NITROGEN METABOLISM: An Overview

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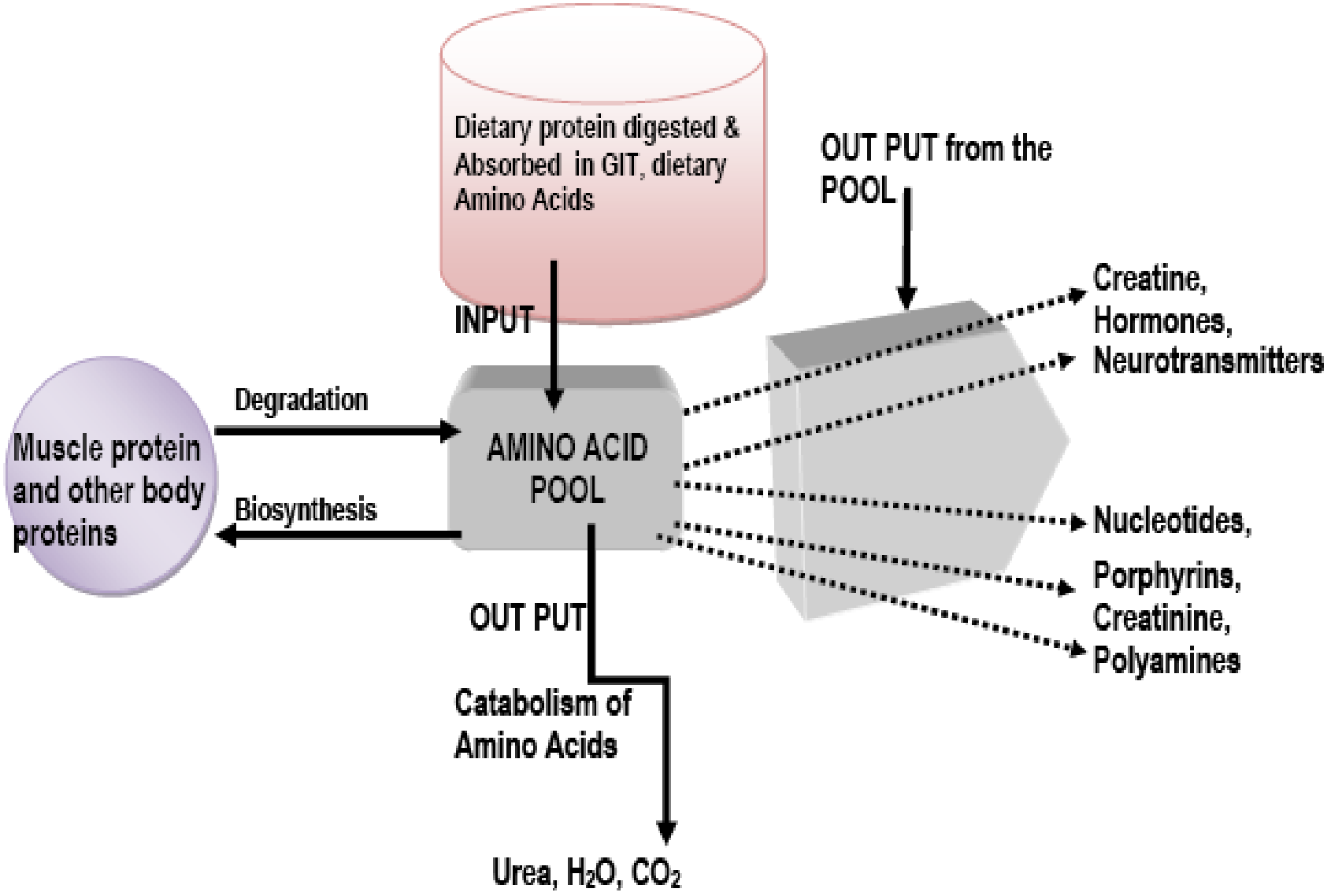
How are nitrogen-containing compounds stored in the body?

- Nitrogen-containing compounds are not stored in the body,
- Amino Acids are the major sources of Nitrogen,
- It is assumed that the body contains an "**Amino Acids Pool**"
- Amino Acid "**Pool**" consist of compartments with different combinations and concentrations of amino acids;
  - Pool is not located in any specific tissues or organs
- **Fig 1:** Schematic diagram of oversimplification of **Amino Acid "Pool"**

Fig. 1: Schematic diagram of Amino Acid Pool



What ate the Input and Output sources of Amino Acid Pool?

- Major Inputs into Amino Acid Pool:
  - Dietary Protein,
  - Degradation of Cellular Proteins,
- Outputs from Amino Acid Pool:
  - Protein Biosynthesis, the major drain on the pool,
  - Urea formation from amino acid catabolism,
  - Biosynthesis of Nitrogen containing compounds,

IMPORTANT TO NOTE

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

What are the Essential Amino Acids?

- Essential Amino Acids (EAA):
  - Amino Acids whose Carbon Skeletons cannot be synthesized in the body;
  - They must be obtained from the Diet;
- Acronyms for EAA are:
- **TV TILL PM**
  - **8 EAA** for humans including healthy infants,
- **PVT TIM HALL**
  - 10 EAA for Albino Rats,

Why do some infants need 9 EAA?

- **Arginine** is the **Ninth EAA** for Premature Infants (**Why?**)
  - Arginine is synthesize in Urea cycle,
  - Premature Infants: 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;
- Acronym of the **9 EAA** for Premature infants:
  - **A TV TILL PM**

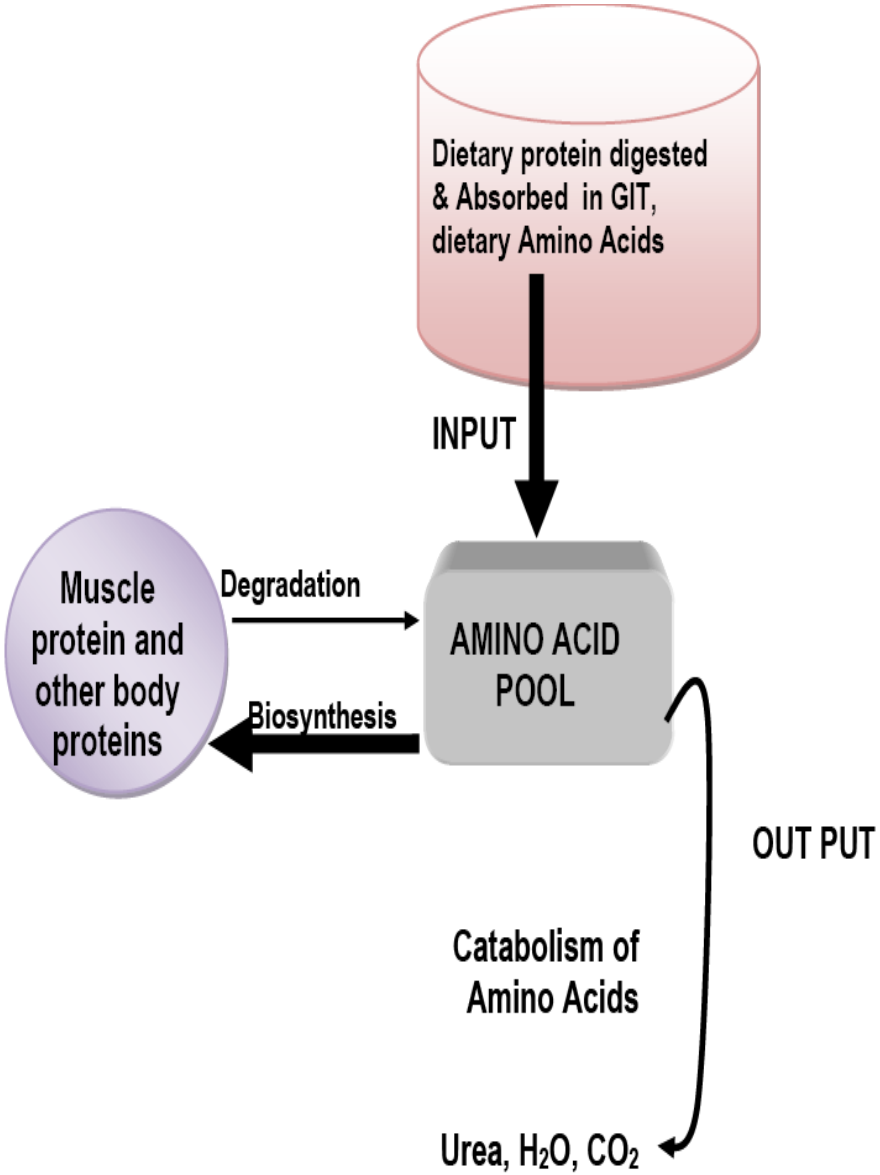
What is Nitrogen Balance?

- **Nitrogen balance:**
  - Total daily intake of Nitrogen **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 be Positive or Negative**;

  - **Note:** Prolonged Negative Nitrogen Balance is dangerous and sometimes fatal if loss of body protein reaches about one-third total body protein;

- **Positive Nitrogen Balance (Figs 2a & 2b):**
  - Total daily intake of nitrogen, **mainly as Protein** in the diet, **is greater than** total daily nitrogen losses mainly as Urea urine;
    - Eg: An healthy growing children; Normal pregnancy
- **Negative Nitrogen Balance (Figs 3a & 3b)**:
  - Total daily intake of nitrogen, **mainly as protein** in the diet, **is less than** total daily nitrogen losses mainly as Urea in urine;
    - Eg: Tissue wasting, starvation, intake of inadequate dietary protein, lack of dietary EAA,

Figs 2a & 2b: Examples of Positive Nitrogen Balance



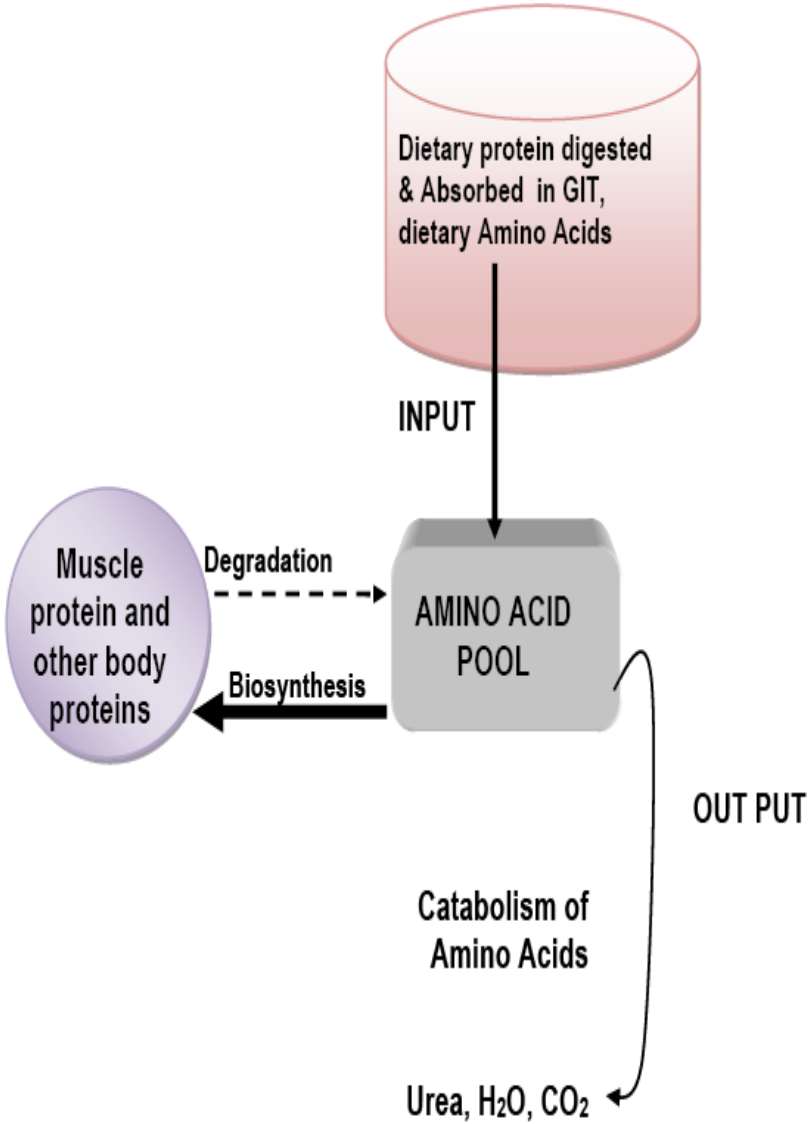
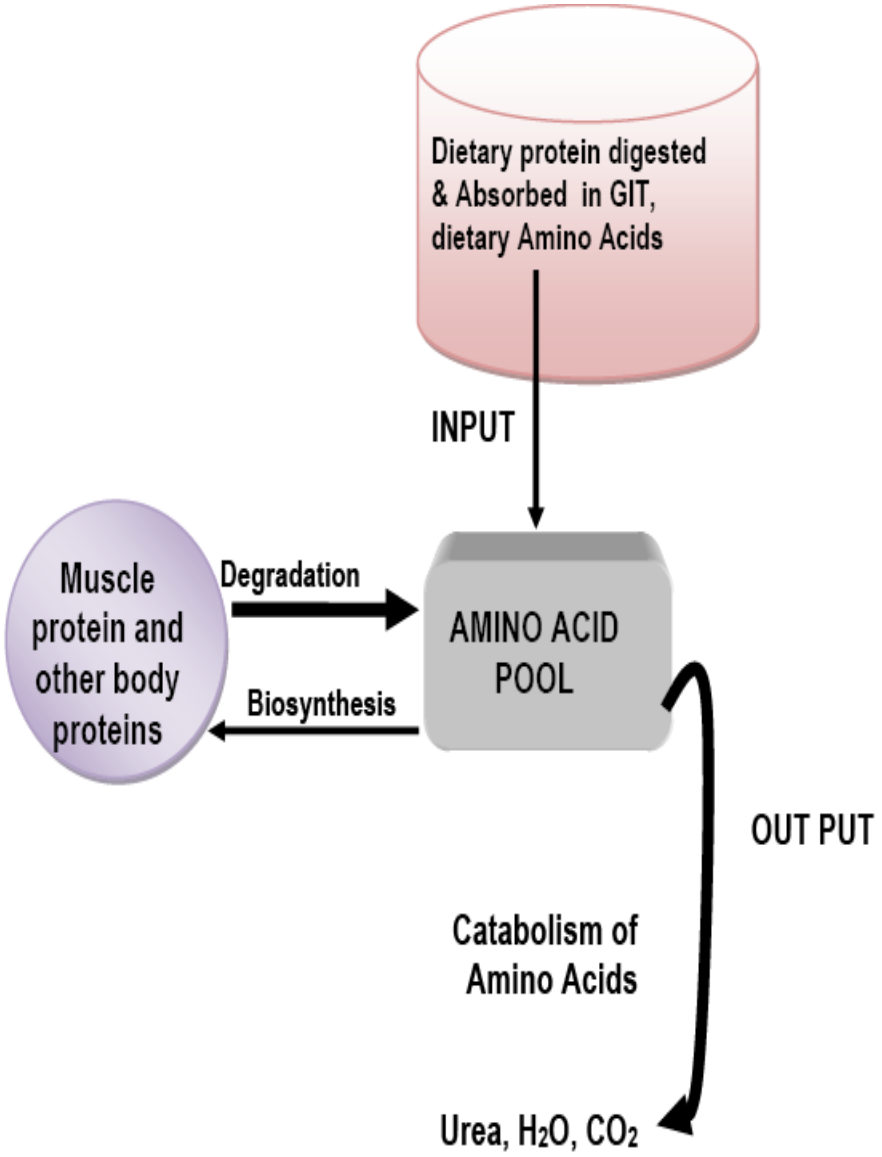
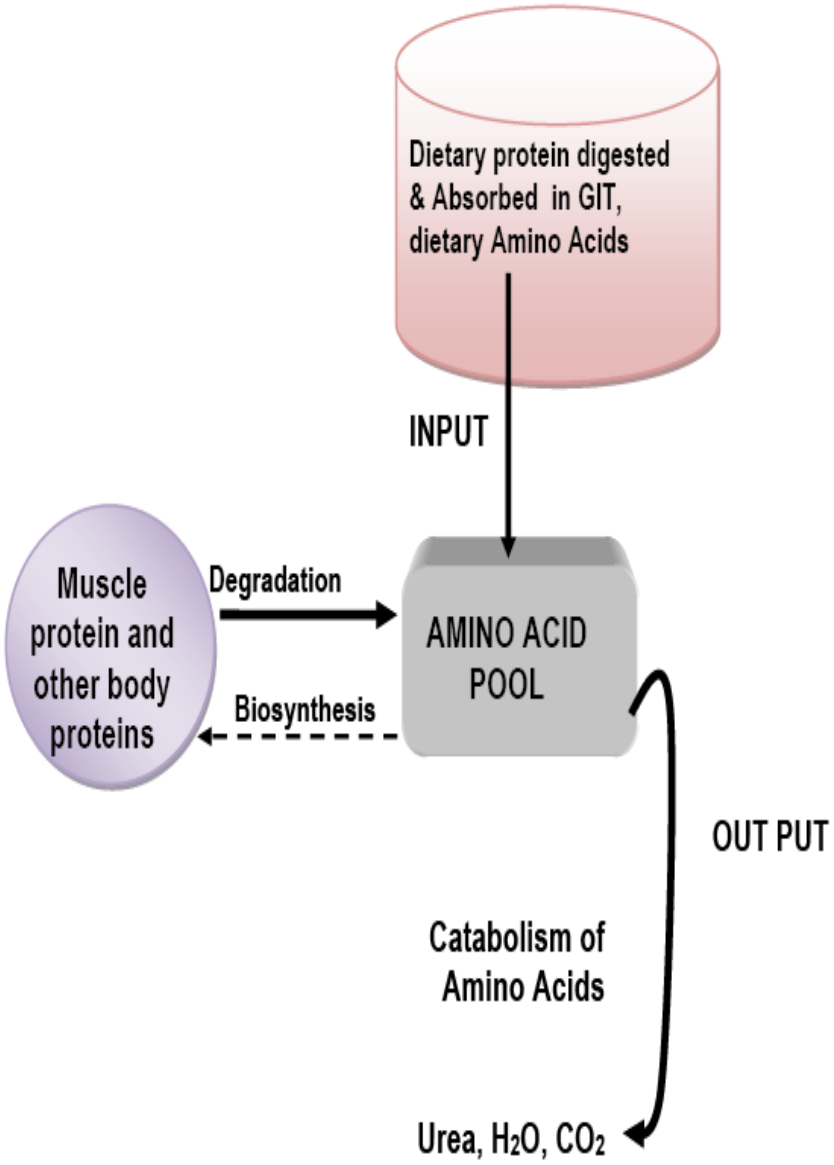


Fig. 3a & 3b: Examples of Negative Nitrogen Balance





How are animals classified under nitrogen metabolism?

- Animals are classified into 3 groups based on end product of Nitrogen Metabolism:
- **Ammonotelic organisms:** Excrete Nitrogen as **Ammonia**
  - Examples: Bony Fish, Teleostean Fish;
- **Uricotelic organisms:** Excrete Nitrogen as **URIC ACID**
  - Uric Acid is relatively insoluble in aqueous medium, thus is excreted as semisolid crystals;
  - Examples: Birds (conserve water for low weight), Reptiles,
- **Ureotelic organisms:** Excrete Nitrogen as **UREA,**
  - Urea is a highly water soluble, nontoxic compound,
  - Examples: Mammals including Humans,

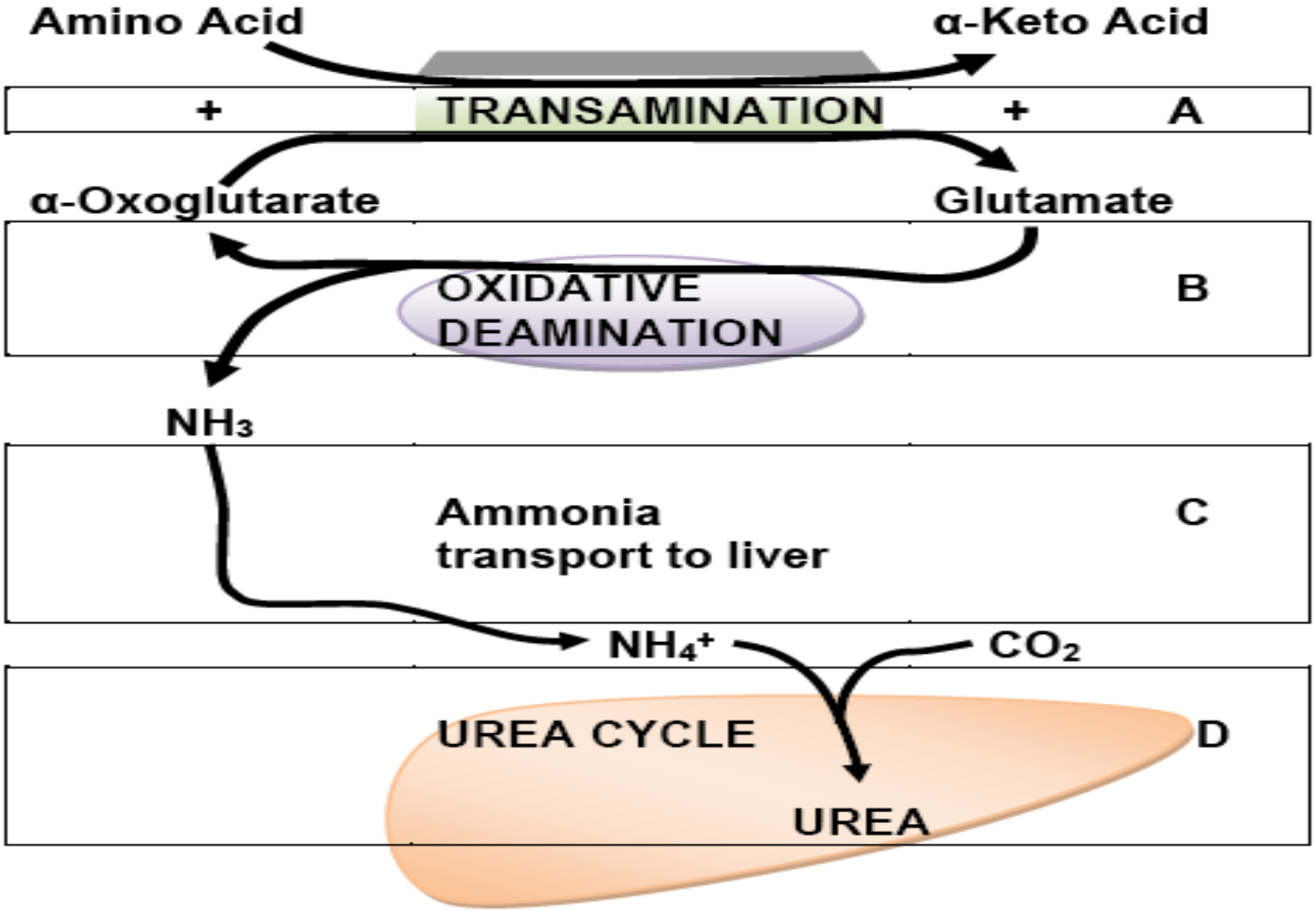
Nitrogen Metabolism in Humans: A Ureotelic Organism

- Primary means of Metabolism of the Nitrogen in Amino Acid is via sequential actions of Enzymes located in different cellular compartments:
  - Cytoplasm (Cytosol)
  - Mitochondria,

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

- **Four major stages** are involved in conversion of Nitrogen in α-Amino Acids to Urea; they are:
  - **Transamination,**
  - **Oxidative Deamination of Glutamate,**
  - **Ammonia Transport,**
  - **Reactions of the Urea Cycle;**
- **Fig 4**: Schematic diagram of **Metabolic Flow of Amino Acid Nitrogen** relating the 4 major stages (A, B, C, D) in catabolism of Amino Acid Nitrogen

Fig. 4: Schematic diagram of **Metabolic Flow of Amino Acid Nitrogen** relating the 4 major stages (A, B, C, D) to catabolism of Amino Acid Nitrogen



TRANSAMINATION: What is Transamination?

- **Transamination**:
  - Transfer of Amino group (NH_4^+) from an **α-Amino Acid** to **α-Keto Acid** to form **New α-Amino Acid** and **New α-Keto acid,**
  - It is Inter-conversion of a Pair of Amino Acids and a pair of Keto acids;
  - Both **α-Keto acids** and **α-Amino acids** are involved,
  - Enzymes involved are: **Transaminases,**
  - Coenzyme involved: **Pyridoxal Phosphate** (B_6PO_4),

Amino Acid A + Keto Acid B ⟵===⟶ Keto Acid A + Amino Acid B

- Not all Amino Acids take part in Transamination,
- α-Amino acids not involve in Transamination are:
  - **Lysine,**
  - **Threonine,**
  - **Cyclic Imino acids: Proline and Hydroxyproline**
- Transamination reactions are Reversible; function in Amino Acid Catabolism and Biosynthesis;
- Each Transaminase enzyme is specific for **one Pair** of substrate but non-specific for the other pair;

Give examples of Transaminase reactions used in diagnosis

1. **Alanine Aminotransferase (ALT)**, Formerly called **Serum Glutamate-Pyruvate Transaminase [SGPT]**,

Alanine + α-Keto Acid ⟷ Pyruvate + Amino Acid

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

Aspartate + α-Oxoglutarate ⟷ Oxaloacetate + Glutamate

3. **Glutamate-α-Oxoglutarate Transaminase (GOT),** also called **Glutamate Transaminase**) catalyzes:

Glutamate + α-Keto Acid ⟷ α-Oxoglutarate + Amino Acid

What is the significance of 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 high rate;**
  - All Amino Nitrogen from Amino Acids that can undergo Transamination can be concentrated in Glutamate by the **Glutamate Transaminase** reaction;
- Alanine is substrate for Glutamate Transaminase reaction thus, those Amino Acids that **cannot react directly** with **α-Oxoglutarate** can react with Pyruvate (**ALT** reaction)
- Formation of **NH_4^+** ion from α-Amino groups occurs mainly via conversion to α-Amino Nitrogen in Glutamate;

OXIDATIVE DEAMINATION: What is Oxidative Deamination?

- **Oxidative Deamination:** Reaction catalyzed by the enzyme **Glutamate Dehydrogenase** (**GDH**);
- Oxidative Deamination is the Oxidative removal of Ammonium ion (**NH_4^+**) from Glutamate;
- GDH reaction occurs in the Mitochondria;
- α-Amino groups of most amino acids are ultimately transferred to **α-Oxoglutarate** by Transamination to form **Glutamate**; the Amino group is then removed as Ammonia by Oxidation;
- The **GDH REACTION**:

- Enzyme is: **Glutamate Dehydrogenase (GDH**)
- Coenzymes are: NADH or NADPH

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

- Reaction is freely reversible,
- It is involved in Amino Acid biosynthesis and catabolism;
  - For biosynthesis, it catalyzes addition of **NH_4^+** ion to α-Oxoglutarate from TCA cycle;
  - For catabolism, it channels Amino 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,
  - Blood (excluding Portal Blood): 0.05mM;
- Ammonia concentration in Blood is lower than in most Tissues,
  - It indicates that Ammonia as such is not the main form in which excess NH_3 in tissues is transported to Liver;

How is NH_3 transported from TISSUES TO LIVER?

- Within cells of tissues other than Liver, NH_3 can be removed by **two** reactions:
- **First: Glutamate Dehydrogenase (GDH) reaction:**

α-Oxoglutarate + NH_4^+ + NADH + H^+ ⇐⇒ Glutamate + NAD

- **Second: Glutamine Synthetase reaction**:

Glutamate + NH_3 + ATP ⇒ Glutamine + ADP + P_i

- Glutamate in GDH reaction then Transaminates with suitable Keto acid to give an amino acid;

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

Glutamate + Pyruvate ⟵⟶ α-Oxoglutarate + Alanine;

- Thus, **NH_3 is transported** as **Amino Acids** having a total concentration in Blood plasma of between 3.0mM to 4.OmM;
  - Most plentiful of these Amino Acids are **Glutamine** and **Alanine** that penetrate into the Liver most easily:
    - **Glutamine (0.4mM)**,
    - Glutamate (0.23mM), and
    - **Alanine (0.4mM);**

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 high activity of **Adenylate Deaminase** that catalyzed the reaction:

$$\textbf{AMP + } \textbf{H}_2\textbf{O} \text{ ====} \rightarrow \textbf{ IMP + } \textbf{NH}_3$$

- NH_3 from **Muscle** enters blood and is transported as such to Liver,
- NH_3 from **Brain** is converted to Glutamine because of High activity of **Glutamine Synthetase,**
- Glutamine so formed is 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:

  1. **Portal Blood as Free NH_3 and Amino Acids,**
     - Amount of Amino Acids and of Free NH_3 in Portal Blood depends on the diet,

  2. **Systemic Blood as Amino Acids:**
     - As Glutamine and Alanine,

{NOTE: α-KG = α-Ketoglutarate (α-Oxoglutarate)}

- **In Liver NH_3 can arise from the enzymes**:
  - **Glutaminase:** Catalyzes formation of NH_3 from Glutamine:

    Glutamine + H_2O =====➔ Glutamate + NH_3

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

    AMP + H_2O ====➔ IMP + NH_3

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

Glutamate + NAD ⬅==➔ α-Oxoglutarate + NADH +H^+ + NH_4^+

How is excess ammonia removed from metabolic system?

First: formation of Glutamate from Alpha-Oxoglutarate and Ammonia, by **GDH** reaction:

α-KG+ NH_3 + NADH + H^+ ⟵⟶ Glutamate + NAD + H_2O

Second: ALT reactions:

Glutamate + Pyruvate ⟵⟶ α-Oxoglutarate + Alanine;

Third: formation of Glutamine by Glutamate and **NH_3** by **Glutamine Synthetase** reaction:

Glutamate + NH_3 + ATP ======> Glutamine + ADP + P_i

- Excess $\mathbf{NH_3}$ is removed as Glutamate, Alanine and Glutamine in blood;
- Extensive utilization of Alpha-Oxoglutarate for removal of excess Ammonia can deplete TCA cycle Intermediates and affects energy supply to the cerebral tissue, unless mechanisms to replenishing TCA-cycle intermediates are available;
- Mechanism include Anaplerotic reactions to replace the depleted intermediates in TCA-cycle;

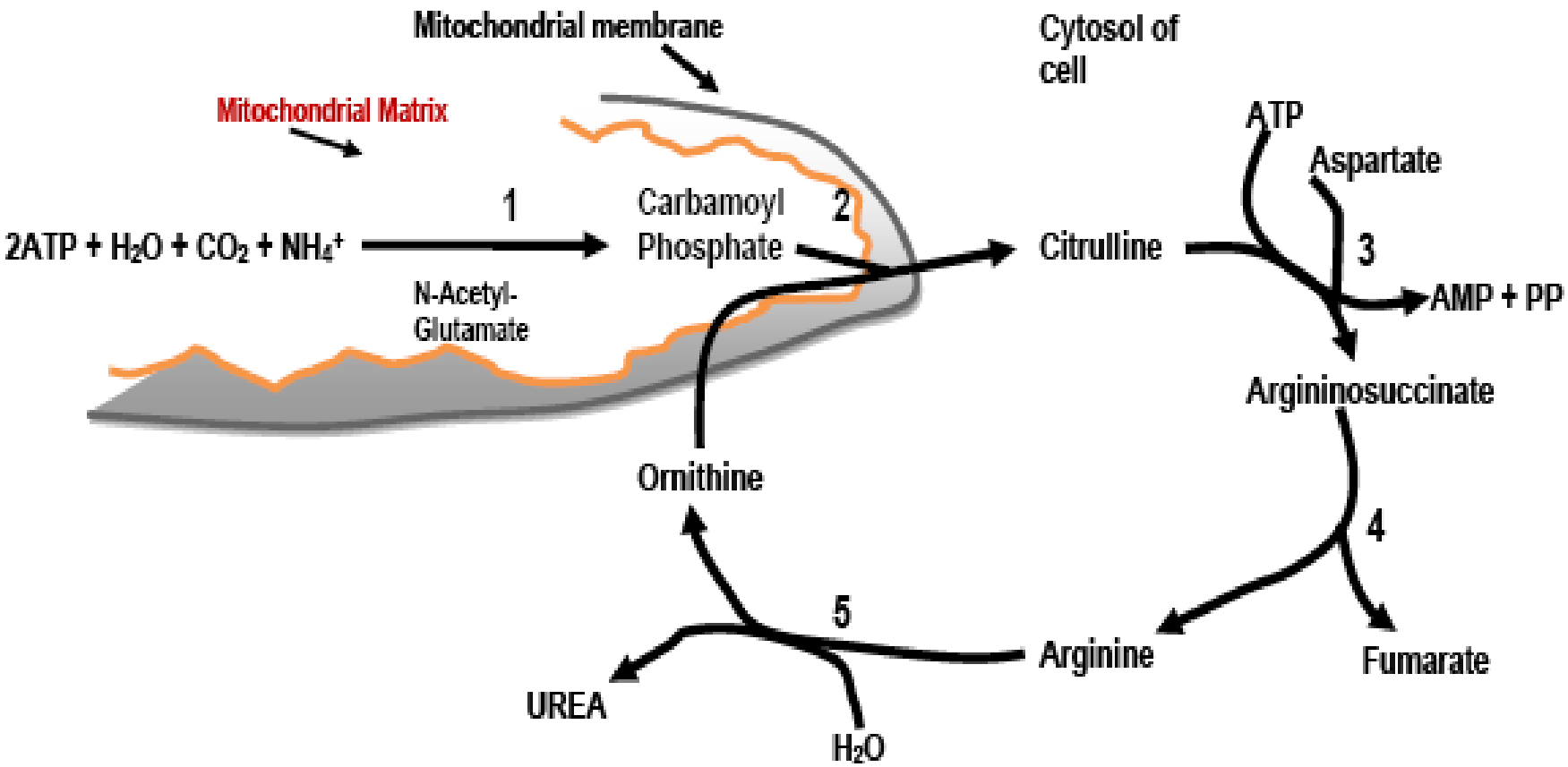
REACTIONS OF UREA CYCLE:

Give a brief outline of the Urea Cycle.

- Urea cycle can be separated in Five major reactions;
- Biosynthesis of Urea requires the following:
  - Three molecules of ATP,
  - Ammonium ion (NH_3) of amino acid being degraded;
  - CO_2
  - α-Amino Nitrogen of Aspartate;
- First & Second reactions occur in Mitochondrial matrix, because of location of the **Two** urea cycle enzymes:
  - **Carbamoyl Phosphate Synthetase,**
  - **Ornithine Transcarbamoylase (Citrulline Synthetase**)

- Three reactions occur in Cytosol that contains the enzymes:
  - **Argininosuccinate Synthetase,**
  - **Argininosuccinate Lyase,**
  - **Arginase,**
- Urea contains 2 Nitrogen atoms:(**H_2N-CO-NH_2)**
  - One derived from NH_3 directly,
  - One from Aspartate,
- **Fig. 5:** Schematic diagram of UREA CYCLE
- Compartmentalization of Urea cycle enzymes requires that certain urea cycle intermediates be **transported across Mitochondrial membrane,**

Fig 5: Simplified schematic diagram of Urea Cycle



FIVE MAJOR ENZYMES IN UREA CYCLE

1. Carbamoyl Phosphate Synthase
2. Ornithine Transcarbamoylase
3. Argininosuccinate Synthase
4. Argininosuccinate Lyase
5. Arginase

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 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 according to the Metabolic Fate of their Carbon Skeleton;
- **Ketogenic Amino Acids:**
  - Amino Acids whose Carbon Skeletons can be converted to **Acetyl-CoA** or **Acetoacetyl-CoA** and used for biosynthesis of **Ketone bodies,**
  - Purely Ketogenic amino acids: **Leucine** and **Lysine**

- **Glucogenic Amino Acids**:
  - Amino Acids whose Carbon Skeletons can be converted to **Pyruvate** or to Intermediates in TCA-cycle and used for biosynthesis of Glucose,
  - Almost all amino acids are Glucogenic,
- Some Amino Acids are both Ketogenic and Glucogenic: Examples:
  - Isoleucine,
  - Phenylalanine,
  - Tyrosine, and
  - Tryptophan

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