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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

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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

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:

\[ R_1\text{-C-} \text{coo}(-) + R_2\text{-C-} \text{coo}(-) \rightarrow R_1\text{-C-} \text{coo}(-) + R_2\text{-C-} \text{coo}(-) \]

\[ \overset{\alpha\text{-keto}}{\overset{\text{acid}}{\overset{\text{amino}}{\overset{\text{acid}}{\overset{\text{(typically}}{\overset{\text{alpha-ketoglutarate)}}}}}} \]

\[ \overset{\alpha\text{-keto}}{\overset{\text{acid}}{\overset{\text{amino}}{\overset{\text{acid}}{\overset{\text{(typically glutamate)}}}}} \]

Details of reaction mechanism:

1. Amino acid
2. \[ R_1\text{-C-} \text{coo}(-) \]
3. \[ R_2\text{-C-} \text{coo}(-) \]
4. Pyridoxal phosphate
5. H\(^+\)
6. Water
7. 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:**

\[
\text{glutamate} \rightarrow \text{NAD}^+ \rightarrow \alpha\text{-ketoglutarate} + \text{ammonia}
\]

**Glutamine Synthetase:**

\[
\text{glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamine} + \text{ADP} + \text{P}_i
\]
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} + \text{Oxaloacetate} \rightarrow \text{Aspartate} + \text{\(\alpha\)-Keto glutarate} \]
2ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate

2ADP + P₃ → ATP + AMP + P₃

Citrulline → Ornithine

Liver mitochondrion

Liver cytoplasm

Ornithine → Argininosuccinate

Argininosuccinate → Urea

Urea → Fumarate

ATP → Aspartate

Aspartate → Ornithine

Citrulline
Carbamoyl phosphate synthetase I

bicarbonate \[\text{HO-C-O}^{(-)}\] → carbonyl phosphate \[\text{HO-C-O}^{\text{P}}\] → carbamate \[\text{HO-C-NH}_2\] → carbamoyl phosphate \[\text{PO-C-NH}_2\]
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

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

Arginine → Urea → Ornithine
Urea Cycle Connects to TCA Cycle

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

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

Aspartate:
- $\text{H} \left( -\text{C}_2\text{H}_2\text{C}_2\text{C}_2\text{NH}_2 \right)$
Getting Amines Into the Liver

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

Glutamine Synthetase:
\[
\text{Glutamate} \quad \overset{(\text{ATP} + \text{NH}_3)}{\text{glutamate}} \quad \overset{(\text{ADP} + P_i)}{\text{glutamine}}
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbonyl phosphate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbamate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbamoyl phosphate} \\
\text{ADP} & \quad \text{ADP} & \quad \text{ADP} & \\
\end{align*}
\]
Complicating the picture: Other tissues may be involved

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

Intestine:
- Glutamine
- Alanine → NH₄⁺ → Citrulline

Kidney:
- Glutamine
- NH₃
- Arginine
- Citrulline

Liver:
- Glutamine
- Alanine
- Glu → Aspartate
- NH₄⁺ → Urea

Arginine
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>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>CPSD</strong></td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
</tr>
<tr>
<td><strong>OTCD</strong></td>
<td>Elevated CP causes synthesis of Orotate</td>
</tr>
<tr>
<td><strong>ASD</strong></td>
<td>Elevated citrulline</td>
</tr>
<tr>
<td><strong>ALD</strong></td>
<td>Elevated argininosuccinate</td>
</tr>
<tr>
<td><strong>AD</strong></td>
<td>Elevated arginine</td>
</tr>
</tbody>
</table>
CPS I is Stimulated by NAG

\[
\text{(-)}\quad \text{ooc} \quad \text{c} \quad \text{ch}_{2} \text{ch}_{2} \text{c} \quad \text{=} \quad \text{h} \\
\text{NH}_{3} \\
\text{(+)}
\]

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

(repeating the figure from page 3 of your handout)

\[
\text{bicarbonate} \quad \text{ATP} \quad \text{ADP} \quad \text{carbonyl phosphate} \quad \text{ATP} \quad \text{carbamoyl phosphate}
\]
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:

\[ \text{Aspartate} \quad \text{glutaminate} \quad \text{oxaloacetate} \]
\[ \text{Glutamate} \quad \text{α-ketoglutarate} \]
\[ \text{Alanine} \quad \text{Pyruvate} \]

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 $\alpha$-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

**Glycine Synthase:**

\[
(-)\text{OOC} - \overset{\text{H}}{\text{C}} - \overset{\text{NH}_3}{\text{H}}^{(+)}
\]

\[
\xrightarrow{\text{THF}} \overset{\text{CO}_2 + \text{NH}_4^{(+)}\text{N}_5^\text{N}_0\text{methylene THF}}{\xleftarrow{\text{NAD}^{+}}} \overset{\text{NADH}}{\xrightarrow{\text{H}_2\text{O}}}
\]

**Serine Hydroxymethyltransferase:**

\[
(-)\text{OOC} - \overset{\text{CH}}{\text{C}} - \overset{\text{NH}_3}{\text{H}}^{(+)} \overset{\text{CH}_2\text{OH}}{\text{H}_2\text{O}}
\]

\[
\xrightarrow{\text{THF}} \overset{\text{N}_5^\text{N}_0\text{methylene THF}}{\xrightarrow{\text{Glycine}}} \overset{\text{Serine}}{\xleftarrow{\text{NAD}^{+}}} \overset{\text{NADH}}{\xrightarrow{\text{H}_2\text{O}}}
\]

**Serine Dehydratase:**

\[
(-)\text{OOC} - \overset{\text{CH}}{\text{C}} - \overset{\text{NH}_3}{\text{H}}^{(+)} \overset{\text{CH}_2\text{OH}}{\text{H}_2\text{O}}
\]

\[
\xrightarrow{\text{H}_2\text{O}} \overset{\text{H}^+}{\xrightarrow{\text{CH}_3}} \overset{\text{CH}_3}{\xleftarrow{\text{H}_2\text{O}}} \overset{\text{H}^+}{\xrightarrow{\text{CH}_3}} \overset{\text{H}_2\text{O}}{\xrightarrow{\text{H}^+}} \overset{\text{OOC} - \overset{\text{C}}{\text{C}} - \overset{\text{O}}{\text{H}}^{(+)} \overset{\text{CH}_3}{\text{CH}_3}}{\text{CO}_2 + \text{NH}_4^{(+)}\text{N}_5^\text{N}_0\text{methylene THF}}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine

\[ \text{NH}_3 \]

\[ \text{NH}_3 \]

Tetrahydrobiopterin + O\(_2\)

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

Enzyme: Phenylalanine hydroxylase

\[ \text{OH} \]

Tyrosine

\[ \text{NH}_3 \]

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: Homogentisate dioxygenase

(You don’t need to know the rest)

Phenylpyruvate

Phenylketonuria

(no phenylalanine hydroxylase)
Branched Chain Amino Acids

Isoleucine

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{CH} \quad \text{C} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{NH}_3^{(+)} \\
\end{align*}
\]

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

\[\text{Glu}\]

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{C} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \\
\end{align*}
\]

Leucine

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{CH} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{NH}_3^{(+)} \\
\end{align*}
\]

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

\[\text{Glu}\]

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{C} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \\
\end{align*}
\]

Valine

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{CH} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \text{NH}_3^{(+)} \\
\end{align*}
\]

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

\[\text{Glu}\]

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{C} \quad \text{COO}^{(-)} \\
\text{CH}_3 & \quad \\
\end{align*}
\]

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

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

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

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

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \\
\end{align*}
\]

\[\text{NADH} + \text{CO}_2 \quad 2\]

\[
\begin{align*}
\text{CH}_3\text{CHCH}_2 & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \\
\end{align*}
\]

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

\[
\begin{align*}
\text{CH}_3\text{CH} & \quad \text{C} \quad \text{S-CoA} \\
\text{CH}_3 & \quad \\
\end{align*}
\]

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

(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
Synthesis of Bioactive Amines

- Glutamate \( \rightarrow \) \( \gamma \)-aminobutyric acid (GABA) via Glutamate decarboxylase (PLP-dependent)
- Histidine \( \rightarrow \) Histamine via Histidine decarboxylase (PLP-dependent)
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