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:

\[ R_1\text{C} - \text{coo}^- + R_2\text{C} - \text{coo}^- \rightarrow R_1\text{C} - \text{coo}^- + \text{NH}_2 + R_2\text{C} - \text{coo}^- \]

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

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

Details of reaction mechanism:

\[ \text{amino acid} + \text{H}^+ \rightarrow \text{Pyridoxal phosphate} \]

\[ \text{Pyridoxal phosphate} + \text{H}_2O \rightarrow \text{Pyridoxamine phosphate} \]
Transfer the amine back to an acceptor α-keto acid

\[
\text{pyridoxamine} \quad + \quad \alpha\text{-keto acid} \quad \rightarrow \quad \text{pyridoxal} \quad + \quad \text{amino acid}
\]
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):

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

Glutamate Dehydrogenase:

\[
\text{Glutamate} \xrightarrow{\text{NAD}(P)} \text{NAD}(P)\text{H} \xrightarrow{\text{mito}} \text{\(\Delta\text{O}_2\text{CCH}_2\text{CH}_2\text{C}-\text{CO}_2\text{NH}_2\)} \rightarrow \text{\(\Delta\text{O}_2\text{CCH}_2\text{CH}_2\text{C}-\text{CO}_2\text{NH}_3\)} + \text{\(\Delta\text{O}_2\text{CCH}_2\text{CH}_2\text{C}-\text{CO}_2\text{NH}_3\)}
\]

Glutamine Synthetase:

\[
\text{Glutamate} \xrightarrow{\text{ATP+NH}_3} \text{\(\text{OOC-C-CH}_2\text{CH}_2\text{COO}^-\)} \xrightarrow{\text{ADP+P}_i} \text{\(\text{OOC-C-CH}_2\text{CH}_2\text{C}=\text{NH}_2\)} + \text{\(\text{OOC-C-CH}_2\text{CH}_2\text{COO}^-\)}
\]
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

bicarbonate $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{NH}_3} \text{carbamate} \xrightarrow{\text{ATP}} \text{carbamoyl phosphate}$
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

\[
\begin{align*}
\text{Argininosuccinate} & \quad \text{Citrulline} \\
\text{ATP} & \quad \text{AMP + PP}_{i}
\end{align*}
\]
Argininosuccinate lyase

Argininosuccinate → Arginine

Fumarate
Arginase

Arginine $\xrightarrow{\text{H}_2\text{O}}$ Ornithine

Urea
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \rightarrow & \quad \text{carbonyl phosphate} & \quad \rightarrow & \quad \text{carbamate} & \quad \rightarrow & \quad \text{carbamoyl phosphate} \\
& \quad \text{ATP} & \quad \text{ADP} & \quad \text{P}_i & \quad \text{ADP} & \quad \text{ATP}
\end{align*}
\]
Complicating the picture: Other tissues may be involved

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

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

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

Liver:
- Glutamine
  - NH$_4$
  - Arginine
  - Urea
  - Aspartate
  - Glu
  - Alanine

Urea
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>
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<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

\[
\begin{align*}
\text{(-)} \quad & \quad \text{OOC} - \text{C} - \text{CH}_2 \text{CH}_2 \text{C} - \text{O} & + & \quad \text{CoA} - \text{C} = \text{O} \\
\text{glutamate} & & \text{acetyl CoA} & \text{N-acetyl glutamate} \\
\text{(NAG)} & & & \\
\end{align*}
\]

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{HO} - \text{C} - \text{O}^{(-)} \\
& \quad \xrightarrow{\text{ADP}} \quad \text{HO} - \text{C} - \text{O}^{\text{P}} \\
& \quad \xrightarrow{\text{NH}_3} \quad \text{HO} - \text{C} - \text{NH}_2 \\
& \quad \xrightarrow{\text{ATP}} \quad \text{Pi} - \text{O} - \text{C} - \text{NH}_2 \\
\end{align*}
\]
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:

\[
\begin{align*}
\text{Glutamine} & \quad \xrightarrow{\text{glutaminase}} \quad \text{glutamate} + \text{ammonia} \\
\text{Asparagine} & \quad \xrightarrow{\text{asparaginase}} \quad \text{aspartate} + \text{ammonia}
\end{align*}
\]
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:

\[
\begin{align*}
\text{Glycine} & \quad \text{THF} \quad \mathbf{N^\delta - N^\epsilon - \text{methylene THF}} \\
\text{Glycine} & \quad \text{CO}_2 \quad + \quad \text{NH}_4^{(+)}
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Serine} & \quad \text{THF} \quad \mathbf{N^\delta - N^\epsilon - \text{methylene THF}} \\
\text{Glycine} & \quad \text{THF} \quad \mathbf{N^\delta - N^\epsilon - \text{methylene THF}}
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{H}_2\text{O}
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups

Methionine

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

Methionine

\[ \text{ATP} + \text{H}_2\text{O} \rightarrow \text{PPi} + \text{Pi} \]

S-Adenosyl Methionine

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

S-Adenosyl Homocysteine

\[ \text{S} - \text{Adenosyl Homocysteine} \]

Methylated acceptor

Biosynthetic Methylation reaction

\[ \text{Methyl acceptor} \]

\[ \text{see examples} \]

Homocysteine

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

Homocysteine

\[ \text{NS methyl tetrahydrofolate} \]

tetrahydrofolate

\[ \text{tetrahydrofolate} \]

[NS methyl]

\[ \text{tetrahydrofolate} \]

Cysteine

\( (\text{remainder of homocysteine degraded for energy}) \)

Serine

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

Serine

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

Cysteine

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

Cysteine

\[ \text{NS methyl tetrahydrofolate} \]
Deficiency:
Alkaptonuria
"Ochronosis"

Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine

Tetrahydrobiopterin + O₂
Dihydrobiopterin + H₂O

Enzyme: Phenylalanine hydroxylase

(+)
NH₃

Phenylpyruvate

Tyrosine

Homogentisate

Deficiency: Alkaptonuria "Ochronosis"

Enzyme: homogentisate dioxygenase

(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 $\xRightarrow{}$ Norepinephrine $\xRightarrow{}$ Epinephrine
Synthesis of Bioactive Amines

Tryptophan → 5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase

PLP-dependent decarboxylation

NAD+ → CO₂
Synthesis of Bioactive Amines

Glutamate

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

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

Histidine

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

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