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**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

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:

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

\[
\rightarrow R_1\text{C--coo}^{(-)} + R_2\text{C--coo}^{(-)} \qquad \text{amino acid (typically glutamate)}
\]

Details of reaction mechanism:

\[
\text{amino acid} \quad R\text{C--coo}^{(-)} \quad \text{H}_2\text{O} \quad \text{pyridoxal phosphate}\]

\[
\rightarrow \text{H}^+ \quad \text{N} \quad \text{C--coo}^{(-)} \quad \text{pyridoxamine phosphate}
\]
Transfer the amine back to an acceptor α-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:**

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

**Glutamine Synthetase:**

\[
\text{glutamate} \xrightarrow{\text{ATP}+\text{NH}_3} \text{ADP}+\text{P}_i \xrightarrow{\text{glutamine}} \text{\(\text{\(-\text{OOC}\text{C}--\text{CH}_2\text{CH}_2\text{COO}^-\)}}\text{\(\text{\(+\)}\text{\(+\)}\text{\(+\)}} \rightarrow \text{\(\text{\(-\text{OOC}\text{C}--\text{CH}_2\text{CH}_2\text{C}--\text{NH}_2\)}}\text{\(\text{\(+\)}\text{\(+\)}\text{\(+\)}}
\]

\[
\text{glutamate} \xrightarrow{\text{ATP}+\text{NH}_3} \text{ADP}+\text{P}_i \xrightarrow{\text{glutamine}} \text{\(\text{\(-\text{OOC}\text{C}--\text{CH}_2\text{CH}_2\text{C}--\text{NH}_2\)}}\text{\(\text{\(+\)}\text{\(+\)}\text{\(+\)}}
\]

\[
\text{glutamate} \xrightarrow{\text{ATP}+\text{NH}_3} \text{ADP}+\text{P}_i \xrightarrow{\text{glutamine}} \text{\(\text{\(-\text{OOC}\text{C}--\text{CH}_2\text{CH}_2\text{C}--\text{NH}_2\)}}\text{\(\text{\(+\)}\text{\(+\)}\text{\(+\)}}
\]
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 $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{NH}_3}$ carbamate $\xrightarrow{\text{ATP}}$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

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

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

Arginine $\rightarrow$ Ornithine

Arginine $\rightarrow$ Urea $\rightarrow$ Ornithine

$\text{H}_2\text{O}$
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[ \text{H} \quad + \quad \text{NAD(P)} \quad \rightarrow \quad \text{NAD(P)H} \quad + \quad \text{NH}_3 \quad \]

\[ \text{(-O}_2 \text{C-CH}_2 \text{CH}_2 \text{C=}\text{CO}_2\text{NH}_2} \quad \rightarrow \quad \text{(-O}_2 \text{C-CH}_2 \text{CH}_2 \text{C=}\text{CO}_2 \quad + \quad \text{NH}_3 \]

Glutamine Synthetase:

\[ \text{H} \quad + \quad \text{ATP} + \text{NH}_3 \quad \rightarrow \quad \text{ADP} + \text{P}_i \]

\[ \text{(-O}_2 \text{C-CH}_2 \text{CH}_2 \text{C}=\text{CO}_2\text{NH}_3} \quad \rightarrow \quad \text{(-O}_2 \text{C-CH}_2 \text{CH}_2 \text{C}=\text{CO}_2 \quad + \quad \text{NH}_3 \]

\[ \text{H} \quad + \quad \text{glutamate} \quad \rightarrow \quad \text{ADP} + \text{P}_i \]

\[ \text{NH}_3 \quad + \quad \text{glutamine} \quad \rightarrow \quad \text{ATP} + \text{NH}_3 \]

\[ \text{(+)} \quad \text{(+)} \quad \text{(+)} \quad \text{(+)} \]
CPS I is Stimulated by NAG

Glutamate + Acetyl CoA \rightarrow \text{N-acetyl glutamate (NAG)}

(repeating the figure from page 3 of your handout)

bicarbonate → carboxylic phosphate → carbamate → 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></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSD</td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
</tr>
<tr>
<td>OTCO</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>
2 ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate

2 ADP + P₃ → P₄

Ornithine → Citrulline

Liver mitochondrion → Liver cytoplasm

Ornithine → Citrulline

Urea

Arginine

Argininosuccinate

Fumarate
**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 $\rightarrow$ carbonyl phosphate $\rightarrow$ carbamate $\rightarrow$ carbamoyl phosphate
1. Carbamoyl phosphate + ATP $\rightarrow$ OCPH$_2$NH$_2$ + ADP + P$_i$

2. OCPH$_2$NH$_2$ + ATP $\rightarrow$ Citrulline + AMP + P$_i$

3. Citrulline $\rightarrow$ Argininosuccinate + NH$_3$

4. Argininosuccinate + H$_2$O $\rightarrow$ Arginine + Fumarate

5. Ornithine + NH$_3$ + H$_2$O $\rightarrow$ Urea + CO$_2$
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:

\[ \text{Glycine Synthase:} \quad \text{Glycine} \quad \xrightarrow{\text{NAD}^+} \quad \text{CO}_2 \quad + \quad \text{NH}_4^+ \]

\[ \text{THF} \quad \text{N}^+\text{N}^0\text{methylene THF} \]

Serine Hydroxymethyltransferase:

\[ \text{Serine} \quad \xrightarrow{\text{THF}} \quad \text{Glycine} \quad \xrightarrow{\text{N}^+\text{N}^0\text{methylene THF}} \]

Serine Dehydratase:

\[ \text{Serine} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{Serine} \quad \xrightarrow{\text{NH}_4^+} \quad \text{Glycine} \]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Deficiency:
Alkaptonuria
“Ochronosis”

Phenylalanine

(+)
NH₃

Tetrahydrobiopterin + O₂

Dihydrobiopterin + H₂O

(-)

Phenylalanine hydroxylase

Enzyme:

HO

CH₂

CH

COO

Tyrosine

(+)
NH₃

Homogentisate

Deficiency:
Alkaptonuria
“Ochronosis”

Enzyme:

Enzyme:

Homogentisate dioxygenase

(you don’t need to know the rest)

Phenylpyruvate

Phenylketonuria
(no phenylalanine hydroxylase)
Branched Chain Amino Acids

Isoleucine

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

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

\[ \alpha-KG \]

\[ \text{Glu} \]

\[ \text{CH}_3\text{CH}_2\text{CH}(\text{−})\text{CH}_2\text{C}=\text{O} \]

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

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

\[ \text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{SCoA} \]

Leucine

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

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

\[ \alpha-KG \]

\[ \text{Glu} \]

\[ \text{CH}_3\text{CH}(\text{−})\text{CH}_2\text{CH}(\text{−})\text{CO} \]

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

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

\[ \text{CH}_3\text{CH}(\text{−})\text{CH}_2\text{CH}(\text{−})\text{SCoA} \]

Valine

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

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

\[ \alpha-KG \]

\[ \text{Glu} \]

\[ \text{CH}_3\text{CH}(\text{−})\text{CH}\text{C}=\text{O} \]

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

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

\[ \text{CH}_3\text{CH}(\text{−})\text{CH}\text{SCoA} \]

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

Tyrosine $\rightarrow$ Dihydroxyphenylalanine (L-DOPA)

Tyrosine hydroxylase

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

Tryptophan \[\rightarrow\] 5-hydroxytryptophan

\[\text{Tryptophan hydroxylase}\]

5-hydroxytryptophan \[\rightarrow\] Serotonin

\[\text{PLP-dependent decarboxylation}\]

Serotonin \[\rightarrow\] NAD+

\[\text{CO}_2\]
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