

# **SHRIMATI INDIRA GANDHI COLLEGE**

(NATIONALLY ACCREDITED AT “A” GRADE (3<sup>RD</sup> CYCLE) BY NAAC)

**TIRUCHIRAPPALLI-2**

## **TUTORIAL MATERIAL**

### **BIOENERGETICS AND METABOLISM**

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# Cellular Respiration

### 1. Define Bioenergetics

Bioenergetics is a field in biochemistry and cell biology that concerns energy flow through living systems. This is an active area of biological research that includes the study of the transformation of energy in living organisms and the study of thousands of different cellular processes such as cellular respiration and the many other metabolic and enzymatic processes that lead to production and utilization of energy in forms such as adenosine triphosphate (ATP) molecules. That is, the goal of bioenergetics is to describe how living organisms acquire and transform energy in order to perform biological work. The study of metabolic pathways is thus essential to bioenergetics.

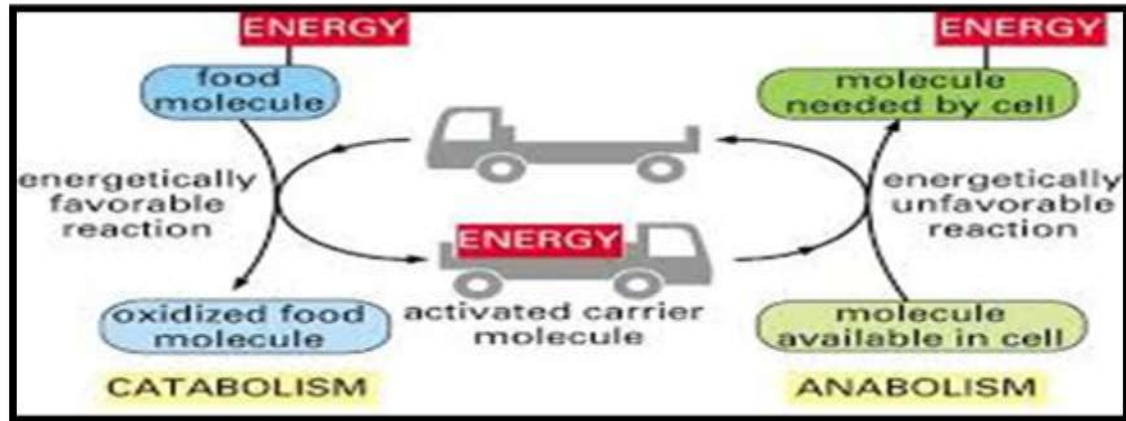
### 2. Define Metabolism

Metabolism is a term that is used to describe all chemical reactions involved in maintaining the living state of the cells and the organism. Metabolism can be conveniently divided into two categories:

- Catabolism - the breakdown of molecules to obtain energy
- Anabolism - the synthesis of all compounds needed by the cells

Metabolism is closely linked to nutrition and the availability of nutrients. Energy formation is one of the vital components of metabolism.

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#### 3. What are the types of cell respiration?

There are two types of cell respiration: aerobic cell respiration, a reaction with the participation of molecular oxygen ( $O_2$ ); and anaerobic cell respiration, without the participation of molecular oxygen and which uses other inorganic molecules as an oxidant instead.

#### 4. How many ATP molecules are produced for each glucose molecule used in aerobic respiration?

In fermentation, two ATP molecules are produced from one glucose molecule. In aerobic respiration, a much more productive process, 38 ATP molecules are made from one glucose molecule.

#### 5. What are the three phases of cell respiration?

The three phases of aerobic cell respiration are glycolysis, the Krebs cycle and the respiratory chain (also known as the electron transport chain).

#### 6. Define Entropy

**Entropy**, the measure of a system's thermal energy per unit temperature that is unavailable for doing useful work. Because work is obtained from ordered molecular motion, the amount of **entropy** is also a measure of the molecular disorder, or randomness, of a system.

### 7. Enthalpy

Enthalpy is defined as a state function that depends only on the prevailing equilibrium state identified by the system's internal energy, pressure, and volume. It is an extensive quantity.

Enthalpy is the preferred expression of system energy changes in many chemical, biological, and physical measurements at constant pressure, because it simplifies the description of energy transfer. At constant pressure, the enthalpy change equals the energy transferred from the environment through heating or work other than expansion work.

The total enthalpy,  $H$ , of a system cannot be measured directly. The same situation exists in classical mechanics: only a change or difference in energy carries physical meaning. Enthalpy itself is a thermodynamic potential, so in order to measure the enthalpy of a system, we must refer to a defined reference point; therefore what we measure is the change in enthalpy,  $\Delta H$ . The  $\Delta H$  is a positive change in endothermic reactions and negative in heat-releasing exothermic processes.

### 8. Gibbs Standard free energy

The Gibbs free energy of a system at any moment in time is defined as the enthalpy of the system minus the product of the temperature times the entropy of the system.

$$G = H - TS$$

The Gibbs free energy of the system is a state function because it is defined in terms of thermodynamic properties that are state functions. The change in the Gibbs free energy of the system that occurs during a reaction is therefore equal to the change in the enthalpy of the system minus the change in the product of the temperature times the entropy of the system.

$$\Delta G = \Delta H - \Delta(TS)$$

If the reaction is run at constant temperature, this equation can be written as follows.

$$\Delta G = \Delta H - T\Delta S$$

The change in the free energy of a system that occurs during a reaction can be measured under any set of conditions. If the data are collected under standard-state conditions, the result is the **standard-state free energy of reaction** ( $\Delta G^\circ$ ).

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

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### Redox Reaction

An oxidation-reduction (redox) reaction is a type of chemical reaction that involves a transfer of electrons between two species. An oxidation-reduction reaction is any chemical reaction in which the oxidation number of a molecule, atom, or ion changes by gaining or losing an electron.

In these reactions, electrons are transferred from one reactant to another. So, simply put, electrons are lost from one substance and gained by another. **Oxidation** refers to the loss of electrons from a substance, while **reduction** refers to the gain of electrons by a substance. Redox reactions are common and vital to some of the basic functions of life, including photosynthesis, respiration, combustion, and corrosion or rusting.

### Define High Energy Phosphate

ATP, or adenosine triphosphate, is formed after the binding of one phosphate molecule (phosphorylation) to one ADP (adenosine diphosphate) molecule. This is a process that stores energy in the produced ATP molecule. When ATP provides energy to the cellular metabolism, it releases one of its phosphate ions and ADP reappears.

ADP can also release more phosphate ions and generate AMP (adenosine monophosphate) or even non-phosphorylated adenosine. Adenosine production from ATP is used in tissues that need urgent supply oxygen, such as in the heart during a myocardial infarction (heart attack). This is because adenosine creates a local vasodilator effect, thus providing faster vasodilation than other physiological methods.

### Oxidative phosphorylation

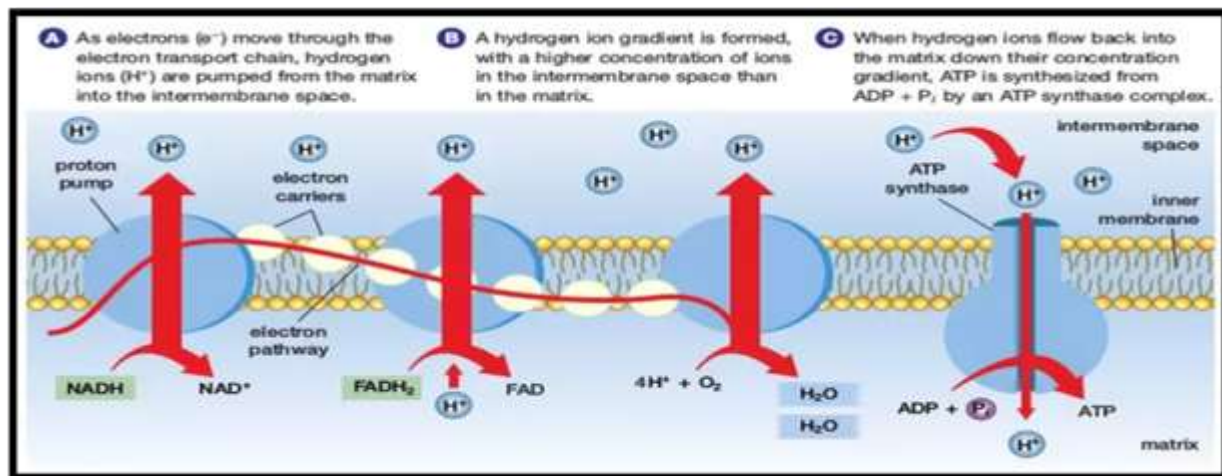
This is the process in which ATP is formed as a result of the transfer of electrons from NADH or FADH<sub>2</sub> to O<sub>2</sub> by a series of electron carriers. Electrons are passed from one member of the transport chain to another in a series of redox reactions.

Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called **chemiosmosis**. Together, the electron transport chain and chemiosmosis



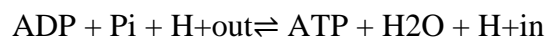
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make up **oxidative phosphorylation**. This process, which takes place in mitochondria, is the major source of ATP in aerobic organisms.



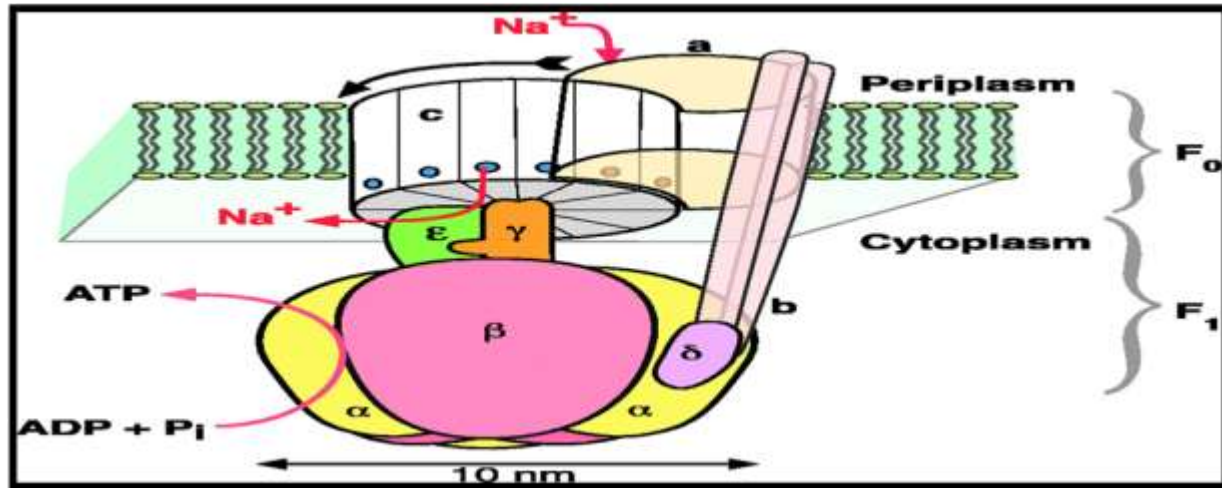
#### What is the role of ATP Synthase?

ATP synthase is an enzyme that creates the energy storage molecule adenosine triphosphate (ATP). ATP is the most commonly used "energy currency" of cells for all organisms. It is formed from adenosine diphosphate (ADP) and inorganic phosphate ( $P_i$ ). The overall reaction catalyzed by ATP synthase is:



The formation of ATP from ADP and  $P_i$  is energetically unfavorable and would normally proceed in the reverse direction. In order to drive this reaction forward, ATP synthase consists of two main subunits,  $F_0$  and  $F_1$ , which has a rotational motor mechanism allowing for ATP production. Because of its rotating subunit, ATP synthase is a molecular machine.





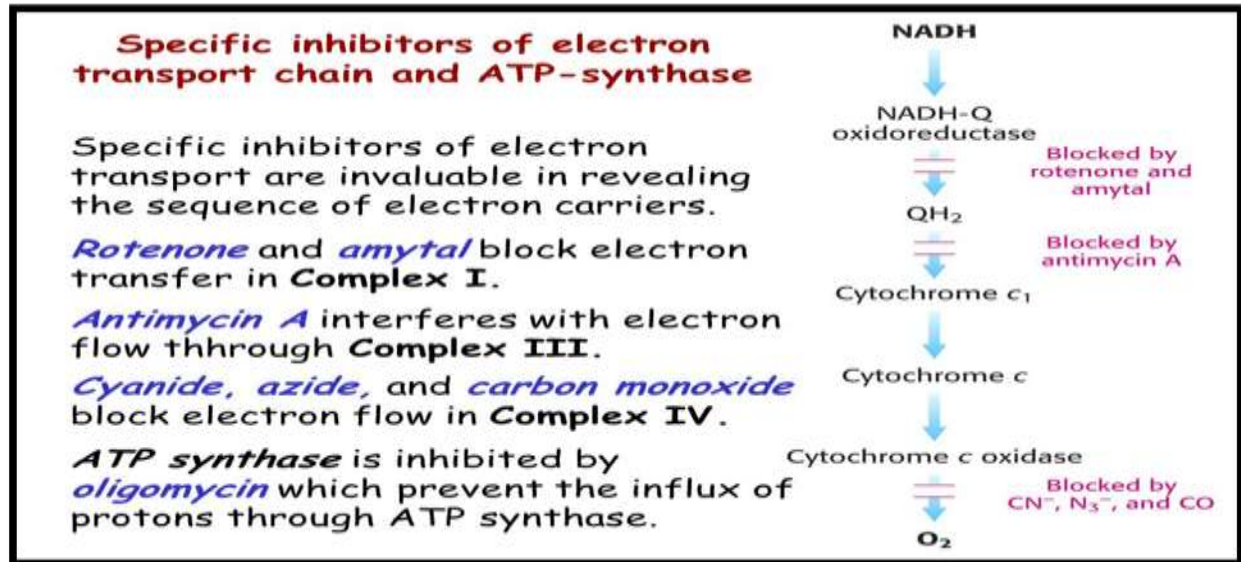
#### What is the importance of electron transport chain?

The electron transport chain is a system of molecules through which electrons are transferred to generate ATP. It has an important role in both photosynthesis and cellular respiration. It has an important role in both photosynthesis and cellular respiration that is responsible for producing: Water (with the help of oxygen we breathe) up to 38 ATP (thanks to the proton gradient) NAD and FAD (which are recycled to be used again in the Citric acid cycle and Glycolysis).

#### What are the Inhibitors of the Electron Transport Chain?

The most important known inhibitors of the ETC are Amytal, Rotenone, Antimycin A, CO, Sodium Azide, and Cyanides. Amytal, a barbiturate, and Rotenone, a plant product used as insecticide and pesticide, block the ETC between NADH dehydrogenase.

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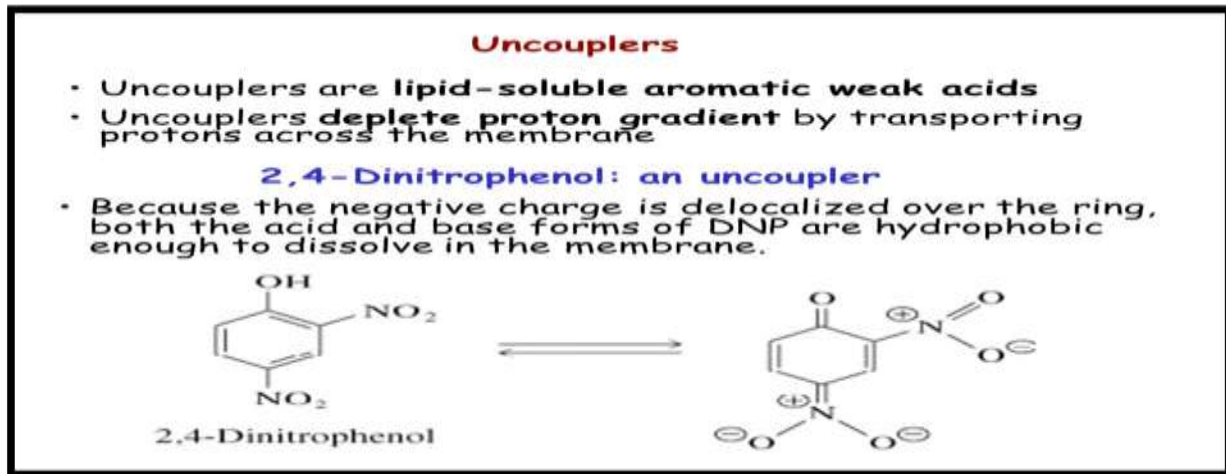


### Definition of Uncoupler

It is an agent that dissociates two integrated series of chemical reactions especially: one that prevents the formation of ATP in oxidative phosphorylation in mitochondria by dissociating the

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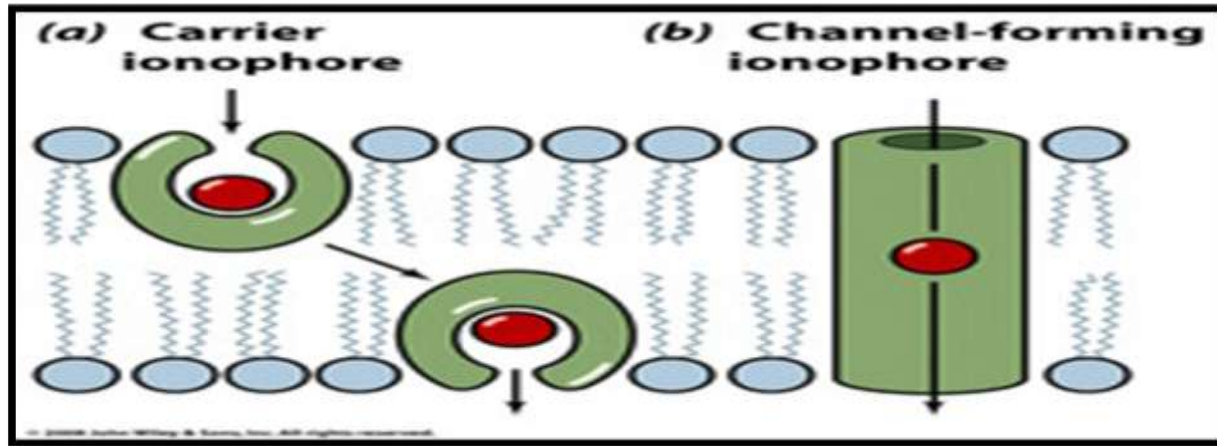
reactions of phosphorylation from those concerned with electron transport and oxidation.



### What is the role of Ionophores?

Ionophores means "ion carrier" as these compounds catalyze ion transport across hydrophobic membranes such as liquid polymeric membranes (carrier-based ion selective electrodes) or lipid bilayers found in the living cells or synthetic vesicles (liposomes). Carrier ionophores bind to a particular ion and change its charge from the surrounding environment. This makes it easier for the ion to pass through the hydrophobic interior of the lipid membrane.

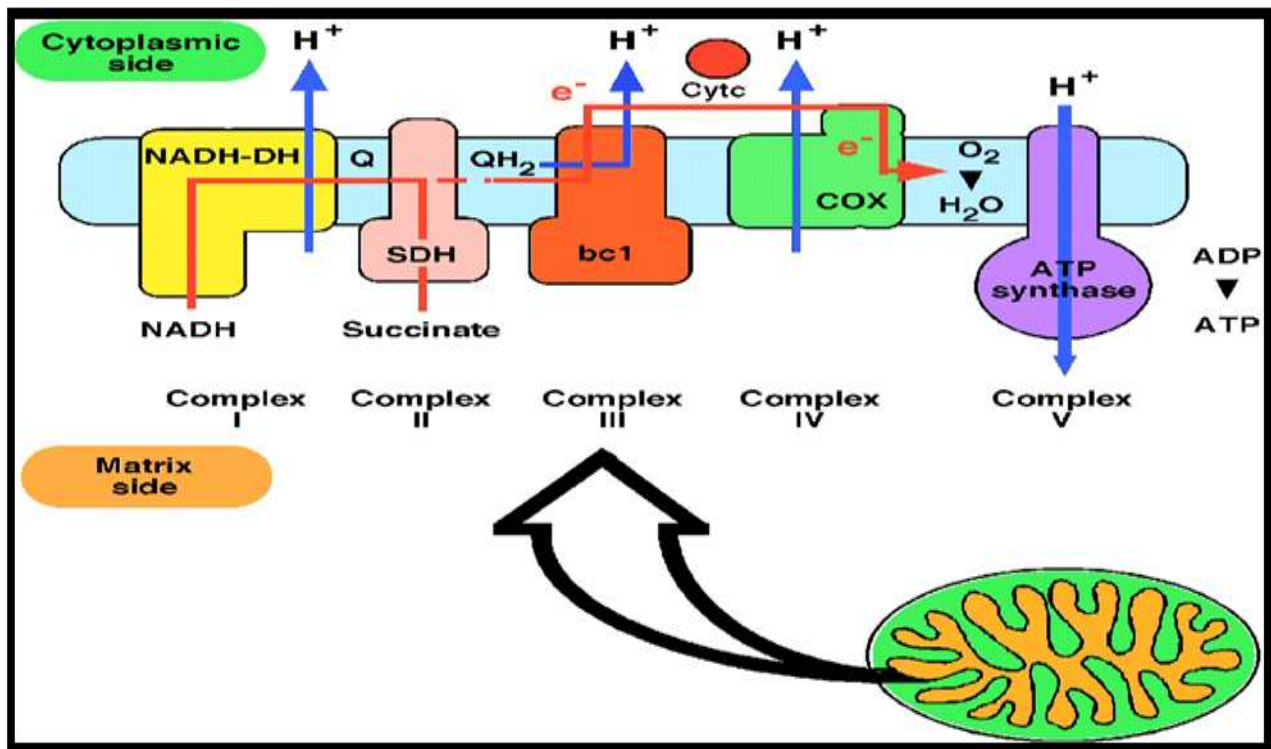
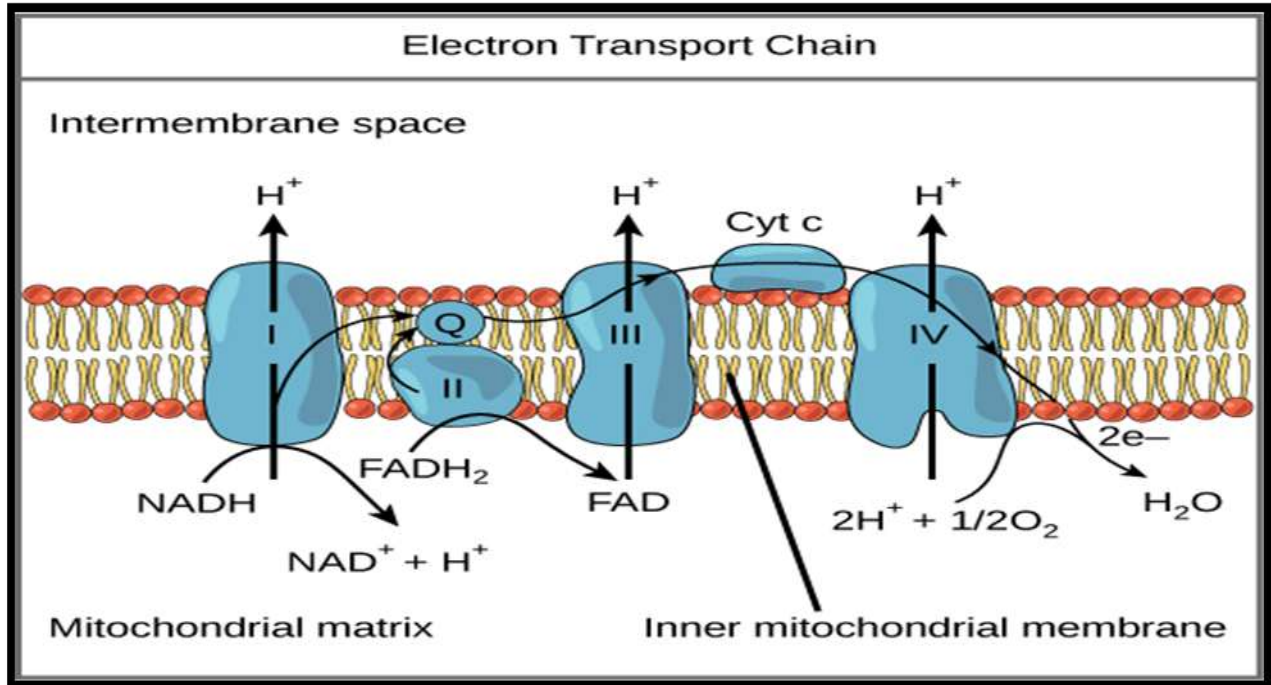
- An example of a carrier ionophore is valinomycin, a molecule that transports a single potassium cation. Carrier ionophores may be proteins or other molecules.
- Channel formers that introduce a hydrophilic pore into the membrane, allowing ions to pass through without coming into contact with the membrane's hydrophobic interior. An example of a channel former is gramicidin A. Channel forming ionophores are usually large proteins.



#### Electron Transport Chain

An electron transport chain (ETC) is a series of complexes that transfer electrons from electron donors to electron acceptors via redox (both reduction and oxidation occurring simultaneously) reactions, and couples this electron transfer with the transfer of protons ( $H^+$  ions) across a membrane.

- This creates an electrochemical proton gradient that drives the synthesis of adenosine triphosphate (ATP), a molecule that stores energy chemically in the form of highly strained bonds.
- The molecules of the chain include peptides, enzymes (which are proteins or protein complexes), and others.
- The final acceptor of electrons in the electron transport chain during aerobic respiration is molecular oxygen although a variety of acceptors other than oxygen such as sulfate exist in anaerobic respiration.



# Metabolism of Carbohydrate

### Define Glycolysis

**Glycolysis** is an oxygen-independent metabolic pathway that converts glucose  $C_6H_{12}O_6$ , into pyruvate,  $CH_3COCOO^- + H^+$ . The free energy released in this process is used to form the high-energy molecules ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide).

The glycolysis pathway can be separated into two phases:

- The Preparatory/Investment Phase – wherein ATP is consumed
- The Pay Off Phase – wherein ATP is produced.

### What is an Amphibolic pathway?

**Amphibolic pathway** is the biochemical pathway that serves both anabolic and catabolic processes. An important example of an amphibolic pathway is the krebcycle which involves both the catabolism of carbohydrates and fatty acids and the synthesis of anabolic precursors for amino-acid synthesis (e.g.  $\alpha$ -ketoglutarate and oxaloacetate).

### Why is TCA cycle Amphibolic in nature?

In aerobic organisms, the citric acid cycle is an Amphibolic pathway. Since the citric acid does both synthesis (anabolic) and breakdown (catabolic) activities; it is called an amphibolic pathway.

### What is the role of the NAD molecule in glycolysis?

NAD (nicotinamide adenine dinucleotide) is a hydrogen acceptor and necessary reductant (to receive hydrogen) in some reactions, in which it is reduced and converted into  $NADH_2$ . During glycolysis, two NAD molecules gain hydrogen ions released after an intermediate reaction, thus forming  $NADH_2$ .



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### Define gluconeogenesis

**Gluconeogenesis** is a metabolic pathway that leads to the synthesis of glucose from pyruvate and other non-carbohydrate precursors, even in non-photosynthetic organisms. It occurs in all microorganisms, fungi, plants and animals, and the reactions are essentially the same, leading to the synthesis of one glucose molecule from two pyruvate molecules. Therefore, it is in essence glycolysis in reverse, which instead goes from glucose to pyruvate, and shares seven enzymes with it.

### What is the purpose of oxidative decarboxylation?

**Pyruvate decarboxylation** or **pyruvate oxidation** is the conversion of pyruvate into acetyl-CoA (activated acetate) by the enzyme pyruvate dehydrogenase. Pyruvate dehydrogenase complex reaction is:  $1 \text{ pyruvate} + 1 \text{ NAD}^+ + \text{CoA} \rightarrow 1 \text{ acetyl-CoA} + \text{NADH} + \text{CO}_2 + \text{H}^+$

**Pyruvate dehydrogenase** is an enzyme complex which contains- TPP (thiamine pyrophosphate), lipoic acid, decarboxylase, transacetylase and  $\text{Mg}^{2+}$  ions.

In the citric acid cycle it is used three times to generate  $\text{CO}_2$  whilst also reducing  $\text{NAD}^+$  to NADH. Firstly, pyruvate is converted by a pyruvate dehydrogenase complex to Acetyl CoA. Secondly, using the enzyme Isocitrate dehydrogenase to  $\alpha$ -Ketoglutarate and finally  $\alpha$ -Ketoglutarate dehydrogenase is used to convert  $\alpha$ -Ketoglutarate to Succinyl CoA

### Significance of Pentose-Phosphate-Pathway:

- (i) It provides alternative route for carbohydrate breakdown.
- (ii) It generates NADPH molecules which are used as reductants in biosynthetic processes under conditions when NADPH molecules are not generated by photosynthesis.
- (iii) It provides Ribose sugars for the synthesis of nucleic acids.



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(iv) It plays important role in fixation of CO<sub>2</sub> in photosynthesis through Ribulose-5-Phosphate. (Ribulose 1, 5-bisphosphate derived from Ribulose-5-Phosphate is the primary acceptor of CO<sub>2</sub> in photosynthesis).

(v) It provides Erythrose-4-phosphate which is required for the synthesis of shikimic acid. The latter is precursor of aromatic ring compounds.

### **Importance of Glyoxylate Cycle:**

(1) During the germination of fatty seeds, the fats which are insoluble are hydrolysed into fatty acids and glycerol. Fatty acids after P-oxidation produce acetyl-CoA units which synthesize sucrose (which is soluble) through glyoxylate cycle. Soluble sucrose is then supplied to different growing regions of the young germinating seedling till it develops its own photosynthetic system.

(2) Those micro-organisms which can grow on ethyl alcohol or acetate as a sole source of energy and carbon make use of this cycle in synthesizing longer carbon chains.

(3) The Glyoxylate cycle is an example of gluconeogenesis

### **Define glucuronic acid cycle**

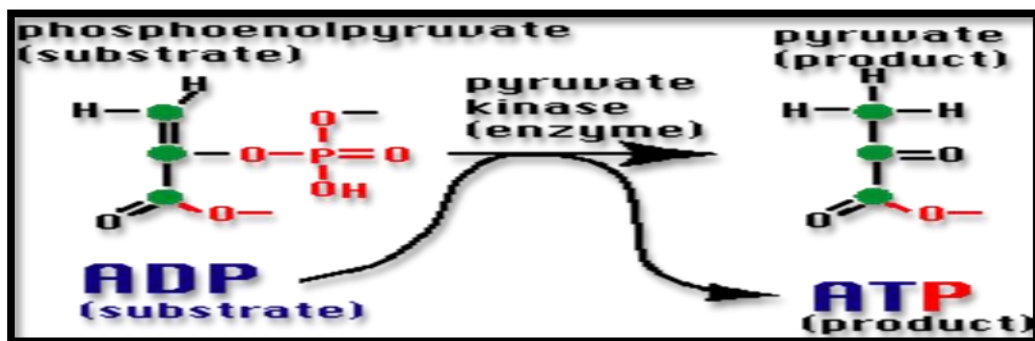
Glucuronic acid is an uronic acid that was first isolated from urine (hence the name). It is found in many gums such as gum arabic (c. 18%) and xanthan, and is important for the metabolism of microorganisms, plants and animals. Glucuronic acid is a sugar acid derived from glucose, with its sixth carbon atom oxidized to a carboxylic acid. In living beings, this primary oxidation occurs with UDP- $\alpha$ -D-glucose (UDPG).

It has 3 Function

- Detoxification of poisonous Substance through Conjugation And subsequent Elimination
- Transport of hormones, other important substances are transported through conjugation and subsequent release at the target location, tissue etc.
- Intermediate in the biosynthesis of ascorbic acid

#### What is an example of substrate level phosphorylation?

The term substrate level phosphorylation refers to the synthesis of ATP by reactions in which ADP is one of several substrates and ATP is one of several products of an enzyme catalyzed reaction. In the example below the substrates are phosphoenolpyruvate and ADP. These two molecules bind to the active site of the enzyme pyruvate kinase. During catalysis, the phosphate group of the phosphoenolpyruvate is transferred to the ADP molecule producing ATP and pyruvate. Thus the products of the reaction are pyruvate and ATP.



#### What's the difference between substrate level phosphorylation and oxidative phosphorylation?

**Substrate-level phosphorylation** is directly phosphorylating ADP with a phosphate and energy provided from a coupled reaction. **Oxidative phosphorylation** is when ATP is generated from the **oxidation** of NADH and FADH<sub>2</sub> and the subsequent transfer of electrons and pumping of protons.

#### What is the metabolic fate of UDP-Galactose?

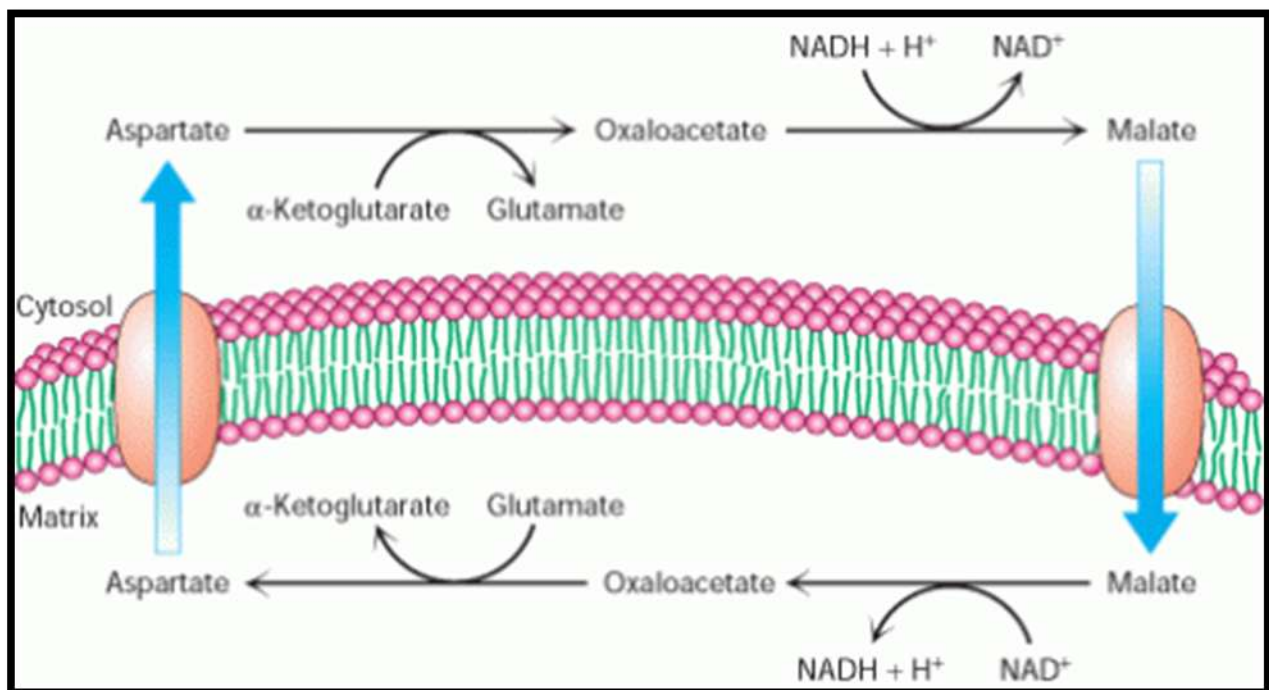
- UDP-Gal is an important precursor in the synthesis of glycolipids, such as gangliosides and galactocerebrosides, sphingolipids, mucopolysaccharides, and membrane glycoproteins.
- In the adult mammary gland, under the influence of prolactin, UDP-Gal can be joined to glucose to give milk sugar.

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#### What is the role of malate aspartate shuttle?

The malate-aspartate shuttle is a biochemical system for translocating electrons produced during glycolysis across the semipermeable inner membrane of the mitochondrion for oxidative phosphorylation in eukaryotes.

These electrons enter the electron transport chain of the mitochondria via reduction equivalents to generate ATP. The shuttle system is required because the mitochondrial inner membrane is impermeable to NADH, the primary reducing equivalent of the electron transport chain. To circumvent this, malate carries the reducing equivalents across the membrane.



#### Define Glycogenesis

Glycogenesis is defined as “Synthesis of Glycogen from Glucose. It takes place in the Cytosol and requires ATP and UTP, besides Glucose.” The goal of glycolysis, glycogenolysis, and the citric acid cycle is to conserve energy as ATP from the catabolism of carbohydrates. If the cells have sufficient supplies of ATP, then these pathways and cycles are inhibited. Under these

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conditions of excess ATP, the liver will attempt to convert a variety of excess molecules into glucose and/or glycogen.

### **What is the end product of glycogenolysis in the liver?**

Glucose-6-phosphate is synthesized directly from glucose or as the end product of gluconeogenesis. Glycogenolysis: In glycogenolysis, glycogen stored in the liver and muscles, is converted first to glucose-1-phosphate and then into glucose-6-phosphate.

### **Why is gluconeogenesis important?**

**Gluconeogenesis** is a metabolic pathway that leads to the synthesis of glucose from pyruvate and other non-carbohydrate precursors, such as lactate, glycerol, and glycogenic amino acids..

It ensures the maintenance of appropriate blood glucose levels when the liver glycogen is almost depleted and no carbohydrates are ingested.

Maintaining blood glucose within the normal range, 3.3 to 5.5 mmol/L (60 and 99 mg/dL), is essential because many cells and tissues depend, largely or entirely, on glucose to meet their ATP demands; examples are red blood cells, neurons, skeletal muscle working under low oxygen conditions, the medulla of the kidney, the testes, the lens and the cornea of the eye, and embryonic tissues.

For example, glucose requirement of the brain is about 120 g/die that are equal to:

- over 50% of the total body stores of the monosaccharide, about 210 g, of which 190 g are stored as muscle and liver glycogen, and 20 g are found in free form in body fluids;
- About 75% of the daily glucose requirement, about 160 g.

During fasting, as in between meals or overnight, the blood glucose levels are maintained within the normal range due to hepatic glycogenolysis, and to the release of fatty acids from adipose tissue and ketone bodies by the liver. Fatty acids and ketone bodies are preferably used by skeletal muscle, thus sparing glucose for cells and tissues that depend on it, primarily red blood cells and neurons.

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#### **Write note on Pyruvate dehydrogenase complex**

Pyruvate dehydrogenase complex (PDC) is a complex of three enzymes that converts pyruvate into acetyl-CoA by a process called pyruvate decarboxylation. Acetyl-CoA may then be used in the citric acid cycle to carry out cellular respiration, and this complex links the glycolysis metabolic pathway to the citric acid cycle.

Pyruvate dehydrogenase is inhibited when one or more of the three following ratios are increased: ATP/ADP, NADH/NAD<sup>+</sup> and acetyl-CoA/CoA.

In eukaryotes PDC is tightly regulated by its own specific pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP), deactivating and activating it respectively.

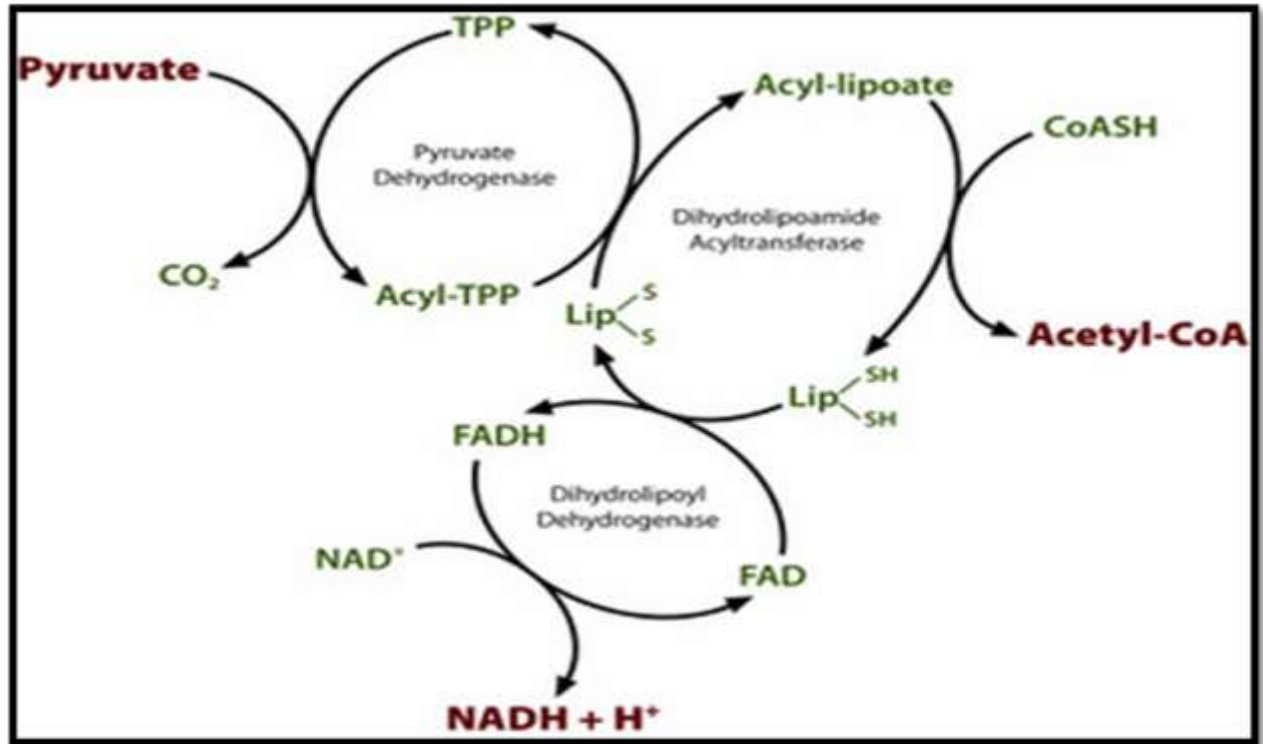
- PDK phosphorylates three specific serine residues on E1 with different affinities. Phosphorylation of any one of them renders E1 inactive. Dephosphorylation of E1 by PDP reinstates complex activity.

- Products of the reaction act as allosteric inhibitors of the PDC, because they activate PDK. Substrates in turn inhibit PDK, and thus, reactivating PDC. During starvation, PDK increases in amount in most tissues, including skeletal muscle, via increased gene transcription.

- Under the same conditions, the amount of PDP decreases. The resulting inhibition of PDC prevents muscle and other tissues from catabolizing glucose and gluconeogenesis precursors.

Metabolism shifts toward fat utilization, while muscle protein breakdown to supply gluconeogenesis precursors is minimized, and available glucose is spared for use by the brain.

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# Metabolism of Protein

### Define -Essential Amino Acid

Amino acids are organic compounds composed of nitrogen, carbon, hydrogen and oxygen, along with a variable side chain group. Your body needs 20 different amino acids to grow and function properly. Though all 20 of these are important for your health, only nine amino acids are classified as essential.

These are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The best sources of essential amino acids are animal proteins like meat, eggs and poultry.

### Why ammonia is toxic to the body?

When ammonia levels in the brain cells are high, lot of ammonia combines with lot of alpha-ketoglutarate to form lot of glutamine in brain cells. This results in lack of alpha-ketoglutarate and accumulation of glutamine in the brain cells.

This leads to two events-

A. TCA- Lack of alpha-ketoglutarate for TCA cycle provides the impairment of energy in brain cells. B. Glutamine has osmotic property. When glutamine increased in the brain cells, excess of water are entered into the cells due to osmosis. This results in swelling of the brain cells. Hence energy deficiency and cell swelling result in impaired functioning of brain cells in ammonia toxicity. This specially affects astrocytes in brain.

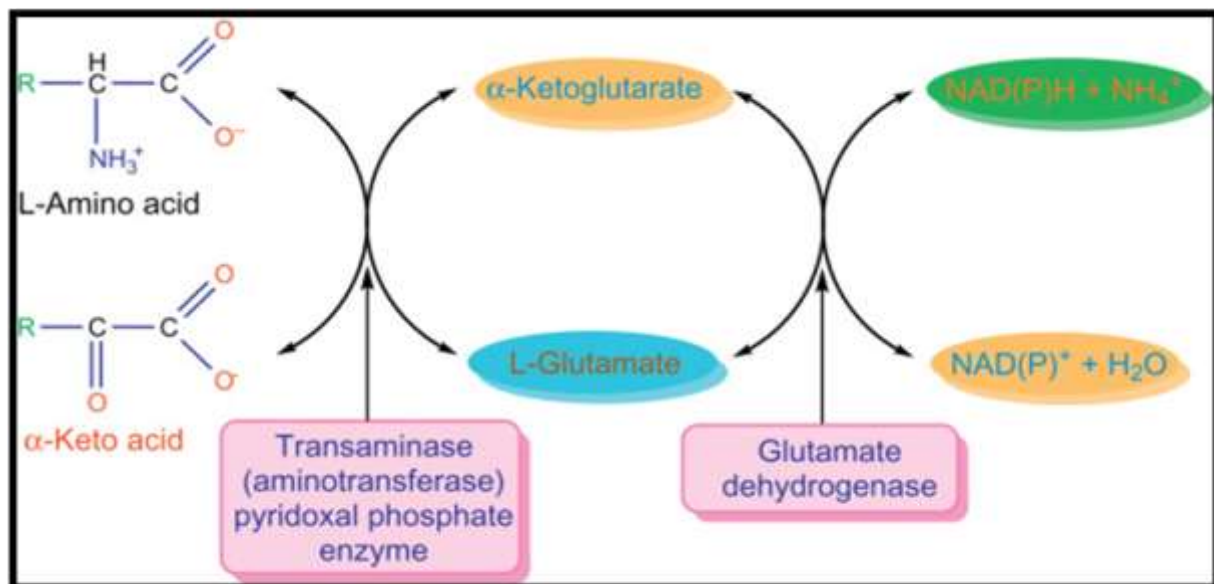
### Explain-Dehydrogenation of L-Glutamate

Glutamate dehydrogenase plays a major role in amino acid metabolism. It is a zinc protein; requires NAD<sup>+</sup> or NADP<sup>+</sup> as a coenzyme; and is present in high concentrations in the mitochondria of liver, heart, muscle, and kidney.



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- It catalyzes the (reversible) oxidative deamination of L-glutamate to  $\alpha$ -ketoglutarate and  $\text{NH}_3$ . The initial step probably involves formation of  $\alpha$ -iminoglutarate by dehydrogenation. This step is followed by hydrolysis of the imino acid to a keto acid and  $\text{NH}_3$ .
- Glutamate dehydrogenase is an allosteric protein modulated positively by ADP, GDP, and some amino acids, and negatively by ATP, GTP, and NADH. Its activity is affected by thyroxine and some steroid hormones in vitro.
- Glutamate dehydrogenase is the only amino acid dehydrogenase present in most cells. It participates with appropriate transaminases (aminotransferases) in the deamination of other amino acids shown here:



#### What is the mechanism of Transamination?

It is a chemical reaction that transfers an amino group to a ketoacid to form new amino acids. This is one of the major degradation pathways which convert essential amino acids to nonessential amino acids (amino acids that can be synthesized de novo by the organism).

Transamination in biochemistry is accomplished by enzymes called transaminases or aminotransferases.  $\alpha$ -ketoglutarate acts as the predominant amino-group acceptor and produces glutamate as the new amino acid.

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Amino acid +  $\alpha$ -ketoglutarate  $\leftrightarrow$   $\alpha$ -keto acid + Glutamate

Glutamate's amino group, in turn, is transferred to oxaloacetate in a second transamination reaction yielding aspartate.

Glutamate + oxaloacetate  $\leftrightarrow$   $\alpha$ -ketoglutarate + aspartate

### **Give a short note on Synthesis of Serine, Glycine, and Cysteine Are Derived from 3-Phosphoglycerate**

In the first step the hydroxyl group of 3-phosphoglycerate is oxidized by NAD<sup>+</sup> to yield 3-phosphohydroxypyruvate. Transamination from glutamate yields 3-phosphoserine, which undergoes hydrolysis by phosphoserine phosphatase to yield free serine.

- The three-carbon amino acid serine is the precursor of the two-carbon glycine through removal of one carbon atom by serine hydroxyl methyl transferase.
- Tetrahydrofolate is the acceptor of the  $\beta$ -carbon atom of serine during its cleavage to yield glycine. This carbon atom forms a methylene bridge between N-5 and N-10 of tetrahydrofolate to yield N5,N10 methylenetetrahydrofolate
- In mammals, cysteine is made from two other amino acids: In the first reaction methionine is converted into S-adenosylmethionine. After the enzymatic transfer of the methyl group to any of a number of different acceptors, S-adenosylhomocysteine, the demethylated product, is hydrolyzed to free homocysteine.
- Homocysteine next reacts with serine in a reaction catalyzed by cystathionine  $\beta$ -synthase to yield cystathionine in the last step cystathionine- $\gamma$ -lyase, a PLP-requiring enzyme, catalyzes the removal of ammonia and cleavage of cystathionine to yield free cysteine.

### **What is the Significance of the Urea Cycle?**

1. Detoxification of NH<sub>3</sub>: Major biological role of this pathway is the detoxification of NH<sub>3</sub>. Toxic ammonia is converted into a nontoxic substance urea and excreted in urine.

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2. Biosynthesis of arginine: The urea cycle also serves for the biosynthesis of arginine from ornithine in liver, kidney and intestinal mucosa. Kidney and intestinal mucosa probably contribute most of the body arginine because they possess all the urea cycle enzymes except arginase. Hence they can form up to arginine and cannot form urea. The arginine is used for protein synthesis.

### **Discuss about Urea Cycle:**

The urea cycle (also known as the ornithine cycle) is a cycle of biochemical reactions that produces urea ((NH<sub>2</sub>)<sub>2</sub>CO) from ammonia (NH<sub>3</sub>). This cycle occurs in ureotelic organisms. The urea cycle converts highly toxic ammonia to urea for excretion. This cycle was the first metabolic cycle to be discovered (Hans Krebs and Kurt Henseleit, 1932), five years before the discovery of the TCA cycle. The urea cycle takes place primarily in the liver and, to a lesser extent, in the kidneys.

A. Steps of Urea Cycle:- 1. Formation of Carbamoyl Phosphate 2.Synthesis of Citrulline 3.Synthesis of Argininosuccinate 4.Cleavage of Argininosuccinate 5. Cleavage of Arginine

### A. Steps of Urea Cycle:

#### 1. Formation of Carbamoyl Phosphate:

Condensation of ammonium ion with bicarbonate ion results in the formation of carbamoyl phosphate by the help of the enzyme carbamoyl phosphate synthase-I present in the liver mitochondria. It requires Mg<sup>2+</sup> and a dicarboxylic acid i.e. N-acetyl glutamate. This step requires 2 ATPs.

#### 2. Synthesis of Citrulline:

Carbamoyl phosphate formed in the first step combines with ornithine resulting in the synthesis of citrulline aided by the enzyme citrulline synthase or ornithine transcarbamoylase. Citrulline is easily permeable to the mitochondrial membrane and hence it diffuses into the cytosol.

#### 3. Synthesis of Argininosuccinate:

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In the cytosol, citrulline combines with the amino acid aspartate forming argininosuccinate catalysed by the enzyme argininosuccinate synthase. It requires ATP which is hydrolysed to AMP resulting in utilization of two high energy bonds.  $Mg^{2+}$  acts as cofactor.

#### 4. Cleavage of Argininosuccinate:

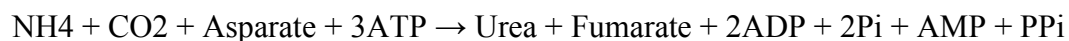
The enzyme argininosuccinase acts reversibly to cleave arginino-succinate into Arginine and fumarate. Fumarate enters the TCA cycle (the linkage between TCA and urea cycle is known as Krebs bi-cycle).

#### 5. Cleavage of Arginine:

Arginine is lysed into ornithine and urea under the influence of the enzyme arginase. Hence arginine is known as semi-essential amino acid i.e. though it is synthesized in the body it is not available for protein synthesis. Ornithine is regenerated in this step and the urea cycle completes by the formation of urea. Ornithine and lysine are potent inhibitors of the enzyme arginase.

Arginase is also present in testis, renal tubules, mammary gland and skin in minute quantities. The intermediate amino acids formed in the urea cycle i.e. ornithine, citrulline and argininosuccinate are known as non-protein amino acids.

The overall equation of urea formation is:



The urea cycle brings two amino groups and  $HCO_3^-$  together to form urea. Thus toxic, insoluble ammonia is converted into non-toxic, water soluble, excretable urea. Hence, urea cycle disposes two waste products i.e.  $NH_4$  and  $HCO_3^-$ . This fact suggests that urea cycle participates in the regulation of blood pH, which depends on the  $HCO_3^-/H_2CO_3$ . Though 3 ATPs are utilized, the ultimate cost of making a molecule of urea is 4 ATPs (one ATP is converted into AMP).

#### **Write note on Deamination**

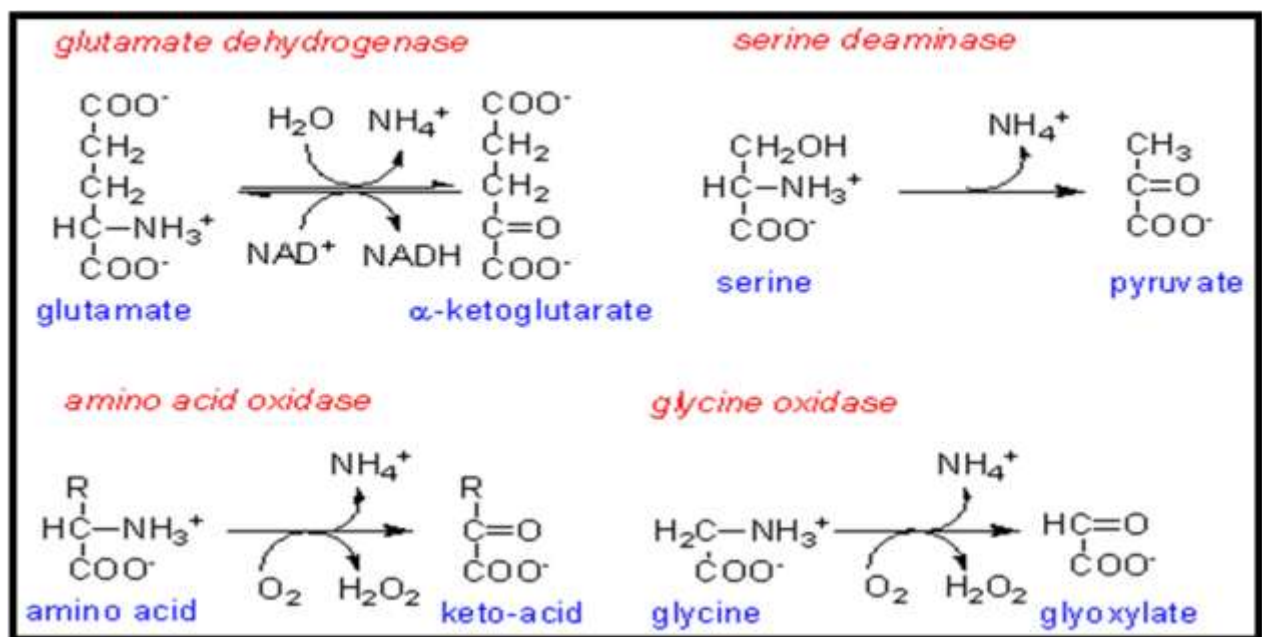
Deamination is the removal of an amino group from a molecule. Enzymes that catalyze this reaction are called deaminases. In the human body, deamination takes place primarily in the

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liver, and however glutamate is also deaminated in the kidneys. In situations of excess protein intake, deamination is used to break down amino acids for energy. The amino group is removed from the amino acid and converted to ammonia.

The amine group is first converted into ammonia (which is toxic) and then converted into urea. Urea is non-toxic and excreted from the body by the kidneys (it is a component of urine)

The amine group can also be transferred via transamination to make new amino acids. These amino acids are non-essential – as they can be synthesized by the body. The remaining carbon skeleton is recycled to produce compounds that can be oxidized for energy. This includes the formation of glucose, ketone bodies and acetyl CoA



# Metabolism of Lipid

### What is the role of Carnitine Transporter?

The carnitine shuttle consists of three enzymes (carnitine palmitoyltransferase 1 (CPT1A and CPT1B), carnitine acylcarnitine translocase (SLC25A20), carnitine palmitoyl-transferase 2 (CPT2)) and a small, soluble molecule, carnitine, to transport fatty acids as their long-chain fatty acylcarnitine esters. The carnitine shuttle is responsible for transferring long-chain fatty acids across the barrier of the inner mitochondrial membrane to gain access to the enzymes of beta-oxidation.

### Define Lipogenesis

Lipogenesis is defined as the synthesis of fatty acids from non-lipid precursors. It is a pathway for metabolism of excess carbohydrate and is activated by high carbohydrate availability. In energy sufficient states, such as in the postprandial state, glucose is converted to pyruvate through glycolysis and pyruvate is imported into the mitochondria to join TCA cycle.

Citrate formed in the TCA cycle is transported into the cytosol where it is converted to acetyl-CoA by ATP citrate lyase. De novo synthesis of fatty acids in liver begins with ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase 1 (ACC1).

Malonyl-CoA which serves as a two-carbon donor is added to the acetyl-CoA primer by a multifunctional enzyme complex, the fatty acid synthase (FAS). Thus, the fatty chain grows by the attachment of acyl residue with elongation by two carbon subunits each cycle. Palmitic acid (16:0) is the predominant fatty acid generated through de novo lipogenesis and the reactions can be expressed as:

Acetyl-



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### What is the Importance of lipogenesis?

The significance of lipogenesis in brown adipose tissue has been discussed. The capacity for lipogenesis in brown adipose tissue is high.

- Rather, it could be considered to represent a “refilling” reaction that occurs in the tissue after cessation of the thermogenic stimulus. Thus, during the perinatal period, the activity of lipogenesis is low when thermogenesis is high.
- This reverse correlation is probably due to the effect of suckling (i.e., a change from a carbohydrate “diet” in the fetal state to a high-lipid diet).
- Several amino acids have propionyl-CoA as the end product of oxidation. This three-carbon fatty acid cannot be degraded through the normal  $\beta$ -oxidation pathway for fatty acid derivatives, so its accumulation could result in sequestration of all mitochondrial CoA, with consequent inhibition of thermogenesis.
- This effect may explain why the mitochondria have a high activity level of a propionyl-CoA hydrolase with unusual regulatory properties. This hydrolase could function to recover the CoA otherwise bound up in propionyl-CoA.

### Define Lipolysis

Lipolysis is the catabolic process leading to the breakdown of triacylglycerol (TAGs) into FFAs and glycerol. After release into the blood, FFAs are transported and taken up by other tissues to be utilized for  $\beta$ -oxidation and subsequent ATP generation. Some FFAs do not leave the fat cell and are reesterified into intracellular TAG.

During lipolysis, intracellular TAG undergoes hydrolysis through the action of three major lipases: adipose triglyceride lipase, HSL, and monoacylglycerol (MGL) lipase. ATGL hydrolyses TAGs into diacylglycerol (DAG) and one FA, followed by HSL converting DAG into monoacylglycerol (MAG) plus one FA, MGL then hydrolyses MAG to produce glycerol. Lipolysis is regulated by the ANS and by several humoral factors, such as catecholamines (phosphorylation of HSL), glucocorticoids (upregulation of ATGL), natriuretic peptides, and growth hormone.



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### **What is a role of Acetyl Coenzyme A Carboxylase in Controlling Fatty Acid Metabolism?**

Fatty acid metabolism is stringently controlled so that synthesis and degradation are highly responsive to physiological needs. Fatty acid synthesis is maximal when carbohydrate and energy are plentiful and when fatty acids are scarce.

Acetyl CoA carboxylase plays an essential role in regulating fatty acid synthesis and degradation. Recall that this enzyme catalyzes the committed step in fatty acid synthesis: the production of malonyl CoA (the activated two-carbon donor). The carboxylase is controlled by three global signals—glucagon, epinephrine, and insulin—that correspond to the overall energy status of the organism.

Insulin stimulates fatty acid synthesis by activating the carboxylase, whereas glucagon and epinephrine have the reverse effect. The levels of citrate, palmitoyl CoA, and AMP also exert control. Citrate, a signal that building blocks and energy are abundant, activates the carboxylase. Palmitoyl CoA and AMP, in contrast, lead to the inhibition of the carboxylase. Thus, this important enzyme is subject to both global and local regulation in a cell.

### **Define Ketogenesis**

Ketogenesis is the biochemical process by which organisms produce a group of substances collectively known as ketone bodies by the breakdown of fatty acids and ketogenic amino acids.

The three ketone bodies, each synthesized from acetyl-CoA molecules, are:

- Acetoacetate, which can be converted by the liver into  $\beta$ -hydroxybutyrate, or spontaneously turn into acetone
- Acetone, is generated through the decarboxylation of acetoacetate, either spontaneously or through the enzyme acetoacetate decarboxylase..
- $\beta$ -hydroxy butyrate is generated through the action of the enzyme D- $\beta$ -hydroxy butyrate dehydrogenase on acetoacetate.

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### **Explain-Triacylglycerol**

A triglyceride (TG) is an ester derived from glycerol and three fatty acids (from tri- and glyceride). Triglycerides are the main constituents of body fat in humans and other animals, as well as vegetable fat.

There are many different types of triglyceride, with the main division between saturated and unsaturated types. Saturated fats are "saturated" with hydrogen — all available places where hydrogen atoms could be bonded to carbon atoms are occupied. Unsaturated fats have double bonds between some of the carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. These have a lower melting point and are more likely to be liquid at room temperature.

### **What is meant by Polyunsaturated Fatty Acids?**

Polyunsaturated fatty acids (PUFAs) are fatty acids that contain more than one double bond in their backbone. PUFA are also known as essential fatty acids, because these are not formed in the body and are obtained through dietary means. PUFAs have two subdivisions:

- Omega-3(*n*-3) fatty acids:  $\alpha$ -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Fatty fishes and fish oils are the sources of EPA and DHA. Principal sources of ALA are mainly flaxseed, soybeans, canola, and some nuts.
- Omega-6(*n*-6) fatty acids: linoleic acid (LA),  $\gamma$ -linolenic acid (GLA), and arachidonic acid (ARA). LA occurs mainly in vegetable oils of corn, safflower, soybean, and sunflower. ARA is found in animal products such as meat, poultry, and eggs.
- PUFAs are essential for healthy status, being essential components of cell membranes influencing fluidity, permeability, and microdomain composition. Moreover, they are involved in several membrane receptors signaling, lipid metabolism, and regulation of gene expression.

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### **What are the major functions of cholesterol?**

Cholesterol can both be produced by the body itself and obtained from food sources. Cholesterol plays an important part in the human body. Its functions are as follows:

**Hormone production.** Cholesterol plays a part in producing hormones such as estrogen, testosterone, progesterone, aldosterone and cortisone.

**Vitamin D production.** Vitamin D is produced when the sun's ultraviolet rays reach the human skin surface.

**Bile production.** Cholesterol produces bile acids which aid in digestion and vitamin absorption.

**Cell membrane support.** Cholesterol plays a very important part in both the creation and maintenance of human cell membrane.

### **What are the symptoms of bad cholesterol?**

High cholesterol can cause atherosclerosis, a dangerous accumulation of cholesterol and other deposits on the walls of your arteries. These deposits (plaques) can reduce blood flow through your arteries, which can cause complications, such as:

**Chest pain.** If the arteries that supply your heart with blood (coronary arteries) are affected, you may have chest pain (angina) and other symptoms of coronary artery disease.

**Heart attack.** If plaques tear or rupture, a blood clot may form at the plaque-rupture site — blocking the flow of blood or breaking free and plugging an artery downstream. If blood flow to part of your heart stops, you'll have a heart attack.

**Stroke.** Similar to a heart attack, if blood flow to part of your brain is blocked by a blood clot, a stroke occurs.

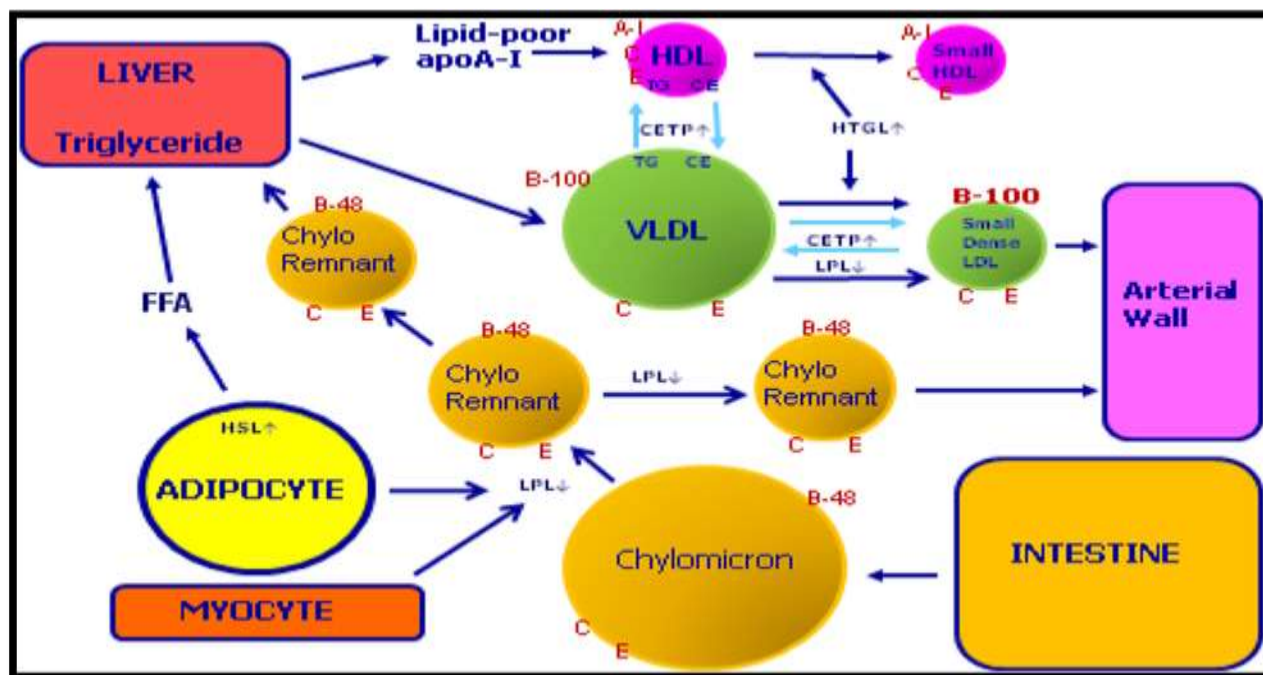
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#### Define Lipoprotein

Lipoproteins are basically a core full of fat and cholesterol, along with a lipid membrane that contains proteins called apolipoproteins. There are many types of lipoproteins, but the two most important ones are called LDL (Low Density Lipoprotein) and HDL (High Density Lipoprotein).

A lipoprotein is a biochemical assembly whose primary purpose is to transport hydrophobic lipid (a.k.a. fat) molecules in water, as in blood or extracellular fluid. They have a single-layer phospholipid and cholesterol outer shell, with the hydrophilic portions oriented outward toward the surrounding water and lipophilic portions of each molecule oriented inwards toward the lipids molecules within the particles.

Apolipoproteins are embedded in the membrane, both stabilising the complex and giving it functional identity determining its fate. Thus the complex serves to emulsify the fats. Many enzymes, transporters, structural proteins, antigens, adhesions, and toxins are lipoproteins. Examples include the plasma lipoprotein particles classified as HDL, LDL, IDL, VLDL and ULDL (a.k.a. chylomicrons) lipoproteins, according to density / size (an inverse relationship), compared with the surrounding plasma water.



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### **What is the function of the bile?**

Bile or gall is a dark green to yellowish brown fluid, produced by the liver, that aids the digestion of lipids in the small intestine. In humans, bile is produced continuously by the liver (liver bile), and stored and concentrated in the gallbladder. Bile contains bile acids, which are critical for digestion and absorption of fats and fat-soluble vitamins in the small intestine. Many waste products, including bilirubin, are eliminated from the body by secretion into bile and elimination in feces.

### **What are the function of bile salts?**

Bile salts are composed of the salts of four different kinds of free bile acids (cholic, deoxycholic, chenodeoxycholic, and lithocholic acids); each of these acids may in turn combine with glycine or taurine to form more complex acids and salts.

Bile salts help with the digestion of fats in our bodies. They also help us to absorb fat-soluble vitamins like A, D, E, and K. In addition to bile salts, bile contains cholesterol, water, bile acids, the pigment bilirubin and eliminate waste products

### **What are the role of phospholipids?**

Phospholipids are a class of lipids that are a major component of all cell membranes. They can form lipid bilayers because of their amphiphilic characteristic. The structure of the phospholipid molecule generally consists of two hydrophobic fatty acid "tails" and a hydrophilic "head" consisting of a phosphate group. The two components are joined together by a glycerol molecule. The phosphate groups can be modified with simple organic molecules such as **choline, ethanolamine or serine.**

Phospholipids play multiple roles in cells in forming the permeability barrier of the cell membrane and intracellular organelles, in providing the supporting matrix and surface for many catalytic processes, in actively participating in signal transduction in response to both external and internal stimuli, and in providing precursors for signaling processes and macro molecular synthesis

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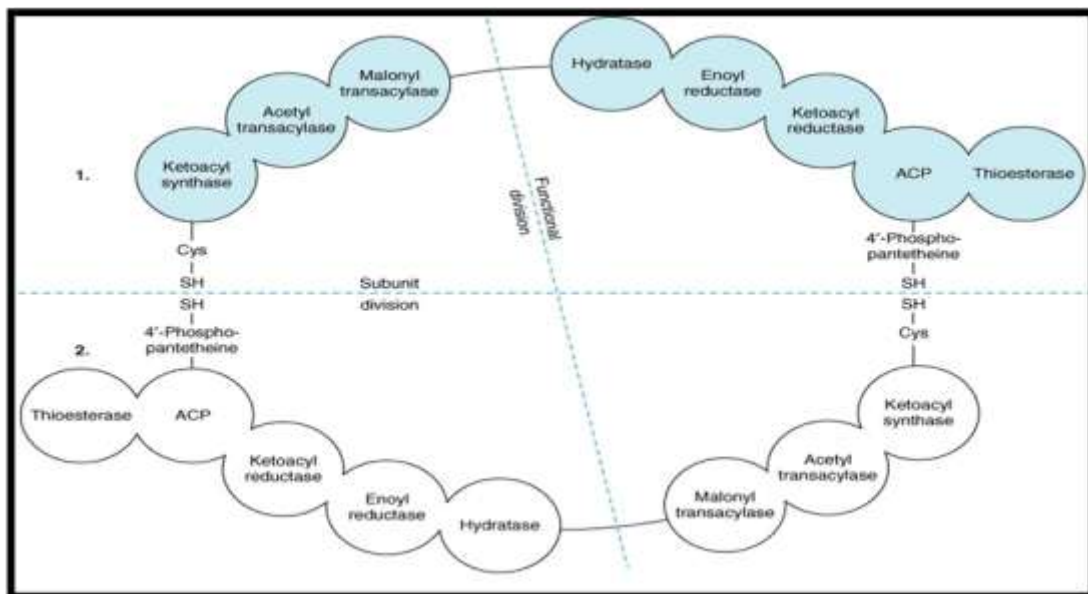
#### Explain-Fatty Acid Synthesis

Fatty acid synthesis is the creation of fatty acids from acetyl-CoA and NADPH through the action of enzymes called fatty acid synthases. This process takes place in the cytoplasm of the cell. Most of the acetyl-CoA which is converted into fatty acids is derived from carbohydrates via the glycolytic pathway.

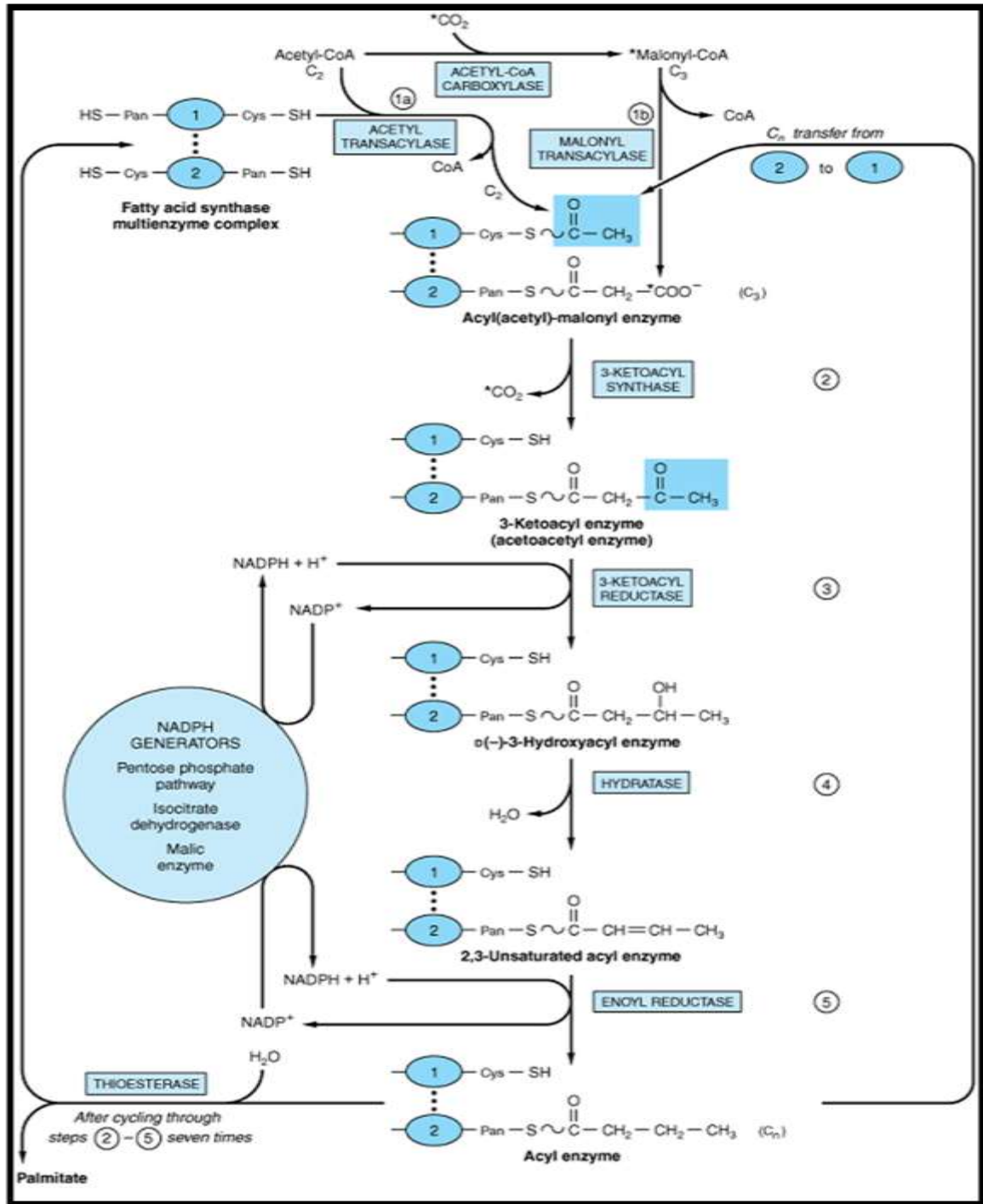
In fatty acid synthesis, acetyl-coA is the direct precursor only of the methyl end of the growing fatty acid chain. All the other carbons come from the acetyl group of acetyl-CoA but only after it is modified to provide the actual substrate for fatty acid synthase, malonyl-CoA.

Malonyl-CoA contains a 3-carbon dicarboxylic acid, malonate, bound to Coenzyme A. Malonate is formed from acetyl-CoA by the addition of CO<sub>2</sub> using the biotin cofactor of the enzyme acetyl-CoA carboxylase.

Formation of malonyl-CoA is the commitment step for fatty acid synthesis, because malonyl-CoA has no metabolic role other than serving as a precursor to fatty acids.. FAS is a large multienzyme complex. In mammals, FAS contains two subunits, each containing multiple enzyme activities.



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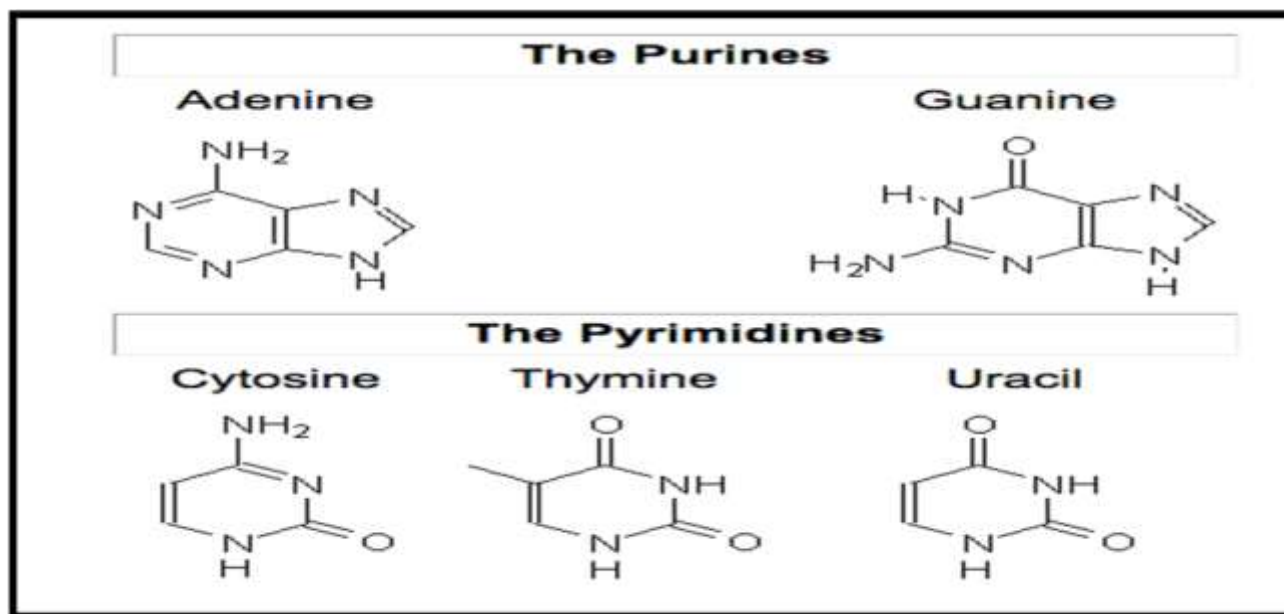


#### Metabolism of Purine & Pyrimidine Nucleotide

##### Draw the structure of nitrogenous base

Purines and Pyrimidines are nitrogenous bases that make up the two different kinds of nucleotide bases in DNA and RNA. The two-carbon nitrogen ring bases (adenine and guanine) are purines, while the one-carbon nitrogen ring bases (thymine and cytosine) are pyrimidines.

They include the nucleobases adenine (2) and guanine (3). In DNA, these bases form hydrogen bonds with their complementary pyrimidines, thymine and cytosine, respectively. This is called complementary base pairing. In RNA, the complement of adenine is uracil instead of thymine.



##### Explain-Salvage Pathways Mechanism of Nucleotides

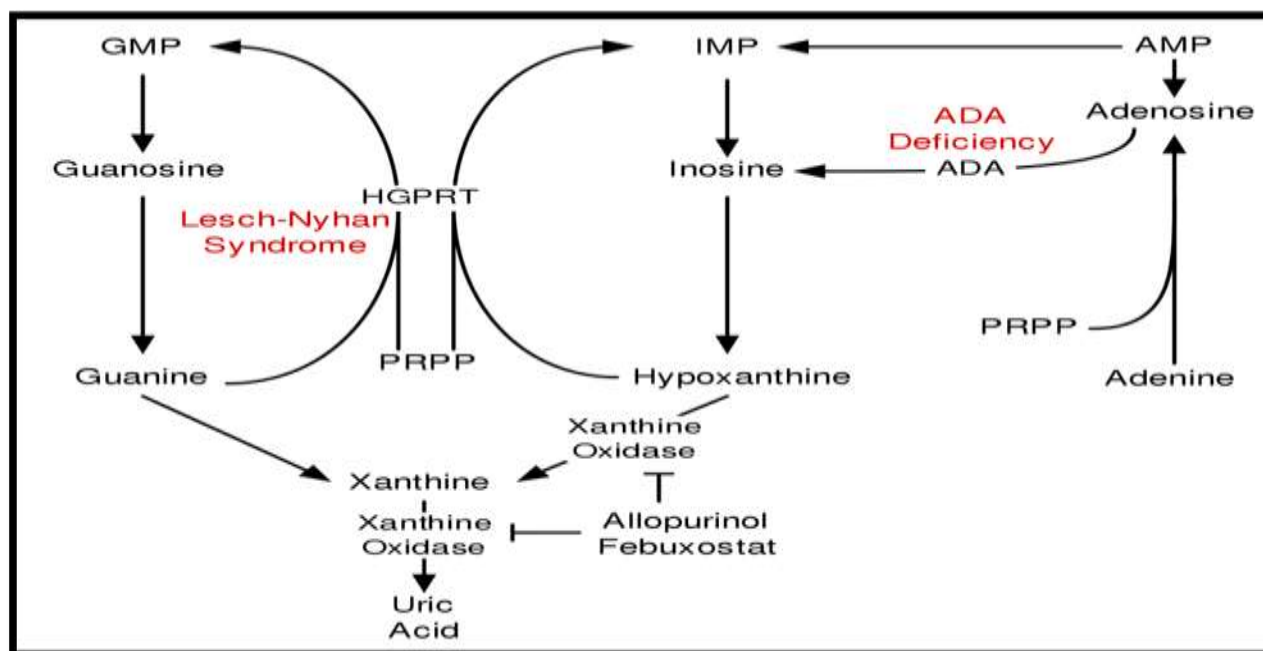
The nucleotide and nucleosides of a cell are continually in flux. For example, DNA and RNA chains are being synthesized in the cell. Even though the overall DNA content of a cell is constant, small stretches are continually being repaired.

Free purines and pyrimidines are converted back into nucleoside triphosphate monomers to be reincorporated into DNA. A common step in this pathway is the reaction of free bases with

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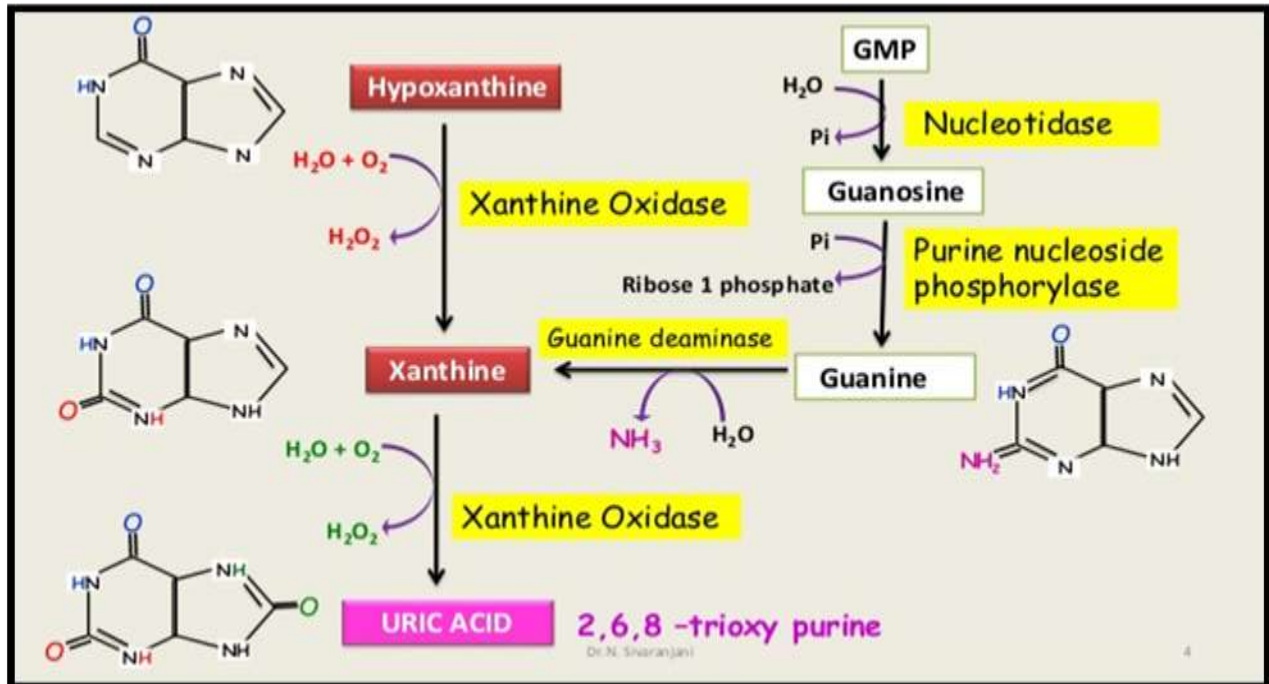
phosphoribosyl pyrophosphate (PRPP) to yield nucleotides. PRPP is a general activator of nitrogen ring compounds.

For example, PRPP is added to anthranilate during the biosynthesis of tryptophan in bacteria. PRPP is made by the activation of ribose-5-phosphate. Ribose-5-phosphate can be made through the pentose phosphate pathway. Apparently, two enzymes exist in all systems—one for purines and one for pyrimidines. The synthesis of the glycosidic bond uses the 1'-pyrophosphate of PRPP as an energy source, and either enzyme transfers the free base to the 1' position of the ribose, making a nucleotide.



#### Discuss-Metabolism of Purine Nucleotide

The end product of purine catabolism is uric acid in humans. Nitrogen excreted as uric acid is very little in humans, as humans are ureotelic (nitrogen is excreted as urea). In birds, amphibians and reptiles are uricotelic – they excrete uric acid as major end product of purine and amino acid catabolism. Lower primates and some mammals have the enzyme uricase which converts uric acid to allantoin. Normal serum Uric acid concentration: • 3 - 7 mg /dl in males, 2 - 5 mg/dl in females



#### What is the Mechanism of allopurinol?

The drug allopurinol, which is an inhibitor of xanthine oxidase, effectively treats gout. Allopurinol is structurally similar to hypoxanthine, except that the 5-membered ring has the positions of the carbon and nitrogen reversed.

Xanthine oxidase is able to bind allopurinol and catalyze one oxidation, converting it to a compound that is similar to xanthine. However, after that conversion, the enzyme is trapped in an inactive oxidation state and can't carry out its normal function of forming uric acid. Additionally, allopurinol inhibits the de novo (new, from other compounds; not recycled) synthesis of purines, further decreasing the amount of uric acid formed in the blood.

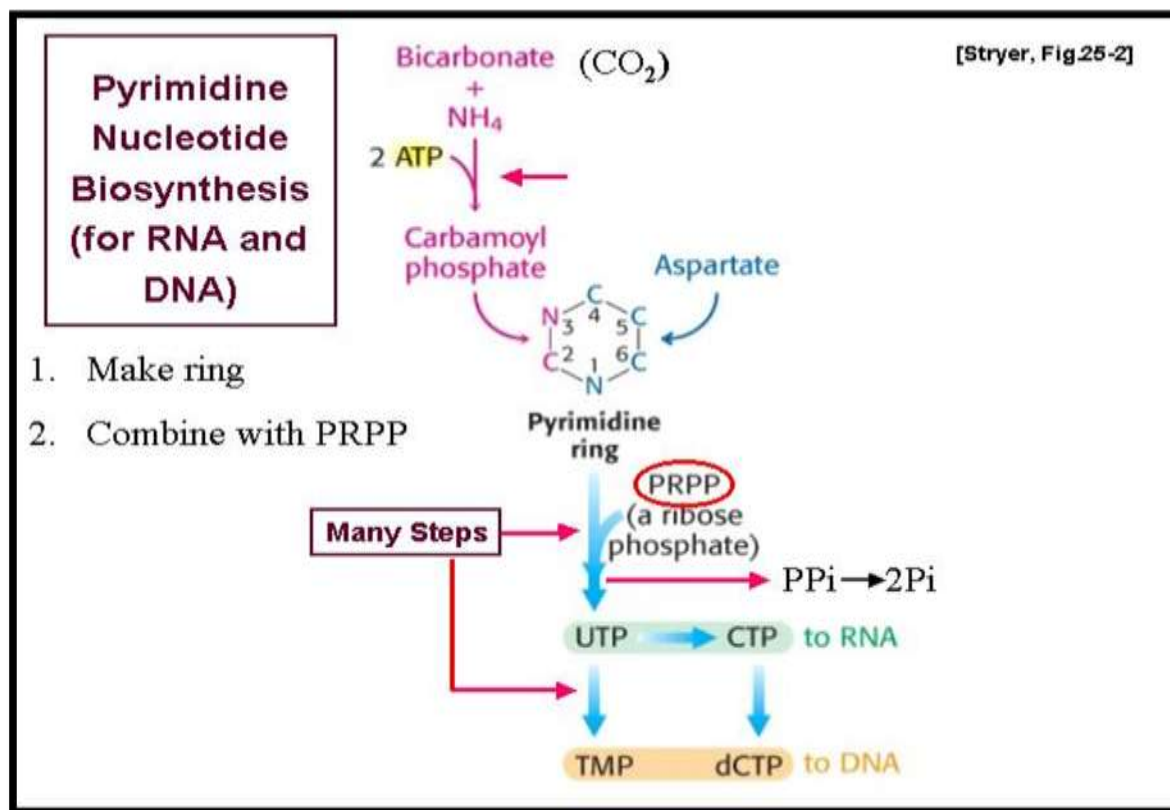
#### Biosynthesis of Pyrimidine Nucleotides:

Pyrimidine nucleotide biosynthesis takes place in a different manner from that of purine nucleotides. The six membered pyrimidine ring is made first and then attached to ribose phosphate. The synthesis begins with carbon dioxide and ammonia combining to form carbamoyl phosphate catalysed by the cytosolic enzyme carbamoyl phosphate synthetase-II.

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Carbamoyl phosphate combines with aspartate to form carbamoyl aspartate aided by the enzyme aspartate transcarbamoylase. Dihydroorotate is formed from carbamoyl aspartate by removal of water and closure of the ring under the influence of the enzyme dihydroorotase.

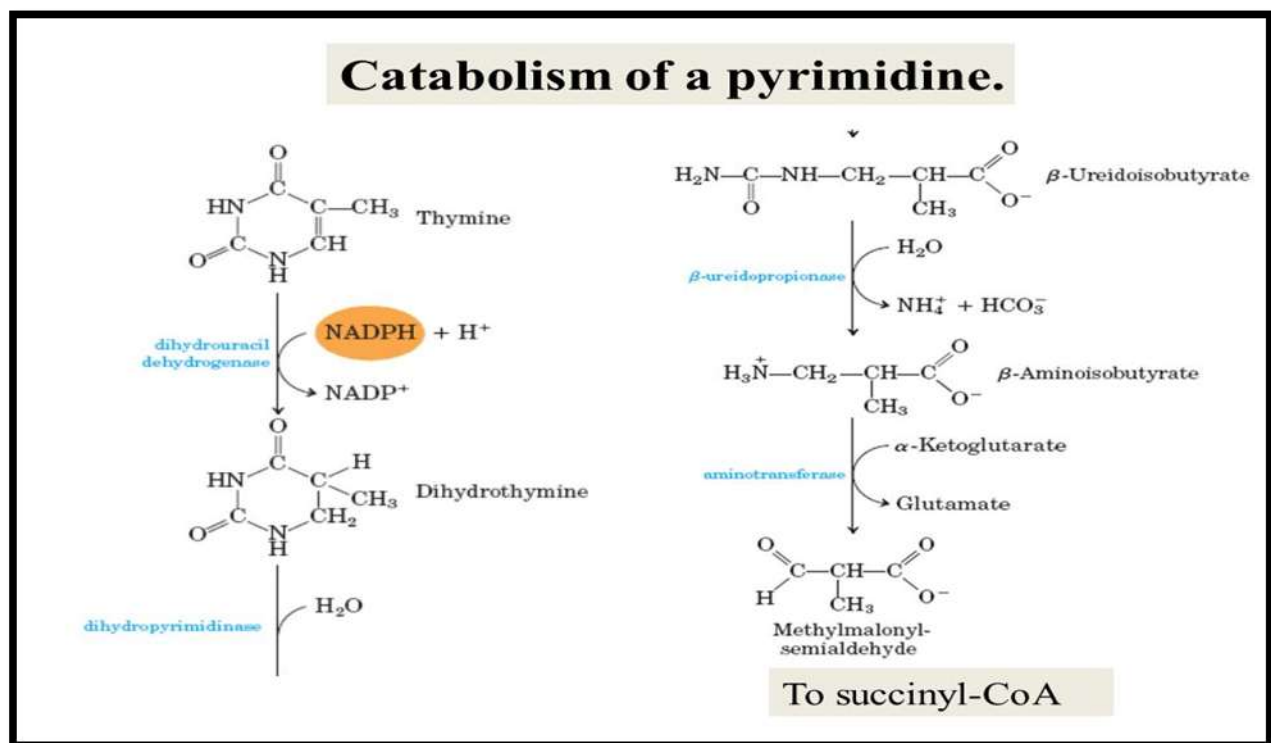
Dihydroorotate is oxidized to orotic acid by dehydrogenase which uses  $\text{NAD}^+$  as the electron acceptor. Orotic acid is attached to ribose to yield orotidylic acid. Orotidylate is then decarboxylated to form uridylylate. Uridylylate is then converted to all the other pyrimidine nucleotides viz., CMP, UMP & TMP.



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#### Give a note on Catabolism of Pyrimidine:

- i. Liver is the main site for the catabolism of pyrimidines.
- ii. CO<sub>2</sub> is released from the pyrimidine nucleus representing a major pathway for the catabolism of uracil, cytosine, and thymine.
- iii. The major end products of cytosine, uracil and thymine are β-alanine and β-amino-isobutyric acid, respectively.
- iv. Thymine is the precursor of β-aminoisobutyric acid in humans and in animals. β-aminoisobutyric acid is excreted more in leukemia. This is due to increased destruction of cells and their DNA.
- v. The β-aminoisobutyric acid is converted into methylmalonic semi-aldehyde and then to propionate which turns to succinate.



### Disorder of Pyrimidine Nucleotides

The pyrimidines (like purines) can also serve as precursors in the salvage pathway to be converted to the respective nucleotides.

This reaction is catalysed by pyrimidine phosphoribosyltransferase which utilizes PRPP as the source of ribose 5-phosphate.

Orotic aciduria: This is a rare metabolic disorder and is characterized by the excretion of orotic acid in urine, severe anemia & retarded growth.

Enzyme deficiency: Orotate phosphoribosyl transferase & OMP decarboxylase of pyrimidine synthesis.

### **Disorders of pyrimidine metabolism**

- ⊙ **Orotic aciduria:**
- ⊙ **This is a rare metabolic disorder.**
- ⊙ **Characterized by the excretion of orotic acid in urine, severe anemia & retarded growth.**
- ⊙ **Enzyme deficiency: Orotate phosphoribosyl transferase & OMP decarboxylase of pyrimidine synthesis**