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Viewer discretion advised: Material may contain medical images that may be disturbing to some viewers.
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:

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

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

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

Details of reaction mechanism:

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

\(\text{pyridoxal phosphate}\)

\(\text{pyridoxamine phosphate}\)
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 + $\alpha$-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:**

\[
\text{Glutamate} \quad \text{NAD(P)} \quad \text{O} \quad \text{NH}_3
\]

Glutamate → NAD(P)H → α-ketoglutarate + ammonia

**Glutamine Synthetase:**

\[
\text{Glutamate} \quad \text{ATP} + \text{NH}_3 \quad \text{ADP} + \text{P}_i
\]

Glutamate + ATP + NH₃ → Glutamine
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

\[ (-\text{NH}_3) + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{NH}_3 + \text{H}^+ \]

Glutamine   glutamate

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

Glutamate donates its amino group to form aspartate:

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

bicarbonate + ATP → carbonyl phosphate + ADP

carbonyl phosphate + NH₃ → carbamate + Pi

carbamate + ATP → carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[ \text{Ornithine} \quad \text{Citrulline} \]

\[ \text{Ornithine Transcarbamoylase} \]
Argininosuccinate synthetase

\[
\begin{align*}
\text{Citrulline} & \rightarrow \text{Argininosuccinate} \\
\end{align*}
\]
Argininosuccinate lyase

Argininosuccinate → Fumarate → Arginine
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Arginine
- Citrulline
- Argininosuccinate

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

Urea:
- Urea
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

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

**Glutamine Synthetase:**

\[ \text{glutamate} \xrightarrow{\text{ATP+NH}_3} \text{ADP+P}_i \xrightarrow{\text{glutamine}} \]
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)

\[
\text{bicarbonate} \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
\]
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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
bicarbonate & \quad \text{ATP} & \quad \text{carbonyl phosphate} & \quad \text{carbamic anhydride} & \quad \text{carbamoyl phosphate} \\
\text{ADP} & \quad \text{NH}_3 & \quad \text{P}_i & \quad \text{ADP} & \quad \text{P}_i \\
\end{align*}
\]
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:

We also already know how to degrade Glutamine:

\[
\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}
\]

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

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
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:

\[
\begin{align*}
\text{Glycine} & \quad \text{NAD}^+ \quad \text{NADH} \\
\text{THF} & \quad \text{N}^6-\text{N}^\circ-\text{methylene THF} \\
\text{CO}_2 + \text{NH}_4^+ & \\
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Glycine} & \quad \text{THF} \\
\text{N}^6-\text{N}^\circ-\text{methylene THF} & \\
\text{Serine} & \\
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{NH}_4^+ \\
\text{Glycine} & \\
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine

\[ \text{Phenylalanine} \rightarrow \text{Tetrahydrobiopterin} + O_2 \rightarrow \text{Dihydrobiopterin} + H_2O \rightarrow \text{Enzyme: Phenylalanine hydroxylase} \rightarrow \text{Tyrosine} \]

Phenylketonuria (no phenylalanine hydroxylase)

Phenylpyruvate

Deficiency: Alkaptonuria “Ochronosis”

Homogentisate

Enzyme: homogentisate dioxygenase

(you don’t need to know the rest)
Branched Chain Amino Acids

Isoleucine  Leucine  Valine

\[
\text{CH}_3\text{CH}_2\text{CH} - \text{CH} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \text{NH}_3^{(+)}
\]
\[
\alpha-\text{KG}
\]
\[
\text{Glu}
\]
\[
\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{COO}^{(-)}
\]
\[
\text{CH}_3
\]
\[
\text{NAD}^+\text{CoASH}
\]
\[
\text{NADH} + \text{CO}_2
\]
\[
\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{S-CoA}
\]

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

\[
\text{CH}_3\text{CHCH}_2 - \text{CH} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \text{NH}_3^{(+)}
\]
\[
\alpha-\text{KG}
\]
\[
\text{Glu}
\]
\[
\text{CH}_3\text{CHCH}_2 - \text{C} - \text{COO}^{(-)}
\]
\[
\text{CH}_3
\]
\[
\text{NAD}^+\text{CoASH}
\]
\[
\text{NADH} + \text{CO}_2
\]
\[
\text{CH}_3\text{CHCH}_2 - \text{C} - \text{S-CoA}
\]

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

\[
\text{CH}_3\text{CH} - \text{C} - \text{COO}^{(-)}
\]
\[
\text{CH}_3 \text{NH}_3^{(+)}
\]
\[
\alpha-\text{KG}
\]
\[
\text{Glu}
\]
\[
\text{CH}_3\text{CH} - \text{C} - \text{S-CoA}
\]
\[
\text{CH}_3
\]
\[
\text{NAD}^+\text{CoASH}
\]
\[
\text{NADH} + \text{CO}_2
\]
\[
\text{CH}_3\text{CH} - \text{C} - \text{S-CoA}
\]

(continues on to degradation path similar to \(\beta\)-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

\( \text{Tryptophan hydroxylase} \)

\( \text{PLP-dependent decarboxylation} \)

\( \text{CO}_2 \)
Synthesis of Bioactive Amines

Glutamate

\[ \text{COO} \text{-CH}_2 \text{CH}_2 \text{CH} \text{-COO} \]

Glutamate decarboxylase (PLP-dependent)

\[ \text{COO} \text{-CH}_2 \text{CH}_2 \text{CH}_2 \text{-NH}_3 \]

\( \gamma \)-aminobutyric acid (GABA)

Histidine

\[ \text{CH}_2 \text{-CH} \text{-COO} \]

Histidine decarboxylase (PLP-dependent)

\[ \text{CH}_2 \text{-CH}_2 \text{-NH}_3 \]

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