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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:

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

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

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

Details of reaction mechanism:

[Diagram showing the reaction mechanism involving pyridoxal phosphate and its intermediates]
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 → 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:**

Glutamate $\rightarrow$ NAD(P) $\rightarrow$ NAD(P)H $\rightarrow$ α-ketoglutarate + NH$_3$

**Glutamine Synthetase:**

Glutamate + ATP + NH$_3$ $\rightarrow$ Glutamine + ADP + P$_i$
Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{glutamine} & \quad \begin{array}{c}
\overset{\text{H}}{\overset{\text{NH}_3}{\overset{\text{(-)}}{\overset{\text{OOC-CH}_2CH_2CO}}}}
\end{array} \\
\quad \text{H}_2\text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{glutamate} & \quad \begin{array}{c}
\overset{\text{H}}{\overset{\text{NH}_3}{\overset{\text{(-)}}{\overset{\text{OOC-CH}_2CH_2CO}}}}
\end{array} \\
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:}
\begin{align*}
\overset{\text{H}}{\overset{\text{NH}_3}{\overset{\text{(-)}}{\overset{\text{O}_2\text{C-CH}_2\text{C}-\text{CO}_2\text{(-)}}}}}} & \quad \overset{\text{H}}{\overset{\text{NH}_3}{\overset{\text{(-)}}{\overset{\text{O}_2\text{C-CH}_2\text{C}-\text{CO}_2\text{(-)}}}}}} \\
\quad \text{Glutamate} & \quad \text{oxaloacetate} \\
\end{align*}
\]

\[
\begin{align*}
\overset{\text{H}}{\overset{\text{NH}_3}{\overset{\text{(-)}}{\overset{\text{O}_2\text{C-CH}_2\text{C}-\text{CO}_2\text{(-)}}}}}} & \quad \overset{\text{H}}{\overset{\text{NH}_3}{\overset{\text{(-)}}{\overset{\text{O}_2\text{C-CH}_2\text{C}-\text{CO}_2\text{(-)}}}}}} \\
\overset{\text{\alpha\text{-keto glutarate}}}{\overset{\text{aspartate}}{\overset{\text{NH}_3}{\overset{\text{(-)}}{\overset{\text{O}_2\text{C-CH}_2\text{C}-\text{CO}_2\text{(-)}}}}}}}
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate $\rightarrow$ carbonyl phosphate $\rightarrow$ carbamoyl phosphate $\rightarrow$ carbamate $\rightarrow$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

\[
\text{Citrulline} \rightarrow \text{Argininosuccinate}
\]

\[
\text{aspartate}
\]

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

Argininosuccinate \( \xrightarrow{\text{Argininosuccinate lyase}} \) Arginine

Fumarate
Arginase

\[
\text{Arginine} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{Urea} \quad \xrightarrow{\text{Arginase}} \quad \text{Ornithine}
\]
2ATP + HCO₃⁻ + NH₃ → \text{Carbamoyl phosphate} → 2ADP + Pi → \text{Ornithine} → \text{Citrulline} → \text{Urea} → \text{Arginine} → \text{Argininosuccinate} → \text{Fumarate}

Liver mitochondrion → Liver cytoplasm
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Argininosuccinate
- Arginine
- Aspartate
- Oxaloacetate
- Malate
- Fumarate
- Citrate
- α-Ketoglutarate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[
\text{glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamine} \xrightarrow{\text{ADP} + \text{P}_i} \text{NH}_3
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

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

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

(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:

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

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

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparagine:

\[ \text{Asparagine} \overset{\text{asparaginase}}{\rightarrow} \text{aspartate} + \text{ammonia} \]
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:**

\[
\text{Glycine} \xrightarrow{\text{NAD}^+} \text{CO}_2 + \text{NH}_4^+ \\
\]

\[
\text{N}_5 - \text{N}_1 - \text{methylene THF} \xrightarrow{\text{NADH}} \text{THF} \\
\]

**Serine Hydroxymethyltransferase:**

\[
\text{Serine} \xrightarrow{\text{NAD}^+} \text{Glycine} \\
\]

\[
\text{N}_5 - \text{N}_1 - \text{methylene THF} \xrightarrow{\text{NADH}} \text{THF} \\
\]

**Serine Dehydratase:**

\[
\text{Serine} \xrightarrow{\text{H}_2\text{O}} \text{Glycine} \\
\]

\[
\text{N}_5 - \text{N}_1 - \text{methylene THF} \xrightarrow{\text{NADH}} \text{THF} \\
\]
Methionine Cycle
And Biological Methyl Groups
Deficiency: Alkaptonuria

"Ochronosis"

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

Tyrosine

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

Homogentisate

Deficiency: Alkaptonuria
“Ochronosis”

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

Phenylpyruvate

Enzyme: Homogentisate dioxygenase

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

Isoleucine

$\text{CH}_3\text{CH}_2\text{CH} - \text{CH} - \text{COO}^{(-)}$
$\text{CH}_3\text{NH}_3^{(+)}$

$\alpha$-KG

Glu

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

$\text{NAD}^+, \text{CoASH}$

$\text{NADH} + \text{CO}_2$

Leucine

$\text{CH}_3\text{CHCH}_2 - \text{CH} - \text{COO}^{(-)}$
$\text{CH}_3\text{NH}_3^{(+)}$

$\alpha$-KG

Glu

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

$\text{NAD}^+, \text{CoASH}$

$\text{NADH} + \text{CO}_2$

Valine

$\text{CH}_3\text{CH} - \text{CH} - \text{COO}^{(-)}$
$\text{CH}_3\text{NH}_3^{(+)}$

$\alpha$-KG

Glu

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

$\text{NAD}^+, \text{CoASH}$

$\text{NADH} + \text{CO}_2$

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

Tryptophan → 5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase

PLP-dependent decarboxylation

NAD⁺
Synthesis of Bioactive Amines

- Glutamate $\rightarrow$ GABA
- Histidine $\rightarrow$ 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