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NITROGEN METABOLISM – An Overview

How are nitrogen-containing compounds stored in the body?

- Nitrogen-containing compounds are not stored in the body
- Amino Acids are the major sources of Nitrogen in the body
- **Figure 1** shows schematic diagram of Amino acid utilization
  - Diagram is oversimplification of **Amino Acid "Pool**"
- Amino Acid "Pool" is made up of several compartments that vary in patterns of amino acids as well as concentrations

What ate the Input and Output processes of the Pool?

- Major Inputs into Amino Acid Pool:
  - Dietary Protein, Degradation of Cellular Proteins

- Outputs from Amino Acid Pool usually occur from
  - Protein Biosynthesis, which is major drain on the pool,
  - Urea formation from amino acid catabolism,
  - Biosynthesis of Nitrogen containing compounds

TAKE NOTE:

- Amount of some Free Amino Acids in Intracellular Compartments is considerably higher than in Extracellular Compartments
- Movement of Amino Acids into Intracellular compartments is by active transport that requires energy from ATP
- Total amount of Free Amino Acids in the body is about 100g
- Glutamate and Glutamine constitute about 50% of Total Free Amino Acids
- Essential Amino Acids constitute about 10% Free Amino Acids

What are dietary Essential Amino Acids?

- Amino Acids whose Carbon Skeleton cannot be synthesized in the body are called Dietary Essential Amino Acids
- Dietary essential Amino Acids must be obtained from the Diet
- Acronyms for essential amino acids are:
  - **TV TILL PM** (8 Essential amino acids for humans including healthy infants)
  - **PVT TIM HALL** (10 Essential amino acids for Albino Rats)

Why do some infants need 9 essential amino acids?

- Arginine is the Ninth essential amino acid for Premature Infants (Why?)
  - Arginine is synthesize in the Urea cycle
  - In Premature babies Urea cycle is not fully functional
  - **Arginine** may not be synthesized in amounts adequate enough to meet the requirements for both protein biosynthesis and urea cycle function;
- Arginine becomes an essential amino acid for Premature babies

What is Nitrogen Balance?

- **Nitrogen balance is when total daily Nitrogen Intake, mainly as Protein in the diet, is equal to total daily Nitrogen losses mainly as Urea in urine**
  - Example: A "healthy" adequately nourished adult

- **Nitrogen balance can also be either Positive or Negative**:
- **Positive Nitrogen Balance**:
  - Positive Nitrogen Balance its when total daily nitrogen intake, mainly as Protein in the diet, **is greater than** total daily nitrogen losses mainly as Urea urine
    - Examples: An healthy growing children; Normal pregnancy

- **Negative Nitrogen Balance**:
  - Negative Nitrogen Balance is when total daily nitrogen intake, mainly as protein in the diet, **is less than** total daily nitrogen losses mainly as Urea in urine
    - Examples: Diseases involving tissue wasting or in starvation or intake of inadequate dietary protein or lack of an essential amino acid

- Prolonged periods of Negative Nitrogen Balance are dangerous and sometimes fatal if the loss of body protein reaches about one-third total body protein

How are animals classified under nitrogen metabolism?

- Animals are classified into 3 groups based on end product of Nitrogen Metabolism
- **Ammonotelic organisms:**
  - Animals that excrete Nitrogen as Ammonia
    - Examples: Bony Fish, Teleostean Fish

- **Uricotelic organisms:**
  - Animals that excrete Nitrogen as **URIC ACID**
  - Uric Acid is relatively insoluble in aqueous medium, thus is excreted as semisolid crystals. Examples: Birds (they conserve water and maintain low weight)

- **Ureotelic organisms:**
  - Animals that excrete Nitrogen as **UREA,** which is a highly water soluble, nontoxic compound. Examples: Mammals including Humans

Nitrogen Metabolism in Humans – A Ureotelic Organism:

- Primary means of Metabolism of Nitrogen in Amino Acid is via sequential action of Enzymes located in different cellular compartments

What are the stages involved in conversion of Nitrogen in Amino Acids to Urea in Ureotelic organisms?

- **Four stages** are involved in conversion of Nitrogen in α-Amino Acids to Urea
  - **Transamination;**
  - **Oxidative Deamination of Glutamate;**
  - **Ammonia transport;**
  - **Reactions of the Urea cycle**

- **Figure 2**: Schematic diagram of **Metabolic Flow of Amino Acid Nitrogen** relates the Four stages to Overall Catabolism of Amino Acid Nitrogen

TRANSAMINATION:

What is Transamination?

- Transamination involves the transfer of an Amino group from an α-Amino Acid to a α-Keto Acid to form a New α-Amino Acid and a New α-Keto acid
- Transamination is Inter-conversion of a Pair of Amino Acids and a Aair of Keto acids
  - Both α-Keto acids and α-Amino acids are involved

- **Not all Amino Acids can take part in Transamination reaction**
- α-Amino acids that cannot undergo Transamination include:
  - **Lysine, Threonine, Cyclic Imino acids: Proline and Hydroxyproline**
- Transamination reactions are **Reversible,** therefore they can function in Amino Acid Catabolism and Biosynthesis
- Enzymes that catalyze Transamination reactions are called T**ransaminases** or **Amino Transferases**
- **Pyridoxal Phosphate** (Coenzyme **B_6PO_4**) is Coenzyme
- General reaction catalyzed by Transaminase (Amino Transferase) can be written as follows:

$$\text{Amino Acid A + Keto Acid B} \xleftarrow{B_6\ PO_4} \text{Keto Acid A + Amino Acid B}$$

- Each Transaminase enzyme is specific for one Pair of substrate but non-specific for the other pair

Give examples of some Transaminase reactions used in diagnosis?

- Examples of Transaminases (Amino Transferases) used in diagnosis:
- **Alanine Aminotransferase (ALT, Formerly called Serum Glutamate-Pyruvate Transaminase [SGPT]) catalyzes the reaction:**

Alanine + α-Keto Acid ⟵======⟶ Pyruvate + Amino Acid

- **Aspartate Aminotransferase (AST, Formerly called Serum Glutamate-Oxaloacetate Transaminase [SGOT])** catalyzes the reaction:

  Aspartate + α-Oxoglutarate ⟵⟶ Oxaloacetate + Glutamate

- **Glutamate-α-Oxoglutarate Transaminase (also called Glutamate Transaminase) catalyzes the reaction:**

  Glutamate + α-Keto Acid ⟵⟶ α-Oxoglutarate + Amino Acid

What is the significance of the Glutamate Transaminase reaction?

- Glutamate Transaminase reactions is significant in Nitrogen metabolism because **Glutamate is the Only Amino Acid in Mammalian tissues that undergoes Oxidative Deamination at an appreciable rate**
- **All Amino Nitrogen from Amino Acids that can undergo Transamination can be concentrated in Glutamate** by the Glutamate Transaminase reactions
- **Since Alanine is a substrate for Glutamate Transaminase reaction, those** Amino Acids that cannot react directly with **α-Oxoglutarate** can react with Pyruvate in the Alanine Transaminase (ALT) reaction
- **Formation of Ammonia from α-Amino groups occurs mainly via conversion to the α-Amino Nitrogen of L-Glutamate**

OXIDATIVE DEAMINATION

What is Oxidative Deamination?

- **Glutamate Dehydrogenase reaction is called Oxidative Deamination**
- Oxidative Deamination is the Oxidative removal of Ammonia from Glutamate
  - α-Amino groups of most amino acids are ultimately transferred to α-Oxoglutarate by Transamination, forming Glutamate, the Amino group is then removed as Ammonia by Oxidation

- Reaction occurs in Mitochondrial matrix:

Glutamate + NAD ⟵⟶ α-Oxoglutarate + NH_4^+ + NADH + H^+

- **Enzyme is: Glutamate Dehydrogenase** (**GDH**)
- Coenzymes are: NADH or NADPH
- Reaction is freely reversible and takes part in Amino Acid Biosynthesis and Catabolism
  - For biosynthesis, it catalyzes Amination of α-Oxoglutarate by Free Ammonium ion
  - For catabolism, it channels Nitrogen from Glutamate to Urea

AMMONIA TRANSPORT

What is the range of Ammonia in Tissues including blood?

- Range of NH_3 concentration in tissues:
  - Cardiac Muscle: 0.2mM
  - Abdominal Muscle and Kidneys: 0.9mM
  - Brain and Thigh muscle: 0.3mM
  - Liver: 0.7mM

- Concentration of NH_3 in Blood (excluding Portal Blood): 0.05mM
  - **Ammonia concentration in Blood is lower than in most Tissues**

- Indicating that Ammonia as such is not the main form in which excess NH_3 in tissues is transported to the Liver

How is NH_3 transported from the TISSUES TO LIVER?

- A series of reactions can be used to answers this question:
- Within the cells of tissues other than Liver, NH_3 can be removed by two reactions:

- **First** is the **Glutamate Dehydrogenase (GDH) reaction:**

$$\alpha\text{-Oxoglutarate} + NH_4^+ + NADH + H^+ \leftrightarrows \text{Glutamate} + NAD$$

- **Second** is the non-reversible **Glutamine Synthetase reaction**:

$$\text{Glutamate} + NH_3 + ATP \longrightarrow \text{Glutamine} + ADP + P_i$$

- Glutamate formed in GDH reaction can Transaminate with any suitable Keto acid to yield a different amino acid

- Example: Pyruvate can Transaminate with Glutamate to form Alanine:
- Enzyme: ALT

$$\text{Glutamate} + \text{Pyruvate} \leftrightarrows \alpha\text{-Oxoglutarate} + \text{Alanine}$$

- NH_3 is transported therefore, as Amino Acids having a Total Concentration in Blood plasma of between 3.0mM to 4.OmM.
- Glutamine (0.4mM), Glutamate (0.23mM) and Alanine (0.4mM) are the most plentiful and of these Glutamine and Alanine penetrate into the Liver most easily

How is NH_3 produced in Brain and Muscle and transported to the Liver?

- High amount of NH_3 is produced in **Muscle** and **Brain** because of relatively high activity of **Adenylate Deaminase** that catalyzed the following reaction:

$$\textbf{AMP + } H_2O \textbf{ ====➔ IMP + } NH_3$$

- NH_3 produced in **Muscle** enters blood and is transported as such to Liver
- NH_3 produced in **Brain** is converted to Glutamine because of High activity of **Glutamine Synthetase**
- Glutamine so formed is then transported in the blood to the Liver

What are the sources of NH_3 in the Liver?

- Liver receives NH_3 by two main routes
- From Portal Blood as Free NH_3 and Amino Acids
    - Amount of Amino Acids in Portal Blood and of Free NH_3 (formed by bacterial action in GIT) depends on the diet

- From Systemic Blood as Amino Acids (mainly Glutamine and Alanine)

- **In Liver NH_3 can arise from the following enzymes**:
    - **Glutaminase: C**atalyzes formation of NH_3 from Glutamine:

$$\textbf{Glutamine + } H_2O \textbf{ =====➔ Glutamate + } NH_3$$

- **Adenylate Deaminase:** Catalyzes formation of NH_3 from AMP:

$$\textbf{AMP + } H_2O \textbf{ ====➔ IMP + } NH_3$$

- **Glutamate Dehydrogenase:** Catalyzes the reversible reaction:

$$\textbf{Glutamate + NAD + } H_2O \textbf{ ⬅==➔ } \alpha\textbf{-Oxoglutarate + NADH + } H^+ \textbf{ + } NH_4^+$$

How is excess ammonia removed from the metabolic system?

- During **Ammonia overloading**, excess Ammonia is removed from by forming Glutamate (GDH reaction), which then Transaminate with Pyruvate to form Alanine
- Combination of GDH and Transaminase systems results in an overall buffering of the NH_3 concentration within the Liver
- Schematic diagram below shows the Link between Transamination and Oxidative Deamination:

Use schematic diagram to show the link between Transamination and Oxidative Deamination (See diagram below)

- Glutamate Dehydrogenase (GDH) catalyzes Oxidative Deamination of α-Amino Acids
- GDH operating in conjunction with Transaminases can convert α-Amino group of most Amino Acids to Free Ammonia
- Alanine Transaminase can act as a Link between GDH and those Amino Acids that cannot Transaminate directly with α-Oxoglutarate

REACTIONS OF THE UREA CYCLE:

Give a brief outline of the Urea Cycle

- Synthesis of Urea requires 3 ATP, NH_3, CO_2 and α-Amino Nitrogen of Aspartate
- First two reactions of Urea cycle occur in Mitochondrial matrix, which contain **Two** of the urea cycle enzymes:
  - **Carbamoyl Phosphate Synthetase**
  - **Ornithine Transcarbamylase (also called Citrulline Synthetase**)

- Three reactions occur in the Cytosol that contains the enzymes:
  - **Argininosuccinate Synthetase**
  - **Argininosuccinate Lyase**
  - **Arginase**

- Urea contains two atoms of Nitrogen, one derived from NH_3 directly and the other from Aspartate
- Compartmentalization of Urea cycle enzymes requires that certain urea cycle intermediates be **transported across Mitochondrial membrane**

How is Urea Cycle regulated?

- Levels of urea cycle enzymes fluctuate with changes in feeding patterns:
  - With protein-free diets: (i.e., low or very low amount of protein in the diet)
  - Urea excretion accounts for only about 60% of total urinary nitrogen compared to about 80% in a normal diet
  - Levels of all urea cycle enzymes decline

- With high-protein diets or during starvation (when Gluconeogenesis from Amino Acids is high) levels of all urea cycle enzymes increase

What is the fate of Carbon skeletons of Amino Acids?

- Amino acids can be classified on Metabolic Fate of Carbon Skeleton
- **Ketogenic Amino Acids:**
  - Amino Acids whose Carbon Skeleton can be converted to either Acetyl-CoA or Acetoacetyl-CoA and used for biosynthesis of Ketone bodies
    - The only purely Keogenic amino acids are Leucine and Lysine

- **Glucogenic Amino Acids:**
  - Amino Acids whose Carbon Skeleton can be converted to Pyruvate or to Intermediates in TCA-cycle and used for biosynthesis of Glucose
    - Almost all amino acids with few exceptions are Glucogenic

- Some Amino Acids are classified as both Ketogenic and Glucogenic:
  - Example: Isoleucine, Phenylalanine, Tyrosine, and Tryptophan