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, NH$_3$

Urea

Folate metabolism

Methylene THF

Met Cycle

TCA Cycle

oxaloacetate

fumarate

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:

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

α-keto acid (typically α-ketoglutarate)

Details of reaction mechanism:

Amino acid

\[
R\text{--C--coo}^\text{(-)} + \text{H}^+ + \text{pyridoxal phosphate} \rightarrow \text{H_2O}
\]

α-keto acid

\[
R\text{--C--coo}^\text{(-)} + \text{H}^+ + \text{pyridoxamine phosphate} \rightarrow \text{H_2O}
\]
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):

\[
\text{Glutamate} + \text{oxaloacetate} \rightarrow \alpha\text{-ketoglutarate} + \text{aspartate}
\]
Getting Amines Into the Liver

Glutamate Dehydrogenase:
\[
\text{glutamate} \xrightarrow{\text{NAD(P)}} \text{α-ketoglutarate} + \text{NH}_3
\]

Glutamine Synthetase:
\[
\text{glutamate} \xleftarrow{\text{ATP}+\text{NH}_3} \text{glutamine}
\]
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:

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

\[
\text{Glutamate} + \text{oxaloacetate} \rightarrow \text{α-keto glutarate} + \text{aspartate}
\]
1. 2ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate
2. 2ADP + P₃ → Ornithine
3. Ornithine + Citrulline → Urea
4. Argininosuccinate → Fumarate
5. ATP → aspartate
Carbamoyl phosphate synthetase I

\[
\begin{align*}
\text{bicarbonate} & \xrightarrow{\text{ATP}} \text{carbonyl phosphate} \\
\text{carbonyl phosphate} & \xrightarrow{\text{NH}_3} \text{carbamate} \\
\text{carbamate} & \xrightarrow{\text{ATP}} \text{carbamoyl phosphate}
\end{align*}
\]
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

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

Argininosuccinate $\xrightarrow{\text{Argininosuccinate lyase}}$ Arginine + Fumarate
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:

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} \quad \text{ATP} & \quad \text{carbonoyl phosphate} \quad \text{ATP} \\
\text{ADP} & \quad \text{carbamate} \quad \text{ADP} \\
\text{Pi} & \quad \text{carbamoyl phosphate} \\
\end{align*}
\]
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>
<th></th>
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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

\[
\text{(-) ooc} \quad \text{(-) ooc}
\]

\[
\text{NH}_2
\]

\[
\text{H}
\]

\[
\text{CH}_2\text{CH}_2\text{C}
\]

\[
\text{=O}
\]

\[
\text{OH}
\]

\[
\text{glutamate}
\]

\[
\text{H}
\]

\[
\text{=O}
\]

\[
\text{CH}_3
\]

\[
\text{N-acyl glutamate}
\]

\[
\text{(NAG)}
\]

\[
\text{CoA-}\text{c} \quad \text{CoA-}\text{c}
\]

\[
\text{=O}
\]

\[
\text{CH}_3
\]

\[
\text{synthetase}
\]

\[
\text{N-acetyl glutamate}
\]

\[
\text{glutamate}
\]

\[
\text{acetyl CoA}
\]

(repeating the figure from page 3 of your handout)

\[
\text{bicarbonate}
\]

\[
\text{ATP}
\]

\[
\text{ADP}
\]

\[
\text{carbonyl phosphate}
\]

\[
\text{ATP}
\]

\[
\text{Pi}
\]

\[
\text{carbamate}
\]

\[
\text{ATP}
\]

\[
\text{carbamoyl phosphate}
\]
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:

\[
\begin{align*}
\text{H} & \quad \text{transamination} & \quad \text{H} \\
(-\text{O}_2\text{CCH}_2\text{C}^-\text{CO}_2\text{(-)} & \quad \text{oxaloacetate} & \quad (-\text{O}_2\text{CCH}_2\text{C}^-\text{CO}_2\text{(-)} \\
\text{NH}_3 & \quad \text{transamination} & \quad \text{NH}_3 \\
\text{aspartate} & \quad \text{aspartate} & \quad \text{glutamate} \\
\text{H} & \quad \text{transamination} & \quad \text{H} \\
(-\text{O}_2\text{CCH}_2\text{C}^-\text{CO}_2\text{(-)} & \quad \text{a-ketoglutarate} & \quad (-\text{O}_2\text{CCH}_2\text{C}^-\text{CO}_2\text{(-)} \\
\text{NH}_3 & \quad \text{transamination} & \quad \text{NH}_3 \\
\text{glutamate} & \quad \text{glutamate} & \quad \text{alanine} \\
\text{H} & \quad \text{transamination} & \quad \text{H} \\
\text{CH}_3^-\text{C}^-\text{CO}_2\text{(-)} & \quad \text{pyruvate} & \quad \text{CH}_3^-\text{C}^-\text{CO}_2\text{(-)} \\
\text{NH}_3 & \quad \text{transamination} & \quad \text{NH}_3 \\
\text{alanine} & \quad \text{alanine} & \quad \text{alanine}
\end{align*}
\]

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, 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:

\[ \text{Glycine} \xrightarrow{\text{Glycine Synthase}} \text{Glycine} + \text{CO}_2 + \text{NH}_4^+ \]

Serine Hydroxymethyltransferase:

\[ \text{Serine} \xrightarrow{\text{Serine Hydroxymethyltransferase}} \text{Glycine} \]

Serine Dehydratase:

\[ \text{Serine} \xrightarrow{\text{Serine Dehydratase}} \text{Serine} \xrightarrow{\text{Water}} \text{Serine} \xrightarrow{\text{Further Reactions}} \text{Glycine} \]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine + Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O

Enzyme: Phenylalanine hydroxylase

Tyrosine

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

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 $\xrightarrow{\text{Monoamine oxidase}}$ Norepinephrine $\xrightarrow{\text{Deaminase}}$ Epinephrine
Synthesis of Bioactive Amines

Tryptophan

\[ \text{NH}_3 \]^{(+)} \quad \text{Trypophan hydroxylase} \quad \text{5-hydroxytryptophan} \quad \text{NH}_3 \]^{(+)}

PLP-dependent decarboxylation

\[ \text{CO}_2 \]

\[ \text{HO} \quad \text{CH}_2 \quad \text{CH} \quad \text{NH}_3 \]^{(+)}

Serotonin
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

Histidine decarboxylase (PLP-dependent)

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