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:

\[
R_1\text{C} - \text{coo}^\text{(-)} + \alpha\text{-keto acid (typically alpha-ketoglutarate)} \rightarrow R_1\text{C} - \text{coo}^\text{(-)} + R_2\text{C} - \text{coo}^\text{(-)}
\]

Details of reaction mechanism:

pyridoxal phosphate

\[
\text{amino acid} \rightarrow \text{amino acid} + \text{H}_2\text{O}
\]
Transfer the amine back to an acceptor α-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 → 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:**

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

**Glutamine Synthetase:**

\[ \text{glutamate} \xrightarrow{\text{ATP} + \text{NH}_3} \text{glutamine} \]

\[ \text{ATP} \rightarrow \text{ADP} + P_i \]
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:

\[
\text{Glutamate-aspartate aminotransferase}:
\text{Glutamate} + \text{oxaloacetate} \rightarrow \alpha\text{-keto glutarate} + \text{aspartate}
\]
Carbamoyl phosphate synthetase I

bicarbonate + ATP → carbonyl phosphate + ADP

carbonyl phosphate + NH$_3$ → carbamate + Pi

carbamate + ATP → carbamoyl phosphate + ADP
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[ \text{(-)OOC} \quad \text{C} \quad \text{(-)NH}_3 \quad \text{NH}_2^{(+)} \]

Ornithine

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

Citrulline

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

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

\[ \text{(-)OOC} \quad \text{C} \quad \text{H}_2 \quad \text{H}_2 \quad \text{H}_2 \quad \text{NH} \quad \text{C} \quad \text{NH}_2 \]
Argininosuccinate synthetase

Citrulline

Argininosuccinate

aspartate
Argininosuccinate lyase

Argininosuccinate $\xrightarrow{\text{Argininosuccinate lyase}}$ Arginine + Fumarate
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

Glutamate $\rightarrow$ NAD(P)$^+$ + $\alpha$-ketoglutarate + ammonia

Glutamate $\rightarrow$ NAD(P)H

**Glutamine Synthetase:**

Glutamate + ATP + NH$_3$ $\rightarrow$ Glutamine + ADP + P$_i$
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)
Complicating the picture: Other tissues may be involved

Muscle:
- Amino acids: Transamination, Deamination
- Alanine $\rightarrow$ Glutamate $\rightarrow$ Glutamine
  - NH$_4$ (purine deamination)

Intestine:
- Glutamine
- Alanine $\rightarrow$ NH$_4$ $\rightarrow$ Citrulline

Kidney:
- Glutamine $\rightarrow$ NH$_3$
  - NH$_4$ $\rightarrow$ Arginine
  - Citrulline

Liver:
- Glutamine
  - NH$_4$
  - Arginine $\rightarrow$ Urea
  - Aspartate $\rightarrow$ Glu $\rightarrow$ Alanine
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>CPSD</th>
<th>No elevation except ammonia; diagnosed by elimination.</th>
</tr>
</thead>
<tbody>
<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

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

(repeating the figure from page 3 of your handout)

\[
\text{bicarbonate} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
\]
1. 2ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate

2. 2ADP + P₁ → P₂ + Ornithine → Citrulline

3. ATP + AMP + P₂ → Argininosuccinate

4. Argininosuccinate → Fumarate

5. Urea → Ornithine → Citrulline
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: $\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}$

Asparagine: $\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}$

We also already know how to degrade Glutamine:

$\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}$

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

$\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}$
Amino Acids are categorized as ‘Glucogenic’ or ‘ketogenic’ or both.

Many amino acids are purely glucogenic:
- Glutamate, aspartate, alanine, glutamine, asparagin,…

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:

Glycine

Serine Hydroxymethyltransferase:

Serine

Serine Dehydratase:

Serine
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

\[
\text{Phenylalanine} \xrightarrow{\text{Phenylalanine hydroxylase}} \text{Tyrosine} \xrightarrow{\text{Enzyme: homogentisate dioxygenase}} \text{Homogentisate}
\]

Deficiency: Alkaptonuria

“Ochronosis”

You don’t need to know the rest.
Branched Chain Amino Acids

Isoleucine

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

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

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

-- Transamination --

\[
\text{Glu}
\]

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

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

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

Leucine

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

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

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

Valine

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Tyrosine → Dihydroxyphenylalanine (L-DOPA) via Tyrosine hydroxylase

Dopamine → Norepinephrine → Epinephrine

Chemical structures of Tyrosine, Dihydroxyphenylalanine (L-DOPA), Dopamine, Norepinephrine, and Epinephrine are depicted.
Synthesis of Bioactive Amines

Tryptophan $\rightarrow$ 5-hydroxytryptophan $\rightarrow$ Serotonin

Tryptophan hydroxylase

PLP-dependent decarboxylation

NAD$^+$
Synthesis of Bioactive Amines

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

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