University of Papua New Guinea
School of Medicine and Health Sciences
Division of Basic Medical Sciences
Discipline of Biochemistry and Molecular Biology

CARBOHYDRATE METABOLISM

Why is Glycolysis important?

- Glycolysis is major pathway for metabolism of Glucose, Fructose and Galactose in cells
- Glycolysis can occur either in the present of oxygen (Aerobic condition) or in the absence of oxygen (Anaerobic condition)

What are the different types of Glycolysis?

- Anaerobic and Aerobic Glycolysis
- **Anaerobic Glycolysis**:
    - Occurs in the absence of Oxygen,
    - Produces 2 molecules of Lactate and a Net of 2 ATP per molecule of Glucose
    - Lactate is the end product of Anaerobic Glycolysis

- **Aerobic Glycolysis**:
    - Occurs in the presence of Oxygen,
    - Produces 2 molecules of Pyruvate and a Net of 6 ATP per molecule of Glucose
    - Pyruvate is end product of Aerobic Glycolysis

What are the major functions of Glycolysis?

- Major functions of Glycolysis include:
- Production of energy by substrate level Phosphorylation and via supplying substrates to Citric Acid Cycle (Krebs Cycle) and Oxidative Phosphorylation,
- Production of Intermediates for other biosynthetic pathways
- Major biochemical significance of Glycolysis is the ability to provide ATP under Anaerobic condition
- It allows Skeletal muscle to perform at very high level under Anaerobic conditions
- It also allows tissues with significance Glycolytic ability to survive Anoxic Episodes

Give a brief description of the Glycolytic Pathway (Fig. 1)

- Glucose is converted to Glucose-6-phosphate (G-6-P)
    - Enzyme: Hexokinase or Glucokinase,
    - ATP is required, reaction is not reversible
- G-6-P is converted to Fructose-6-Phosphate (F-6-P)
- F-6-P to F-1, 6-Bisphosphate (F-1, 6-BP) catalyzed by Phosphofructokinase (PFK)
    - ATP is required, reaction is not reversible
    - Reaction is the rate limiting step in Glycolysis
    - PFK is the regulatory enzyme of Glycolysis
- F-1, 6-BP is split into Two Triose-sugars by Aldolase
    - Dihydroxyacetone Phosphate (DHAP) and Glyceraldehyde-3-Phosphate (G-3-P)

- DHAP is converted to G-3-P {gives a total of 2 G-3-P}
- G-3-P are converted to 1,3-Bisphosphoglycerate (1,3-BPG)
    - 2 molecules of NAD is converted to 2 NADH + 2 H^+
    - 1,3-BPG is a High Energy compound
- 1,3-BPG is converted to 3-Phosphoglycerate (3-PG)
    - 2 ADP is converted to 2 ATP
    - **Substrate Level Phosphorylation**
- 3-PG converted to 2-PG
- 2-PG converted to Phosphoenolpyruvate (PEP)
    - PEP is a High Energy compound
- PEP is converted to Pyruvate
    - 2 ADP is converted to 2 ATP
    - **Substrate Level Phosphorylation**

What is the fate of Pyruvate during Glycolysis?

- Fate of Pyruvate is determined by **Redox** state of Tissues
- Two possible conditions: Anaerobic and Aerobic conditions
- **Under Anaerobic Conditions**:
    - Pyruvate is converted to Lactate: **Lactate Dehydrogenase (LDH)**
    - PYRUVATE + NADH + H^+ ←====→ LACTATE + NAD
        - Reaction is essential step in Anaerobic Glycolysis,
        - It is the anaerobic means of converting NADH to NAD
        - Ensures that NAD required for continuation of Glycolysis is available
    - Lactate is produced in active muscle tissues under Anaerobic conditions
        - Lactate released into the blood, taken up by the Liver, converted back to Glucose by Gluconeogenesis
        - LDH is used mainly for conversion of NADH to NAD,
        - LDH allows Glycolysis to continue, and ATP to be produced under Anaerobic conditions
- **Under Aerobic conditions**:
    - Pyruvate is taken up into Mitochondria and converted to Acetyl-CoA
    - Reaction catalyzed by Pyruvate Dehydrogenase complex
    - Acetyl-CoA enters TCA cycle and is oxidized to CO_2
    - NADH from Glycolysis and TCA cycle are taken up by Mitochondria for oxidation via Electron Transport Chain to produce ATP

What are the Total and Net amounts of ATP formed when One Molecule of Glucose is metabolized via Anaerobic Glycolysis?

- Amount of ATP molecules used up = **2 ATP**
- Total amount of ATP produced = **4 ATP** (formed at Substrate Level)
- Net amount of ATP produced equals: 4 ATP – 2 ATP = **2 ATP**

What are the Total and Net amounts of ATP formed when One Molecule of Glucose is metabolized via Aerobic Glycolysis?

- Amount of ATP molecules used up = **2 ATP**
- Amount of ATP produced:
  - At substrate level: **4 ATP**
  - 2 Molecules of NADH produced are transported to Mitochondria
  - In Electron Transport Chain **2 NADH** gives **6 ATP** molecules
- **Total** amount of ATP formed equals: 4 ATP + 6 ATP **= 10 ATP**
- **Net** amount of ATP formed equals: 10 ATP – 2 ATP = **8 ATP**

How is Glycolysis in mammalian RBC different from Glycolysis in the muscle tissues (Fig 2)?

Glycolysis in Red Blood Cell is called 2,3-BisPhosphoglycerate Shunt (2,3-BPG Shunt)
Anaerobic Glycolysis occurs in RBC
Mature RBC in mammals do not contain Mitochondria
Glycolysis in RBC is mainly for production of 2,3-BPG
Conversion of 1,3-BPG to 3-PG catalyzed by Phosphoglycerate Kinase is bypassed
1,3-BPG is converted to 2,3-BPG by **Bis-Phosphoglycerate Mutase** (not in muscle)
2,3-BPG is then converted to 3-PG by 2,3-BPG Phosphatase
High-energy in 1,3-BPG is loss because no ATP is formed

What is the function of 2,3-BPG in RBC?

2,3-BPG combines with Hemoglobin (Hb),
Causes a decrease in the affinity of Hb for Oxygen
Displaces Oxygen from Oxy-hemoglobin (HbO_4)
Presence of 2,3-BPG in RBC helps Oxy-hemoglobin to unload Oxygen to tissues

What is the function of Pyruvate Dehydrogenase (PDH) Complex?

- PDH complex is located in Mitochondrial matrix
  - It is the link between Glycolysis and TCA cycle, under Aerobic condition
- PDH catalyzes the Oxidative Decaboxylation of Pyruvate to Acetyl-CoA
- Cofactors required are: Thiamine Pyrophosphate (TPP) Lipoic Acid; NAD; FAD; CoASH
- Reaction catalyzed:
  - **Pyruvate + NAD + CoASH =====➔ Acetyl-CoA + NADH + H^+ + CO_2**
- NADH formed enters ETC to produce 3 ATP

Give a brief description of TCA cycle (Fig. 3)?

- TCA cycle (Citric Acid Cycle, Kreb's Cycle) is a series of enzymatic reactions responsible for catabolism of Acetyl-CoA
- Acetyl-CoA is an ester of Coenzyme-A, which is the biologically active form of water-soluble vitamin **Pantothenic acid**
- TCA cycle occurs within Mitochondrial matrix under **Aerobic** condition
- Essentially TCA cycle comprises of combination of Acetyl-CoA with Oxaloacetate to give the Six-Carbon Tri-carboxylic acid compound called Citric acid (Citrate)
- Series of reactions then followed during which 2 molecules of CO_2 are given off and reducing equivalents (3 NADH, $FADH_2$) and GTP are formed
- Reducing equivalents enter the ETC to generate energy (ATP) via Oxidative Phosphorylation
- Oxygen is required as the final oxidant of the reducing equivalents
- Absence of Oxygen (Anoxia) or partial deficiency of oxygen (Hypoxia) results in either total or partial inhibition of TCA cycle

What is the significance of TCA cycle?

TCA cycle is an Amphibolic pathway

It is involved in both Anabolic and Catabolic processes

Intermediates of TCA cycle are used as precursors in biosynthesis of compounds

TCA cycle acts as final common pathway for Oxidation of Carbohydrate, Fats and Proteins

Glucose, Fatty Acids and Amino Acids are metabolized to Acetyl-CoA or intermediates of TCA cycle

TCA cycle plays major role in Gluconeogenesis, Lipogenesis, Transamination and Deamination of most Amino Acids

TCA cycle provides much of the energy for respiration

NADH and $FADH_2$ generated in the cycle are transferred to the ETC for formation of ATP via Oxidative Phosphorylation

How many molecules of ATP are produced when One Molecule of Acetyl-CoA goes through the TCA cycle?

- When one Molecule of Acetyl-CoA goes through TCA cycle:
  - **NADH** (Isocitrate Dehydrogenase reaction) to ETC = **3 ATP**
  - **NADH** (Alpha-Oxoglutarate Dehydrogenase reaction) to ETC = **3 ATP**
  - **GTP** (Substrate level Phosphorylation) = **1 ATP**
  - **$FADH_2$** (Succinate Dehydrogenase reaction) to ETC = **2 ATP**
  - **NADH** (Malate Dehydrogenase reaction) to ETC = **3 ATP**

Total equals: 12 ATP molecules

What is Gluconeogenesis?

- Gluconeogenesis is synthesis of Glucose from non-carbohydrates sources
- Gluconeogenesis occurs mainly in the liver and to a lesser extent in the kidney
- Most enzymes of Gluconeogenesis are present in Cytosol, but
    - Pyruvate Carboxylase is located in Mitochondrial matrix, whereas
    - Glucose-6Phosphatase is bound to the smooth endoplasmic reticulum

What is the significance of Gluconeogenesis?

- Gluconeogenesis produces glucose when carbohydrate is not available in sufficient amounts from the diet
- Glucose produced is for maintenance of blood glucose levels during starvation or during vigorous exercise
- Brain and Red Blood Cells depend almost entirely on blood glucose as energy source
    - Supply of glucose is necessary as a source of energy, especially for the nervous system and RBC

- Glucose is required in Adipose Tissues as a source of Glycerol,
- Under Anaerobic conditions glucose is the major fuel for energy production in the Skeletal muscle
- Glucose is the precursor of Lactate in Mammary gland

How is the Gluconeogenic pathway related to Glycolysis?

- Gluconeogenesis is not exactly the reversal of Glycolysis
- Three Irreversible reactions in Glycolysis must be bypassed for Gluconeogenesis to occur
- Three Irreversible reactions are:
    - Hexokinase (or Glucokinase) reaction
    - Phosphofructokinase reaction
    - Pyruvate Kinase reaction

- During Gluconeogenesis only these three reactions are circumvented by special reactions, all the other reactions in Glycolysis are reversible

The following points must be noted about the pathway of Gluconeogenesis:

- Glucose-6-Phosphatase (G-6-Phosphatase):
    - Present in **Liver** and **Kidney**, but
    - ABSENT in **Skeletal Muscle** and **Adipose Tissues**
- G-6-Phosphatase allows tissue to release Glucose into the blood
- Reaction catalyzed by G-6-Phosphatase is as follows:

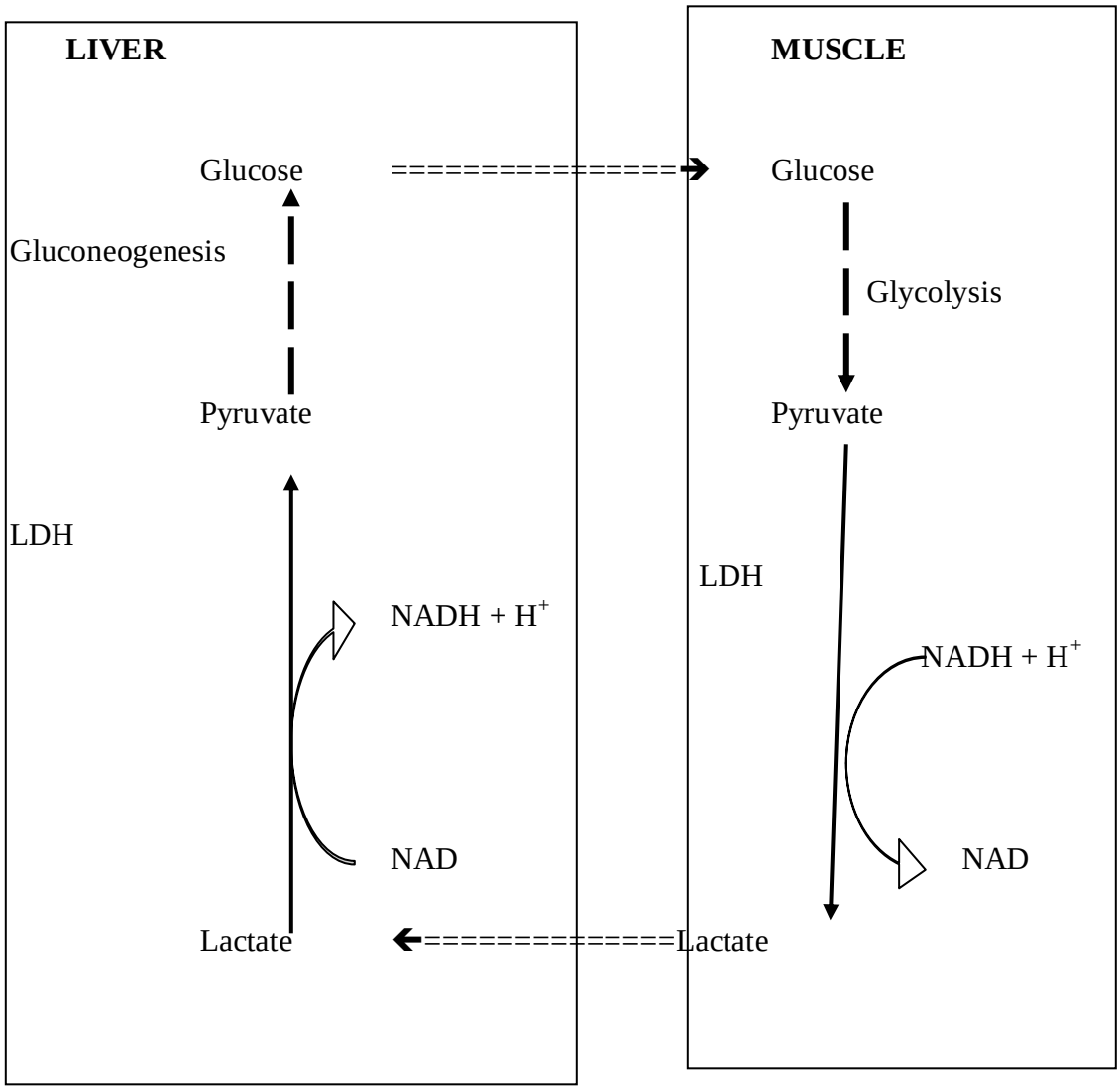
    - **G-6-Phosphate + H_2O =====➔ Glucose + Pi**

- G-6-Phosphatase is present in Liver and Kidney, thus they can both release Glucose into blood
- G-6-Phosphatase in NOT present in skeletal muscle and adipose tissue, thus they cannot release glucose into the blood

- Fructose-I, 6-Bisphosphate is converted to Fructose-6-Phosphate by the enzyme Fructose-1,6-Bisphosphatase
- Fructose-1,6-Bisphosphatase is NOT present in Heart muscle and Smooth muscle
- Some of the substrates for Gluconeogenesis include Pyruvate, Lactate, Glucogenic Amino Acids and Glycerol

What is the Cori cycle (Lactic acid cycle)?

Cori Cycle also called Lactic Acid cycle is presented in **Figure below**



How is Fructose metabolized?

Two pathways for metabolism of Fructose

Metabolism of Fructose in Muscle and Adipose Tissue:

Hexokinase Phosphorylate Fructose

Fructose + ATP ======➔ Fructose-6-Phosphate

Fructose-6-Phosphate enters Glycolytic pathway

Metabolism of Fructose in Liver is via Fructose-1-Phosphatre pathway, that utilizes Fructokinase reaction: **See Figure: 4**

Fructokinase is not affected by fasting

Fructokinase is not affected by **Insulin:**

Fructose metabolism occurs normally in patients with Diabetic Mellitus

Essential Fructosuria (Fructose in urine) is due to:

Defect in activity of Fructokinase

Hereditary Fructose Intolerance is due to:

Defect in Fructose-1-Phosphate Aldolase

How is Galactose metabolized?

- **See Figure 5**

What conditions occur when there is defect in Galactose metabolism?

- Galactokinase catalyzes formation of Galactose-1-phosphate
- Defect in Galactokinase causes accumulation of Galactose in blood and tissues
  - Galactose accumulating in Lens is converted to Galacitol (Dulcitol) by Aldose Reductase
  - Dulcitol accumulation causes Cataracts
- **Galactosemia** (Galactose accumulation in blood) is due to
  - Defect in Galactose-1-Phosphate Uridyl Transferase
- Galactose and Galactose-1-phosphate accumulate in blood and tissues

What are some of the consequence of Galactosemia in Children?

- After consuming milk:
  - Severe vomiting and Diarrhea, Enlarged Liver, Jaundice, Cataract due to accumulation of Dulcitol, Mental retardation
- Using Galactose-free diets can control the condition

What is the Pentose Phosphate Pathway (HMP Shunt)?

- Alternative pathway for the metabolism of glucose
- Biosynthetic pathway that does not generate ATP
- **Glucose-6-Phosphate Dehydrogenase (G-6-PD)** catalyzes first reaction

    - **G-6-PD catalyzes the formation of NADPH**

- Produces **NADPH** for reductive biosynthesis of Fatty acids, Steroids
- Produces of **Ribose-5-phosphate** for Nucleotide and Nucleic acid synthesis
- Major route for use of Pentose and their conversion to Fructose-6-phosphate and Glyceraldehyde 3-phosphate
- HMP shunt occurs most actively in Liver, Mammary glands, Adipose tissues, RBC, Testis, Adrenal Cortex
- Core reaction of HMP shunt can be summarize as:

G-6-P + 2 NADP + H_2O ===➔ Ribose-5-phosphate + 2 NADPH + $2H^+$ + CO_2

What is the major role of HMP shunt in Red Blood Cells?

- To provides NADPH for Reduction of Oxidized Glutathione (GSSG) to Reduced Glutathione (GSH)
    - Reaction is catalyzed by Glutathione Reductase

    - **GSSG + NADPH + H^+ ======➔ 2 GSH + NADP**

- **GSH** then removes Hydrogen Peroxide (**H_2O_2**) from RBC
- Reaction is catalyzed by Glutathione Peroxidase that utilizes Selenium

    - **2 GSH + H_2O_2 ===➔ GSSG + 2 H_2O**

- Function of Hb is stops when Hydrogen Peroxide accumulates in RBC
- Hb is converted to MetHb that cannot transport Oxygen

Why does a person with G-6-PD deficiency suffer from hemolytic anemia?

- G-6-PD catalyzes formation of NADPH + H^+ in HMP shunt
- Hemolytic anemia usually occur in individuals with G-6-PD deficiency
- HMP shunt will not produce enough NADPH required to maintain high level of GSH to protect RBC from Oxidative damage
- Hemolysis of RBC will occur because Oxidants will accumulate in RBC and damage the membrane
- Certain drugs and chemicals that act as oxidizing substances (e.g., Aspirin, Sulfonamides and Anti-malarial drug Primaquine) can cause Hemolytic Anemia
- Such drugs or compounds converts GSH to GSSG and increase the demand for HMP shunt to produce more NADPH needed to convert GSSG back to GSH

How is the body store of Glycogen accounted for?

- Liver and Skeletal muscle are major storage sites for Glycogen
- Liver stores about 6.0% by weight of Glycogen
- Skeletal muscle stores about 1.0% by weigh of Glycogen
- Skeletal muscle contains about 3 to 4 times more Glycogen store than Liver
    - Mass of skeletal muscle is much greater than mass of Liver

- Liver Glycogen stores in humans are only adequate for about 12 to 18 hours of fasting, after which the Liver becomes almost totally depleted of Glycogen and require Gluconeogenesis to maintain blood glucose level
- Muscle glycogen is only depleted significantly after prolonged vigorous exercise

What are some of the functions of Glycogen?

- Glycogen in Liver maintains blood glucose level between meals
    - Essential to supply easily metabolizable energy source to particularly the brain, which uses only glucose as the major substrate for energy production
- Muscle glycogen serves as readily available source of glucose for the energy needs of skeletal muscle

How is Glycogen degraded (Glycogenolysis)? (Fig. 6)

Glycogenolysis is the pathway for Degradation of Glycogen
Two Stage involved:

Stage 1: Cleavage of terminal alpha-1,4-Glycosidic bond in Glycogen
Enzyme & Co-enzyme:
Glycogen Phosphorylase (**Phosphorylase**) and Pyridoxal Phosphate (B_6PO_4)

Glycogen + Pi ←=====→ Glycogen (n-1 residues) + G-1-P

Glycogen Phosphorylase catalyzes cleavage of terminal Glycosidic bond in Glycogen to yield G-1-P and Glycogen molecule shorter by one-glucose unit
Reaction occurs sequentially until it gets close to the branch point in Glycogen

Stage 2: Removal of branch chains:
Glycogen-Debranching enzyme system catalyzes removal of branch-points in Glycogen
Catalyzes cleavage of alpha-I, 6-Glycosidic bond in Glycogen
Combined action of Glycogen Phosphorylase and Glycogen-Debranching enzyme system leads to the complete degradation of Glycogen

What happens to G-1-P?

Glucose-I-P is converted to Glucose-6-phosphate by Phosphoglucomutase

G-1-P ⬅=====➔ G-6-P

What happens to G-6-P in Liver, Kidneys and Skeletal Muscle?

- **LIVER and KIDNEYS**:
  - Glucose-6-Phosphatase catalyzes conversion of G-6-P to Glucose

G-6-P + H_2O =======➔ Glucose + Pi

  - Free Glucose formed diffuses into the blood to maintain blood glucose concentration

- **SKELETAL MUSCLE**:
- **Glucose-6-Phosphatase is not ABSENT in Skeletal Muscle**
  - Skeletal muscle cannot convert G-6-P to Glucose
- Glycogenolysis in skeletal muscle is to produce energy for muscle contraction and not to increase blood glucose concentration

How is Glycogen synthesized? (Fig. 7)

- Glycogenesis is biosynthesis of Glycogen
- Requires 3 enzymes and "**Glycogen primer** (Glycogenin)"
  - Enzymes required are as follows:
  - **UDP-Glucose Pyrophosphorylase**:
    - Catalyzes formation of UDP-Glucose from UTP and G-1-P
  - **Glycogen Synthase**:
    - Uses UDP-Glucose as substrate to synthesize Glycogen by adding one residue at a time to "Glycogen primer" forming alpha-1 4-Glycosidic bond between Glycosyl residues
  - **Branching enzyme**:
    - Catalyzes formation of branch-points (alpha-1, 6-Glycosidic bonds at the branch points)

How is metabolism of Glycogen regulated?

- Allosteric regulation and Hormones control Glycogenolysis and Glycogenesis
- Glycogen metabolism is controlled by Glycogen Phosphorylase and Glycogen Synthase
  - Both enzymes are partly controlled by cyclic AMP (c AMP)
- Cyclic AMP causes **Activation** of **Glycogen Phosphorylase** and at the same time causes **Inhibition** of **Glycogen Synthase**
  - Increasing concentration of cyclic AMP results in Increasing rate Glycogenolysis and decreasing Glycogenesis
- Adenylate Cyclase catalyzes formation of Cyclic AMP from ATP
  - Epinephrine (Adrenaline), Norepinephrine (Noradrenaline) and Glucagon activate Adenylate cyclase, thus causing it to increasing the formation of cyclic AMP
- Phosphodiesterase catalyzes degradation of Cyclic AMP

- Insulin can increase the activity of Phosphodiesterase in the Liver, thereby reducing the concentration of cyclic AMP

How is Glycogen metabolism regulated in Skeletal Muscle?

- Epinephrine promotes Glycogenolysis and inhibits Glycogenesis (**Why?**)
    - Because epinephrine stimulates formation of cyclic AMP by activating Adenylate cyclase

- In an emergency Epinephrine is released, it acts on the muscle cell membrane to increased Glycogenolysis so as to provide energy for muscle contraction and at the same time inhibits the Glycogenesis
- Insulin inhibits Glycogenolysis and promotes Glycogenesis (Why?)
- Because insulin reduces the level of cyclic AMP via the activation of Phosphodiesterase
- Note that insulin enhances the entry of glucose into the muscle ceils

How is Glycogen metabolism regulated in the Liver?

- Glucagon promotes Glycogenolysis and inhibits Glycogenesis (Why)
    - Because Glucagon activates Adenylate cyclase, thus increasing the level of cyclic AMP
- Insulin increases Glycogenesis in the Liver by increasing the activity of Glycogen Synthase

STUDY QUESTIONS:

1. Why is Glycolysis important?
2. What are the different types of Glycolysis?
3. What are the major functions of Glycolysis?
4. What is the fate of Pyruvate (a) Under Anaerobic conditions, (B) Under Aerobic conditions?
5. Give the total and net amounts of ATP formed when One Glucose is metabolized via Anaerobic Glycolysis?
6. What are the Total and Net amounts of ATP formed when One Molecule of Glucose is metabolized via Aerobic Glycolysis?
7. What is the function of 2,3-BPG in RBC?
8. What is the function of Pyruvate Dehydrogenase (PDH) Complex?
9. What is the significance of TCA cycle?
10. How many molecules of ATP are produced when One Acetyl-CoA goes through the TCA cycle?
11. What is the significance of Gluconeogenesis?
12. What is the Cori cycle (Lactic acid cycle)?
13. What is the major role of HMP shunt in Red Blood Cells?
14. Why does a person with G-6-PD deficiency suffer from hemolytic anemia?
15. What are some of the functions of Glycogen?
16. What happens to G-6-P in Liver, Kidneys and Skeletal Muscle?

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METABOLISM OF LIPIDS

- Lipids act as energy stores (Triglycerides) and as important structural components of cells (Cholesterol and Phospholipids)
- Fat provides about half the energy for resting muscle and Heart

What are the major differences and similarities between biosynthesis and degradation of fatty acids?

- Biosynthesis of fatty acids occur in Cytoplasm
- Degradation (beta-oxidation) of fatty acids occur in mitochondria
- Co-factor for Biosynthesis is NADPH obtained from HMP-pathway
- Co-factors for beta-oxidation are: FAD and NAD
- Acetyl-CoA is involved in both biosynthesis and beta-oxidation
- Acetyl-CoA in fatty acid biosynthesis exists temporarily bound to the enzyme complex as Malonyl-CoA

Biosynthesis of Saturated Fatty Acids:

How is fatty acid synthesized?

- Pathway for biosynthesis of fatty acid is presented in **Fig. 1**
  - Acetyl-CoA is the precursor for biosynthesis of fatty acid
- Biosynthesis of fatty acids is carried out by Fatty Acid Synthase (FAS)
- Fatty Acid Synthase is a complex enzyme with multiple enzyme activities
- Biosynthesis of fatty acid can be separated into **Two Stages**:

Stage One: involves synthesis of Malonyl-ACP and Acetyl-ACP (**Fig. 1**)

- Three enzymes are involved:
  - Acetyl-CoA Carboxylase, (requires Biotin as coenzyme)
  - Malonyl Transacylase and Acetyl Transacylase

Stage Two: It is a Cyclic Process involving 4 Enzymatic activities: (**Fig. 1**)

- These are:
  - Beta-keto-ACP Synthase (3-Ketoacyl Synthase)
  - Beta-keto-ACP Reductase, (3-Ketoacyl Synthase)
  - 3-Hydroxy Acyl-ACP Dehydratase
  - Enoyl-ACP Reductase

- During these reactions NADPH is oxidation to NADP
- The cyclic process is repeated to obtain Palmitic Acid (C 16 saturated fatty acid) **Why?**
  - Because Palmitic acid is the fatty acid molecule that is synthesized by the enzyme Fatty Acid Synthase
- Palmitic acid is released from the enzyme and can then undergo separate elongation to yield other fatty acid molecules

Beta Oxidation of Fatty Acid (Degradation of fatty acids):

What is beta-oxidation?

- Oxidation (degradation) of Fatty Acid is termed Beta-oxidation because,
  - Oxidation occurs via sequential removal of 2-Carbon units by oxidation at the Beta-Carbon position of the Fatty Acyl-CoA molecule

$$\text{R-CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \underset{\beta}{\mathbf{CH_2}} - \underset{\alpha}{\text{CH}_2} - \text{COCoA}$$

Fatty Acyl-CoA

- Fatty acids must be activated in the Cytoplasm and Transported into the Mitochondria before beta-oxidation can occur

How is fatty acid activated in the cytoplasm?

- Long chain fatty acids in cytoplasm are activated by Fatty Acyl-CoA Ligase (also called Acyl-CoA Synthetase or **Thiokinase**)
- Activation process involves utilization of 2 high-energy bonds in ATP (this is equivalent to utilization of 2 molecules of ATP)
- Net reaction can be represented as:

Thiokinase

Fatty acid + ATP + CoA ==========➔ Fatty-Acyl-CoA + PPi + AMP

How is activated fatty acid transported into mitochondria?

- Transport of activated fatty acid (Fatty-Acyl-CoA) into mitochondria is via the Carnitine Transport system that forms Acyl-Carnitine Intermediate, (**Fig. 2**)
- Acyl-Carnitine is formed by the action of Carnitine Acyl-Transferase I, an enzyme located in the Outer Mitochondrial Membrane
- Acyl-Carnitine molecule is transported into the mitochondria where Carnitine Acyltransferase II catalyses the regeneration of Fatty Acyl-CoA molecule

(**Fig. 2**)

Briefly outline the pathway for beta-oxidation of saturated fatty acids

- Beta-oxidation of activated saturated fatty acid can be separated into a Cyclic Process made up of Four reactions (**Fig. 3**):
  - Oxidation reaction: catalysed by Acyl-CoA Dehydrogenase (requires FAD)
  - Hydration reaction: catalysed by Enoyl-CoA Hydratase
  - Oxidation reaction: catalysed by Hydroxy-Acyl-CoA Dehydrogenase (requires NAD)
  - Thiolysis reaction: catalysed by Keto-Acyl-CoA Thiolase (requires CoA-SH)

- Each Cycle of Beta-Oxidation produces: (**Fig. 3**)
  - One mole of NADH, One mole of FADH and One mole of Acetyl-CoA
- An Acyl-CoA molecule containing two carbon atom shorter than the Acyl-CoA at the start of the cycle
- Total number of beta-oxidation cycles depend on the length of the Saturated fatty acid

What happens to $FADH_2$, NADH, Acetyl-CoA and Acyl-CoA produced at the end of each beta-oxidation cycle?

- FADH and NADH enter the Respiratory Chain to produce ATP
- Acetyl-CoA enters the TCA cycle where it is further oxidized

<u>Biosynthesis of Triglycerides or Triacylglycerols</u>:

- Triglyceride is made up of Glycerol to which 3 fatty acid residues are esterified
- Glycerol is the major building block for biosynthesis of Triacylglycerols, in tissues other than Adipose Tissue

How is Glycerol activated in tissues other than Adipose tissue?

- In tissues other than Adipose tissue Glycerol is activated in a reaction catalysed by Glycerol Kinase
- It involves Phosphorylation of Glycerol at C-3 position

Glycerol + ATP ========➔ Glycerol-3-Phosphate + ADP

How is Glycerol-3-Phosphate (Glycerol-3-P) produced in Adipose Tissue?

- Glycerol Kinase is not present in Adipose Tissue
- Dihydroxyacetone Phosphate (DHAP), produced via Glycolysis, is converted to Glycerol-3-P in a reaction catalysed by **Glycerol-3-Phosphate Dehydrogenase** that utilizes NADH (**Fig. 4**)

Glycolysis = = = ➔ DHAP + NADH + H^+ ➔==➔ Glycerol-3-P + NAD

Why does adipose tissue require glucose for biosynthesis of Triglycerides?

- Adipose tissue does not contain Glycerol Kinase, therefore it cannot directly convert Glycerol to Glycerol-3-P, which is the precursor for biosynthesis of Triglycerides
- Adipose tissue metabolises Glucose via Glycolysis to form DHAP, which is then converted to Glycerol-3-P by Glycerol-3-P Dehydrogenase

How is Phosphatidic Acid synthesized using either Glycerol-3-P or DHAP as precursor? (**Fig. 5**)

- Fatty acids used in biosynthesis of Triglycerides are activated by Acyl-CoA synthetase before they are used
- Two Acyl-CoA are esterified to Glycerol-3-P to yield 1, 2-Diacylglycerol-phosphate (**Phosphatidic acid**) {**Fig. 5**}

How is Triacylglycerol synthesized from Phosphatidic Acid? (**Fig. 6**)

- Phosphate is then removed, by Phosphatidic Acid Phosphatase, to yield 1,2-Diacylglycerol, to which the Third Fatty Acyl-CoA is added **(Fig. 6)**
- Intestinal Monoacylglycerols, derived from hydrolysis of dietary fats, can also serve as substrates for biosynthesis of 1,2-diacylglycerols

<u>Degradation of Triacylglycerols</u>:

Outline the pathway for degradation of Triacylglycerol

- Fatty acids in Triacylglycerols are released from Glycerol backbone by the action of specific Lipases **(Fig 7)**
- Enzymes involved in the degradation of Triacylglycerols are:

(a) **Hormone-sensitive Triacylglycerol Lipase**: Catalyses the reaction:

Triacylglycerol ====➔ Di-acylglycerol + Free Fatty Acid

(b) **Di-acylglycerol Lipase**: Catalyses the reaction:

Di-acylglycerol =======➔ Mono-acylglycerol + Free Fatty Acid

(c) Mono-acylglycerol Lipase: Catalyses the reaction:

Mono-acylglycerol ======➔ Glycerol + Free Fatty Acid

- Free fatty acids formed are degraded by beta-oxidation to produce energy
- Glycerol is converted to Dihydroxyacetone phosphate and used in Glycolysis

Biosynthesis of Phospholipids:

What mechanisms are used in the biosynthesis of Phospholipids?

- Phospholipids can be synthesized by two mechanisms (**Fig. 8**)
- First mechanism:

- Utilizes Cytidine Diphosphate (CDP) Activated Polar Head Group for attachment to the Phosphate of Phosphatidic Acid:
    - Examples: Biosynthesis of: Phosphatidyl-Choline and Phosphatidyl-Serine

- Second mechanism:
    - Utilizes CDP Activated 1,2-Diacylglycerol and Inactivated Polar Head Group
    - Examples: Biosynthesis of: Phosphatidyl-Inositol and Cardiolipin

How is Phosphatidyl-Choline biosynthesised?

- **Phosphatidyl-Choline (PC):** also called Lecithin
- PC is made up of Glycerol backbone, with:
    - Either Palmitic or Stearic acid attached to Sn-1 position of Glycerol
    - Either Oleic, Linoleic or Linolenic acid attached to Sn-2 of Glycerol
    - Phosphate group and Choline attached to Sn-3 of Glycerol

- **Biosynthesis of PC**:
    - Choline is activated first by Phosphorylation and then by coupling to CDP prior to attachment to Phosphatidic acid
    - PC can also be synthesized by addition of CDP-activated Choline to 1, 2-Diacylglycerol

How is Phosphatidyl-Ethanolamine biosynthesised?

- **Phosphatidyl Ethanolamines (PE)**:
- PE is made up of Glycerol backbone, with:
    - Either Palmitic or Stearic acid attached to Sn-1 of Glycerol
    - Long chain unsaturated fatty acid (e.g. 18:2, 20:4 and 22:6) attached to Sn-2 of Glycerol
    - Phosphate and Ethanolamine attached to Sn-3 of Glycerol

- **Biosynthesis of PE**:
    - Ethanolamine is activated by Phosphorylation and then by coupling to CDP
    - Ethanolamine is then transferred from CDP-Ethanolamine to 1, 2-Diacylglycerol to form PE

How significant is Phosphatidyl-Inositol?

- **Phosphatidyl Inositol (PI)** contain almost exclusively Stearic acid at Sn-1 and Arachidonic acid at Sn-2 positions of Glycerol
- PI exist in membranes with various levels of Phosphate esterified to the Hydroxyls of the Inositol
- Molecules with Phosphorylated Inositol are termed **Poly-Phosphoinositides**
- Poly-Phosphoinositides are important Intracellular Transducers of Signals emanating from Plasma Membrane
- Example:

- Phosphatidyl-Inositol 4,5-bisphosphate (PIP2) is a critically important membrane Phospholipid involved in Transmission of Signals for Cell Growth and Differentiation from outside the cell to inside

- Biosynthesis of PI involves CDP-activated 1,2-Diacylglycerol condensing with Myo-inositol

How are Phospholipids degraded?

- Phospholipids are degraded by Phospholipases
- Four Phospholipases involve in degradation of Phospholipids are:
  - Phospholipase A1
  - Phospholipase A2
  - Phospholipase C
  - Phospholipase D

- Phospholipase exhibits substrate specificity for specific position in Phospholipid (**Fig. 9**)
- Action of Phospholipase A2 on a phospholipid produces a Lyso-phospholipid that can:
  - Act as substrate for Acyl-Transferases that utilize different Acyl-CoA groups
  - Accept acyl groups from other Phospholipids in exchange reaction catalysed by Lysolecithin: Lecithin Acyltransferase (LLAT)

- **Phospholipase A2** is an important enzyme, whose activity is responsible for the release of **Arachidonic acid** from the C-2 position of membrane phospholipids
- Arachidonic acid is then used as substrate for biosynthesis of Prostaglandins and Leukotrienes

How are the Ketone bodies synthesized?

- Ketone bodies are the following three compounds together:
  - Acetoacetic acid, Beta-hydroxy-butyric acid and Acetone
- Acetyl-CoA is the precursor for biosynthesis of Ketone bodies
- Excess Acetyl-CoA molecules produced from beta-oxidation of fatty acids are usually used to synthesize the ketone bodies (**Fig. 10**)
- Biosynthesis of ketone bodies occur via formation of HMG-CoA (3-Hydorxy-3-Methyl-Glutaryl-CoA)

Cholesterol Metabolism:

- Cholesterol:
  - Plays important role in membrane structure
  - Is a precursor for biosynthesis of Steroid Hormones and Bile Acids
- Both dietary cholesterol and that synthesized de novo are transported in Lipoprotein particles in blood
- Cholesteryl esters are the forms in which cholesterol is stored in cells
- Biosynthesis and utilization of cholesterol must be tightly regulated in order to prevent over-accumulation and abnormal deposition within the body
- Of particular importance clinically is the abnormal deposition of cholesterol and cholesterol-rich lipoproteins in the coronary arteries
  - Such deposition, eventually leading to atherosclerosis, is the leading contributory factor in diseases of the coronary arteries

How is Cholesterol synthesised?

- Acetyl-CoA is the precursor and NADPH is the cofactor for biosynthesis of Cholesterol
- Biosynthesis of Cholesterol can be conveniently separated into Five major steps:
- These are:
  - Acetyl-CoA is converted to 3-Hydroxy-3-Methyl-Glutaryl-CoA (HMG-CoA),
  - HMG-CoA is converted to Mevalonate
  - Mevalonate is converted to Isoprene-based molecule, Isopentenyl Pyrophosphate (IPP), with loss of CO_2
  - Isopentenyl Pyrophosphate (IPP) is then converted to Squalene
  - Squalene is converted to Cholesterol

Calculate the amount of ATP produced when One Molecule of Palmitic acid (C 16 fatty acid) is completely oxidized to CO_2 and H_2O.

- Oxidation of Palmitic acid is via beta-oxidation
- Palmitic acid is first activated in the cytoplasm to Palmitoyl-CoA
  - Note: 2 ATP molecules are used for activation of Palmitic Acid

Thiokinase

Palmitic Acid + ATP + CoA =========➔ Palmitoyl-CoA + PPi + AMP

- Palmitoyl-CoA is then transported into the mitochondria were beta-oxidation occurs
- Beta-oxidation of Palmityl-CoA can be summarise as follows:

Palmitoyl-CoA + 7 CoA-SH + 7 FAD + 7 NAD^+ =====➔ 8 Acetyl-CoA + 7 $FADH_2$ + 7 NADH

- $FADH_2$, NADH and Acetyl-CoA are used for energy production

Production of Energy by $FADH_2$ and NADH:

- The 7 $FADH_2$ and 7 NADH goes to the Electron transport chain (ETC) to produce ATP
  - A total of 7 $FADH_2$ will yield 14 ATP in the ETC
  - A total of 7 NADH + 7 H^+ will yield 21 ATP in the ETC

- This gives a total of 35 ATP molecules
- Net amount of ATP produce will be 35 ATP – 2 ATP = 33 ATP

Production of Energy by Acetyl-CoA:

- Acetyl-CoA from beta-oxidation goes to the TCA cycle
- When One Acetyl-CoA goes through the TCA cycle a total of 12 ATP are produced
- Therefore 8 Acetyl-CoA will give 12 x 8 ATP = 96 ATP

Total amount of ATP produced when one molecule of Palmitic acid is completely oxidized to H_2O and CO_2 = **33 ATP + 96 ATP = 129 ATP molecules**

Study Questions:

1. How are fatty acids activated in the cytoplasm?
2. List the five major steps in the biosynthesis of cholesterol
3. What are the stages in the biosynthesis of fatty acids?
4. What are the four stages in beta-oxidation of fatty acids?
5. Write the structure of Phosphatidic acid.
6. Write the reaction for the biosynthesis of Triacylglycerol from Phosphatidic acid.
7. Write the reaction for the degradation of Triacylglycerol.
8. Use a diagram to show the point of action of the different Phospholipase enzymes on a phospholipid.
9. What are ketone bodies? Name the ketone bodies.
10. How ketone bodies synthesised?
11. Why does adipose tissue require glucose for biosynthesis of Triacylglycerol?

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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:**

$$\text{Alanine + α-Keto Acid} \rightleftarrows \text{Pyruvate + Amino Acid}$$

- **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:

$$\mathbf{AMP + H_2O ====\rightarrow IMP + NH_3}$$

- 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:

$$\mathbf{Glutamine + H_2O =====\rightarrow Glutamate + NH_3}$$

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

$$\mathbf{AMP + H_2O ====\rightarrow IMP + NH_3}$$

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

$$\mathbf{Glutamate + NAD + H_2O \leftarrow==\rightarrow \alpha\text{-Oxoglutarate + NADH + H^+ + NH_4^+}}$$

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

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INTRODUCTION TO HORMONES AND HORMONAL CONTROL

What are Hormones?

- In multi-cellular organisms cells communicate with one another in order to coordinate their growth and metabolism.
- One of the principle ways by which cells communicate with each other is by means of Extracellular signaling molecules or Hormones.
- Hormones are substances that carry information from **Sensor Cells**, which sense changes in the environment, to **Target Cells** that respond to changes
- Hormones also help to coordinate various metabolic processes in the body.

How are hormones classified?

- Hormones can be classified according to the various criteria:
    - By the proximity of their site of synthesis to their site of action,
    - By their chemical structure, and
    - By their degree of solubility in aqueous medium.

How can hormones be classified by proximity of their site of synthesis to their site of action?

- **Hormones can be classified into 3 distinct types based on the distance between the Site of Synthesis and Site of Action of the hormone (**Fig. 1**).**
    - **Autocrine Hormones**: these are hormones that affect the same cells that synthesize them.
    - **Paracrine Hormones**: these are hormones that are synthesized very close to their site of action.
    - **Endocrine Hormones**: these are hormones that are synthesized by endocrine glands and transported a considerable distance by the blood to target cells that contain the appropriate receptors

How can hormones be classified according to their Chemical Structure?

- Hormones can be separated into Four broad classes on the basis of their structure:
    - **Peptide or Protein hormones**: these are synthesized as larger precursors that undergo processing and secretion. Examples include the Hypothalamic factor called Thyrotropin releasing hormone (TRH), which is made up of three amino acid residues; Insulin made up of 51 amino acid residues; Pituitary Gonadotrophins, which are large molecules (Glycoproteins with subunits).
    - **Amino acid derivatives**: e.g., Adrenaline, Catecholamines, Thyroid Hormones
    - **Fatty acid derivatives**: such as the Eicosanoids (e.g., Prostaglandins).
    - **Steroid hormones**: These are all derivatives of Cholesterol.

How can hormones be classified according to their solubility in aqueous medium in the cells?

- Hormones can be separated into Two Classes on the basis of their solubility in aqueous medium;
- Location of receptor for each class of hormone is also different.

    - **Hydrophilic Hormones (Lipo-phobic Hormones):**
        - Hormones that are soluble in aqueous medium
        - They cannot cross the cell membrane, therefore they bind to **receptor molecules on the outer surface** of target cells, initiating reactions within the cell that ultimately modifies the functions of the cells

    - **Lipophilic Hormones (Hydrophobic Hormones):**
        - Hormones that are not soluble in aqueous medium
        - They can easily cross the cell membrane, therefore they can enter target cells and bind to **intracellular receptors**

Acronyms of some Hormones

Hormones	Acronym
AdrenoCorticoTrophic Hormone (Corticotrophin)	ACTH
Arginine Vasopressin (Anti-Diuretic Hormone)	AVP (ADH)
Corticotrophin Releasing Hormone	CRH
Follicular Stimulating Hormone	FSH
Gonadotrophin Releasing Hormone	GnRH
Growth Hormone	GH (HGH)
Growth Hormone Releasing Hormone	**GHRH**
Luteinizing Hormone	LH
Parathyroid Hormone	PTH
Thyroid Stimulating Hormone	TSH
Thyrotrophin Releasing Hormone	TRH
Thyroxine Tri-iodothyronine	T_4 T_3

How do hormones exist in blood plasma?

- Hormones are normally present in blood plasma at very low concentrations
- In blood, hormone generally binds to specific plasma carrier protein, forming a complex, which is then transported in the plasma to distant target cells
- Plasma carrier proteins exist for all classes of endocrine hormones

What are the functions of carrier proteins for hormones?

- Carrier proteins for:

- Peptide Hormones prevent the destruction of the peptide hormones by Protease enzymes in plasma
- Steroid Hormones and Thyroid Hormones allow these very hydrophobic compounds to be present in the plasma at concentrations several hundred-fold greater than their solubility in water would permit
- Small, Hydrophilic Amino Acids – derived hormones prevent their filtration by the kidneys, thus greatly prolonging their circulating half-life

TAKE NOTE:

- Because of the very low concentrations of hormones in blood plasma, sensitive protein receptors have evolved in target tissues to detect and interact with hormones
- All tissues that are capable of responding to hormones have two properties in common:
  - They posses specific receptors with very high binding affinity for specific hormone
  - Each receptor is usually coupled to a process that regulates metabolism of the target cells

What is the mechanism of action of Hydrophilic hormones with receptors in target cells?

- Mechanism of action of Hydrophilic hormones with receptors in target cells is called "Second Messenger".
- Receptors for Hydrophilic Hormones (Amino Acid – derived Hormones and Peptide Hormones) are located on the Plasma membrane of target cells
- Hormone (**First messenger**) interacts with the receptor on the cell membrane,
- Hormone-receptor complex causes conformational change in membrane proteins that results in production within the cell of compounds (**Second Messenger)**, such as Cyclic-AMP, or Cyclic-GMP
- Elevation in the cellular level of one or other of these second messengers leads to a rapid alteration in cellular function.
  - For example, the action of the hormone called Glucagon on Glycogen metabolism is carried out through the Second Messenger cyclic-AMP.

What are some of the properties of receptors for Hydrophilic hormones?

- Receptors for hydrophilic hormones are large, integral membrane proteins with specificity and high affinity for a given hormone. (Some hormone receptors may be Transmembrane-protein with enzymatic activity).
- Binding between the hormone and receptor is reversible, and the hormonal action declines as the plasma level of the hormone declines
- Hydrophilic hormones can initiate a response without entering target cells
- Hydrophilic hormones tend to cause a more rapid response and have a shorter duration of action than lipophilic hormones
- Action of hydrophilic hormone can last from seconds to hours
- G – proteins are associated with hormone receptors on the cytosolic side of the cell membrane. (G – protein is a protein that bound either GTP or GDP)

What is the mechanism of action of Lipophilic (Hydrophobic) hormones with receptors in target cells?

- Lipophilic hormone (e.g., Steroid hormones, Thyroid hormones) can move across cell membrane to bind with intracellular receptor, forming the so called Hormone-Receptor Complex
- Hormone-Receptor Complex then bind to Specific Sequence of Nucleotide Bases in the DNA called the Hormone Response Element (HRE)
- Binding of Hormone-Receptor Complex to HRE results in the production of Messenger-RNA (Transcription of Specific Genes) required for biosynthesis of specific protein (**Fig. 2**)
- Lipophilic hormones are slower to act and have a longer duration of action than hydrophilic hormones. Their duration of action ranges from hours to days.

What is Negative-Feedback Mechanism for Regulation of Hormone secretion?

- Regulation of the secretion of some hormones from their endocrine glands is controlled through Negative-Feedback Mechanism, (Long-Loop and Short-Loop negative Feedback, **Figs. 3a. 3b**)
- Hormone released from one endocrine gland often regulates the release of another hormone from a second gland, which in turn controls hormonal production in and release from the first gland
- In addition, plasma concentration of the hormone itself or of a substance produced by the target tissue in response to the hormone regulates the further release of the hormone from the gland

What are some of the factors controlling hormone secretion?

- Hormone secretion is under a variety of influences:
    - Stimulatory and Inhibitory agents, such as: Hypothalamic Peptides or Neurotransmitters may influence hormone synthesis or release.
    - Many hormones, such as Gonadotropin Releasing Hormone (GnRH), are released in a pulsatile fashion
    - Some hormones exhibit a Circadian Rhythm, {e.g., AdrenoCorticoTrophic Hormone (ACTH), and Cortisol}; note that Prolactin, Thyroid Stimulating Hormone (TSH), Growth Hormone (GH) and even Parathyroid Hormone (PTH) have peak secretion at different times during the day or night.
    - Stress can increase hormone synthesis and release, e.g., are ACTH, GH and Prolactin.
    - Hormones synthesized by target cells may feed back to the endocrine glands (Negative Feed Back control).
    - Changes in metabolic products as a result of hormone action may likewise exert feedback control.
    - Other hormones or drugs may modulate normal endocrine responses.

<u>TAKE NOTE</u>:

- Concentration of hormones in blood plasma are usually not constant, Thus:
    - A single measurement of a hormone in peripheral plasma may suggest, incorrectly, that there is abnormal endocrine function

- Dynamic tests have been developed to give clearer information about endocrine activity in the patient, especially those with suspected Pituitary or Adrenocortical disorders

OVERVIEW OF SOME SPECIFIC HORMONES

INSULIN:

- Insulin is a Protein Hormone that is secreted by Beta cell in the Islets of Langerhans in the Pancreas
- Insulin is the Principal hormone affecting Blood Glucose level, thus an understanding of its mode of action is an important prerequisite to the study of the condition called Diabetes Mellitus (Sweet Urine)
- Insulin acts through membrane receptors and its main target tissues are Skeletal Muscle and Adipose tissue
- Overall effect of Insulin is to promote cellular uptake and storage of metabolic fuels and these actions can be categorize as follows:
  - Metabolic functions that are enhanced in the presence of Insulin:
    - These include – Glucose uptake in muscle and adipose tissue, Glycolysis, Glycogenesis, Protein synthesis, and Cellular uptake of ions especially K^+ ions and PO_4^{3-} ions.
  - Metabolic functions that are decreased in the presence of insulin:
    - These include – Gluconeogenesis, Glycogenolysis, Lipolysis, Ketogenesis, and Proteolysis.

- Insulin Stimulates Biosynthesis of: Glycogen, Fats and Proteins,
- Insulin at the same time Inhibits Degradation of: Glycogen, Fat and Proteins.
- Insulin affects the uptake of Glucose into Muscle cells, Adipose tissue, Connective tissues and White blood cells
- Insulin DOES NOT affects uptake of Glucose into the Brain, Liver and Kidneys
- Insulin regulates metabolism of Glucose in the Liver
- Insulin Counter Regulatory Hormones, such as, Glucagon, Epinephrine, Glucocorticoids, and Growth hormone oppose the Actions of Insulin.
- Homeostatic Regulation of Glucose concentration in blood is the result of Balance between the Actions of Insulin and the Insulin Counter-Regulatory Hormones (**Fig. 4: Stop – Go – Reactions**)

GLUCAGON:

- Glucagon is a hormone produced in the Alpha cells of the Pancreas
- Glucagon is an Insulin Counter-Regulatory Hormone, whose action is to increase Blood Glucose Level from Low to Normal
- Glucagon acts primarily in the Liver to stimulate the breakdown of Glycogen to Glucose, which is then released into the blood
- Glucagon also signals the breakdown of Fats in Adipose Tissue and the conversion of Fatty Acids to Glucose (Gluconeogenesis) in the Liver
- Production and release of Glucagon is stimulated by Falling Glucose Level (Hypoglycemia) and by Increase Absorption of Amino Acids into the blood (as arise after a protein-rich meal)
- High Blood Glucose Level Inhibits the production and release of Glucagon

THYROID HORMONES:

What are the Thyroid Hormones?

- Thyroxine (T_4) and Tri-Iodothyronine (T_3) are together known as the "Thyroid Hormones"
- Thyroid hormones are unique because they contain the Trace element Iodine
- T_4 contains 4 Iodine atoms
- T_3 contains 3 Iodine atoms

Where are the Thyroid hormones produced?

- Thyroid hormones are produced by Thyroid Gland
- Thyroid gland secretes mostly T_4
- T_4 is usually called the Pro-hormone because it is later converted to T_3
- T_4 is converted to T_3 by removal of an Iodine atom (De-Iodination)
- Peripheral tissues, especially the Liver and Kidney, De-Iodinate T_4 to produce approximately two-thirds of the circulating T_3, present in blood plasma
- T_4 can be metabolised to reverse T_3 (rT_3), which is biologically inactive.

How do Thyroid hormones exist in blood plasma?

- Both T_4 and T_3 circulate in plasma bound to two specific binding proteins:
  - **Thyroxine Binding Globulin (TBG) and**
  - **Transthyretin (also called Thyroxine-Binding Pre-Albumin or TBPA**)
  - In plasma TBG is quantitatively the most important binding protein for the Thyroid hormones
  - TBG is synthesized in the Liver
  - Thyroid hormones are also bound to Plasma Albumin

What form of Thyroid hormone is biologically active?

- T_3 is the biological active form of the Thyroid hormones
- T_3 binds to receptors and triggers the end-organ effects of the Thyroid hormones
  - It is the Unbound, or "Free" T_3 concentration that are important for the biological effects of the Thyroid hormone, including the feedback to the Pituitary and Hypothalamus

How are Thyroid hormones secretion regulated?

- Thyroid hormone secretion are regulated by Negative-Feedback mechanism
- Feedback regulation of Thyroid hormones occurs via the Hypothalamic-Pituitary-Thyroid axis (HPT axis)
- Thyrotropin Releasing Hormone (TRH) is secreted by Hypothalamus
- TRH then stimulates the Anterior Pituitary to produce Thyroid Stimulating Hormone (TSH)
- TSH stimulates the Thyroid glands to produce Thyroid hormones (**Fig. 5**)
- Excess amount of Thyroid hormones will feedback inhibits the Anterior Pituitary and Hypothalamus (Long-loop feedback)
- In addition, excess amount of TSH will feedback inhibits the Hypothalamus to stop the production of TRH (Short-loop feedback)

What are some of the actions of Thyroid hormones?

- Thyroid hormones act at the cellular level and affect whole body metabolism

- Thyroid hormones affect protein, fat and carbohydrate metabolism
- Thyroid hormones regulate Gene Expression, Tissue Differentiation, and general metabolism and development
- Thyroid hormones are essential for the Normal Maturation and Metabolism of all the tissues in the body
- Hypothyroid children may have Delayed Skeletal Maturation, Short Stature and Delayed Puberty

Study Questions:

1. Classify hormone using the following criteria: Site of synthesis to site of action; Chemical structure of the hormones, Solubility of hormones in aqueous medium
2. What are the functions of carrier protein for hormones?
3. What are the properties of the receptors for hydrophilic hormones?
4. What is the mechanism of action of hydrophilic hormones with receptors in target cells?
5. What is the mechanism of action of Lipophilic (Hydrophobic) hormones with receptors in target cells?
6. What is the function of the Hormone Response Element (HRE)?
7. Use a fully labeled diagram to explain the Negative-Feedback mechanism for regulation of endocrine secretion
8. What are the Thyroid hormones?
9. How is the secretion of Thyroid hormone regulated?
10. What are some of the actions of the Thyroid hormones?
11. What hormones are called Insulin Counter Regulatory Hormones?
12. What are the functions of Insulin?

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LECTURE NOTES: XENOBIOTICS – An **Overview**

What are Xenobiotics?

- Xenobiotic is a chemical that is found in the body but which is not normally produced or expected to be present in the body
- Xenobiotic also refers to substances that are present in much higher quantity than is normally found in the body: e.g., drugs such as antibiotics are xenobiotics in humans, because the human body does not produce them, nor would they be expected to be present as part of a normal diet.
- Xenobiotic is a term usually used in the context of toxicants and pollutants such as Dioxins and Polychlorinated Biphenyls, etc.

What are some of our daily sources of Xenobiotics?

- Most foreign compounds (Xenobiotics) in our diet are natural plant or animal products, or are formed during cooking of foods
- Most essential nutrients may act as Xenobiotics if consumed unwisely
- Poorly regulated food and chemical industries tend to create new opportunities for diet-related poisonings, that act as carcinogens
- Our ability to metabolism dietary Xenobiotics is often dependent on our nutritional status, and our exposure to other dietary compounds
- Diets high in fruits and vegetables tend to decrease cancer risk, because of the anti-oxidant properties of compounds like Vitamin C, Beta-Carotene and Phytochemicals
- Current knowledge indicates that Phytochemicals as the most effective anti-carcinogenic components in fruits and vegetables
- Many plant products that appear to decrease the risk of cancer or Cardiovascular Disease (CVD) are marketed as "Antioxidants", however the Phytochemicals tend to function through modulation of Xenobiotic metabolism
- Chronic exposure to xenobiotics such as ethanol, or other drugs, has a direct effect on nutritional status

What is Biotransformation?

- Biotransformation is the process of converting foreign compounds (Xenobiotics) that are hydrophobic to hydrophilic compounds that can be excreted from the body
- Several enzyme systems, are able to Biotransform foreign compounds (Xenobiotics) and endogenous metabolic waste products that are hydrophobic to more water-soluble (hydrophilic) compounds that can be readily excreted
- Biotransformation reactions are usually categorized by the normal sequence (phases) with which they tend to react with Xenobiotics

Example: (**Fig 1**)

- Phase I reaction: Benzene is Biotransform to Phenol by Oxidation
- Phase II: Phenyl is Biotransform to Phenyl Sulfate by Conjugation

What are the Phases of Biotransformation reactions?

- Biotransformation reactions can be categorized into Two Phases: Phase I and Phase II

What is Phase I Reaction (of Biotransformation)?

- Phase I reactions of Biotransformation usually involves:
  - Reactions that modify the xenobiotic by adding polar groups to the compound
  - Modification the xenobiotic by Oxidation, Reduction, Hydrolysis, Hydration, Dehydro-chlorination or other reactions catalyzed by enzymes in either the Cytosol, Endoplasmic Reticulum (Microsomal enzymes) or of other cell Organelles.

What is Phase II Reaction of Biotransformation?

- Phase II reactions of Biotransformation involves conjugation of the Polar compounds obtained in Phase I reactions with another compound that will make the xenobiotic more soluble and therefore more easily eliminated from the body
- The conjugated products are larger, have poor ability to cross cell membranes and more polar in nature, thus they are readily excreted from the body via the bile or urine

What is Phase III Reaction (of Biotransformation)?

- Further metabolism of conjugated metabolites produced by Phase II reactions: it may result in the production of toxic derivatives.

<u>TAKE NOTE</u>:

- Constant and unavoidable exposure to xenobiotics (food additives, chemicals, therapeutic agents, etc) is part of our daily routine
- The property (lipophilictity), which enables these xenobiotics to be absorbed, is also the major problem for their elimination.
- The rate of elimination from the body is often determined by their conversion to water-soluble chemicals (known as Biotransformation) by enzymes in the Liver and other tissues, which facilitate their elimination

- Xenobiotics that are soluble in fat (highly lipophilic) usually have the tendency to accumulate in the body

  - Their accumulation results from the ability of highly lipophilic substances to dissolve in the lipid membrane of epithelial cells and to be retained in the body via their passive transport back across the epithelium
  - Biotransformation is the mechanism available in the body for the excretion of these highly lipophilic compounds

What are the mechanisms involved in Phase I reactions of Biotransformation?

- Phase I reactions involves conversion of xenobiotics from Lipophilic state to Polar

- During Phase I reactions the xenobiotic is altered by the introduction of a polar group such as: Hydroxyl (- OH), Carboxyl (-COOH), or Amino ($-NH_2$)
- Phase I reaction may unmasking one of these Polar groups in the xenobiotic
- Addition or unmasking of polar groups by Phase I reaction may be as a result of Oxidation, Reduction or Hydrolysis reaction.
- The specific reaction that occurs is usually determined by the structure of the xenobiotic

Use appropriate examples to briefly describe the various types of Phase I reactions

Oxidation Reactions: is the most important of the Phase I reactions

- Examples include the following:
  - Hydroxylation reactions of various Aromatic and Aliphatic compounds
  - Substrates for oxidative reactions include the Alkyl-amino compounds (e.g. Nicotine and Morphine)
  - N-alkyl groups in particular can be removed by Oxidative Dealkylation
  - O-alkyl groups (especially methyl groups) can also be removed
  - Compounds with a Thio-ether group are readily oxidized to Sulfoxides
  - Alkyl groups are also readily oxidized and undergo fairly rapid Hydroxylation
  - Oxidation of Aromatic compounds leads to Phenolic compounds

Mixed-Function Oxidase (MFO) System:

- Cytochrome P-450 is an enzyme the plays significant role in the Oxidation of Xenobiotics that are highly lipophilic
- Cytochrome P-450 is part of an enzyme system called "Mixed-Function Oxidase (MFO),
  - MFO system refers to the ability of the enzyme to incorporate molecular Oxygen into the substrate and to reduce the other atom of Oxygen to H_2O
  - MFO system is made up of:
    - Cytochrome P-450 occupying a key position
    - Flavo-proteins that utilizes NADPH and NADH to produce reducing equivalents:
    - Example of reaction catalyzed by MFO

$$\mathbf{SH + NADPH + H^+ + O_2 \text{ ======} \rightarrow SOH + NADP^+ + H_2O}$$

(Where SH is the Substrate to be oxidized and SOH is the Hydroxylation product)

- Several Isoenzymes of Cytochrome P-450 with different types of substrates

Reduction reactions:

- Reduction reactions are not very common
- Example:
  - Reduction can occur across N=N double bonds ("Azo" compounds) or "Nitro" groups (NO_2)
  - Reduction of Nitro (NO_2) group produces the corresponding "Amines" (NH_2) group

Biotransformation of Nitrobenzene to Aniline (Fig. 2)

Hydrolysis reactions:

- **Hydrolysis** refers to the cleavage of a foreign compound by the addition of H_2O
- Example: Conversion of Benzene to Phenol

    - **Examples: Biotransformation of Procaine a local anesthetic (Fig 3)**

How is Biotransformation related to Bioinactivation and Bioactivation?

Biotransformation can be separated into two different processes: Bioinactivation and Bioactivation

- Biotransformation often leads to changes in a Xenobiotic molecule that increases its solubility in water and improves its excretion
- Biotransformation therefore, tends to reduce the duration of the toxic effect of the Xenobiotic, because, there is usually a relationship between the concentration of a Xenobiotic and the intensity of its toxic effect
    - Many Biotransformation reactions may be considered as **Bioinactivation** or **Detoxification** reactions
    - Bioinactivation generally means decrease in the intensity of the toxic effects of Xenobiotic compounds

- Biotransformation reactions that yield products having higher toxicity than the parent compound, are referred to as **Bioactivation** reactions

How are Phase I reactions of Biotransformation relate to Bioinactivation and Bioactivation?

- Some Phase I reactions can cause Bioactivation by increase the Toxicity of a compound, because the introduction of a Polar group in a compound can also increase the likelihood of the polar compound to interact with components of the biological system (Proteins or DNA)

- **Example**:

    - Conversion of the Insecticide Parathion to Paraoxon
        - Parathion is one of the Organothiophosphates that are Neurotoxic, because they can inhibit the enzyme Acetyl-cholinesterase (AchE) in the nervous system
        - Affinity of this enzyme for Paraoxon is many times higher than it is for the parent compound Parathion
        - Thus, the oxidation reaction required to make the Parathion more water soluble leads to a bioactivation product (Paraoxon)
        - In a subsequent reaction Paraoxon can be hydrolyzed, to produce a compound that has no toxic effect on AchE

- In this example:
    - The first Biotransformation reaction, (Oxidation to Paraoxon), results in Bioactivation
    - The second reaction, the Hydrolysis, causes Bioinactivation

- Another example of a Bioactivation product being formed is in the conversion of Benzene to Phenol:
    - A highly reactive intermediate called an Epoxide is formed during the conversion of Benzene to Phenol
    - The reactive Epoxide intermediate is very short-lived but can at times interact with Nucleophilic groups in macromolecules, such as, Proteins and DNA

Give a brief summary of Phase I reactions of Biotransformation:

- Organisms are able to change the biological activity (Biotransformation) of xenobiotics by enzymatic reactions that make the xenobiotics more polar and thus more easily excreted from the body
- First step in Biotransformation process is the addition of a Polar handle by a Phase I reaction.
- Phase I reactions often take place under the control of enzymes from the Mixed Function Oxidase (MFO) system, which is a collection of enzymes capable of catalyzing the oxidation of many xenobiotics.
- Biotransformation of xenobiotics leads to changes in their biological activity:
    - Toxicity of the Xenobiotic is usually reduced (Bioinactivation), but some Bioactivation reactions can also occur, especially among oxidative reactions
- Introduction of a polar group to a xenobiotic may give the compound sufficient Hydrophilic character for rapid excretion,
    - For most substances, a subsequent reaction (Phase II reaction) is required

Phase II Reactions: Conjugations

- During Phase II reactions of Biotransformation, Polar products formed during Phase I reactions are combined (Conjugated) with endogenous Hydrophilic compounds, to produce highly Hydrophilic products that can be rapidly excretion
- Endogenous metabolites, such as, Glucuronic acid, Sulfate, Glycine and Glutathione are use for the "Conjugation reactions"
- Some of the Enzymes involved in Phase II reactions are:
    - Glucuronyl Transferase; Sulfotransferase; Glutathione-S-transferase; Epoxide Hydrolase

- Phase II reactions are usually designed to Bioinactivate Xenobiotics, however there are a few notable exceptions where the water-soluble products formed are more Bioactive or more Toxic than the parent compounds

Use appropriate examples to briefly describe the various types of Phase II reactions
Glucuronyl Transferase:

- Formation of Glucuronides is quantitatively the most important conjugation reaction, involved in Phase II reactions
- UDP-Glucuronyl Transferase catalyses the reaction, by attaching two molecules of Glucuronic acid to polar groups on a xenobiotic compound
    - Conjugation of polar groups with Glucuronic acid occurs only after activation of Glucuronic acid to form Uridine Diphosphate Glucuronic Acid (UDPGA)

Example: Glucuronide conjugation of Aniline to Aniline-N-Glucuronide (Fig 4)

Sulfotransferase:

- Another very common Phase II reaction is Sulfate Conjugation
- Sulfate needs to be activated before the conjugation reaction can take place.
    - Activation of Sulfate:
        - Sulfate is first converted into Adenosine-5'-PhosphoSulfate (APS)
        - APS is then Metabolized into 3'-PhosphoAdenosine-5'-PhosphoSulfate (PAPS), which is the activated Sulfate

- Activated Sulfate (PAPS) is then Conjugated with the Polar Xenobiotic compound, (e.g., Phenol) which becomes more water soluble and more easily excreted

Glutathione (GSH) Conjugation (Mercapturic Acid formation):

- Glutathione (GSH) conjugation usually results in the formation of Mercapturic acids
- Xenobiotics containing Halogen atoms are the usual substrate for this reaction
    - Examples of such Xenobiotics include: Di-Chloro-Nitro-Benzene and Bromo-Cyclo-Hexane
    - These substances eventually form Mercapturic acids and are excreted

- Epoxides can also be metabolized to Mercapturic acids by Glutathione conjugation (e.g., Benzene metabolism, producing the reactive intermediate Benzene Epoxide)

Epoxide Hydrolase:

- An enzyme that is responsible for the detoxification of Epoxides by Hydration
    - Reaction proceeds via activation of H_2O rather than activation of Epoxide ring

How are Phase II reactions related to Bioinactivation and Bioactivation of Xenobiotics?

- Products of Phase II reactions usually have very low biological activity, highly water-soluble and readily excreted
    - Most Phase II reactions are Bioinactivation or Detoxification reactions

- Some Phase II reactions can produce Bioactive products:
- Examples:
    - Sulfation may produce unstable compounds such as the Sulfate conjugates of Benzyl alcohols and Hydroxamic acids, which are highly electrophilic
    - Highly electrophilic ions can react readily with biological macromolecules (Proteins, RNA, and DNA), causing cell death or tumor formation
    - Glucuronides of Aromatic Amines are unstable compounds
        - Aromatic Amines may be bioactivated (in the liver) to form N-Hydroxyl derivatives, after which they can be bioinactivated by forming N-Glucuronides

Give a brief summary of Phase II Reactions of Biotransformation:

- In Phase II reactions, Polar Xenobiotics are conjugated with endogenous substances making them more water soluble and more easily excreted:
    - Examples of the Phase II reactions are: Glucuronidation, Sulfation, and Mercapturic Acid formation

- Glucuronidation and Sulfation proceed via enzymatic reactions using activated Glucuronic acid and Sulfate
- Mercapturic acid formation is an enzymatic process, which results from the conjugation of reactive intermediates with Glutathione (GSH). The conjugated product is then converted into a Mercapturic acid and excreted

- Phase II reactions generally result in the formation of compounds that are less biologically active (Bioinactivation) although sometimes they yield products with a higher bioactivity (Bioactivation)

Summary of Biotransformation Reactions:

- Biotransformation reactions are enzymatic conversions of lipophilic foreign substances and endogenous metabolic waste products.
- These reactions convert lipophilic (non-polar) compounds into polar compounds that are water soluble and easily excreted.

- Biotransformation can generally be seen as divided into two steps:
    - In the phase I reactions the MFO system plays an important role (oxidation reactions), with Cytochrome P-450 playing the key in this system.
    - Phase II reactions consist mostly of conjugation reactions with, for example Glucuronic acid, Sulfate, or Glutathione.

- Biotransformation reactions can lead to considerable changes in the biological activity of xenobiotics.
- If the activity is decreased the reaction is considered a Bioinactivation or Detoxification reaction.
- If the toxicity is increased it is referred to as a Bioactivation reaction.

Study questions:

1. What is Biotransformation?
2. What is Phase I reaction of Biotransformation?
3. What is Phase II reaction of Biotransformation?
4. How is Biotransformation related to Bioinactivation and Bioactivation?
5. How are Phase I and Phase II reactions related to Bioinactivation and Bioactivation?
6. Give a brief summary of Phase I reaction of Biotransformation
7. Give a brief summary of Phase II reaction of Biotransformation