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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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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:

\[
\begin{align*}
R_1\text{-}C\text{-}\text{coo}^- (\text{amino acid}) + R_2\text{-}C\text{-}\text{coo}^- (\text{amino acid}) & \rightarrow R_1\text{-}C\text{-}\text{coo}^- (\text{alpha-ketoglutarate}) + R_2\text{-}C\text{-}\text{coo}^- (\text{amino acid}) \\
\end{align*}
\]

#### Details of reaction mechanism:

\[
\begin{align*}
\text{amino acid} & \rightarrow \text{pyridoxal phosphate} + \text{H}_2\text{O} \\
H & \rightarrow N \rightarrow \text{H}^+ \\
\text{alpha-keto acid} & \rightarrow \text{pyridoxamine phosphate} \\
\end{align*}
\]
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 + 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:

\[
\begin{align*}
\text{glutamate} & \quad \text{NAD}(P) \quad \text{(mito)} \\
& \quad \text{NAD}(P)H \\
& \quad \alpha\text{-keto glutarate} \quad \text{ammonia}
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} \quad \text{ATP}+\text{NH}_3 & \quad \text{ADP}+\text{P}_i \\
& \quad \text{glutamine}
\end{align*}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{H} \quad \text{NH}_3 \quad \text{H} \\
\text{NH} \quad \text{H} \quad \text{H}
\]

Glutaminate is hydrolyzed to glutamate and ammonia:

\[
\text{H} \quad \text{NH}_3 \quad \text{H} \\
\text{NH} \quad \text{H} \quad \text{H}
\]

Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamate donates its amino group to form aspartate:

\[
\text{Glutamate-aspartate aminotransferase:}
\]

\[
\text{H} \quad \text{NH}_3 \quad \text{H} \\
\text{NH} \quad \text{H} \quad \text{H}
\]

Glutamate oxaloacetate α-keto glutarate aspartate
Carbamoyl phosphate synthetase I

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

ATP, ADP, $\text{NH}_3$, $\text{Pi}$
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline

aspartate

ATP

AMP + PP_i

Argininosuccinate
Argininosuccinate lyase

Argininosuccinate

Fumarate

Arginine
Arginase

\[
\begin{align*}
(-)\text{OOC} & \text{-C-CH}_2\text{CH}_2\text{CH}_2\text{NH-} & \text{NH}_2 \\
\text{H} & \text{++} & \text{H} \\
\text{NH}_3 & \text{++} & \text{NH}_3 \\
\text{Arginine} & \text{H}_2\text{O} & \text{Ornithine}
\end{align*}
\]

Urea
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\text{glutamate} \xrightarrow{\text{NAD}(P)} \text{NAD}(P)H \xrightarrow{\text{mito}} \text{α-ketoglutarate} + \text{NH}_3
\]

**Glutamine Synthetase:**

\[
\text{glutamate} \xrightarrow{\text{ATP}+\text{NH}_3} \text{glutamine} \xrightarrow{\text{ADP}+P_i} \text{glutamine}
\]
CPS I is Stimulated by NAG

Glutamate + Acetyl CoA → N-acetyl glutamate (NAG)

(repeating the figure from page 3 of your handout)

Bicarbonate + ATP + ADP → Carbamoyl phosphate + ADP + P_i

Carbamoyl phosphate + ATP → Carbamoyl phosphate
Glucose → Pyruvate → Glutamate → α-ketoglutarate → Alanine

Muscle → (Amines) → Amino acids

Blood transport

Glucose → Pyruvate → Glutamate → α-ketoglutarate → Urea

Liver → Alanine → α-ketoglutarate

Glutamate → N₂
Complicating the picture: Other tissues may be involved

Muscle:
- Amino acids: Transamination, Deamination
- Alamine $\rightarrow$ Glutamate $\rightarrow$ Glutamine $\rightarrow$ NH$_4^{(+)}$
- Purine deamination:

Intestine:
- Glutamine
- Alamine $\rightarrow$ NH$_4^{(+)}$ Citrulline

Kidney:
- Glutamine
- NH$_3$
- NH$_4^{(+)}$
- Citrulline
- Arginine

Liver:
- Glutamine
- Alamine $\rightarrow$ Glu $\rightarrow$ Aspartate $\rightarrow$ NH$_4^{(+)}$ Urea
- Arginine

Urea
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>
<th></th>
</tr>
</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

![Chemical reaction diagram]

(repeating the figure from page 3 of your handout)

![Chemical reaction diagram]
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:

\[
\begin{align*}
\text{Glutamine} & \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia} \\
\text{Asparagine} & \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\end{align*}
\]
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:**

\[
\begin{align*}
(-)\text{OOC} & - C - \text{NH}_3 \\
\text{H} & \quad \text{NAD}^+ \quad \text{NADH} \\
\text{H} & \quad \text{CO}_2 + \text{NH}_4^+
\end{align*}
\]

**Serine Hydroxymethyltransferase:**

\[
\begin{align*}
(-)\text{OOC} & - \text{CH} - \text{NH}_3 \\
\text{CH}_2\text{OH} & \quad \text{THF} \quad \text{N}^6 - \text{N}^0 - \text{methylene THF} \\
\end{align*}
\]

**Serine Dehydratase:**

\[
\begin{align*}
(-)\text{OOC} & - \text{C} - \text{NH}_3 \\
\text{CH}_2\text{OH} & \quad \text{H}_2\text{O} \\
\end{align*}
\]

\[
\begin{align*}
(-)\text{OOC} & - \text{C} - \text{NH}_3 \\
\text{C} & \quad \text{NH}_4^+ \\
\text{CH}_3 & \quad \text{H}_2\text{O}
\end{align*}
\]

\[
\begin{align*}
(-)\text{OOC} & - \text{C} - \text{NH}_2 \\
\text{CH}_3 & \quad \text{H}_2\text{O}
\end{align*}
\]

\[
\begin{align*}
(-)\text{OOC} & - \text{C} - \text{O} \\
\text{CH}_3 & \quad \text{H}_2\text{O}
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

\[
\text{Phenylalanine} \rightarrow \text{Tetrahydrobiopterin} + O_2 \rightarrow \text{Dihydrobiopterin} + H_2O \rightarrow \text{Enzyme: Phenylalanine hydroxylase} \rightarrow \text{Tyrosine} \rightarrow \text{Homogentisate}
\]

- Phenylketonuria
  - (no phenylalanine hydroxylase)
- Phenylpyruvate
  - Deficiency: Alkaptonuria “Ochronosis”
  - Enzyme: homogentisate dioxygenase
  - (you don’t need to know the rest)
Branched Chain Amino Acids

Isoleucine

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

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

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

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

Leucine

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

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

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

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

Valine

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

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

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

\[
\text{Glu}
\]

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

\[
\text{CH}_3
\]

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

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

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

(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 $\xrightarrow{\text{Conversion}}$ Norepinephrine $\xrightarrow{\text{Conversion}}$ Epinephrine
Synthesis of Bioactive Amines

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

Glutamate decarboxylase (PLP-dependent) → γ-aminobutyric acid (GABA)

Histidine decarboxylase (PLP-dependent) → Histamine
Glutamate, aspartate, alanine, glutamine, asparagine, (proline), glycine, serine (cysteine, tyrosine)

NON-Essential Amino Acids:

Essential Amino Acids:

Arginine (!), phenylalanine, methionine, histidine, Isoleucine, leucine, valine, threonine, tryptophan, lysine