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:

\[
R_1\text{C}^\text{NH}_2 + R_2\text{C}^\text{COO}^- \rightarrow R_1\text{C}^\text{COO}^- + R_2\text{C}^\text{NH}_2
\]

Alpha-keto acid (typically alpha-ketoglutarate)

Details of reaction mechanism:

1. Amino acid
2. \(H^+\)
3. Pyridoxal phosphate
4. Pyridoxamine phosphate
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:

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

Glutamine Synthetase:

\[
\text{Glutamate} + \text{NH}_3 + \text{ATP} \quad \xrightarrow{\text{ADP} + P_i} \quad \text{Glutamine}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{Glutamine} \xrightarrow{\text{H}_2\text{O}} \text{Glutamate} + \text{NH}_3
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:}
\text{Glutamate} + \text{Oxaloacetate} \xrightarrow{\text{Ketoglutarate}} \text{Aspartate} + \text{α-Ketoglutarate}
\]
Carbamoyl phosphate synthetase I

bicarbonate + ATP → carbonyl phosphate + ADP

carbonyl phosphate + NH₃ → carbamate + Pi

carbamate + ATP → carbamoyl phosphate + ADP
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Arginine $\rightarrow$ Fumarate

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

\[ \text{H} \]

\[ \text{NH}_2 \]

\[ (+) \]

\[ \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} = \text{NH}_2^{(+)} \]

\[ \text{H} \]

\[ \text{NH}_3 \]

\[ (+) \]

\[ (-)\text{O}_2\text{C} - \text{C} = \text{NH}_2^{(-)} \rightarrow \text{H} \]

\[ \text{H} \]

\[ \text{NH}_2 \]

\[ (+) \]

\[ \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} - \text{NH}_2 \]

\[ \text{NH}_3 \]

\[ (+) \]
Arginase

Arginine $\xrightarrow{\text{H$_2$O}}$ Ornithine

$\xrightarrow{\text{Urea}}$
Urea Cycle Connects to TCA Cycle

- Ornithine → Citrulline
- Arginine
- Argininosuccinate
- Aspartate
- Oxaloacetate
- Malate
- Fumarate
- TCA Cycle
- Citrate
- α-Ketoglutarate

Urea Cycle
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

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

**Glutamine Synthetase:**

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

\[
\begin{align*}
\text{(-) } & \quad \text{OOC} - \text{C} - \text{CH}_2\text{CH}_2\text{C} - \text{O} - \text{NH}_3 \\
\text{ (+) } & \quad \text{OOC} - \text{C} - \text{CH}_2\text{CH}_2\text{C} - \text{O} - \text{H} \\
\end{align*}
\]

\text{glutamate} \quad \text{acetyl CoA} \\

\text{N-acetyl glutamate (NAG)}

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{HO} - \text{C} - \text{O}^{(-)} & \quad \text{ATP} \\
\text{bicarbonate} & \quad \text{ADP} \\
\text{HO} - \text{C} - \text{O} & \quad \text{NH}_3 \\
\text{carbonyl phosphate} & \quad \text{ADP} \\
\text{HO} - \text{C} - \text{NH}_1 & \quad \text{ATP} \\
\text{carbamate} & \quad \text{ADP} \\
\text{PO} - \text{C} - \text{NH}_2 & \quad \text{carbamoyl phosphate}
\end{align*}
\]
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.

<p>| | |</p>
<table>
<thead>
<tr>
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<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>
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</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)

\[
\text{bicarbonate} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
\]
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:

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:

Serine Hydroxymethyltransferase:

Serine Dehydratase:
Methionine Cycle
And Biological Methyl Groups
Deficiency:
Alkaptonuria
"Ochronosis"

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

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

Tetrahydrobiopterin + O\(_2\)
\[ \text{Dihydrobiopterin} + \text{H}_2\text{O} \]

Enzyme: Phenylalanine hydroxylase

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

Homogentisate

Deficiency:
Alkaptonuria
"Ochronosis"

Enzyme: homogentisate dioxygenase

Phenylpyruvate
\[ \text{CH}_2 - \text{C} - \text{COO}^{(-)} \]
Branched Chain Amino Acids

Isoleucine

Leucine

Valine

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

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

(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 → 5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase
PLP-dependent decarboxylation

NH₃
NAD⁺
Synthesis of Bioactive Amines

Glutamate

- \(\text{COO} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH} \quad \text{COO} \quad \text{NH}_3\)

(+)

Glutamate decarboxylase (PLP-dependent)

GABA

\(\text{COO} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH} \quad \text{NH}_3\)

(+)

Histidine

- \(\text{NH}_3\)

(+)

Histidine decarboxylase (PLP-dependent)

Histamine

- \(\text{NH}_3\)
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