH}_2\text{CH} \_\text{CH} \_\text{COO}^{(-)} \\
\text{CH}_3 \text{NH}_3^{(+)} \quad \alpha \text{-KG} \\
\rightarrow \text{Glu} \\
\text{CH}_3\text{CH}_2\text{CH} \_\text{C} \_\text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NAD}^+ \text{CoASH} \\
\rightarrow \text{NADH} + \text{CO}_2
\]

Leucine
\[
\text{CH}_3\text{CHCH}_2 \_\text{CH} \_\text{COO}^{(-)} \\
\text{CH}_3 \text{NH}_3^{(+)} \quad \alpha \text{-KG} \\
\rightarrow \text{Glu} \\
\text{CH}_3\text{CHCH}_2 \_\text{C} \_\text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NAD}^+ \text{CoASH} \\
\rightarrow \text{NADH} + \text{CO}_2
\]

Valine
\[
\text{CH}_3\text{CH} \_\text{CH} \_\text{COO}^{(-)} \\
\text{CH}_3 \text{NH}_3^{(+)} \quad \alpha \text{-KG} \\
\rightarrow \text{Glu} \\
\text{CH}_3\text{CH} \_\text{C} \_\text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NAD}^+ \text{CoASH} \\
\rightarrow \text{NADH} + \text{CO}_2
\]

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

----------------- Branched-chain \(\alpha\)-keto acid dehydrogenase -----------------
Synthesis of Bioactive Amines

Tyrosine → Dihydroxyphenylalanine (L-DOPA)

Dopamine → Norepinephrine → Epinephrine
Synthesis of Bioactive Amines

Tryptophan

\[ \text{Tryptophan hydroxylase} \]

\[ \text{5-hydroxytryptophan} \]

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

\[ \text{CO}_2 \]

\[ \text{Serotonin} \]
Synthesis of Bioactive Amines

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

\[
\text{Histidine} \quad \xrightarrow{\text{Histidine decarboxylase (PLP-dependent)}} \quad \text{Histamine}
\]
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