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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

Glu, Gln, Asp, NH₃

Urea

Folate metabolism

Methylene THF

Met Cycle

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:

\[
\begin{align*}
R_1\text{C} & \text{coo}^- + R_2\text{C} & \text{coo}^- \\
\text{NH}_2 & & \text{NH}_2
\end{align*}
\]

\[
\xrightarrow{\alpha\text{-keto acid}}
\]

\[
\begin{align*}
R_1\text{C} & \text{coo}^- + R_2\text{C} & \text{coo}^-
\end{align*}
\]

\[
\text{NH}_2 & & \text{NH}_2
\]

\[
\xrightarrow{\alpha\text{-keto acid}}
\]

\[
\text{amino acid} \quad \text{alpha-ketoglutarate}
\]

\[
\text{typically glutamate}
\]

Details of reaction mechanism:

\[
\begin{align*}
\text{amino acid} \\
R\text{C} \text{coo}^- + \text{H}_2\text{O} \\
\text{pyridoxal phosphate} \\
\text{H}^+ \\
\text{pyridoxamine phosphate}
\end{align*}
\]
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:**

\[
\text{Glutamate} \rightarrow (\text{NAD}(P) \rightarrow \text{O}_2\text{CCH}_2\text{CH}_2\text{C} = \text{CO}_2^{\text{NH}_2} \rightarrow \text{NH}_3 + \text{NAD}(P)_\text{H} \rightarrow \text{O}_2\text{CCH}_2\text{CH}_2\text{C} = \text{CO}_2^{\text{NH}_2} \rightarrow \text{O}_2\text{CCH}_2\text{CH}_2\text{C} = \text{CO}_2^{\text{NH}_2} + \text{NH}_3
\]

**Glutamine Synthetase:**

\[
\text{Glutamate} \rightarrow (\text{ATP} + \text{NH}_3 \rightarrow \text{ADP} + \text{Pi} \rightarrow \text{NH}_3 \rightarrow \text{Glutamine}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{Glutamine} \xrightarrow{\text{H}^+} \text{Glutamate} + \text{NH}_3
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} + \text{oxaloacetate} \xrightarrow{\text{Enzyme}} \text{α-keto glutarate} + \text{aspartate}
\]
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{ATP} \text{carbonyl phosphate} \xrightarrow{NH_3} \text{carbamate} \xrightarrow{ATP} \text{carbamoyl phosphate}$
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

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

\[
\begin{align*}
\text{Arginine} & \quad \overset{H_2O}{\longrightarrow} \quad \text{Ornithine} \\
\text{Urea} & 
\end{align*}
\]
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\text{glutamate} \xrightarrow{\text{NAD}(P)} \alpha\text{-ketoglutarate} + \text{NH}_3
\]

**Glutamine Synthetase:**

\[
\text{glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamine} + \text{ADP} + \text{P}_i
\]
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)

\[
\text{bicarbonate} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
\]
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>
<th></th>
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</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

Glutamate + Acetyl CoA → N-Acetyl Glutamate (NAG)

(repeating the figure from page 3 of your handout)

Bicarbonate + ATP → Carboxyl Phosphate

Carboxyl Phosphate + NH₃ → Carbamate

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:

\[
\begin{align*}
\text{Glutamine:} & \quad \text{glutaminase} \\
\text{Asparagine:} & \quad \text{asparaginase}
\end{align*}
\]

We also already know how to degrade Glutamine:

\[
\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}
\]

…and by analogy, how to degrade Asparagine:

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
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:

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

\[
\text{THF} \quad \text{N}^5-\text{N}^\prime-\text{methylene} \text{ THF}
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine} \quad \xrightarrow{\text{THF} \quad \text{N}^5-\text{N}^\prime-\text{methylene} \text{ THF}} \quad \text{Glycine}
\]

Serine Dehydratase:

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

\[
\text{THF} \quad \text{N}^5-\text{N}^\prime-\text{methylene} \text{ THF}
\]
Phenylalanine and Tyrosine

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

Phenylalanine → Tetrahydrobiopterin + O₂

(+)

NH₃

(--)

Dihydrobiopterin + H₂O

(--)

NH₃

Enzyme: Phenylalanine hydroxylase

Phenylalanine hydroxylase → Tyrosine

(--)

Enzyme: homogentisate dioxygenase

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Phenylpyruvate

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

Isoleucine

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

\[
\text{CH}_3 \text{NH}_{3}^{(+)}
\]

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

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

\[
\text{CH}_3
\]

\[
(\text{continues on to degradation path similar to } \beta\text{-oxidation of fatty acids})
\]

Leucine

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

\[
\text{CH}_3 \text{NH}_{3}^{(+)}
\]

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

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

\[
\text{CH}_3
\]

\[
(\text{continues on to degradation path similar to } \beta\text{-oxidation of fatty acids})
\]

Valine

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

\[
\text{CH}_3 \text{NH}_{3}^{(+)}
\]

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

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

\[
\text{CH}_3
\]

\[
(\text{continues on to degradation path similar to } \beta\text{-oxidation of fatty acids})
\]
Synthesis of Bioactive Amines

Tyrosine $\xrightarrow{\text{Tyrosine hydroxylase}}$ Dihydroxyphenylalanine (L-DOPA)

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

Tryptophan $\rightarrow$ NAD$^+$

Tryptophan hydroxylase

5-hydroxytryptophan $\rightarrow$ CO$_2$

PLP-dependent decarboxylation

Serotonin
Synthesis of Bioactive Amines

\[
\text{Glutamate} \quad \xrightarrow{\text{Glutamate decarboxylase (PLP-dependent)}} \quad \text{\(\gamma\)-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