Author: Robert Lyons, Ph.D., 2008

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M1 Renal:
Nitrogen Metabolism (and Related Topics)

- Amino Acid Metabolism (Nitrogen metabolism)
- Folate Metabolism (“One-Carbon pathways”)
- Nucleotide Metabolism

Dr. Robert Lyons
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Director, DNA Sequencing Core
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Fall 2008
Amino Acid Metabolism (Nitrogen Metabolism)  Dec 12-14 2006  Dr. Robert Lyons

See: http://seqcore.brcf.med.umich.edu/mcb500 for supplementary (non-required) course materials.

Medical relevance of amino acid metabolism pathways:
What is nitrogen balance, and what affects it?
Role of vitamins: pyridoxamine (VitB6), folic acid
Understanding a critical function of the liver: nitrogen metabolism
Which amino acids are essential?
Inborn errors of metabolism: amino acid breakdown, urea cycle
Pharmacologic manipulation of neurotransmitters (e.g. Parkinson’s Syndrome)

I. Protein degradation/Nitrogen balance

A. Cells constantly turn over proteins

It’s a normal process, balanced by protein intake.
Proteins can be degraded if they are:
...
Amino Acid metabolism

Glu, Gln, Asp, NH$_3$

Urea

Folate metabolism

Methylene

THF

Met Cycle

oxaloacetate

fumarate

TCA Cycle

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:

\[ \text{R}_1\text{C} - \text{COO}^{(-)} + \text{R}_2\text{C} - \text{COO}^{(-)} \quad \text{α-keto acid (typically α-ketoglutarate)} \]

Details of reaction mechanism:

\[ \text{H} \quad \text{R-C-COO}^{(-)} \quad \text{NH}_2 \quad + \quad \text{O} \quad \text{CH} \quad \text{H}_2\text{O} \quad \rightarrow \quad \text{H} \quad \text{R-C-COO}^{(-)} \quad \text{N} \quad \text{H} \quad \text{R-C-COO}^{(-)} \quad \text{R-C-COO}^{(-)} \quad \text{R-C-COO}^{(-)} \quad \alpha\text{-keto acid} \quad \text{pyridoxal phosphate} \quad \text{pyridoxamine phosphate} \]
Transfer the amine back to an acceptor α-keto acid

pyridoxamine phosphate + $\alpha$-keto acid \rightarrow pyridoxal phosphate + 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:

\[
\text{Some amino acid} + \alpha\text{-ketoglutarate} \rightarrow \text{some alpha keto acid} + \text{Glutamate}
\]
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 $\rightarrow$ α-ketoglutarate + aspartate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{Glutamine} & \rightarrow \text{Glutamate} + \text{NH}_3 \\
H_2C(OOC)CH(NH_2) & \rightarrow H_2C(OOC)CH_2NH_3^+ + \text{NH}_3
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

Glutamate-aspaltate aminotransferase:

\[
\begin{align*}
\text{Glutamate} + \text{Oxaloacetate} & \rightarrow \text{Aspartate} + \alpha\text{-Ketogluutarate} \\
(-)^{14}O_2CCH_2CH_2(OOC)NH_2 & + (-)^{14}O_2CCH_2C(O)CO_2 \rightarrow (-)^{14}O_2CCH_2CH_2(OOC)NH_2 + (-)^{14}O_2CCH_2C(O)CO_2 + \text{NH}_2
\end{align*}
\]
Carbamoyl phosphate synthetase I

bicarbonate + ATP → carbonyl phosphate + ADP

carbonyl phosphate + NH₃ → carbamate + Pi

carbamate + ATP → carbamoyl phosphate + ADP
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline

R. Lyons
Argininosuccinate synthetase

\[
\begin{align*}
\text{Argininosuccinate} & \rightarrow \text{Citrulline} \\
\text{ATP} & \rightarrow \text{AMP} + \text{PP}_i
\end{align*}
\]
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Arginine $\rightarrow$ Fumarate

R. Lyons
Arginase

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

$\text{H}_2\text{O}$

Urea

R. Lyons
Liver mitochondrion

Liver cytoplasm

2ATP + HCO₃⁻ + NH₄⁺ → Carbamoyl phosphate

2ADP + Pᵢ → Ornithine

Ornithine + Citrulline → Urea

Argininosuccinate → Fumarate

Arginine → Aspartate
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Arginine
- Citrulline
- Argininosuccinate

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

Aspartate:
- $(-)\text{C}_2\text{H}_4\text{C}-\text{CO}_2\text{(-)}$
- $\text{NH}_2$

Fumarate:
- $(-)\text{C}_2\text{H}_4\text{C}-\text{CO}_2\text{(-)}$
- $\text{H}$
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

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

**Glutamine Synthetase:**

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

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

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

(repeating the figure from page 3 of your handout)
Muscle

Glucose → Pyruvate

Glutamate → α-ketoglutarate

Alanine

(Amines)

Amino acids

Liver

Glucose → Pyruvate

Glutamate → NH₃

Urea

Alanine → α-ketoglutarate

blood transport
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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</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

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

(repeating the figure from page 3 of your handout)
Liver mitochondrion

Liver cytoplasm

Ornithine

Citrulline

Argininosuccinate

Fumarate

Arginine

Urea

HCO$_3^-$

NH$_3$

2ATP + HCO$_3^-$ + NH$_3$ → Carbamoyl phosphate

2ADP + P$_i$ + ATP

Ornithine

Citrulline

Arginino succinate

Fumarate

NH$_2$-OPO$_4^{3-}$

L. Lyons
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:

\[ \text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} \quad \text{asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} \]

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:

\[
\text{Glycine Synthase:} \quad \text{H} \quad \text{NH}_3^{(+)} \quad \text{THF} \quad \text{N}^5-N^{10}-\text{methylene} \quad \text{THF}
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine Hydroxymethyltransferase:} \quad \text{H}_2\text{O} \quad \text{H} \quad \text{NH}_3^{(+)} \quad \text{THF} \quad \text{N}^5-N^{10}-\text{methylene} \quad \text{THF} \quad \text{Glycine}
\]

Serine Dehydratase:

\[
\text{Serine Dehydratase:} \quad \text{H}_2\text{O} \quad \text{H} \quad \text{NH}_3^{(+)} \quad \text{THF} \quad \text{N}^5-N^{10}-\text{methylene} \quad \text{THF} \quad \text{Glycine}
\]
Methionine Cycle
And Biological Methyl Groups

**Methionine**
1. $\text{CH}_3\text{SCH}_2\text{CH}_2\text{COO}^(-)$
2. $\text{ATP} + \text{H}_2\text{O}$ $\rightarrow$ $\text{PPi} + \text{Pi}$

**S-Adenosyl Methionine**
1. $\text{CH}_3\text{SCH}_2\text{CH}_2\text{COO}^(-)$
2. Adenine

**Homocysteine**
1. $\text{HSCH}_2\text{CH}_2\text{COO}^(-)$

**S-Adenosyl Homocysteine**
1. $\text{HSCH}_2\text{CH}_2\text{COO}^(-)$

**Serine**
1. $\text{HOCH}_2\text{C}^\text{OH}$

**Cysteine**
1. $\text{HSCH}_2\text{CH}_2\text{COO}^(-)$

(Remainder of homocysteine degraded for energy)
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine

\( \text{CH}_2\text{CH} \text{NH}_3 \text{COO} \)

(+)

Tetrahydrobiopterin + O\(_2\)

Dihydrobiopterin + H\(_2\)O

Enzyme: Phenylalanine hydroxylase

Tyrosine

\( \text{HO-CH}_2\text{CH} \text{COO} \)

(+)

NH\(_3\)

Enzyme: Homogentisate dioxygenase

Homogentisate

Deficiency: Alkaptonuria “Ochronosis”

Phenylketonuria (no phenylalanine hydroxylase)

Phenylpyruvate

(you don’t need to know the rest)

Phenylpyruvate
Branched Chain Amino Acids

Isol cune

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \_\_\text{CH} \_\_\text{COO}^{(-)} \\
\text{CH}_3 & \text{NH}^{(+)}_3
\end{align*}
\]

\[\alpha-KG\]

\[\text{Glu}\]

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \_\_\text{C} \_\_\text{COO}^{(-)} \\
\text{CH}_3
\end{align*}
\]

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

\[\text{NADH} + 2\text{CO}\

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \_\_\text{C} \_\_\text{S-CoA} \\
\text{CH}_3
\end{align*}
\]

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

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH} & \_\_\text{C} \_\_\text{S-CoA} \\
\text{CH}_3
\end{align*}
\]

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

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

Tyrosine $\xrightarrow{\text{Tyrosine hydroxylase}}$ Dihydroxyphenylalanine (L-DOPA)

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

Tryptophan \[\rightarrow\] NAD⁺

Tryptophan hydroxylase \[\rightarrow\] 5-hydroxytryptophan

PLP-dependent decarboxylation \[\rightarrow\] Serotonin
Synthesis of Bioactive Amines

Glutamate (PLP-dependent) → GABA

Histidine (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
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