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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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Director, DNA Sequencing Core
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:

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

\[
\alpha\text{-keto acid (typically alpha-ketoglutarate)}
\]

\[
\alpha\text{-keto acid (typically glutamate)}
\]

Details of reaction mechanism:

[Diagram showing the steps of the transamination reaction involving amino acids and pyridoxal 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 + α-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:

\[
\begin{align*}
\text{glutamate} & \rightarrow \text{NAD}(P)H + \text{NH}_3 + \alpha\text{-keto glutarate} \\
\text{(mito)} & \\
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} + \text{ATP} + \text{NH}_3 & \rightarrow \text{glutamine} + \text{ADP} + \text{Pi} \\
\text{(+) NH}_3 & \rightarrow \\
\end{align*}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\text{H} \quad (\text{C}\text{C}\text{H}_2\text{H}_2\text{N}) \rightarrow \text{H} \quad (\text{C}\text{C}\text{H}_2\text{H}_2\text{N} + \text{NH}_3)
\]

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

Glutamate donates its amino group to form aspartate:

\[
\text{H} \quad \text{C}\text{C}\text{N} + \text{H} \quad (\text{C}\text{C}\text{H}_2\text{H}_2\text{CO}) \rightarrow \text{H} \quad (\text{C}\text{C}\text{H}_2\text{H}_2\text{CO} + \text{H}_2\text{O})
\]
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{ATP} \text{carbonyl phosphate} \xrightarrow{NH_3} \text{carbamate} \xrightarrow{ATP} \text{carbamoyl phosphate}$
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline

aspartate

ATP

AMP + PP_{i}

Argininosuccinate
Argininosuccinate lyase

\[
\begin{align*}
\text{Argininosuccinate} & \quad \xrightarrow{\text{Argininosuccinate lyase}} \quad \text{Arginine} \\
(-)^{\text{OOOC}} \text{C} & \quad \text{CH}_2 \text{CH}_2 \text{NH}^- \quad \text{C} = \text{NH}_2^{(+)} \\
& \quad \downarrow \quad \text{NH}_3^{(+)} \\
& \quad \text{H} \\
& \quad (-)^{\text{O}_2 \text{CCH}_2\text{C} - \text{CO}_2^{(-)}} \\
\end{align*}
\]

\[
\begin{align*}
\text{Fumarate} & \quad \text{H} \\
& \quad \uparrow \\
& \quad (-)^{\text{O}_2 \text{C} = \text{C} - \text{CO}_2^{(-)}} \\
\end{align*}
\]
Arginase

\[
\begin{align*}
\text{Arginine} & \quad \text{H}_2\text{O} \quad \text{Urea} \\
\text{Ornithine} & \quad \text{H} \\
\end{align*}
\]
Urea Cycle Connects to TCA Cycle

- Ornithine
- Citrulline
- Argininosuccinate
- Arginine
- Urea

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

(-b\text{CCH}_2\text{C}-\text{CO}_2\text{-NH}_2)

(-b\text{C}-\text{C}-\text{CO}_2\text{-})
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\text{glutamate} \xrightarrow{\text{NAD}(P)} \underbrace{\text{\(-O_2CCH_2CH_2C-\text{CO}_2\)}}_{\text{mito}} \text{\(-O_2CCH_2CH_2C-\text{CO}_2\)} + \text{NH}_3
\]

Glutamine Synthetase:

\[
\text{glutamate} \xrightarrow{\text{ATP} + \text{NH}_3} \underbrace{\text{\(-OOC-C-\text{CH}_2\text{CH}_2\text{COO}^-\)}}_{\text{ADP} + P_i} \text{\(-OOC-C-\text{CH}_2\text{CH}_2\text{C}^-\)} + \text{NH}_3
\]
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)

bicarbonate $\rightarrow$ carbonyl phosphate $\rightarrow$ carbamate $\rightarrow$ 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></th>
<th>Description</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

\[
\begin{align*}
(-) \quad & \text{ooc} \quad \text{H} \quad \text{CH}_2 \text{CH}_2 \quad (\text{+}) \quad \text{NH}_3 \\
\text{glutamate} & + \quad \text{CoA} \quad \text{c} \quad \text{O} \\
\text{acetyl CoA} & \quad \text{N-acetyl glutamate} \\
\text{(NAG)} & \quad \text{N-acetyl glutamate synthetase}
\end{align*}
\]

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \rightarrow \text{carbonyl phosphate} \\
\text{ATP} & \rightarrow \text{ADP} \\
\text{NH}_3 & \rightarrow \text{carbamate} \\
\text{ATP} & \rightarrow \text{carbamoyl phosphate}
\end{align*}
\]
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:

\[
\text{Glutamine} \rightarrow \text{glutamate} + \text{ammonia}
\]

\[
\text{Asparagine} \rightarrow \text{aspartate} + \text{ammonia}
\]

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, 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 $\alpha$-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

```
(-)OOC-C-NH(+)_3
\   \      \   
|     |      |     |
H     H      NAD(H)
```

Glycine

```
\   \      \   
|     |      |     |
NAD(+)       NADH
```

```
\   \      \   
|     |      |     |
CO_2  +  NH_4(+)
```

THF  N^6-N^0-methylene THF

Serine Hydroxymethyltransferase:

```
(-)OOC-CH-NH(+)_3
\   \      \   
|     |      |     |
CH_2OH
```

Serine

```
\   \      \   
|     |      |     |
N^6-N^0-methylene THF  Glycine
```

Serine Dehydratase:

```
(-)OOC-CH-NH(+)_3
\   \      \   
CH_2OH
```

```
\   \      \   
|     |      |     |
H_2O
```

```
(-)OOC-C-NH(+)_3
```

```
\   \      \   
|     |      |     |
H_2C
```

```
(-)OOC-C=NH(+)_3
```

```
\   \      \   
|     |      |     |
CH_3
```

```
(-)OOC-C=NH_2
```

```
\   \      \   
|     |      |     |
H_2O
```

```
(-)OOC-C=O
```

```
\   \      \   
|     |      |     |
CH_3
```

```
(-)OOC-C=O
```

```
\   \      \   
|     |      |     |
NH_4(+)
```

```
(-)OOC-C=O
```

```
\   \      \   
|     |      |     |
CH_3
```
Methionine Cycle And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine

\[
\begin{align*}
\text{(+) Tetrahydrobiopterin} + O_2 \\
\text{Dihydrobiopterin} + H_2O
\end{align*}
\]

Enzyme: Phenylalanine hydroxylase

Tyrosine

\[
\begin{align*}
\text{(+) Tetrahydrobiopterin} + O_2 \\
\text{Dihydrobiopterin} + H_2O
\end{align*}
\]

Enzyme: Homogentisate dioxygenase

Homogentisate

Deficiency: Alkaptonuria, "Ochronosis"

Phenylpyruvate

Deficiency: Alkaptonuria (no phenylalanine hydroxylase)
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 $\rightarrow$ 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+ → CO₂
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