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

oxaloacetate

fumarate

TCA 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:

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

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

Details of reaction mechanism:

\text{amino acid}

\[ \text{H} \]

\[ \text{R} - \text{C} - \text{coo}^{(-)} \]

\[ \text{NH}_2 \]

\[ \text{O} \]

\[ \text{CH}_2 \]

\[ \text{pyridoxal phosphate} \]

\[ \text{H}_2\text{O} \]

\[ \text{H}^+ \]

\[ \text{R} - \text{C} - \text{coo}^{(-)} \]

\[ \text{NH}_2 \]

\[ \text{H} \]

\[ \text{R} - \text{C} - \text{coo}^{(-)} \]

\[ \text{NH}_2 \]

\[ \text{H} \]

\[ \text{R} - \text{C} - \text{coo}^{(-)} \]

\[ \text{H} \]

\[ \text{R} - \text{C} - \text{coo}^{(-)} \]

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

Glutamate + oxaloacetate $\rightarrow$ $\alpha$-ketoglutarate + aspartate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[
\text{ATP} + \text{NH}_3 \rightarrow \text{GTP} + \text{Glutamine}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{H} \quad \overset{\text{H}_2\text{O}}{\text{NH}_3} \quad \overset{\text{H^+}}{\text{H}_2\text{O}} \quad \overset{\text{NH}_3}{\text{H^+}} \quad \overset{\text{H}_2\text{O}}{\text{H^+}} \quad \overset{\text{NH}_3}{\text{H^+}} \quad \overset{\text{H}_2\text{O}}{\text{H^+}} \quad \overset{\text{NH}_3}{\text{H^+}} \quad \overset{\text{H}_2\text{O}}{\text{H^+}} \quad \overset{\text{NH}_3}{\text{H^+}}
\]

Glutamine

Glutamate

Glutamate dehydrogenase reaction and several other reactions as well.

Glutamate donates its amino group to form aspartate:

\[
\text{H} \quad \overset{\text{H}_2\text{O}}{\text{NH}_3} \quad \overset{\text{H^+}}{\text{H}_2\text{O}} \quad \overset{\text{NH}_3}{\text{H^+}} \quad \overset{\text{H}_2\text{O}}{\text{H^+}} \quad \overset{\text{NH}_3}{\text{H^+}} \quad \overset{\text{H}_2\text{O}}{\text{H^+}} \quad \overset{\text{NH}_3}{\text{H^+}} \quad \overset{\text{H}_2\text{O}}{\text{H^+}} \quad \overset{\text{NH}_3}{\text{H^+}}
\]

Glutamate

Oxaloacetate

α-keto glutarate

Aspartate
Carbamoyl phosphate synthetase I

bicarbonate $\rightarrow$ carbamoyl phosphate $\rightarrow$ carbamate $\rightarrow$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

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

Ornithine

\[ \text{(-)} \text{ooc} \text{C} - \text{C} \text{H}_2 \text{H}_2 \text{C} \text{H}_3 \text{NH}_3^{(+)} \]

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

Citrulline

\[ \text{(-)} \text{ooc} \text{C} - \text{C} \text{H}_2 \text{H}_2 \text{C} \text{H}_2 \text{NH} - \text{C} - \text{NH}_2 \]

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

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

Argininosuccinate $\rightarrow$ Arginine $\rightarrow$ Fumarate

$\text{(-ooc} - \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} = \text{NH}_2^{(+)} \text{NH}_3^{(+)} \text{)}$

$\text{(-ooc} - \text{C} - \text{CH}_2\text{CH}_2\text{NH} - \text{C} = \text{NH}_2^{(+)} \text{NH}_3^{(+)} \text{)}$
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Citrulline
- Argininosuccinate
- Arginine

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

Aspartate

Urea
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\text{glutamate} \xrightarrow{\text{NAD}(P)} \text{NAD}(P)H \xrightarrow{\text{mito}} \text{\(\alpha\)-ketoglutarate} + \text{ammonia}
\]

**Glutamine Synthetase:**

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

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

(NAG)

(repeating the figure from page 3 of your handout)
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.

<p>| | |</p>
<table>
<thead>
<tr>
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<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

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

(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:

\[ \text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia} \]

\[ \text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia} \]
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 α-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

\[
\text{Glycine} \quad \xrightarrow{\text{NAD}(+) \text{ or NADH}} \quad \text{CO}_2 \quad + \quad \text{NH}_4^{(+)}
\]

THF \quad \text{N}^5-\text{N}^\text{\textit{o}}\text{-methylene THF}

Serine Hydroxymethyltransferase:

\[
\text{Serine} \quad \xrightarrow{\text{THF}} \quad \text{Glycine}
\]

THF \quad \text{N}^5-\text{N}^\text{\textit{o}}\text{-methylene THF}

Serine Dehydratase:

\[
\text{Serine} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{Glycine}
\]

\[\text{H}_2\text{O} \quad \text{N}^\text{\textit{a}}\text{-methylene THF} \quad \text{Glycine} \]

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

THF \quad \text{N}^5-\text{N}^\text{\textit{o}}\text{-methylene THF}

Serine Hydroxymethyltransferase:

\[
\text{Serine} \quad \xrightarrow{\text{THF}} \quad \text{Glycine}
\]

THF \quad \text{N}^5-\text{N}^\text{\textit{o}}\text{-methylene THF}

Serine Dehydratase:

\[
\text{Serine} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{Glycine}
\]

\[\text{H}_2\text{O} \quad \text{N}^\text{\textit{a}}\text{-methylene THF} \quad \text{Glycine} \]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine: $\text{NH}_3$ $\text{CH}_2\text{CH}(-)\text{COO}$

Enzyme: Phenylalanine hydroxylase

Tyrosine: $\text{NH}_3$ $\text{CH}_2\text{CH}(-)\text{COO}$

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Phenylpyruvate

Tetrahydrobiopterin + $O_2$ $\rightarrow$ Dihydrobiopterin + $H_2O$
Branched Chain Amino Acids

Isoleucine

Leucine

Valine

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

--------------- Branched-chain α-keto acid dehydrogenase ---

(continues on to degradation path similar to β-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 decarboxylase (PLP-dependent)

\[
\text{Glutamate} \rightarrow \text{γ-aminobutyric acid (GABA)}
\]

Histidine decarboxylase (PLP-dependent)

\[
\text{Histidine} \rightarrow \text{Histamine}
\]
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