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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
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:

\[
\text{R}_1\text{C-} \text{coo}^{(-)} + \text{R}_2\text{C-} \text{coo}^{(-)} \rightarrow \text{R}_1\text{C-} \text{coo}^{(-)} + \text{R}_2\text{C-} \text{coo}^{(-)}
\]

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

Details of reaction mechanism:

\[
\text{R-}\text{C-} \text{coo}^{(-)} + \text{H}_2\text{O} \rightarrow \text{H}\text{N} - \text{CH}_3 \rightarrow \text{R}\text{N} - \text{CH}_3 \rightarrow \text{R}\text{N} - \text{CH}_2\text{H} \rightarrow \text{R}\text{N} - \text{CH}_2\text{NH}_2
\]

\[\text{pyridoxal phosphate}\]

\[\text{pyridoxamine phosphate}\]
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):

\[
\text{Glutamate} + \text{oxaloacetate} \rightarrow \alpha\text{-ketoglutarate} + \text{aspartate}
\]
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:

\[
\text{Glutamate} \xrightarrow{\text{ATP+NH}_3} \text{Glutamine} \xrightarrow{\text{ADP+P}_i} \text{Glutamine}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

Glutamate donates its amino group to form aspartate:

Glutamate-aspartate aminotransferase:
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{NH}_3} \text{carbamate} \xrightarrow{\text{ATP}} \text{carbamoyl phosphate}$
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[ \text{NH}_2 - C - O\text{PO}_3^{(-)} \]

Ornithine

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

Citrulline

\[ \text{H}_2\text{C}_2\text{H}_2\text{H}_2\text{NH} - C - \text{NH}_2 \]

\[ \text{Pi} \]
Argininosuccinate synthetase

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

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

Argininosuccinate \( \rightarrow \) Arginine + Fumarate
Arginase

Arginine $\xrightarrow{\text{H}_{2}O} \text{Urea} \xrightarrow{} \text{Ornithine}$
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Arginine
- Argininosuccinate

Urea Cycle

- Aspartate

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

Glutamate Dehydrogenase:

\[
\begin{array}{c}
\text{H} \\
\text{NAD(P)} \\
\text{NAD(P)H} \\
\text{O}
\end{array}
\]

Glutamine Synthetase:

\[
\begin{array}{c}
\text{H} \\
\text{ATP} + \text{NH}_3 \\
\text{ADP} + P_i \\
\text{NH}_3
\end{array}
\]
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)

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

**Muscle:**
- Amino acids: Transamination, Deamination
- Alanine → Glutamate → Glutamine → NH$_4^{(+)}$

**Intestine:**
- Glutamine
- Alanine → NH$_4^{(+)}$ → Citrulline

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

**Liver:**
- Glutamine
- Alanine → Glu → Aspartate → NH$_4^{(+)}$
- 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)
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• 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>Metabolite</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:

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 \( \alpha \)-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

**Glycine Synthase:**

\[ \text{Glycine} \rightarrow \text{THF} \rightarrow \text{N}^5-\text{N}^{10}-\text{methylene THF} \rightarrow \text{CO}_2 + \text{NH}_4^{+} \]

**Serine Hydroxymethyltransferase:**

\[ \text{Serine} \rightarrow \text{THF} \rightarrow \text{N}^5-\text{N}^{10}-\text{methylene THF} \rightarrow \text{Glycine} \]

**Serine Dehydratase:**

\[ \text{Serine} \rightarrow \text{H}_2\text{O} \rightarrow \text{Glycine} \]
Methionine Cycle
And Biological Methyl Groups
Deficiency:
Alkaptonuria
"Ochronosis"

Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

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

Tetrahydrobiopterin + O_2
Dihydrobiopterin + H_2O

Enzyme: Phenylalanine hydroxylase

Phenylketonuria
(no phenylalanine hydroxylase)

[Diagram of biochemical pathway]

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

Homogentisate

Deficiency:
Alkaptonuria
"Ochronosis"

Phenylpyruvate
\[ \text{CH}_2 - \text{C} - \text{COO} \]

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}\(^{-}\)
\]

\[
\text{CH}_3\]

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

\[
\text{NADH} + \text{CoASH} \quad \text{or} \quad \text{NADH} + \text{CO}_2
\]

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

\[
\text{CH}_3\]

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

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}\(^{-}\)
\]

\[
\text{CH}_3\]

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

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

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

\[
\text{CH}_3\]

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}\(^{-}\)
\]

\[
\text{CH}_3\]

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

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

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

\[
\text{CH}_3\]
Synthesis of Bioactive Amines

Tyrosine $\xrightarrow{\text{Tyrosine hydroxylase}}$ Dihydroxyphenylalanine (L-DOPA)

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

Tryptophan $\xrightarrow{\text{Tryptophan hydroxylase}}$ 5-hydroxytryptophan $\xrightarrow{\text{PLP-dependent decarboxylation}}$ Serotonin

$\text{NH}_3$ $\text{NH}_3$ $\text{NAD}^+$ $\text{CO}_2$
Synthesis of Bioactive Amines

Glutamate

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

\[
\text{Glutamate decarboxylase (PLP-dependent)} \quad \gamma\text{-aminobutyric acid (GABA)}
\]

Histidine

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

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