Learning Objectives

1. What Processes Constitute Nitrogen Met.? 
2. How Is Nitrogen Incorporated into Biologically Useful Compounds? 
3. What Role Does Feedback Inhibition Play in Nitrogen Metabolism? 
4. How Are Amino Acids Synthesized? 
5. What Are the Essential Amino Acids? 
6. The Importance of Urea Cycle. 
7. How Are Amino Acids Catabolized?
Catabolic breakdown of amino acids produces citric acid cycle intermediates

Anabolic formation of amino acids uses citric acid cycle intermediates as precursors
Amino Acid Metabolism

• The continuous degradation and synthesis of cellular proteins occur in all forms of life. Each day humans turn over 1–2% of their total body protein, principally muscle protein. Approximately 75% are reutilized. The excess nitrogen forms urea.

• Proteins represent 10-15 % of total energy supply.
Digestion and Absorption of Proteins.
The α-amino group of many amino acids is transferred to α-ketoglutarate to form *glutamate*, which is then oxidatively deaminated to yield ammonium ion (NH4+).
Transamination

Glutamate + $\alpha$-Keto acid $\rightarrow$ $\alpha$-KG + $\alpha$-Amino acid

Glutamate + Oxaloacetate $\rightarrow$ $\alpha$-KG + Aspartate
• All the protein amino acids except lysine, threonine, proline, and hydroxyproline participate in transamination.

• Transamination is readily reversible, and aminotransferases also function in amino acid biosynthesis.

• The coenzyme pyridoxal phosphate (PLP) is present at the catalytic site of aminotransferases.
Aminotransferases

Aspartate aminotransferase (AST), one of the most important of these enzymes, catalyzes the transfer of the amino group of aspartate to $\alpha$-ketoglutarate.

\[
\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}
\]

Alanine aminotransferase (ALT) catalyzes the transfer of the amino group of alanine to $\alpha$-ketoglutarate.

\[
\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}
\]
Glucose - Alanine Cycle

Alanine serves as a carrier of ammonia and of the carbon skeleton of pyruvate from skeletal muscle to liver. The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.
This reaction is catalyzed by \textit{glutamate dehydrogenase}. \textit{This enzyme is unusual in being} able to utilize either NAD+ or NADP+. 
Peripheral Tissues Transport Nitrogen to the Liver

Nitrogen can also be transported as glutamine. Glutamine synthetase catalyzes the synthesis of glutamine from glutamate and NH$_4^+$ in an ATP-dependent reaction:

$$\text{NH}_4^+ + \text{glutamate} + \text{ATP} \xrightarrow{\text{Glutamine synthetase}} \text{glutamine} + \text{ADP} + \text{P}_i$$

The nitrogens of glutamine can be converted into urea in the liver.
Fates of the Carbon Skeletons of Amino Acids

Glucogenic amino acids are shaded red, and ketogenic amino acids are shaded yellow. Most amino acids are both glucogenic and ketogenic.
<table>
<thead>
<tr>
<th>Glucogenic</th>
<th>Ketogenic</th>
<th>Glucogenic and Ketogenic</th>
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<tbody>
<tr>
<td>Aspartate</td>
<td>Leucine</td>
<td>Isoleucine</td>
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<td>Asparagine</td>
<td>Lysine</td>
<td>Phenylalanine</td>
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<td>Alanine</td>
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<td>Tryptophan</td>
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<tr>
<td>Methionine</td>
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Ammonia (NH₃) is a relatively strong base, and at physiological pH values it is mainly present in the form of the ammonium ion NH₄⁺.

NH₃ and NH₄⁺ are toxic, and at higher concentrations cause brain damage in particular. Ammonia therefore has to be effectively inactivated and excreted. This can be carried out in various ways.
If liver function is compromised, as in cirrhosis or hepatitis, elevated blood ammonia levels generate clinical signs and symptoms which may lead to coma “hepatic coma”. Rare metabolic disorders involve each of the five urea cycle enzymes.

- Only traces of ammonia (10–20μg/dL) normally are present in peripheral blood.
Excretion into urine of ammonia produced by renal tubular cells facilitates cation conservation and regulation of acid-base balance. Ammonia production from intracellular renal amino acids, especially glutamine, increases in metabolic acidosis and decreases in metabolic alkalosis.
Aquatic animals can excrete \( \text{NH}_4^+ \) directly. For example, fish excrete \( \text{NH}_4^+ \) via the gills (*ammonotelic animals*).

*Terrestrial vertebrates, including* humans, hardly excrete any \( \text{NH}_3 \), and instead, most ammonia is converted into *urea* before excretion (*ureotelic animals*).

*Birds and reptiles*, form *uric acid*, which is mainly excreted as a solid in order to save water (*uricotelic animals*).
Ammonia as Ammonium ion

Urea

Uric acid

Fish

US

Birds
A kangaroo rat converts some of its waste nitrogen to uric acid.

Reptiles and other desert animals do not usually have much water available, and birds cannot afford to carry the weight of a fluid-filled bladder. These animals do not make urea; rather, they convert all their waste nitrogen to uric acid.
Urea Cycle

Arginine → Argininosuccinate → Ornithine → Citrulline → Carbamoyl phosphate → CO$_2$ + NH$_4^+$ → Urea
Urea Cycle
In the first step, carbamoyl phosphate is formed in the mitochondria from hydrogen carbonate (HCO3–) and NH4+, with two ATP molecules being consumed. In this compound, the carbamoyl residue (–O–CO–NH2) is at a high chemical potential. In hepatic mitochondria, enzyme [1] makes up about 20% of the matrix proteins.
Carbamoyl phosphate synthase I, the rate-limiting enzyme of the urea cycle, is active only in the presence of its allosteric activator \textit{N-acetylglutamate}, which enhances the affinity of the synthase for ATP.

Major changes in diet can increase the concentrations of individual urea cycle enzymes 10-fold to 20-fold. Starvation, for example, elevates enzyme levels to cope with the increased production of ammonia that accompanies enhanced protein degradation.
[2] In the next step, the carbamoyl residue is transferred to the non-proteinogenic amino acid ornithine, converting it into citrulline, which is also non-proteinogenic. This is passed into the cytoplasm via a transporter.
The second NH2 group of the later urea molecule is provided by aspartate, which condenses with citrulline into argininosuccinate. ATP is cleaved into AMP and diphosphate (PPi) for this endergonic reaction. To shift the equilibrium of the reaction to the side of the product, diphosphate is removed from the equilibrium by hydrolysis.
[4] Cleavage of fumarate from argininosuccinate leads to the proteinogenic amino acid arginine, which is synthesized in this way in animal metabolism.

[5] In the final step, urea is released from the guanidinium group of the arginine by hydrolysis, and is immediately rearranged into urea. In addition, ornithine is regenerated and returns via the ornithine transporter into the mitochondria, where it becomes available for the cycle once again.
The rate of urea formation is mainly controlled by reaction [1]. *N*-acetyl glutamate, as an allosteric effector, activates carbamoylphosphate synthase. In turn, the concentration of acetyl glutamate depends on arginine and ATP levels, as well as other factors.
Krebs Bi-cycles
Inherited Defects of the Urea Cycle Cause Hyperammonemia and Can Lead to Brain Damage

All defects in the urea cycle lead to an elevated level of NH4+ in the blood (hyperammonemia). Some of these genetic defects become evident a day or two after birth, when the affected infant becomes lethargic and vomits periodically. Coma and irreversible brain damage may soon follow.
Symptoms of Ammonia Intoxication

This include tremor, slurred speech, blurred vision, coma, and ultimately death.

Ammonia may be toxic to the brain in part because it reacts with α-ketoglutarate to form glutamate. The resulting depleted levels of α-ketoglutarate then impair function of the tricarboxylic acid (TCA) cycle in neurons.
<table>
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<tr>
<th>Essential</th>
<th>Nonessential</th>
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<tbody>
<tr>
<td>Arginine*</td>
<td>Alanine</td>
</tr>
<tr>
<td>Histidine†</td>
<td>Asparagine</td>
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Methionine Metabolism

Methionine + ATP → S-Adenosylmethionine

Transmethylation

S-Adenosylhomocysteine

Ammonia

S-Adenosylmethionine

α-Ketobutyrate

Cystathionine

Serine

Homocysteine

From ser

From met

Cysteine

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END