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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Supplementary study material on the Web:
http://seqcore.brcf.med.umich.edu/mcb500

There are also PDF’s of class handouts with supplemental information available in the table of contents for this course.
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:

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

alpha-keto acid (typically alpha-ketoglutarate)

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

alpha-keto acid (typically glutamate)

Details of reaction mechanism:

amino acid

\[ \text{R--C--COO}^{(-)} + \text{NH}_2 \]

+ O

H

CH

OH

pyridoxal phosphate

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

\[ \rightarrow \text{R--N--COO}^{(-)} \]

\[ \rightarrow \text{R--N--COO}^{(-)} \]

\[ \rightarrow \text{R--N--COO}^{(-)} + \text{H}^+ \]

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

\[ + \text{NH}_2 \]

\[ \text{HCH} \]

\[ \text{BOCH}_2 \text{OH} \]

\[ \text{BOCH}_2 \text{CH}_3 \]

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Transfer the amine back to an acceptor $\alpha$-keto acid
Some amino acid + α-ketoglutarate → some alpha keto acid + Glutamate

In other words, alpha-ketoglutarate is the preferred acceptor, and Glutamate is the resulting amino acid:

In peripheral tissues, transaminases *tend* to form Glutamate when they catabolize amino acids.
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:**

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

**Glutamine Synthetase:**

\[
\begin{align*}
\text{glutamate} & \xrightarrow{\text{ATP} + \text{NH}_3} \text{glutamine} \\
& \xrightarrow{\text{ADP} + P_i} \text{glutamine}
\end{align*}
\]
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:

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

\[
\text{Glutamate} + \text{oxaloacetate} \rightarrow \text{α-keto glutarate} + \text{aspartate}
\]
Carbamoyl phosphate synthetase I
Ornithine Transcarbamoylase

Carbamoyl phosphate

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

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

\[ \text{NH}_2\text{C}\text{H}_2\text{H}_2\text{NH}_3^{(+)} \]
Argininosuccinate synthetase

\[
\text{Citrulline} \xrightarrow{\text{ATP}} \text{Argininosuccinate} \xleftarrow{\text{AMP + PP}_i} \text{Aspartate}
\]
Argininosuccinate lyase

\[
\text{Argininosuccinate} \rightarrow \text{Fumarate} \rightarrow \text{Arginine}
\]
Arginase

\[
\begin{align*}
\text{Arginine} & \quad \text{Ornithine} \\
\text{Urea} & \\
\end{align*}
\]
Urea Cycle Connects to TCA Cycle

- Ornithine → Citrulline
- Citrulline → Argininosuccinate
- Argininosuccinate → Fumarate
- Fumarate → Oxaloacetate
- Oxaloacetate → Malate
- Malate → α-Ketoglutarate
- α-Ketoglutarate → Citrate

Urea Cycle:
- Urea → Ornithine → Citrulline → Argininosuccinate → Urea

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

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

bicarbonate → carbonyl phosphate → carbamate → 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.

<p>| | |</p>
<table>
<thead>
<tr>
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<tr>
<td><strong>CPSD</strong></td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
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<tr>
<td><strong>OTCD</strong></td>
<td>Elevated CP causes synthesis of Orotate</td>
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<tr>
<td><strong>ASD</strong></td>
<td>Elevated citrulline</td>
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<tr>
<td><strong>ALD</strong></td>
<td>Elevated argininosuccinate</td>
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<tr>
<td><strong>AD</strong></td>
<td>Elevated arginine</td>
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</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)

bicarbonate + ATP → carbonyl phosphate + ADP

bicarbonate + ATP → carbamate + ADP

bicarbonate + ATP → carbamoyl phosphate + ADP
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 $\rightarrow$ glutamate + ammonia

Asparagine $\rightarrow$ aspartate + ammonia

We also already know how to degrade Glutamine:

Glutamine $\rightarrow$ glutamate + ammonia

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

Asparagine $\rightarrow$ 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:**

\[
\begin{align*}
(-)^{\text{OOC}} & \quad \text{C} \quad \text{NH}_3^{(+)} \\
\text{Glycine} & \quad \text{THF} \quad \text{N}^5 - \text{N}^{10} - \text{methylene THF} \quad \text{CO}_2 \quad + \quad \text{NH}_4^{(+)}
\end{align*}
\]

**Serine Hydroxymethyltransferase:**

\[
\begin{align*}
(-)^{\text{OOC}} & \quad \text{CH} \quad \text{NH}_3^{(+)} \quad \text{CH}_2\text{OH} \\
\text{Serine} & \quad \text{THF} \quad \text{N}^5 - \text{N}^{10} - \text{methylene THF} \quad \text{Glycine}
\end{align*}
\]

**Serine Dehydratase:**

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

\[
\begin{align*}
\text{NH}_4^{(+)} & \quad \text{H}_2\text{O} \quad \rightarrow \quad (-)^{\text{OOC}} - \text{C} \quad \text{O} \quad \text{CH}_3
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine $\rightarrow$ Dihydrobiopterin + H$_2$O $\rightarrow$ Tetrahydrobiopterin + O$_2$ $\rightarrow$ Tyrosine

Enzyme: Phenylalanine hydroxylase

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Enzyme: homogentisate dioxygenase

Phenylpyruvate

Phenylketonuria (no phenylalanine hydroxylase)

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

Isoleucine

`CH_3CH_2CH(CH_3)COO^(-)`

α-KG

Glu

`CH_3CH_2CH(CH_3)COO`  

NAD^+ CoASH

NADH + CO_2

Valine

`CH_3CH_2CH(CH_3)CH_3`  

α-KG

Glu

`CH_3CH_2CH(CH_3)CH_3`  

NAD^+ CoASH

NADH + CO_2

Leucine

`CH_3CH(CH_3)CH(CH_3)COO^(-)`

α-KG

Glu

`CH_3CH(CH_3)CH(CH_3)COO`  

NAD^+ CoASH

NADH + CO_2

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

Tyrosine → Dihydroxyphenylalanine (L-DOPA) → Dopamine → Norepinephrine → Epinephrine

Tyrosine hydroxylase
Synthesis of Bioactive Amines

Tryptophan → 5-hydroxytryptophan → Serotonin

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

Glutamate

\[
\text{COO} \overset{(-)}{\text{CH}_2 \text{CH}_2 \text{CH} \overset{(-)}{-} \text{COO}} \quad \overset{\text{Glutamate decarboxylase (PLP-dependent)}}{\longrightarrow} \quad \text{COO} \overset{(-)}{\text{CH}_2 \text{CH}_2 \text{CH}_2 \overset{(+)}{-} \text{NH}_3}
\]

\(\gamma\)-aminobutyric acid (GABA)

Histidine

\[
\text{N} \overset{\text{H}}{\text{CH}_2 \overset{(-)}{\text{CH}} \overset{-}{\text{COO}}} \quad \overset{\text{Histidine decarboxylase (PLP-dependent)}}{\longrightarrow} \quad \text{N} \overset{\text{H}}{\text{CH}_2 \overset{-}{\text{CH}_2} \overset{(+)}{-} \text{NH}_3}
\]

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