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:

amino acid

\[
R\text{-C} - \text{coo}^{(-)} + \text{amino acid} \rightarrow \text{pyridoxal phosphate}
\]

\[
\text{pyridoxal phosphate} + \text{H}^+ \rightarrow \text{pyridoxamine phosphate}
\]
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 $\rightarrow$ 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:

\[
\begin{align*}
\text{glutamate} & \quad \text{(mito)} \quad \text{NAD}^+ \\
\text{H} & \quad \text{NAD}^+ \quad \text{O} \\
\text{NH}_2 & \quad \text{NH}_2 \\
\end{align*}
\]

\[
\text{glutamate} \rightarrow \text{NAD}^+ \rightarrow \text{O} \rightarrow \text{NH}_2
\]

\[
\text{NAD}^+ + \text{O} \rightarrow \text{NH}_2
\]

\[
\alpha\text{-ketoglutarate} + \text{ammonia}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} & \quad \text{ATP} + \text{NH}_3 \\
\text{H} & \quad \text{ADP} + P_i \\
\text{NH}_3 & \quad \text{NH}_3
\end{align*}
\]

\[
\text{glutamate} \rightarrow \text{ATP} + \text{NH}_3 \rightarrow \text{ADP} + P_i \rightarrow \text{NH}_3
\]

\[
\text{glutamine}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

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:
Carbamoyl phosphate synthetase I

bicarbonate + ATP → carbonyl phosphate + ADP

carbonyl phosphate + NH₃ → carbamate + P_i

carbamate + ATP → carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

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

\[
\text{arginine} \xrightarrow{\text{H}_2\text{O}} \text{ornithine, urea}
\]
Urea Cycle Connects to TCA Cycle

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

**Glutamate Dehydrogenase:**

\[
\text{H} \quad \text{NAD}(P)H \quad \text{NAD}(P)
\]

\[
\text{glutamate} \quad \text{O} \quad \text{α-ketoglutarate} \quad \text{ammonia}
\]

**Glutamine Synthetase:**

\[
\text{ATP} + \text{NH}_3 \quad \text{ADP} + P_i
\]

\[
\text{glutamate} \quad \text{glutamine}
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

bicarbonate + ATP → carbamoyl phosphate + ADP

Carbamoyl phosphate + ATP → carbamoyl phosphate + ADP
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>
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \rightarrow \text{carbonyl phosphate} \\
\text{carbamide} & \rightarrow \text{carbamoyl phosphate}
\end{align*}
\]
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:

\[
\begin{align*}
\text{Glutamine} & \quad \text{glutaminase} \\
\overset{(-\text{CH}_2\text{C} = \text{CO}_2^\text{(-)}}{\text{NH}_3} & \quad \overset{\text{transamination}}{\longrightarrow} \overset{(-\text{H}_2\text{CCH}_2\text{C} = \text{CO}_2^\text{(-)}}{\text{O}} & \overset{\text{oxaloacetate}}{\text{aspartate}} \\
\overset{(-\text{CH}_2\text{C} = \text{CO}_2^\text{(-)}}{\text{NH}_3} & \quad \overset{\text{transamination}}{\longrightarrow} \overset{(-\text{H}_2\text{CCH}_2\text{C} = \text{CO}_2^\text{(-)}}{\text{O}} & \overset{\text{α-ketoglutarate}}{\text{glutamate}} \\
\overset{\text{CH}_3 = \text{CO}_2^\text{(-)}}{\text{NH}_3} & \quad \overset{\text{transamination}}{\longrightarrow} \overset{\text{CH}_3 = \text{CO}_2^\text{(-)}}{\text{O}} & \overset{\text{pyruvate}}{\text{alanine}}
\end{align*}
\]

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparagine:

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

\[
\text{Glycine} \quad \text{NAD}^+ \quad \text{NADH} \quad \text{THF} \quad \text{N}^6-\text{N}^0-\text{methylene THF} \quad \text{CO}_2 \quad \text{NH}_4^+
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine} \quad \text{THF} \quad \text{N}^6-\text{N}^0-\text{methylene THF} \quad \text{Glycine}
\]

Serine Dehydratase:

\[
\text{Serine} \quad \text{H}_2\text{O} \quad \text{NH}_4^+
\]
Methionine Cycle
And Biological Methyl Groups

Methionine

S-Adenosyl Methionine

Homocysteine

S-Adenosyl Homocysteine

Serine

Cysteine

(remainder of homocysteine degraded for energy)
Deficiency: Alkaptonuria

"Ochronosis"

Phenylalanine and Tyrosine

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

Phenylalanine<br>\[
\begin{array}{c}
\text{NH}_3 \\
\text{CH} \quad \text{CH} \quad \text{COO}^-(+)\\
\end{array}
\]

\[\text{Tetrahydrobiopterin} + \text{O}_2\]

Dihydrobiopterin + \text{H}_2\text{O}

Enzyme: Phenylalanine hydroxylase

Phenylpyruvate

Tetrahydrobiopterin + \text{H}_2\text{O}

Dihydrobiopterin + \text{H}_2\text{O}

Enzyme: Phenylalanine hydroxylase

Tyrosine

\[\begin{array}{c}
\text{HO} \\
\text{CH} \quad \text{CH} \quad \text{COO}^-(+)\\
\end{array}\]

Homogentisate

Deficiency: Alkaptonuria

"Ochronosis"

Enzyme: Homogentisate dioxygenase

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

Isoleucine

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{CH} \quad \text{CH} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{CH} & \quad \text{NH}_3^{(+)} \\
\text{CH}_3 & \quad & \\
\alpha-\text{KG} & \\
\text{Glu} & \\
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{CH} \quad \text{C} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad & \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 & \\
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{CH} \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad & \\
\text{CH}_3 & \quad & \\
\text{continues on to degradation path similar to } \beta\text{-oxidation of fatty acids}
\end{align*}
\]

Leucine

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{CH} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{CH} & \quad \text{NH}_3^{(+)} \\
\text{CH}_3 & \quad & \\
\alpha-\text{KG} & \\
\text{Glu} & \\
\text{CH}_3\text{CHCH}_2 & \quad \text{CH} \quad \text{C} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad & \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 & \\
\text{CH}_3\text{CHCH}_2 & \quad \text{CH} \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad & \\
\text{CH}_3 & \quad & \\
\text{continues on to degradation path similar to } \beta\text{-oxidation of fatty acids}
\end{align*}
\]

Valine

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{CH} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{CH} & \quad \text{NH}_3^{(+)} \\
\text{CH}_3 & \quad & \\
\alpha-\text{KG} & \\
\text{Glu} & \\
\text{CH}_3\text{CH} & \quad \text{CH} \quad \text{C} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad & \\
\text{NAD}^+\text{CoASH} & \\
\text{NADH} + \text{CO}_2 & \\
\text{CH}_3\text{CH} & \quad \text{CH} \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad & \\
\text{CH}_3 & \quad & \\
\text{continues on to degradation path similar to } \beta\text{-oxidation of fatty acids}
\end{align*}
\]

--- Branched-chain \(\alpha\)-keto acid dehydrogenase ---
Synthesis of Bioactive Amines

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

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

Tryptophan → NAD+ → Tryptophan hydroxylase → 5-hydroxytryptophan → PLP-dependent decarboxylation → CO₂ → Serotonin
Synthesis of Bioactive Amines

Glutamate

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

\text{Glutamate decarboxylase (PLP-dependent)}

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

\(\gamma\)-aminobutyric acid (GABA)

Histidine

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

\text{Histidine decarboxylase (PLP-dependent)}

\[ \text{N} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{NH}_3^{(+)} \]

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