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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 \textit{gaining} or \textit{losing} net Nitrogen?
Transaminases Collect Amines

General reaction overview:

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

\( \alpha \)-keto acid (typically \( \alpha \)-ketoglutarate)

Details of reaction mechanism:

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

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

\[ \text{HCH} \rightarrow \text{HCH} \]

\[ \text{NH}_2 \]

\[ \text{pyridoxal phosphate} \]

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

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

**Glutamate Dehydrogenase:**

\[
\text{glutamate} + \text{NAD}(P) \rightarrow \text{\(\gamma\text{-ketoglutarate}\)} + \text{NH}_3
\]

**Glutamine Synthetase:**

\[
\text{glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{glutamine} + \text{ADP} + \text{Pi}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

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

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

Glutamate donates its amino group to form aspartate:

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

bicarbonate + ATP → carbonyl phosphate + ADP

carbonyl phosphate + NH₃ → carbamate + Pi

carbamate + ATP → carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

\[
\text{(-)oCC} \rightarrow \text{C} \rightarrow \text{CH}_2\text{CH}_2\text{NH} \rightarrow \text{C} \rightarrow \text{NH}_2
\]

\[
\text{H} \quad \text{H} \quad \text{H} \quad \text{NH}_2 \quad \text{(+)}
\]

\[
\text{(-)oCC} \rightarrow \text{C} \rightarrow \text{CH}_2\text{CH}_2\text{NH} \rightarrow \text{C} = \text{NH}_2\quad \text{(+)}
\]

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

\[ (-)\text{OOC-C-CH}_2\text{CH}_2\text{NH-C=NH}_2^{(+)} \rightarrow (-)\text{OOC-C-CH}_2\text{CH}_2\text{NH-CH}_2\text{NNH}_2^{(+)} \]

\[ \text{Argininosuccinate} \rightarrow \text{Fumarate} \rightarrow \text{Arginine} \]
Arginase
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Citrulline
- Arginine
- Argininosuccinate

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

Aspartate:
\[ \text{H} \]
\[ \text{H} \]
\[ \text{H} \]
\[ \text{H} \]
\[ \text{H} \]
\[ \text{H} \]
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

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

**Glutamine Synthetase:**

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

\[ \text{glutamine} \xrightarrow{\text{ADP} + P_i} \text{glutamate} \]
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.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Diagnosis</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

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

(repeating the figure from page 3 of your handout)

bicarbonate \[ \xrightarrow{\text{ATP}} \] carbonyl phosphate \[ \xrightarrow{\text{ADP}} \] carbamate \[ \xrightarrow{\text{ATP}} \] 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:

Glutamine $\rightarrow$ glutamate + ammonia

Asparagine $\rightarrow$ aspartate + ammonia

We also already know how to degrade Glutamine:

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

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

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

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)

Phenylalanine: 
\[
\text{NH}_3 + \text{CH}_2 - \text{CH} - \text{COO}^{(-)} \rightarrow \text{Tetrahydrobiopterin} + \text{O}_2 
\]

Dihydrobiopterin + H_2O 

Enzyme: Phenylalanine hydroxylase 

Tyrosine: 
\[
\text{HO} - \text{CH}_2 - \text{CH} - \text{COO}^{(-)} 
\]

Homogentisate 

Deficiency: 

Alkaptonuria

“Ochronosis”

Enzyme: homogentisate dioxygenase

Phenylpyruvate: 
\[
\text{CH}_2 - \text{C} - \text{COO}^{(-)} 
\]
Branched Chain Amino Acids

Isoleucine
\[
\text{CH}_3\text{CH}_2\text{CH} - \text{CH} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \quad \text{NH}_3^{-}
\]
\[
\quad \quad \alpha\text{-KG}
\]

Leucine
\[
\text{CH}_3\text{CHCH}_2 - \text{CH} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \quad \text{NH}_3^{-}
\]
\[
\quad \quad \alpha\text{-KG}
\]

Valine
\[
\text{CH}_3\text{CH} - \text{C} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \quad \text{NH}_3^{-}
\]
\[
\quad \quad \alpha\text{-KG}
\]

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

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

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

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

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

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

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

\[
\text{NADH} + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{NAD}^+ + \text{CO}_2
\]

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

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

Tyrosine $\rightarrow$ Dihydroxyphenylalanine (L-DOPA) via Tyrosine hydroxylase

Dopamine $\rightarrow$ Norepinephrine $\rightarrow$ Epinephrine
Synthesis of Bioactive Amines

Tryptophan → 5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase

PLP-dependent decarboxylation

NAD+
Synthesis of Bioactive Amines

Glutamate

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

Histidine

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