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BIOCHEMISTRY

UNIT 3

TOPIC :

- **Amino acid metabolism**

  General reactions of amino acid metabolism: Transamination, deamination & decarboxylation, urea cycle and its disorders
  Catabolism of phenylalanine and tyrosine and their metabolic disorders (Phenyketonuria, Albinism, alkeptonuria, tyrosinemia)
  Synthesis and significance of biological substances; 5-HT, melatonin, dopamine, noradrenaline, adrenaline
  Catabolism of heme; hyperbilirubinemia and jaundice

Metabolism Of Amino Acids (Proteins)

→ Proteins are the most abundant organic compounds and constitute a major part of the body dry weight (10-12 kg in adults).

→ They perform a wide variety of static (structural) and dynamic (enzymes, hormones, clotting factors, receptors etc.) functions. About half of the body protein (predominantly collagen) is present in the supportive tissue (skeleton and connective) while the other half is intracellular.

→ Amino acid—Proteins are nitrogen-containing macromolecules consisting of LD-amino acids as the repeating units. Of the 20 amino acids found in proteins, half can be synthesized by the body (non-essential) while the rest have to be provided in the diet (essential amino acids).

→ The proteins on degradation (proteolysis) release individual amino acids. Amino acids are not just the structural components of proteins. Each one of the 20 naturally occurring amino acids undergoes its own metabolism and performs specific functions.

→ Some of the amino acids also serve as precursors for the synthesis of many biologically important compounds (e.g., melanin, serotonin, creatine etc.).

→ Certain amino acids may directly act as neurotransmitters (e.g., glycine aspartate, glutamate).

Significance of amino acid metabolism

- It is necessary for proper functioning of body .
- It is very important pharmacologically also .
- Some are important precursor of steroid hormones .
- Some function as Coenzyme .
- Some are act as neurotransmitter (GABA)

General Reactions of Amino Acids and Its Significance

General of Amino Acids are following :

1) **Transamination :** It means movement of an amino (--NH_2) group from an amino acid to a Keto Acid . A group of enzymes called Transaminase catalyses this reaction . Keto acid is a compound that contains a Carboxyl Group (--COOH) and a Ketone Group (>C=O).
2) **Deamination :** Loss of amino group from amino acid is Deamination . It is of two types, Oxidative, Non-Oxidative.
3) **Decarboxilation :** Removal of CO_2 From a compound is called Decarboxilation.

Transamination

- The transfer of an amino (NH_2) group from an amino acid to a keto acid is known as transamination. This process involves the interconversion of a pair of amino acids and a pair of keto acids, catalysed by a group of enzymes called transaminases /aminotransferases.

Silent features of Transamination

- All transaminases require pyridoxal phosphate (PLP), a coenzyme derived from vitamin B6. It is reversible and no free NH_3 liberated, only the transfer of amino group occurs. Transamination diverts the excess amino acids towards energy generation.
- Specific transaminases exist for each pair of amino and keto acids. However, only two— namely, aspartate transaminase and alanine transaminase—make a significant contribution for transamination.
- Transamination is very important for the redistribution of amino groups and production of non-essential amino acids, as per the

requirement of the cell. It involves both catabolism and anabolism of amino acids.

- The amino acids undergo transamination to finally concentrate nitrogen in glutamate. Glutamate is the only amino acid that undergoes oxidative deamination to a significant extent to liberate free NH_3 for urea synthesis.
- All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination. Serum transaminases are important for diagnostic and prognostic purposes.

Mechanism of transamination

- It occurs in two stages
  - Transfer of the amino group to the coenzyme pyridoxal phosphate (bound to the coenzyme) to form pyridoxamine phosphate.
  - The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated.

Deamination

→ The removal of amino group from the amino acids as NH_3 is deamination. Deamination results in the liberation of ammonia for urea synthesis (transamination involves only the shuffling of amino groups). It may be either oxidative or non-oxidative.

1. Oxidative deamination : Oxidative deamination is the liberation of free ammonia from the amino group of amino acids coupled with oxidation. This takes place mostly in liver and kidney. The purpose of oxidative deamination is to provide NH_3 for urea synthesis and D-keto acids for a variety of reactions, including energy generation

- **Oxidation of glutamate-by-glutamate dehydrogenase :** In the transamination process glutamate is form. Now, Glutamate rapidly undergoes oxidative deamination, catalysed by glutamate dehydrogenase (GDH) to liberate ammonia. This enzyme is unique in that it can utilize either NAD+ or NADP+ as a coenzyme. Conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate.
- **Oxidative deamination by amino acid oxidases :** L-Amino acid oxidase and D-amino acid oxidase are flavoproteins, possessing FMN and FAD, respectively. They act on the corresponding amino acids (L or D) to produce D-keto acids and NH_3. In this reaction, oxygen is reduced to H_2O_2, which is later decomposed by catalase.

2. Non-oxidative deamination : Some of the amino acids can be deaminated to liberate NH_3 without undergoing oxidation.

a. Amino acid dehydrases : Serine, threonine and homoserine are the hydroxy amino acids. They undergo non-oxidative deamination catalysed by PLP-dependent dehydrases (dehydratases).

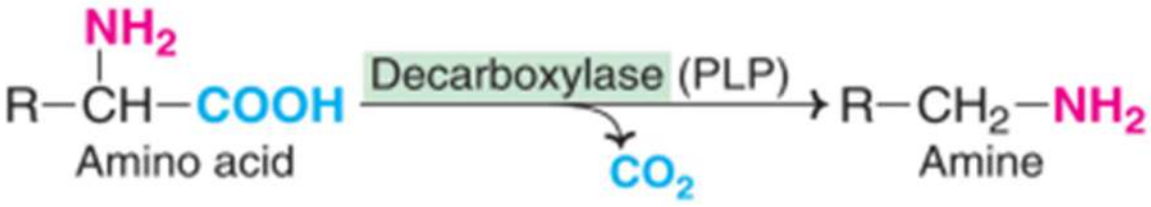
b. Amino acid desulfhydrases : The sulphur amino acids, namely cysteine and homocysteine, undergo deamination coupled with desulfhydration to give keto acids.
c. Deamination of histidine : The enzyme histidase acts on histidine to liberate NH_3 by a non-oxidative deamination process

Decarboxylation

→ Decarboxylation of an amino acid is a chemical reaction in which the carboxyl group (-COOH) of an amino acid is removed, leading to the formation of an amine group (-NH2) and the release of carbon dioxide (CO2). This reaction is catalysed by enzymes called decarboxylases.

→ In general, the decarboxylation of amino acids or their derivatives results in the formation of amines.

→ The decarboxylation of amino acids plays an important role in many biological processes. For example, the amino acid histidine is decarboxylated to form histamine, which is involved in regulating many physiological processes, including digestion and immune response. Similarly, the amino acid glutamic acid is decarboxylated to form the neurotransmitter gamma-aminobutyric acid (GABA), which is involved in the regulation of neuronal activity.

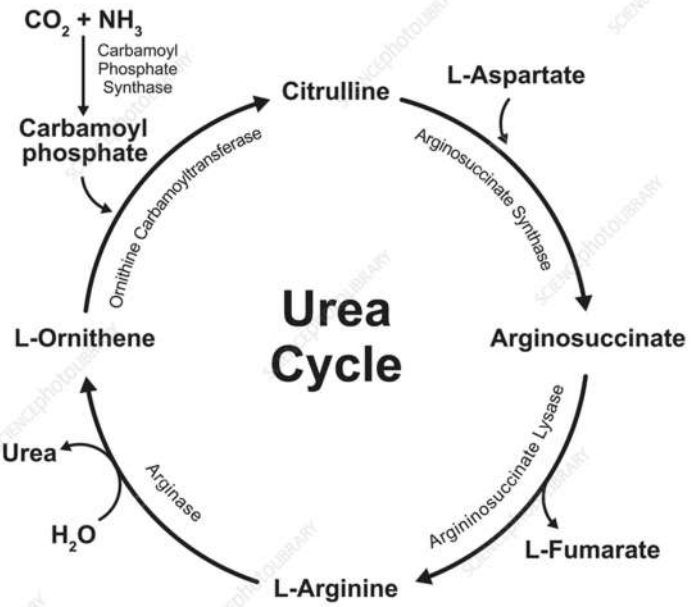


Urea cycle

- Ammonia is constantly being liberated in the metabolism of amino acids (mostly) and other nitrogenous compounds. At the physiological pH, ammonia exists as ammonium (NH_4+) ion. Ammonium ions are very important to maintain acid-base balance of the body.
- The production of NH_3 occurs from the amino acids (transamination and deamination), biogenic amines, amino group of purines and pyrimidines and by the action of intestinal bacteria (urease) on urea.

Introduction about urea cycle

- Urea is the end product of protein metabolism (amino acid metabolism). The nitrogen of amino acids, converted to ammonia, is toxic to the body. It is converted to urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine.
- Urea is synthesized in liver and transported to kidneys for excretion in urine. Urea cycle is the first metabolic cycle that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as Krebs-Henseleit cycle. The individual reactions, however, were described in more detail later on by Ratner and Cohen. Urea has two amino (NH_2) groups, one derived from NH_3 and the other from aspartate. Carbon atom is supplied by CO_2. Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first two enzymes are present in mitochondria while the rest are localized in cytosol



Steps of the Urea Cycle (in Liver):

Step 1: ***Formation of Carbamoyl Phosphate***

- Ammonia + CO_2 + 2 ATP → Carbamoyl phosphate
- Enzyme: **Carbamoyl phosphate synthetase I**

Step 2: ***Formation of Citrulline***

- Carbamoyl phosphate + Ornithine → Citrulline
- Enzyme: **Ornithine transcarbamoylase**

Step 3: ***Formation of Argininosuccinate***

- Citrulline + Aspartate + ATP → Argininosuccinate
- Enzyme: **Argininosuccinate synthetase**

Step 4: ***Cleavage of Argininosuccinate***

- Argininosuccinate → Arginine + Fumarate
- Enzyme: **Argininosuccinase**

*Step 5: **Formation of Urea***

- Arginine → Urea + Ornithine
- Enzyme: **Arginase**

Fate of Urea:

- Released into **blood**, filtered by **kidneys**, excreted via **urine**.

Urea Cycle Disorders (UCDs)

→ UCDs are inherited genetic disorders caused by deficiency or absence of one of the urea cycle enzymes, resulting in accumulation of ammonia (NH_3) in blood—a condition known as hyperammonemia, which is toxic to the brain.

Clinical Features of UCDs:

- **Early Symptoms** (usually in newborns or infants):
  - Poor feeding
  - Vomiting
  - Lethargy
  - Seizures
  - Coma
  - **Hyperammonemia** (ammonia > 150 µmol/L is toxic)
- **Late-Onset Symptoms** (in older children/adults):
  - Delayed development
  - Intellectual disability
  - Behavioral problems
  - Vomiting after high-protein meals

Major UCDs and Enzyme Deficiencies:

Disorder Name	Deficient Enzyme	Features
Carbamoyl Phosphate Synthetase I Deficiency (CPSID)	CPS I	Severe hyperammonemia, no orotic acid in urine
Ornithine Transcarbamoylase Deficiency (OTCD)	OTC	X-linked, most common UCD, high orotic acid in urine
Citrullinemia Type I	Argininosuccinate synthetase	High citrulline in blood
Argininosuccinic Aciduria (ASA)	Argininosuccinate lyase	Elevated argininosuccinate, brittle

		hair
Argininemia	Arginase	Mild hyperammonemia, spasticity, elevated arginine

Diagnosis of UCDs:

1. **Plasma Ammonia Levels**: Very high in UCDs
2. **Plasma Amino Acids**: Elevated citrulline, argininosuccinate, or arginine depending on the defect
3. **Urinary Orotic Acid**:
    - High in OTC deficiency
    - Normal in CPS I deficiency
4. **Genetic Testing**: Confirms the specific enzyme defect
5. **Enzyme Assays**: In liver biopsy (rarely done now)

Treatment of Urea Cycle Disorders

1. *Acute Management of Hyperammonemia:*

- **Hemodialysis:** To rapidly remove ammonia
- **IV Glucose and Lipids:** To reduce protein catabolism
- **Nitrogen-scavenging agents:**
    - **Sodium benzoate**
    - **Sodium phenylacetate/phenylbutyrate**

2. *Long-term Management:*

- **Protein-restricted diet** (avoid excess protein)
- **Supplement essential amino acids**
- **Liver transplantation** in severe or unresponsive cases
- **Arginine or citrulline supplements** (depending on which step is blocked)

Catabolism of Phenylalanine and Tyrosine

Phenylalanine and tyrosine are **aromatic amino acids** that share a common catabolic pathway.

- **Phenylalanine** is first hydroxylated to **tyrosine**.
- **Tyrosine** is then degraded to produce **fumarate** and **acetoacetate**, which enter the **TCA cycle** and **ketone body pathway** respectively.

Step-by-Step Catabolic Pathway:

1. *Phenylalanine → Tyrosine*

- **Enzyme**: **Phenylalanine hydroxylase**
- **Co-factor**: **Tetrahydrobiopterin (BH_4)**
- **Reaction**:

$$\text{Phenylalanine} + BH_4 + O_2 \rightarrow \text{Tyrosine} + BH_2 + H_2O$$

2. *Tyrosine → p-Hydroxyphenylpyruvate*

- **Enzyme: Tyrosine transaminase** (requires PLP)

3. *p-Hydroxyphenylpyruvate → Homogentisate*

- **Enzyme: p-Hydroxyphenylpyruvate dioxygenase**

4. *Homogentisate → Maleylacetoacetate*

- **Enzyme: Homogentisate oxidase**

5. *Maleylacetoacetate → Fumarylacetoacetate*

- **Enzyme: Maleylacetoacetate isomerase**

6. *Fumarylacetoacetate → Fumarate + Acetoacetate*

- **Enzyme: Fumarylacetoacetate hydrolase**

Fumarate enters the **TCA cycle** (glucogenic),
Acetoacetate is a **ketone body** (ketogenic)

Metabolic Disorders

1. Phenylketonuria (PKU)

- Phenylketonuria is a genetic metabolic disorder characterized by the deficiency of the enzyme phenylalanine hydroxylase, leading to the accumulation of phenylalanine and its toxic metabolites in the body.

Cause:

- Deficiency or mutation in phenylalanine hydroxylase enzyme.
- Rarely due to tetrahydrobiopterin (BH_4) deficiency (cofactor of the enzyme).

Biochemical Basis:

- Phenylalanine cannot be converted into tyrosine → excess phenylalanine is converted into phenylpyruvate, phenyllactate, and phenylacetate (neurotoxic).

Symptoms:

- Mental retardation (if untreated)
- Seizures
- Microcephaly
- Musty/mousy body odor
- Hypopigmentation (due to decreased melanin from tyrosine)

Diagnosis:

- **Newborn screening (Guthrie test)**
- Elevated **blood phenylalanine** levels

Treatment:

- ✓ Low-phenylalanine diet
- ✓ Tyrosine supplementation
- ✓ Avoid aspartame

- ✓ BH_4 supplementation in cofactor deficiency

2. Alkaptonuria

- ➢ Alkaptonuria is a rare inherited metabolic disorder caused by the deficiency of homogentisate oxidase, leading to accumulation of homogentisic acid in tissues and urine.

Cause:

- Autosomal recessive deficiency of homogentisate oxidase

Biochemical Basis:

- Homogentisic acid cannot be further broken down → accumulates → excreted in urine and deposited in connective tissues (causing ochronosis).

Symptoms:

- Black urine on standing
- Ochronosis (bluish-black pigmentation of cartilage and sclera)
- Arthritis of spine and large joints (due to pigment deposition)

Diagnosis:

- Urine turns black on standing
- Elevated homogentisic acid in urine (HPLC)

Treatment:

- ✓ Low protein diet (restrict phenylalanine and tyrosine)
- ✓ Vitamin C (delays ochronosis)
- ✓ Pain management and joint replacement if needed

3. Albinism

- Albinism is a group of genetic disorders characterized by complete or partial absence of melanin in the skin, hair, and eyes due to defective melanin synthesis from tyrosine.

Cause:

- Deficiency of tyrosinase, the key enzyme in melanin synthesis from tyrosine.

Types:

- **Oculocutaneous albinism** (affects skin, hair, and eyes)
- **Ocular albinism** (affects eyes only)

Biochemical Basis:

- Tyrosine is not converted into DOPA and then melanin, leading to pigment deficiency.

Symptoms:

- Pale white skin and hair
- Light blue or pink eyes
- Photophobia, nystagmus
- Poor vision
- Increased risk of skin cancers due to UV sensitivity

Diagnosis:

- Clinical observation
- Tyrosinase activity tests
- Genetic testing

Treatment:

- ✓ No specific cure
- ✓ Protective clothing and sunscreen

✓ Visual aids and regular eye checkups

5-HT (Serotonin)

Full Name: 5-Hydroxytryptamine

Synthesis Pathway:

Tryptophan → 5-Hydroxytryptophan → **Serotonin (5-HT)**

1. **Tryptophan hydroxylase**
    - Converts tryptophan to **5-hydroxytryptophan**
    - Requires **tetrahydrobiopterin (BH_4)**
2. **Aromatic L-amino acid decarboxylase**
    - Converts 5-hydroxytryptophan to **5-HT**
    - Requires **pyridoxal phosphate (Vitamin B6)**

Sites of Synthesis:

- CNS (raphe nuclei of brainstem)
- Enterochromaffin cells in the GI tract
- Platelets (store serotonin but don't synthesize it)

Biological Significance:

◈ Regulates mood, sleep, appetite, and pain

◈ Acts as a vasoconstrictor and platelet aggregator

◈ Involved in peristalsis in the gut

◈ Deficiency linked to depression, anxiety, and migraines

Melatonin

Synthesis Pathway:

Tryptophan → 5-HT (serotonin) → **Melatonin**

1. **Serotonin → N-acetyl serotonin**
   - Enzyme: **Serotonin N-acetyltransferase**
2. **N-acetyl serotonin → Melatonin**
   - Enzyme: **Hydroxyindole O-methyltransferase**

Site of Synthesis:

- **Pineal gland** (in the brain)

Biological Significance:

- ◈ Regulates circadian rhythm and sleep-wake cycles
- ◈ Increases in darkness, decreases in light
- ◈ Acts as a powerful antioxidant
- ◈ Modulates immune function and reproductive cycles

Dopamine

Synthesis Pathway:

Tyrosine → L-DOPA → **Dopamine**

1. **Tyrosine hydroxylase**
   - Converts tyrosine to **L-DOPA** (rate-limiting step)
   - Requires **BH_4** as cofactor
2. **DOPA decarboxylase**
   - Converts L-DOPA to **Dopamine**
   - Requires **Vitamin B6**

Sites of Synthesis:

- **Substantia nigra** (midbrain)
- Hypothalamus (tuberoinfundibular pathway)
- Adrenal medulla (minor)

Biological Significance:

- ◈ Controls motor function (Parkinson's disease = ↓ dopamine)
- ◈ Regulates mood, reward, and pleasure (linked to addiction)
- ◈ Inhibits prolactin secretion from anterior pituitary
- ◈ Involved in learning and memory

Noradrenaline (Norepinephrine)

Synthesis Pathway:

Dopamine → Noradrenaline

- **Enzyme**: **Dopamine β-hydroxylase**
- Requires **ascorbic acid (Vitamin C)** and **Cu^{2+}**
- Occurs in **vesicles** of adrenergic neurons

Sites of Synthesis:

- **Locus coeruleus** in brainstem
- **Postganglionic sympathetic neurons**
- **Adrenal medulla**

Biological Significance:

- ◈ Acts as neurotransmitter and hormone
- ◈ Increases heart rate, blood pressure, and alertness
- ◈ Triggers “fight or flight” response
- ◈ Enhances attention and arousal
- ◈ Regulates mood (low levels in depression)

Adrenaline (Epinephrine)

Synthesis Pathway:

Noradrenaline → Adrenaline

- **Enzyme**: **Phenylethanolamine N-methyltransferase (PNMT)**
- Requires **S-adenosylmethionine (SAM)** as methyl donor
- Induced by **cortisol**

Site of Synthesis:

- **Adrenal medulla**

Biological Significance:

- ◈ Enhances sympathetic nervous system activity
- ◈ Increases heart rate, cardiac output, and blood glucose
- ◈ Stimulates glycogenolysis and lipolysis
- ◈ Used clinically in anaphylaxis, asthma, cardiac arrest

Catabolism of Heme

- Heme catabolism is the breakdown of heme molecules, primarily from aged red blood cells (RBCs), into bilirubin, which is ultimately excreted in bile.

Sites:

- Mainly occurs in **reticuloendothelial system (RES):**
  - Liver
  - Spleen
  - Bone marrow

Step-by-Step Pathway of Heme Degradation

1. Hemoglobin Breakdown

- Old RBCs are destroyed in **spleen/liver**.
- Hemoglobin splits into:
  - **Globin** → broken into amino acids.
  - **Heme** → goes for further breakdown.
  - **Iron (Fe^{2+})** → reused or stored.

2. Heme → Biliverdin

- **Enzyme**: Heme oxygenase
- Heme is broken into **Biliverdin** (green color) + **Iron** + **CO**

3. Biliverdin → Bilirubin

- **Enzyme**: Biliverdin reductase
- Biliverdin turns into **Bilirubin** (yellow color)

4. Transport to Liver

- **Unconjugated (indirect) bilirubin** binds to **albumin** and goes to liver.

5. Conjugation in Liver

- **Enzyme**: UDP-glucuronosyltransferase (UGT)
- Bilirubin is made water-soluble by attaching **glucuronic acid**
- Now it's called **Conjugated (direct) bilirubin**

6. Excretion

- Conjugated bilirubin is released into **bile → intestine**

7. In Intestine

- Bacteria convert it to:
  - **Urobilinogen** → Some goes to kidneys → **Urobilin** → gives **yellow color to urine**
  - Some stays in intestine → becomes **Stercobilin** → gives **brown color to stool**

Hyperbilirubinemia

Hyperbilirubinemia is a condition characterized by **increased levels of bilirubin** in the **blood**. It occurs when the **production of bilirubin exceeds its conjugation and excretion**, leading to **accumulation** in body fluids.

- **Normal serum bilirubin**:
  - **Total**: 0.3 – 1.2 mg/dL
  - **Direct (Conjugated)**: < 0.3 mg/dL
  - **Indirect (Unconjugated)**: 0.2 – 0.9 mg/dL
- **Hyperbilirubinemia**:
  - **Total bilirubin > 1.2 mg/dL**
  - **Jaundice** is visible when levels exceed **2–3 mg/dL**

Types of Bilirubin

Type of Bilirubin	Nature	Solubility	Binding	Excretion
Unconjugated	Indirect	Lipid-soluble	Bound to albumin	Not excreted in urine
Conjugated	Direct	Water-soluble	Free	Excreted in urine

Jaundice

Jaundice (also called **icterus**) is a **clinical condition** characterized by **yellowish discoloration of the skin, sclera (white of the eyes), and mucous membranes** due to **increased levels of bilirubin** in the blood (**hyperbilirubinemia**).

- **Visible jaundice** usually occurs when:
  - **Serum bilirubin > 2–3 mg/dL**
  - Normal: **0.3–1.2 mg/dL**

Pathophysiology of Jaundice

- Bilirubin is a yellow pigment formed from the breakdown of hemoglobin in senescent (aged) red blood cells (RBCs).
  It is processed in the liver and excreted in bile.
- If there's a defect in production, uptake, conjugation, or excretion, bilirubin accumulates in the blood → leading to jaundice.

Types of Jaundice (based on site of defect)

Type	Site of Problem	Main Bilirubin Type Elevated
Pre-hepatic (hemolytic)	Before liver (blood/RBCs)	Unconjugated
Hepatic (hepatocellular)	Liver (processing defect)	Mixed (both types)
Post-hepatic (obstructive)	After liver (bile duct blockage)	Conjugated