M1 - Renal, Fall 2007

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Viewer discretion advised: Material may contain medical images that may be disturbing to some viewers.
Nitrogen Metabolism (and Related Topics)

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

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Assistant Professor, Biological Chemistry
Director, DNA Sequencing Core
Supplementary study material on the Web:

http://seqcore.brcf.med.umich.edu/mcb500

There are also PDF’s of class handouts with supplemental information available in the table of contents for this course.
Amino Acid metabolism

- Amino acids
  - Glu, Gln, Asp, NH₃
  - Urea

Folate metabolism

- Methylene THF
  - Met Cycle

TCA Cycle

- oxaloacetate
  - fumarate

Nucleic Acid metabolism

- Purines
  - DNA
  - RNA
  - Pyrimidines
  - (energy)
  - Uric Acid
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:

\[ R_1\text{C-}\text{coo}(-) + R_2\text{C-}\text{coo}(-) \xrightarrow{\alpha\text{-keto acid (typically alpha-ketoglutarate)}} R_1\text{C-}\text{coo}(-) + R_2\text{C-}\text{coo}(-) \]

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

Details of reaction mechanism:

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

\[ R_1\text{C-}\text{coo}(-) + R_2\text{C-}\text{coo}(-) \xrightarrow{\alpha\text{-keto acid (typically glutamate)}} R_1\text{C-}\text{coo}(-) + R_2\text{C-}\text{coo}(-) \]

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

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

**Glutamine Synthetase:**

\[
\text{Glutamate} \quad \xrightarrow{\text{ATP} + \text{NH}_3} \quad \text{ADP} + \text{P}_i \quad \xrightarrow{\text{NH}_3} \quad \text{Glutamine}
\]
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:

\[
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} + \text{Oxaloacetate} \rightarrow \text{Aspartate} + \alpha\text{-Keto glutarate}
\]
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

Citrulline

Argininosuccinate

Aspartate

ATP

AMP + PPi
Argininosuccinate lyase

Argininosuccinate $\xrightarrow{-} \text{Arginine}$

Fumarate $\xrightarrow{-} \text{Arginine}$
Arginase

\[
\begin{align*}
\text{Arginine} & \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{Urea} \\
\text{Ornithine} &
\end{align*}
\]
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Argininosuccinate
- Arginine

Urea Cycle:
- Ornithine → Citrulline → Argininosuccinate → Arginine → Ornithine

TCA Cycle:
- Oxaloacetate → Malate → Fumarate → α-Ketoglutarate → Citrate

Aspartate:
\[ {\text{H}} \]
\[ (-\text{b}_2\text{CCH}_2\text{C}-\text{CO}_2\text{NH}_2) \]

Fumarate:
\[ (-\text{b}_2\text{C}-\text{C}=\text{C}-\text{CO}_2\text{H}) \]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
(-)\text{O}_2\text{CCH}_2\text{CH}_2\text{C}\text{CO}_2^- \xrightarrow{\text{mito}} \text{NH}_2 \xrightarrow{\text{NAD}(P)} \quad \text{H} \quad \xrightarrow{\text{NAD}(P)H} \quad \text{(-)}\text{O}_2\text{CCH}_2\text{CH}_2\text{C}\text{CO}_2^- + \text{NH}_3
\]

Glutamine Synthetase:

\[
\text{(-)}\text{OOC-CH}_2\text{CH}_2\text{COO}^- \xrightarrow{\text{ATP}+\text{NH}_3} \quad \text{H} \quad \xrightarrow{\text{ADP}+\text{P}_i} \quad \text{(-)}\text{OOC-CH}_2\text{CH}_2\text{C} \text{NH}_2
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)
Complicating the picture: Other tissues may be involved

Muscle:
- Amino acids:
  - Transamination
  - Deamination
- Alanine → Glutamate → Glutamine → NH₄⁺
- purine deamination:
  - NH₄⁺

Intestine:
- Glutamine
- Alanine → NH₄⁺ → Citrulline

Kidney:
- Glutamine
- NH₃
- NH₄⁺
- Citrulline
- Arginine

Liver:
- Glutamine
- Arginine
- NH₄⁺
- Urea
- Alanine → Glu → Aspartate
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>
</tr>
</tbody>
</table>
2ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate → NH₃⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-
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)

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:

\[ \text{Glutamine} \rightarrow \text{Glutamate} + \text{Ammonia} \]

\[ \text{Asparagine} \rightarrow \text{Aspartate} + \text{Ammonia} \]

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:

\[
\begin{align*}
\text{Glycine} & \quad \text{THF} \quad \text{N}^6-\text{N}^\circ - \text{methylene THF} \\
\text{Glycine} & \quad \text{THF} \quad \text{N}^6-\text{N}^\circ - \text{methylene THF} \\
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Serine} & \quad \text{THF} \quad \text{N}^6-\text{N}^\circ - \text{methylene THF} \\
\text{Glycine} & \quad \text{THF} \quad \text{N}^6-\text{N}^\circ - \text{methylene THF} \\
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{H}_2\text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{H}_2\text{O} \\
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups

Methionine

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

S-Adenosyl Methionine

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

Homocysteine

\[ \text{HS} - \text{CH}_2 - \text{CH}_2 - \text{C} - \text{COO}^{-} \]

S-Adenosyl Homocysteine

Serine

\[ \text{HO} - \text{CH}_2 - \text{C} - \text{COO}^{-} \]

Cysteine

(remainder of homocysteine degraded for energy)
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

\[
\begin{align*}
\text{Phenylalanine} & \xrightarrow{\text{Tetrahydrobiopterin + } O_2} \text{Dihydrobiopterin + } H_2O \\
& \xrightarrow{\text{Enzyme: Phenylalanine hydroxylase}} \text{Tyrosine} \\
& \xrightarrow{\text{Enzyme: homogentisate dioxygenase}} \text{Homogentisate} \\
& \xrightarrow{\text{Deficiency: Alkaptonuria “Ochronosis”}} \text{Phenylpyruvate}
\end{align*}
\]
Branched Chain Amino Acids

Isoleucine

Leucine

Valine

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

Glu

NAD⁺, CoASH

NAD⁺, CoASH

NAD⁺, CoASH

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

(NADH + CO₂)

NADH + CO₂

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 $\xrightarrow{(+)}$ NAD$^+$

Tryptophan hydroxylase $\rightarrow$ 5-hydroxytryptophan

PLP-dependent decarboxylation $\rightarrow$ CO$_2$

Serotonin $\xrightarrow{(+)}$
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