The Urea Cycle
Nitrogen fixing bacteria

Ammonia NH₃

Bacteria

Nitrite ions (NO₂⁻), Nitrate ions (NO₃⁻)

Plants

Animals

Amino acids-----Proteins

Dietary proteins

Humans

Urea is the waste product produced when the body metabolizes protein

Urea, NH₃
Amino acids

Generated

* Degradation of body proteins
* Degradation of dietary proteins
* Synthesis of non-essential amino acids from simple intermediates of metabolism

Depleted

* Synthesis of body proteins
* Consumed as precursors of essential nitrogen-containing small molecules
* Conversion of amino acids to glucose, fatty acids or CO2
Degradation of body/cellular proteins

Pathways of protein degradation:

Ubiquitin/26S proteasome

Lysosome
  Microautophagy
  Macroautophagy
  Chaperone-mediated microautophagy
Degradation of a target substrate by the ubiquitin pathway involves two steps:

1. **Ubiquitin conjugation**: Ubiquitin is conjugated to the target substrate through a series of enzymes (E1, E2, E3).
2. **26S proteasome-mediated degradation**: The ubiquitinated substrate is recognized by the 26S proteasome, which degrades it into smaller peptides.

The diagram illustrates these processes, showing the steps involved in ubiquitination and proteasome-mediated degradation.
**Autophagy**

**Macroautophagy:** Involves the formation of a crescent-shaped structure (the phagophore) that expands to form the double-membrane autophagosome, capable of fusion with the lysosome.

**Microautophagy:** Lysosomes invaginate and directly sequester cytosolic components.

**CMA:** Involves degradation of selected proteins that have a consensus peptide sequence which is recognized by the binding of a hsc70-containing chaperone/co-chaperone complex for their translocation across the lysosomal membrane.

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**Diagram:**
- **Macroautophagy**
- **Microautophagy**
- **CMA**

**Substrate protein**
- Chaperone
- Amino Acids
A. Digestion of proteins by gastric secretions
*Specificity
*Release of zymogens by Cholecystokinin and secretin
*Activation of zymogens
* Abnormalities in protein digestion
B. Digestion of proteins by pancreatic enzymes
C. Digestion of oligopeptides by enzymes of the small intestine
D. Absorption of amino acids and dipeptides
Free amino acids are taken into the enterocytes up by a Na+-linked secondary transport systems. Di and tripeptides are taken up by H+-linked transporters.
Transport of AA into cells

Seven different transport systems are known that have overlapping specificity for different Amino acids.

The small intestine and proximal tubule of the Kidney have common transport systems for amino acid uptake.

**Cystinuria**------ defective reabsorption of Cystine and also Ornithine, Arginine and Lysine.

**Hartnup disorder**------caused due to the transport of tryptophan.
Essential versus Nonessential Amino Acids

<table>
<thead>
<tr>
<th>Essential</th>
<th>Nonessential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>Alanine</td>
</tr>
<tr>
<td>Histidine</td>
<td>Aspartate</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Leucine</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Lysine</td>
<td>Glycine</td>
</tr>
<tr>
<td>Methionine</td>
<td>Proline</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Serine</td>
</tr>
<tr>
<td>Threonine</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Tryptophan</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td></td>
</tr>
</tbody>
</table>

*a* Arginine is synthesized by mammalian tissues, but the rate is not sufficient to meet the need during growth.

*b* Methionine is required in large amounts to produce cysteine if the latter is not supplied adequately by the diet.

*c* Phenylalanine is needed in larger amounts to form tyrosine if the latter is not supplied adequately by the diet.
Amino Acid Catabolism

1) Removal of $\alpha$-amino group
2) Metabolization of carbon skeleton

- Dietary protein
- Body proteins $\leftrightarrow$ Amino acids
- Nitrogen
- Urea
- NH$_3$
- Fats, sterols
- Acetyl CoA
- Oxygen ($O_2$)
- Carbohydrate intermediates
- Carbon dioxide ($CO_2$), water ($H_2O$), energy
- Glycogen

Coenzymes
- Neurotransmitters
- Phospholipids
- Porphyrins
- Purines
- Pyrimidines
- Other nitrogenous compounds
Transamination: the funneling of amino groups to glutamate

Transfer of amino groups to $\alpha$-ketoglutarate

Almost all amino acids undergo transamination, except lysine and threonine.
Aminotransferase

- Substrate Specific-
  - Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)

↑ AST and ALT - Liver diseases
Nonhepatic diseases

Source of nitrogen in the Urea cycle
Mechanism of action of aminotransferase

1) Transfer amino groups to PLP
2) Resulted pyridoxamine phosphate formed reacts with α-keto acid to form amino acid and aldehyde form of PLP

B \textit{Aspartate aminotransferase}

- Oxaloacetate → Glutamate → Aspartate → α-Ketoglutarate
- Glutamate + α-Ketoglutarate → Pyridoxal phosphate → Aspartate
- Pyridoxamine phosphate
- NH₃
**Glutamate dehydrogenase: The oxidative deamination of amino acids**

(Liver and Kidney)

**Transamination:** Transfer of amino groups

**Deamination:** Liberation of amino group as ammonia

Co-enzymes: NAD+ and NADP+

Directions: Depends on the levels of Glutamate, $\alpha$-ketoglutarate

Allosteric inhibitors: GTP is an inhibitor
ADP is an activator
Amino Acid Catabolism

Amino Acid Metabolism
D-amino acids

• Present in our diet
  * Present in plants
  * Not used for mammalian protein synthesis
  * D-amino acid oxidase enzyme catalyzes deamination of D-AA
Glucose-Alanine cycle serves two purposes:

1) Recycles carbon skeletons between muscle and liver
2) Transports NH3 to the liver and is converted into urea.
Oxidative deamination of Glutamate
By mitochondrial glutamate dehydrogenase
The urea cycle consists of five reactions: two mitochondrial and three cytosolic. The cycle converts two amino groups, one from NH₄⁺ and one from Asp, and a carbon atom from CO₂ to the relatively nontoxic excretion product urea. Requires four "high-energy" phosphate bonds.

<table>
<thead>
<tr>
<th>Step</th>
<th>Reactants</th>
<th>Products</th>
<th>Catalyzed by</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NH₃ + CO₂ + 2 ATP</td>
<td>Carbamoyl Phosphate + 2ADP + pi</td>
<td>Carbamoyl phosphate Synthetase I</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>2</td>
<td>Carbamoyl Phosphate + Ornithine</td>
<td>Citrullin + Pi</td>
<td>Ornithine transcarbamoylase</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>3</td>
<td>Citrullin + Aspartate + ATP</td>
<td>Argininosuccinate + AMP + P Pi</td>
<td>Argininosuccinate synthase</td>
<td>Cytosol</td>
</tr>
<tr>
<td>4</td>
<td>Argininosuccinate</td>
<td>Arginine + Fumarate</td>
<td>Argininosuccinate lyase</td>
<td>Cytosol</td>
</tr>
<tr>
<td>5</td>
<td>Arginine + H₂O</td>
<td>Ornithine + Urea</td>
<td>Arginase</td>
<td>Cytosol</td>
</tr>
</tbody>
</table>
Flow of Nitrogen from amino acids

One Nitrogen of Urea is supplied by ammonia
Second Nitrogen of Urea is supplied by Aspartate
Carbon and oxygen of Urea is derived from CO2

Overall stoichiometry of the urea cycle
Aspartate + NH₃ + CO₂ + 3 ATP →

Urea + fumarate +2ADP +AMP + 2 Pi + Ppi + 3H₂O
Sources of Ammonia

**From Amino acids**

--- in liver by transdeamination reaction

**From glutamine**

--- in kidneys by the action of renal glutaminase and glutamine dehydrogenase

**From urea**

--- Bacterial urease action in the intestine which is then transported into the liver to make urea

**From amines**

--- by the action of amine oxidase

(Amines from diet
Neurotransmitters
Monoamines)

From purines and pyrimidines--- amino groups are released as ammonia
Fate of Urea

Ammonia → Liver → Blood → Kidney → Urea → Excreted in Urine
Diseases caused due to the Urea metabolism/catabolism

Hyperammonemia

Due to the Liver diseases
Blood level of ammonia is increased

Affects CNS and could cause death

Patients with carbamoyl Phosphate synthetase I
Amino acid pool is defined as all the free amino acids in cells and extracellular fluids.

Amino acids are produced by the degradation of body protein, synthesis of nonessential amino acids, and degradation of dietary protein. These processes involve α-keto acids and ammonia and proteolytic enzymes of the GI tract and pancreas.

Amino acid consumption is regulated by ubiquitin, N-terminal amino acids, and PEST sequences.

Protein turnover results in simultaneous synthesis and degradation, leading to the protein turnover process.

Amino acids used in biosynthesis involve transcription and translation factors and biosynthetic pathways.

Metabolism of amino acids involves intermediary metabolism, characterized by hyperammonemia and mental retardation, and treated by drug therapy and reduction of protein intake.

Removal of nitrogen from amino acids occurs because amino acids cannot directly participate in energy metabolism.

Amino groups are removed by transaminases, a sequential reaction mediated by two enzymes: first, transaminases, which result in elevated serum levels and liver damage; second, glutamate dehydrogenase, which results in glutamate oxidatively deaminated to α-ketoglutarate and ammonia, which can be stored and transported as glutamine.

The urea cycle may have nitrogen of aspartate, CO₂, and NH₃ incorporated into urea.