M1 - Renal, Fall 2007

Lyons, R.; Burney, R.

<http://hdl.handle.net/2027.42/64946>
http://hdl.handle.net/2027.42/64946
Nitrogen Metabolism (and Related Topics)

- Amino Acid Metabolism (Nitrogen metabolism)
- Folate Metabolism (“One-Carbon pathways”)
- Nucleotide Metabolism

Dr. Robert Lyons
Assistant Professor, Biological Chemistry
Director, DNA Sequencing Core
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:

\[
R_1\text{-C-}\text{coo}^(-) + R_2\text{-C-}\text{coo}^(-) \rightarrow R_1\text{-}\alpha\text{-keto acid (typically alpha-ketoglutarate)} + R_2\text{-C-}\text{coo}^(-) + \text{NH}_2\text{-amino acid (typically glutamate)}
\]

Details of reaction mechanism:

1. Amino acid
2. \(\text{H}^+\)
3. pyridoxal phosphate
4. \(\text{H}_2\text{O}\)
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):

Glutamate + oxaloacetate $\rightarrow$ $\alpha$-ketoglutarate + aspartate
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\begin{align*}
\text{glutamate} & \quad \xrightarrow{\text{NAD}(P)} \quad \text{NAD}(P)H \\
\text{NAD}(P)H & \quad \xrightarrow{\text{mito}} \quad \text{α-ketoglutarate} \quad \text{ammonia}
\end{align*}
\]

**Glutamine Synthetase:**

\[
\begin{align*}
\text{glutamate} & \quad \xrightarrow{\text{ATP+NH}_3} \quad \text{ADP+P}_i \\
\text{glutamine} & \quad \text{NH}_3
\end{align*}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{glutamine} & \quad \text{glutamate} \\
(-)_{\text{NH}_3}^{\text{H}} \text{C} = \text{CH}_2 \text{CH}_2 \text{O} & \quad (-)_{\text{NH}_3}^{\text{H}} \text{C} = \text{CH}_2 \text{CH}_2 \text{O} \\
\text{glutamate} & \quad \text{NH}_3
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase} & \\
\text{Glutamate} & + \text{oxaloacetate} \quad \text{\rightarrow} \quad \text{aspartate} + \text{\alpha-keto glutarate}
\end{align*}
\]
2ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate

2ADP + P_i → 2NH₃ + H₂O + ATP

Liver mitochondria

Ornithine

Liver cytoplasm

Citrrline

Ornithine

Urea

Argirine

Argininosuccinate

Fumarate

ATP + AMP + P_i → H₂O + NH₃ + aspartate
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{NH}_3} \text{carbamate} \xrightarrow{\text{ATP}}$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

$\text{Ornithine}$

$\text{Citrulline}$
Argininosuccinate synthetase

\[
\begin{align*}
\text{Citrulline} & \rightarrow \text{Argininosuccinate} \\
\text{aspartate} & \rightarrow \text{AMP + PP}_i
\end{align*}
\]
Argininosuccinate lyase

\[
\text{Argininosuccinate} \rightarrow \text{Arginine} \rightarrow \text{Fumarate}
\]
Arginase

\[
\begin{align*}
\text{Arginine} & \quad \overset{\text{H}}{\text{H}} \quad \overset{\text{NH}_3}{\text{NH}}^+ \quad \overset{\text{(-)}}{\text{OOC-C-CH}_2\text{CH}_2\text{CH}_2\text{NH}-\text{C-NH}_2} \quad \text{Arginine} \\
\text{Urea} & \quad \overset{\text{H}_2\text{O}}{\text{O}} \quad \overset{\text{(-)}}{\text{OOC-C-CH}_2\text{CH}_2\text{CH}_2\text{NH}_3^+} \quad \text{Ornithine}
\end{align*}
\]
Urea Cycle Connects to TCA Cycle

1. Ornithine → Citrulline
2. Citrulline → Argininosuccinate
3. Argininosuccinate → Fumarate
   - Fumarate → Oxaloacetate
   - Oxaloacetate → α-Ketoglutarate

Urea Cycle:
- Urea → Arginine
- Arginine → Ornithine

Aspartate:
- Aspartate → Citrulline

TCA Cycle:
- Citrate → α-Ketoglutarate
- α-Ketoglutarate → Fumarate
- Fumarate → Oxaloacetate
- Oxaloacetate → α-Ketoglutarate
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

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

**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)

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

<table>
<thead>
<tr>
<th>Enzyme</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 the synthesis of arginine from ornithine.

1. Carboxamoyl phosphate is formed from ATP, HCO₃⁻, and NH₃.
2. Ornithine is converted to citrulline in the liver mitochondrion.
3. Citrulline is converted to arginine in the liver cytoplasm.
4. Argininosuccinate is converted to fumarate and arginine.
5. Urea is synthesized from arginine in the liver cytoplasm.

The urea cycle is a series of reactions that convert ammonia into urea, which is then excreted from the body.
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbonyl phosphate} \\
\text{carbamate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbamoyl phosphate}
\end{align*}
\]
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:

- Glutamine: \[ \text{Glutamine} \rightarrow \text{glutamate} + \text{ammonia} \]
- Asparagine: \[ \text{Asparagine} \rightarrow \text{aspartate} + \text{ammonia} \]

We also already know how to degrade Glutamine:

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

…and by analogy, how to degrade Asparagine:

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

\[
\text{Glycine} \quad \xrightarrow{\text{Glycine Synthase}} \quad \text{Threonine} + \text{NAD}^+ \rightarrow \text{DHAP} + \text{NH}_4^+ + \text{NADH}
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine} \quad \xrightarrow{\text{Serine Hydroxymethyltransferase}} \quad \text{3-Methylglutaryl-CoA} + \text{NAD}^+ \rightarrow \text{HMG-CoA} + \text{NH}_4^+ + \text{NADH}
\]

Serine Dehydratase:

\[
\text{Serine} \quad \xrightarrow{\text{Serine Dehydratase}} \quad \text{3-Phosphoglycerate} + \text{NH}_4^+ + \text{NAD}^+ \rightarrow \text{Succinate} + \text{CO}_2 + \text{NADH}
\]
Methionine Cycle
And Biological Methyl Groups
Deficiency:
Alkaptonuria
"Ochronosis"

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

Phenylalanine

Tetrahydrobiopterin + O₂
Dihydrobiopterin + H₂O

Enzyme: Phenylalanine hydroxylase

Tyrosine

Homogentisate

Deficiency:
Alkaptonuria
"Ochronosis"

Enzyme: homogentisate dioxygenase

Phenylpyruvate

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

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

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

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

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

\[
\text{Glu} \quad \text{NAD}^{+}\text{CoASH} \quad \text{NADH} + \text{CO}_2
\]

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

\[
\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{S-CoA} \quad \text{NADH} + \text{CO}_2
\]

\[
\text{CH}_3\text{CHCH}_2 - \text{C} - \text{S-CoA} \quad \text{NADH} + \text{CO}_2
\]

\[
\text{CH}_3\text{CH} - \text{C} - \text{S-CoA} \quad \text{NADH} + \text{CO}_2
\]

(continues on to degradation path similar to \(\beta\)-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

NAD+ → CO₂

NH₃ (+) → NH₃ (+)
Synthesis of Bioactive Amines

Glutamate

\[ \text{COO} \rightarrow \text{CH}_2 \text{CH}_2 \text{CH} \rightarrow \text{COO} \]

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

\[ \text{Glutamate decarboxylase (PLP-dependent)} \]

\[ \gamma\text{-aminobutyric acid (GABA)} \]

Histidine

\[ \text{N} \]

\[ \text{CH}_2 \text{CH} \rightarrow \text{COO} \]

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

\[ \text{Histidine decarboxylase (PLP-dependent)} \]

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