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:

\[
\begin{align*}
R_1\text{-C-} &\text{coo}^\text{(-)} + R_2\text{-C-} &\text{coo}^\text{(-)} \\
\text{amino} &\text{acid} &\text{amino} \\
\text{acid} &\text{acid} \\
\text{NH}_2 &\text{NH}_2 \\
\alpha\text{-keto} &\alpha\text{-keto} \\
\text{acid} &\text{acid} \\
\text{(typically)} &\text{(typically)} \\
\text{alpha-ketoglu-} &\text{glutamate})
\end{align*}
\]

Details of reaction mechanism:

\[
\begin{align*}
\text{amino} &\text{acid} \\
\text{acid} &\text{acid} \\
\text{NH}_2 &\text{NH}_2 \\
\text{H} &\text{H} \\
R\text{-C-} &R\text{-C-} \\
\text{COO}^\text{(-)} &\text{COO}^\text{(-)} \\
\text{H}_2\text{O} &\text{H}^+ \\
\text{pyridoxal} &\text{amino} \\
\text{phosphate} &\text{acid}
\end{align*}
\]
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:

\[ \text{Some amino acid} + \alpha\text{-ketoglutarate} \rightarrow \text{some alpha keto acid} + \text{Glutamate} \]
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)} \text{NAD}(P)\text{H} \rightarrow \text{\(\begin{array}{c} - \text{O} \text{C} \text{H}_2 \text{CH}_2 \text{C} - \text{CO}_2(-) \\ \text{NH}_2 \end{array}\)} \rightarrow \text{\(\begin{array}{c} - \text{O} \text{C} \text{H}_2 \text{CH}_2 \text{C} - \text{CO}_2(-) \\ \text{NH}_3 \end{array}\)} + \text{\(\begin{array}{c} - \text{O} \text{C} \text{H}_2 \text{CH}_2 \text{C} - \text{CO}_2(-) \\ \text{O} \end{array}\)}
\]

**Glutamine Synthetase:**

\[
\text{glutamate} \xrightarrow{\text{ATP} + \text{NH}_3} \text{glutamine} \xrightarrow{\text{ADP} + P_i} \text{glutamine}
\]
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:

Glutamate-aspartate aminotransferase:

\[
\text{Glutamate} + \text{oxaloacetate} \rightarrow \text{aspartate} + \text{α-keto glutarate}
\]
Carbamoyl phosphate synthetase I

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

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

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

\[
\text{H} \quad \text{H} \quad \text{O} 
\]

\[
\text{NH}_3 \quad (+) \quad \text{NH}_3 \quad (+) 
\]

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

\[
\begin{align*}
\text{Arginine} & \quad \text{H}_2\text{O} \quad \text{Urea} \quad \text{Ornithine} \\
\text{(-)OOC} & \quad \text{C} \quad \text{CH}_2\text{CH}_2\text{CH}_2\text{NH}^+ \\
\text{NH} & \quad \text{NH}_2 \\
^{(+)} & \\
\end{align*}
\]
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:

\[
\text{Glutamate} \quad \xrightarrow{\text{ATP} + \text{NH}_3} \quad \text{Glutamine}
\]
CPS I is Stimulated by NAG

\[
\begin{align*}
(-)^{\text{OOC}} & \quad \text{glutamate} \\
NH_2 & \\
(+)^{\text{OOC}} & \\
\text{CoA-c} & \quad \text{acetyl CoA} \\
\text{N-acetyl glutamate} & \quad \text{(NAG)}
\end{align*}
\]

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \text{ATP} \\
\text{ADP} & \quad \text{carbonyl phosphate} \\
\text{NH}_3 & \quad \text{carbamate} \\
\text{Pi} & \quad \text{carbamoyl phosphate}
\end{align*}
\]
Complicating the picture: Other tissues may be involved

**Muscle:**
- Amino acids: Transamination, Deamination
- Alanine \(\rightarrow\) Glutamate
- Glutamine \(\rightarrow\) \(\text{NH}_4^{(+)}\)
- \(\text{NH}_4^{(+)}\) (purine deamination)

**Intestine:**
- Glutamine
- Alanine \(\rightarrow\) \(\text{NH}_4^{(+)}\)
- Citrulline

**Kidney:**
- Glutamine
- \(\text{NH}_3\)
- \(\text{NH}_4^{(+)}\)
- Arginine
- Citrulline

**Liver:**
- Arginine
- 
- Glutamine
- Alanine
- Glu \(\rightarrow\) Aspartate
- \(\text{NH}_4^{(+)}\)
- Urea

---
Why is Ammonia Toxic?
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• 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>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

(glutamate + acetyl CoA) → N-acetyl glutamate (NAG)

(repeating the figure from page 3 of your handout)

bicarbonate + ATP → carbamate + ADP

bicarbonate + ATP → carbamoyl phosphate + ADP

bicarbonate + ATP → carbamoyl phosphate + ADP
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 α-ketoglutarate.
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

\[
\begin{align*}
\text{Glycine} & \rightarrow \text{THF} \rightarrow \text{N}^5-\text{N}^{10}-\text{methylene THF} \\
& \rightarrow \text{NAD}^+ \rightarrow \text{CO}_2 + \text{NH}_4^+
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Serine} & \rightarrow \text{THF} \rightarrow \text{N}^5-\text{N}^{10}-\text{methylene THF} \\
& \rightarrow \text{Glycine}
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \rightarrow \text{H}_2\text{O} \\
& \rightarrow \text{NH}_4^+
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine + Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O

Enzyme: Phenylalanine hydroxylase

Phenylalanine → Tyrosine

Deficiency: Alkaptonuria (Ochronosis)

Phenylpyruvate

Homogentisate

Enzyme: Homogentisate dioxygenase

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

Isoleucine

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

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

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

\[ \rightarrow \text{Glu} \]

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

\[ \text{CH}_3 \]

--- Branched-chain \( \alpha \)-keto acid dehydrogenase ---

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

\[ \text{CH}_3 \]

\[ \rightarrow \text{NADH}^+ + 2 \text{H}^+ \]

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

Leucine

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

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

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

\[ \rightarrow \text{Glu} \]

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

\[ \text{CH}_3 \]

Valine

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

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

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

\[ \rightarrow \text{Glu} \]

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

\[ \text{CH}_3 \]

--- Branched-chain \( \alpha \)-keto acid dehydrogenase ---

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

\[ \text{CH}_3 \]

\[ \rightarrow \text{NADH}^+ + 2 \text{H}^+ \]

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

(continues on to degradation path similar to \( \beta \)-oxidation of fatty acids)
Synthesis of Bioactive Amines

Tyrosine → Dihydroxyphenylalanine (L-DOPA) via Tyrosine hydroxylase

Dopamine → Norepinephrine → Epinephrine
Synthesis of Bioactive Amines

Tryptophan $\xrightarrow{\text{Tryptophan hydroxylase}}$ 5-hydroxytryptophan $\xrightarrow{\text{PLP-dependent decarboxylation}}$ Serotonin

$\text{NAD}^+$
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

Histidine decarboxylase (PLP-dependent)

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