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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:

\[
R_1\text{C-COO}^- + R_2\text{C-COO}^- \rightarrow R_1\text{C-COO}^- + R_2\text{C-COO}^- \\
\text{α-keto acid (typically α-ketoglutarate)} \quad \text{α-keto acid (typically glutamate)}
\]

Details of reaction mechanism:

\[
\text{amino acid} \quad \text{amino acid}
\]

\[
R_1\text{C-COO}^- + \text{H}_2\text{O} \rightarrow \text{R} - \text{C} - \text{COO}^- + \text{H}^+ \\
\text{pyridoxal phosphate} \\
\text{pyridoxamine phosphate}
\]
Transfer the amine back to an acceptor $\alpha$-keto acid

pyridoxamine phosphate + $\alpha$-keto acid $\rightarrow$ pyridoxal phosphate + amino 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:**

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

**Glutamine Synthetase:**

\[
\text{Glutamate} + \text{NH}_3 + \text{ATP} \xrightarrow{\text{ADP} + P_i} \text{Glutamine}
\]
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:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase:} \\
\left(\text{aspartate} + \text{glutamate}\right) &\rightarrow \left(\text{oxaloacetate} + \text{α-ketoglutarate}\right)
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate → ATP → carbonyl phosphate → NH₃ → carbamate → ATP → carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

\[ \text{aspartate} \]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\begin{align*}
\text{glutamate} & \quad \xrightarrow{\text{NAD}(P)^+} \quad \text{NAD}(P)_H \\
\xrightarrow{\text{mito}} & \quad \begin{array}{c}
\text{\(\text{\text{\(-\text{O}_2\text{CCH}_2\text{CH}_2\text{C-CO}_2\text{NH}_2\)}}\}\text{\(-\text{O}_2\text{CCH}_2\text{CH}_2\text{C-CO}_2\)}} \\
\end{array}
\end{align*}
\]

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

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} & \quad \xrightarrow{\text{ATP}+\text{NH}_3} \quad \text{ADP}+\text{P}_i \\
\end{align*}
\]

\[
\begin{align*}
\text{glutamine} & \quad \xrightarrow{\text{ADP}+\text{P}_i} \quad \text{NH}_3 \\
\end{align*}
\]
The image depicts a metabolic pathway involving the synthesis and degradation of various amino acids and related compounds. The pathway starts with the synthesis of Carbamoyl phosphate from ATP, HCO₃⁻, and NH₃. This is followed by the formation of Citrulline from Ornithine in the liver mitochondria and cytoplasm. The Citrulline is then converted to Argininosuccinate, which can be further degraded to Fumarate. The overall process involves ATP hydrolysis and the transfer of amino groups and functional groups between molecules.
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)
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>Acronym</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>
The diagram illustrates the urea cycle and arginine biosynthesis pathways in the liver.

1. **Carbamoyl Phosphate Synthetase**: 2ATP + HCO₃⁺ + NH₃ → Carbamoyl phosphate + ADP + Pᵢ

2. **Aspartate Transcarbamoylase**: Carbamoyl phosphate + ornithine → citrulline + ATP

3. **Argininosuccinate Ligase**: Citrulline + 2ATP + H⁺ → argininosuccinate + AMP + PP_i

4. **Argininosuccinate Lyase**: Argininosuccinate → arginine + fumarate

5. **Urea Cycle**: Arginine → ornithine + CO₂ + NH₃

The cycle converts ammonia and carbon dioxide into urea in the liver, playing a crucial role in nitrogen metabolism.
CPS I is Stimulated by NAG

\[
\begin{align*}
(-)\text{ooc} & \quad \text{glutamate} \quad \text{CoA} = 0 \\
\text{NH}_3 & \quad \text{acetyl CoA} \quad \text{N-acetyl glutamate (NAG)}
\end{align*}
\]

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \text{ATP} \\
\text{ADP} & \quad \text{carbonyl phosphate} \\
\text{P}_i & \quad \text{carbamate} \\
\text{ADP} & \quad \text{carbamoyl phosphate}
\end{align*}
\]
The diagram illustrates the urea cycle, a metabolic pathway that occurs in the liver. The cycle begins with the conversion of ammonia to carbamoyl phosphate, followed by the formation of citrulline from ornithine. Citrulline is then converted to argininosuccinate, which is subsequently broken down to fumarate.

1. 2ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate
2. 2ADP + P₃ → Ornithine
3. Ornithine + Citrulline → Argininosuccinate
4. Argininosuccinate → Fumarate

The cycle is completed with the conversion of fumarate to oxaloacetate and the regeneration of ATP.
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:

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparaginase:

\[
\text{Asparaginase} \quad \text{Asparagine} \rightarrow \text{aspartate} + \text{ammonia}
\]
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 $\alpha$-ketoglutarate.
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

\[
\text{Glycine Synthase:} \quad (-OOC-C\text{-NH}_3^+) \quad \xrightarrow{\text{THF}} \quad \text{Glycine} \quad \xrightarrow{\text{NAD}^+} \quad \text{CO}_2 + \text{NH}_4^{(+)}
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine Hydroxymethyltransferase:} \quad (-OOC-C\text{-CH-}\text{-NH}_3^+) \quad \xrightarrow{\text{THF}} \quad \text{Serine} \quad \xrightarrow{\text{N}^5-\text{N}^0-\text{methylene THF}} \quad (-OOC-C\text{-H-}\text{-NH}_3^+)
\]

Serine Dehydratase:

\[
\text{Serine Dehydratase:} \quad (-OOC-C\text{-CH-}\text{-NH}_3^+) \quad \xrightarrow{\text{H}_2\text{O}} \quad (-OOC-C\text{-NH}_3^+) \quad \xrightarrow{\text{H}_2\text{O}} \quad (-OOC-C\text{-NH}_2^+) \quad \xrightarrow{\text{NH}_4^+} \quad (-OOC-C\text{-O})
\]

\[
\text{Serine Dehydratase:} \quad (-OOC-C\text{-CH}_2\text{OH}) \quad \xrightarrow{\text{H}_2\text{O}} \quad (-OOC-C\text{-CH}_2\text{-NH}_3^+) \quad \xrightarrow{\text{H}_2\text{O}} \quad (-OOC-C\text{-CH}_3\text{-NH}_2^+ \quad \xrightarrow{\text{H}_2\text{O}} \quad (-OOC-C\text{-CH}_3)
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

\[
\text{Phenylalanine} \xrightarrow{\text{Tetrahydrobiopterin} + \text{O}_2} \text{Dihydrobiopterin} + \text{H}_2\text{O} \xrightarrow{\text{Enzyme: Phenylalanine hydroxylase}} \text{Tyrosine}
\]

\[
\text{Homogentisate}
\]

Deficiency: Alkaptonuria

(“Ochronosis”)

(Phenylpyruvate)

(Enzyme: homogentisate dioxygenase)

YOU DON’T NEED TO KNOW THE REST
Branched Chain Amino Acids

Isoleucine

Leucine

Valine

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

Glu

------------------- Branched-chain α-keto acid dehydrogenase -------------------

NADH + CO₂

(continues on to degradation path similar to β-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 $\rightarrow$ 5-hydroxytryptophan $\rightarrow$ Serotonin

1. Tryptophan hydroxylase
2. PLP-dependent decarboxylase
3. NAD$^+$
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