Unless otherwise noted, the content of this course material is licensed under a Creative Commons Attribution – Share Alike 3.0 License.

Copyright 2007, Robert Lyons.

The following information is intended to inform and educate and is not a tool for self-diagnosis or a replacement for medical evaluation, advice, diagnosis or treatment by a healthcare professional. You should speak to your physician or make an appointment to be seen if you have questions or concerns about this information or your medical condition. You assume all responsibility for use and potential liability associated with any use of the material.

Material contains copyrighted content, used in accordance with U.S. law. Copyright holders of content included in this material should contact open.michigan@umich.edu with any questions, corrections, or clarifications regarding the use of content. The Regents of the University of Michigan do not license the use of third party content posted to this site unless such a license is specifically granted in connection with particular content objects. Users of content are responsible for their compliance with applicable law. Mention of specific products in this recording solely represents the opinion of the speaker and does not represent an endorsement by the University of Michigan.

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

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
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
- Uric Acid
- (energy)
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:

\[
\begin{align*}
\text{amino acid} & \quad \xrightarrow{\text{H}} \quad \text{amino acid} \\
\text{R-C-}\text{coo}^{(-)} & \quad \xrightarrow{\text{H}} \quad \text{R-C-}\text{coo}^{(-)} \\
\text{NH}_2 & \quad \xrightarrow{\text{O}} \quad \text{NH}_2 \\
\text{H}_2\text{O} & \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{H}_2\text{O} \\
\text{pyridoxal phosphate} & \quad \xrightarrow{\text{pyridoxal phosphate}} \quad \text{pyridoxamine phosphate}
\end{align*}
\]
Transfer the amine back to an acceptor $\alpha$-keto acid

\[
\begin{align*}
\text{pyridoxamine phosphate} & \quad + \quad R-C-COO^- \\
\text{amin}o \text{ acid} & \quad \rightarrow \quad \text{pyridoxal phosphate} & \quad + \quad R-C-COO
\end{align*}
\]
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:**

\[ \text{glutamate} \xrightarrow{\text{NAD}(P)} \text{NAD(P)H} \xrightarrow{\text{mito}} \text{\(\cdot\text{O}_2\text{CCH}_2\text{CH}_2\text{C}-\text{CO}^2\cdot\)} + \text{NH}_3 \]

\[ \text{\(\cdot\text{O}_2\text{CCH}_2\text{CH}_2\text{C}-\text{CO}^2\cdot\)} \]

**Glutamine Synthetase:**

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

\[ \text{glutamine} \xrightarrow{\text{ADP+P}_i} \text{glutamate} \]
Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

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:

\[
\text{Glutamate} + \text{Oxaloacetate} \rightarrow \text{Aspartate} + \text{α-Ketoglutarate}
\]
Carbamoyl phosphate synthetase I

bicarbonate $\rightarrow$ carbonyl phosphate $\rightarrow$ carbamate $\rightarrow$ carbamoyl phosphate

ATP $\rightarrow$ ADP $\rightarrow$ ATP $\rightarrow$ ADP
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline

$\text{NH}_2\text{C}(-)\text{OOC}\text{CH}_2\text{CH}_2\text{NH}_3^+$

$\text{NH}_2\text{C}(-)\text{OOC}\text{CH}_2\text{CH}_2\text{NH}_3^+$

$\text{NH}_2\text{C}(-)\text{OOC}\text{CH}_2\text{CH}_2\text{NH}_3^+$

$\text{NH}_2\text{C}(-)\text{OOC}\text{CH}_2\text{CH}_2\text{NH}_3^+$
Argininosuccinate synthetase

\[ \text{aspartate} \rightarrow \text{Citrulline} \rightarrow \text{Argininosuccinate} \]

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

Argininosuccinate $\xrightarrow{\text{Argininosuccinate lyase}}$ Fumarate $\xrightarrow{\text{Argininosuccinate lyase}}$ Arginine
Arginase

Arginine → Urea → Ornithine
1. Carbamoyl phosphate + ATP + NH₃ → 2ADP + H⁺ + P_I + NH₂C–OPO₃⁻
2. Ornithine + Citrulline → Argininosuccinate
3. Argininosuccinate + ATP → Arginine + Fumarate
4. Arginine + ATP → Urea + H₂O
Urea Cycle Connects to TCA Cycle

Urea Cycle
- Ornithine
- Citrulline
- Argininosuccinate

TCA Cycle
- Aspartate
- Oxaloacetate
- Malate
- Fumarate
- α-Ketoglutarate
- Citrate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\begin{align*}
(\cdot\cdot\cdot_{\text{O}}\text{CCH}_{2}\text{CHC}-\text{CO}_{2}(\cdot) \\
\text{Glutamate} & \xrightarrow{\text{NAD}(P)} \xrightarrow{\text{(mito)}} \text{NH}_{2} \\
\text{glutamate} & \xrightarrow{\text{NAD}(P)H}} \rightarrow \xrightarrow{\alpha-\text{keto glutarate}} + \text{ammonia}
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
(\cdot\cdot\cdot_{\text{OOC}}\text{CCH}_{2}\text{CHCOO}(\cdot) \\
\text{Glutamate} \xrightarrow{\text{ATP}+\text{NH}_{3}} \rightarrow \text{H} \\
\text{glutamate} & \xrightarrow{\text{ADP}+\text{P}_{i}} \rightarrow \xrightarrow{\text{NH}_{3}} \text{glutamine}
\end{align*}
\]
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)

\[
\text{bicarbonate} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
\]
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>Condition</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

\[ \text{glutamate} + \text{acetyl CoA} \rightarrow \text{N-acetyl glutamate (NAG)} \]

(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:

\[
\begin{align*}
\text{Glutamine} & \xrightarrow{\text{aspartate}} \text{Oxaloacetate} \\
\text{Asparagine} & \xrightarrow{\text{aspartate}} \text{A-Ketoglutарате}
\end{align*}
\]

We also already know how to degrade Glutamine:

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

...and by analogy, how to degrade Asparagine:

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate + ammonia}
\]
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 $\alpha$-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

**Glycine Synthase:**

\[
\begin{align*}
\text{Glycine} & \quad \text{NAD}^+ \quad \text{NADH} \\
\text{THF} & \quad \text{N}^6-\text{N}^{\delta}-\text{methylene THF} \\
\text{CO}_2 + \text{NH}_4^+ & 
\end{align*}
\]

**Serine Hydroxymethyltransferase:**

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

**Serine Dehydratase:**

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

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

Phenylalanine + Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O

Phenylalanine hydroxylase

Phenylalanine → Tyrosine

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Phenylalanine → Phenylpyruvate

Enzyme: homogentisate dioxygenase

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

Isoleucine

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

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

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

\[\text{Glu}\]

Leucine

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

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

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

\[\text{Glu}\]

Valine

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

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

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

\[\text{Glu}\]

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

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

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

\[\text{NAD}^+\text{CoASH}\]

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

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

\[\text{NAD}^+\text{CoASH}\]

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

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

\[\text{NAD}^+\text{CoASH}\]

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

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

Tyrosine $\rightarrow$ Dihydroxyphenylalanine (L-DOPA)

Tyrosine hydroxylase

Dopamine $\rightarrow$ Norepinephrine $\rightarrow$ Epinephrine
Synthesis of Bioactive Amines
Synthesis of Bioactive Amines

Glutamate

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

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

\[ \text{NH}_3 \]

\[ (+) \]

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

Histidine

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

\[ \text{Histidine} \]

\[ \text{NH}_3 \]

\[ (+) \]

\[ \text{Histamine} \]
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