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M1 - Renal, Fall 2007

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Nitrogen Metabolism (and Related Topics)

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

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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:

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

\(\text{alpha-keto acid (typically alpha-ketoglutarate)}\)

Details of reaction mechanism:

\[
\begin{align*}
\text{amino acid} & \quad \text{amino acid} \\
R-C-\text{coo}^{(-)} & \quad R-C-\text{coo}^{(-)} \\
\text{H} & \quad \text{H} \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{O} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{pyridoxal phosphate} & \quad \text{pyridoxal phosphate} \\
\end{align*}
\]

\[
\text{H}^{+} \quad \text{H}^{+} \quad \text{H}^{+} \quad \text{H}^{+}
\]

\[
\text{HCH} \quad \text{HCH} \quad \text{HCH} \quad \text{HCH}
\]

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{OCH}_2 & \quad \text{OCH}_2 \\
\text{OH} & \quad \text{OH} \\
\text{H} & \quad \text{H} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{pyridoxamine phosphate} & \quad \text{pyridoxamine phosphate}
\end{align*}
\]
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 + $\alpha$-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:

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

Glutamine Synthetase:

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

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

bicarbonate $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{ADP}}$ carbamoyl phosphate $\xrightarrow{\text{ADP}}$ carbamoyl phosphate
Argininosuccinate synthetase

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

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

\[
\text{aspartate}
\]

\[
\text{(-)}\text{O}_2\text{CCH}_2\text{C} - \text{CO}_2\text{(-)}
\]

\[
\text{(-)}\text{OOC} - \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} = \text{NH}_2^{(+)}
\]

\[
\text{(-)}\text{OOC} - \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} = \text{NH}_2^{(+)}
\]

\[
\text{(-)}\text{O}_2\text{CCH}_2\text{C} - \text{CO}_2\text{(-)}
\]

\[
\text{(-)}\text{OOC} - \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} = \text{NH}_2^{(+)}
\]
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Fumarate $\rightarrow$ Arginine
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine → Citrulline → Argininosuccinate → Arginine

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

**Glutamate Dehydrogenase:**

\[
\begin{align*}
\text{Glutamate} & \quad \text{NH}_2 \\
\quad & \quad \text{mito} \\
\text{NAD(P)} & \quad \text{NAD(P)H} \\
\text{H} & \quad \alpha\text{-ketoglutarate} \\
\text{NH}_3 & + \quad \text{ammonia}
\end{align*}
\]

**Glutamine Synthetase:**

\[
\begin{align*}
\text{Glutamate} & \quad \text{NH}_3 \\
\quad & \quad \text{ATP} + \text{NH}_3 \\
\text{ATP} & \quad \text{ADP} + \text{P}_i \\
\text{Glutamine} & \quad \text{NH}_3
\end{align*}
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

bicarbonate → \text{carbonyl phosphate} → \text{carbamate} → \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>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>
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \text{ATP} \\
\text{carbonyl phosphate} & \quad \text{NH}_3 \\
\text{carbamate} & \quad \text{ATP} \\
\text{carbamoyl phosphate}
\end{align*}
\]
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 $\overset{\text{glutaminase}}{\rightarrow}$ glutamate + ammonia

Asparagine $\overset{\text{asparaginase}}{\rightarrow}$ aspartate + ammonia

We also already know how to degrade Glutamine:

Glutamine $\overset{\text{glutaminase}}{\rightarrow}$ glutamate + ammonia

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

Asparagine $\overset{\text{asparaginase}}{\rightarrow}$ aspartate + 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:**

\[
\begin{align*}
\text{Glycine} & \quad \text{NAD}^+ \quad \text{NADH} \quad \text{CO}_2 \quad \text{NH}_4^+ \\
\text{THF} & \quad \text{N}^5-\text{N}^6-\text{methylene THF}
\end{align*}
\]

**Serine Hydroxymethyltransferase:**

\[
\begin{align*}
\text{Serine} & \quad \text{NAD}^+ \quad \text{NADH} \quad \text{CO}_2 \quad \text{NH}_4^+ \\
\text{THF} & \quad \text{N}^5-\text{N}^6-\text{methylene THF}
\end{align*}
\]

**Serine Dehydratase:**

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \quad \text{NH}_4^+ \quad \text{CO}_2 \quad \text{CH}_3
\end{align*}
\]
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 + NH₃

Homogentisate

Deficiency: Alkaptonuria "Ochronosis"

Phenylpyruvate

Enzyme: homogentisate dioxygenase

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

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

- Leucine
  \[ \text{CH}_3\text{CCH} \rightarrow \text{CH} \rightarrow \text{COO}^{(-)} \]
  \[\text{CH}_3\text{NH}_3^{(+)}\]

- Valine
  \[ \text{CH}_3\text{C} \rightarrow \text{CH} \rightarrow \text{COO}^{(-)} \]
  \[\text{CH}_3\text{NH}_3^{(+)}\]

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

- Glu
  \[ \text{CH}_3\text{CHCH}_2\text{CH} \rightarrow \text{C} \rightarrow \text{COO}^{(-)} \]

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

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

- NAD\(^{+}\) CoASH
  \[ \text{CH}_3\text{CHCH}_2\text{CH} \rightarrow \text{C} \rightarrow \text{S-CoA} \]

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

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

Tyrosine \[ \rightarrow \text{Tyrosine hydroxylase} \rightarrow \text{Dihydroxyphenylalanine (L-DOPA)} \]

Dopamine \[ \rightarrow \text{Norepinephrine} \rightarrow \text{Epinephrine} \]
Synthesis of Bioactive Amines

Tryptophan → Tryptophan hydroxylase → 5-hydroxytryptophan → PLP-dependent decarboxylation → CO₂ → Serotonin
Synthesis of Bioactive Amines

\[
\text{Glutamate} \xrightarrow{\text{Decarboxylase (PLP-dependent)}} \text{\textit{\textgamma}-aminobutyric acid (GABA)}
\]

\[
\text{Histidine} \xrightarrow{\text{Decarboxylase (PLP-dependent)}} \text{Histamine}
\]
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