

Prof . Dr . Samera Alkatib

Introduction in Metabolism

- More than 99% of human body is composed of 6 elements : oxygen , carbon , hydrogen , nitrogen , calcium , and phosphorus .
- Human body contained about 60% H_2O , 15% proteins , 15% lipids , 2% carbohydrates , and 8% minerals .
- Energy is necessary for living systems , it is provided as a chemical compound called adenosine triphosphate (ATP) .
- Living cells used ATP molecules to perform the biochemical and physiological activities such as :

Muscle contraction , nerve excitation , active transport , synthesis of biomolecules .

Note:

The origin of the energy is **photosynthesis** , a process that used by the plants to convert the light energy into chemical energy , which is stored as organic compounds such as glucose, lipids, and proteins

Human used these 3 types of molecules to yield the ATP with the help of enzymes : carbohydrates , lipids , and proteins .

Carbohydrates are broken into glucose , lipids into fatty acids , proteins into amino acids.

- By a series of oxidation-reduction reactions , **mitochondria** hydrolyze these products into several compounds as well as reducing equivalents (NADH and FADH_2) . These reducing equivalents are pass through the **electron transport chain** (**respiratory chain**) and oxidized with consumption O_2 , leading to the synthesis of ATP molecules . This process called **oxidative phosphorylation** . So, Mitochondria is the main site for ATP synthesis .

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- This may explain why during starvation for several days or weeks may end with death or may result in certain metabolic disorders , this can be due to depletion of energy inside the body .
- On other hand , intake energy more than body required , will result in obesity .
- In normal conditions , there is a fine control between the two metabolic processes , energy liberation and energy requirement .

Stages Of Foodstuffs Oxidation

- *First Stage* : The major components of our foods are carbohydrates , lipids , and proteins . These macromolecules are first digested to micromolecules in the gastrointestinal tract (GIT) by digestive enzymes . i.e. carbohydrates to glucose , lipids to fatty acids , proteins to amino acids . This process called digestion or **primary metabolism** .

Second stage

- The products of digestion are absorbed to the tissues , and finally broken down to CO_2 and H_2O in the mitochondria . In this process NADH and FADH_2 are generated . This is name **secondary metabolism** .

Third Stage :

- Finally , these reducing equivalents pass to respiratory chain where energy is released as ATP . This is the **tertiary metabolism** .

Metabolism

The all chemical reactions occur inside the body which include :

- **Catabolism** : Break down of macromolecules into small molecules with liberation energy .
- **Anabolism** : Synthesis of large molecules from small molecules with require energy .

Therefore , the reactions in general are either or :

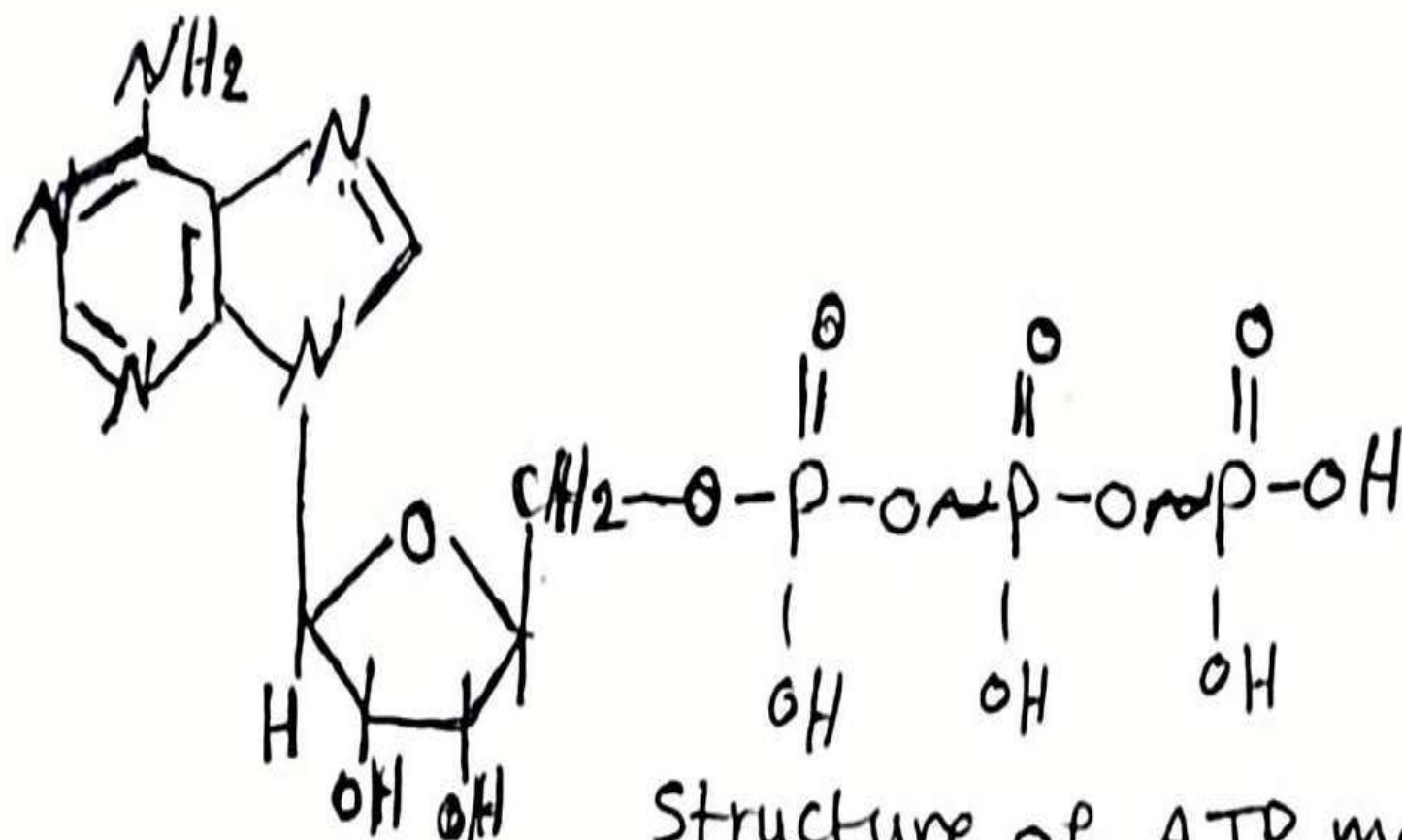
- **Exergonic** , that liberated the energy .
- **Endergonic** , that required energy .
- **Isothermic** , that neither give nor require energy .

Adenosine Triphosphate (ATP)

The energy obtained by the exergonic reactions (catabolism of foodstuffs) are converted in form of chemical energy, called ATP.

Energy in living systems is used as ATP and stored as ATP, so ATP act as the principle **energy currency** inside the cell.

- **The importance of ATP is due to :**
- Stable in aqueous solutions i.e. it cannot be easily hydrolyzed.
- It can be diffuse to any site of the cell that need energy.
- Contain 2 high – energy phosphate bonds, which can easily hydrolyzed and liberate the energy.

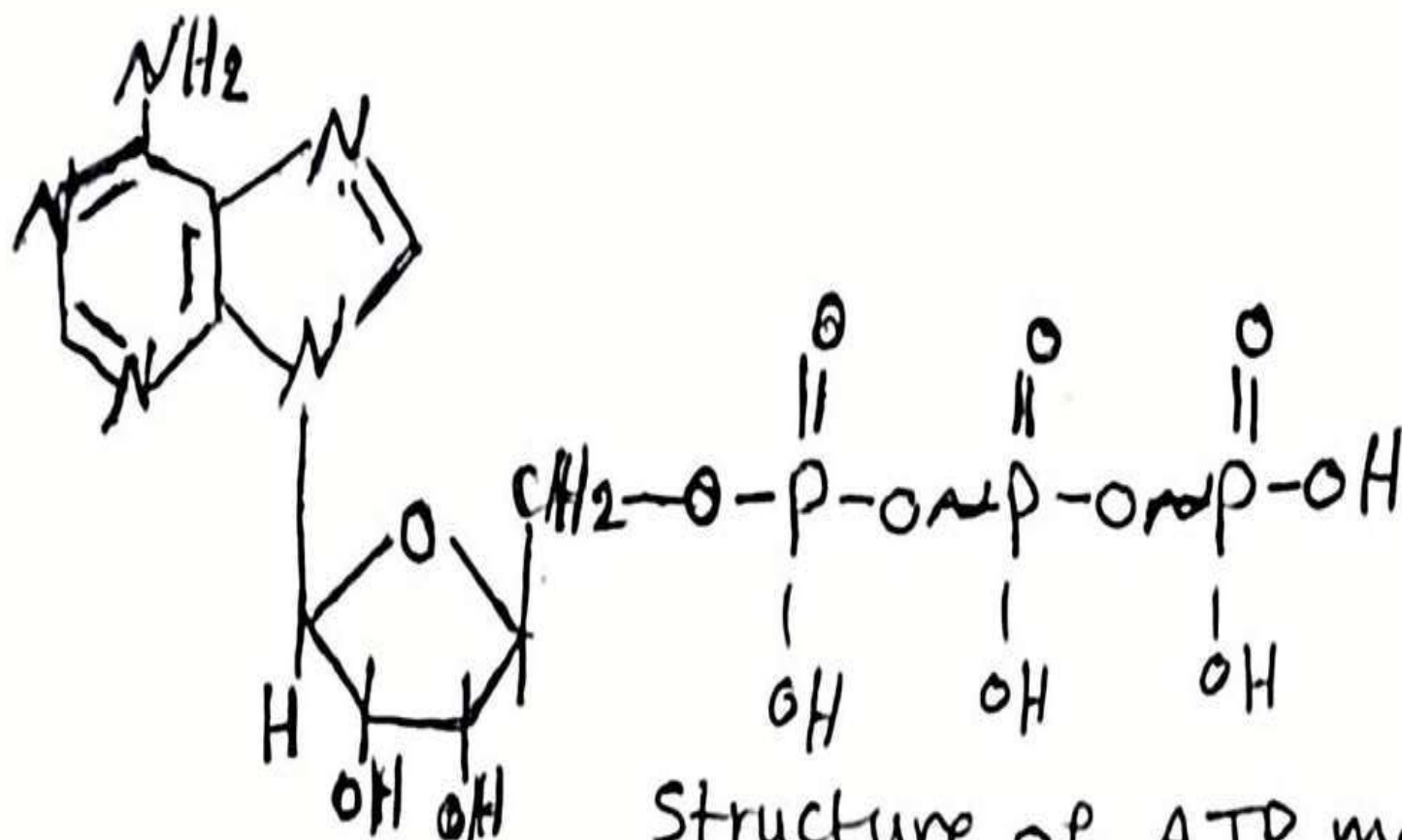


Structure of ATP molecule

ATP molecule made up of 3 parts :

- Nitrogen base (adenine)
- Monosaccharide (ribose)
- Three phosphate groups

The 2 outer phosphate groups are very important , because the bonds in these phosphates are of high energy . The high energy bond in the compounds is usually indicated by squiggle (~)

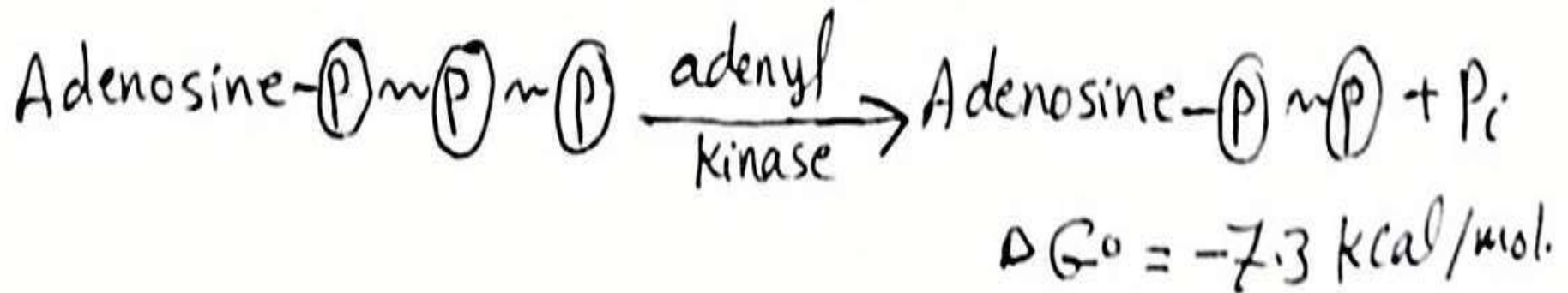


Structure of ATP molecule

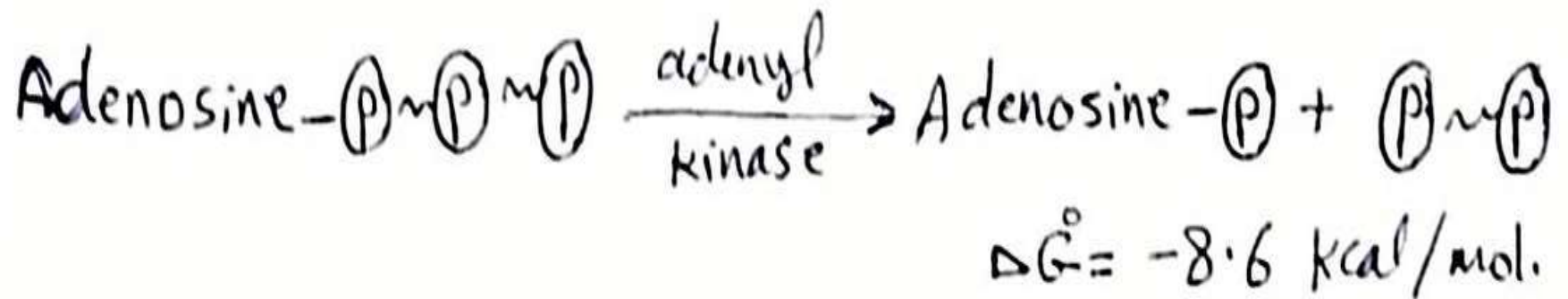
The hydrolysis of ATP to ADP

(adenosine diphosphate)

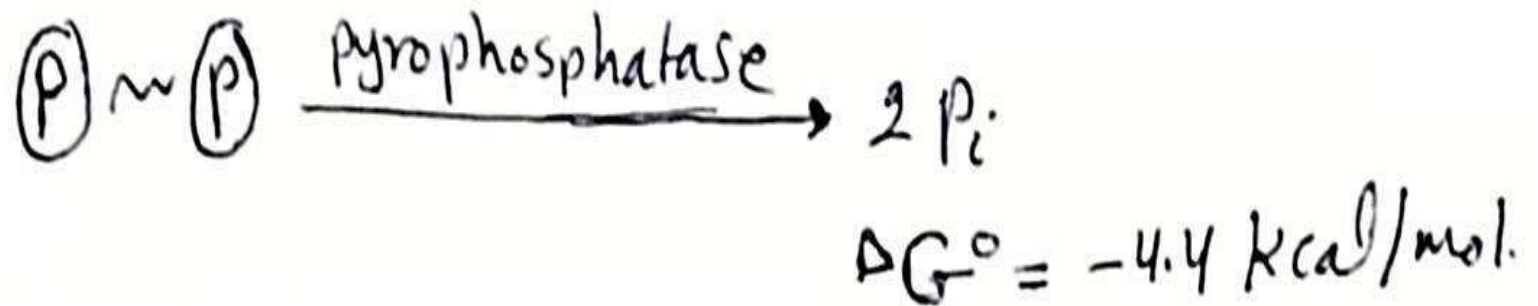
release -7.3Kcal/mol by the action of adenyl kinase .



Under certain conditions , hydrolysis of ATP resulting in the liberation about -8.6Kcal/mol of energy and AMP (adenine monophosphate) .



Hydrolysis of internal phosphate bond will release additional energy of about -4.4Kcal/mol by the action of phosphatase enzyme .



The phosphate group in AMP is considered not high energy phosphate , hence on hydrolysis it will not produce energy .

Kinase : group of enzymes present in all cells ,
transfer the phosphate group from ATP or other
energy phosphate compounds to the substrate .

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Citric Acid Cycle (C.A.C) Or

Kreb's Cycle Or

Tricarboxylic Acid Cycle (TCA)

- Pyruvate derived from glycolysis in cytoplasm can enter the mitochondria and oxidatively decarboxylated to Acetyl CoA
- This is the link between CAC and glycolysis .

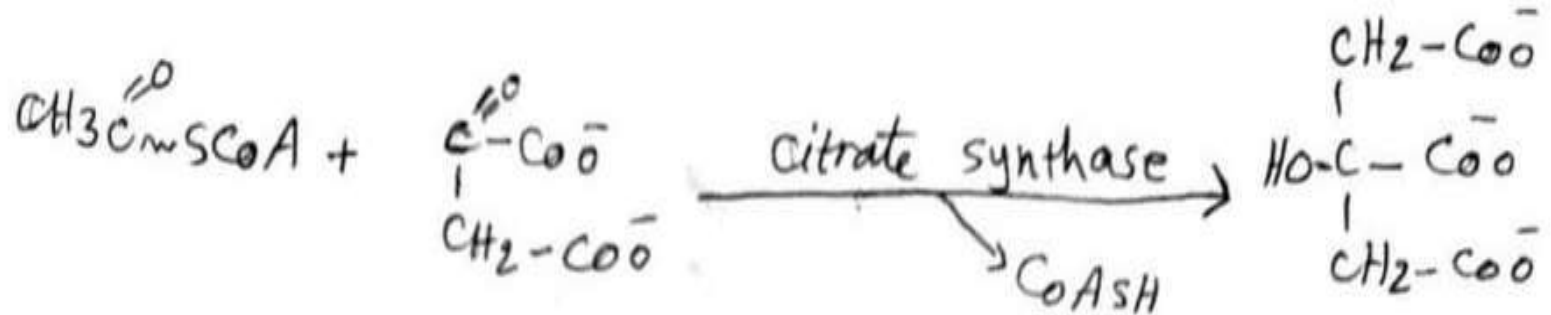
Definition : TCA is the final pathway for metabolism of carbohydrates , lipids , and proteins , which finally produces CO_2 and H_2O , and generated more ATP molecules .

TCA cycle done in the mitochondria of the cell because TCA cycle needs the presence of O_2 , this cycle cannot function in the absence of O_2

All the enzymes of citric acid cycle are located inside the matrix of mitochondria either free or attached to inner membrane . This helps the electrons to transfer among respiratory chain components .

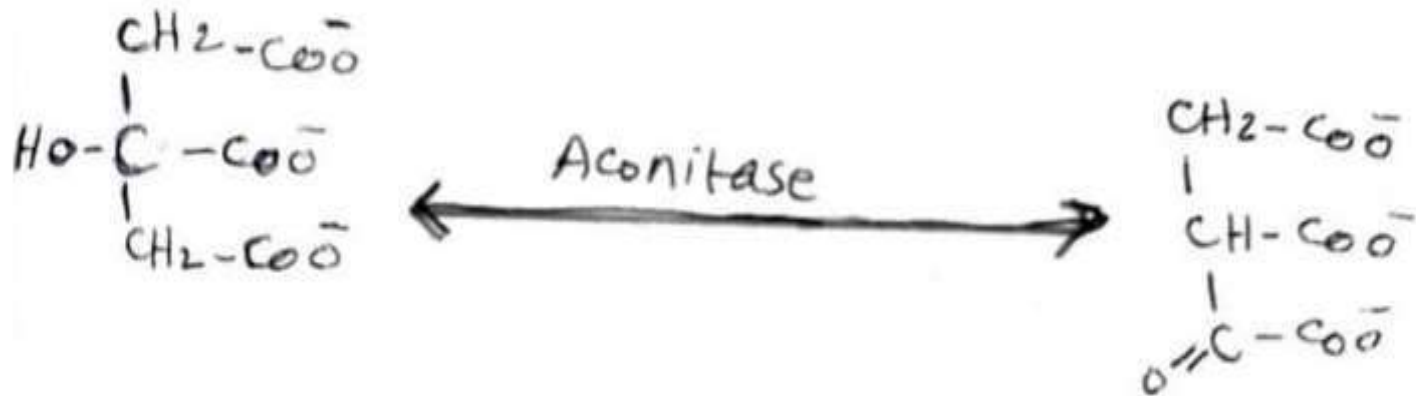
1st step : **Formation of citric acid**

Condensation between acetyl CoA and oxaloacetate by the effect of **citrate synthase** enzyme in an irreversible reaction to form the citrate (a tricarboxylic acid).



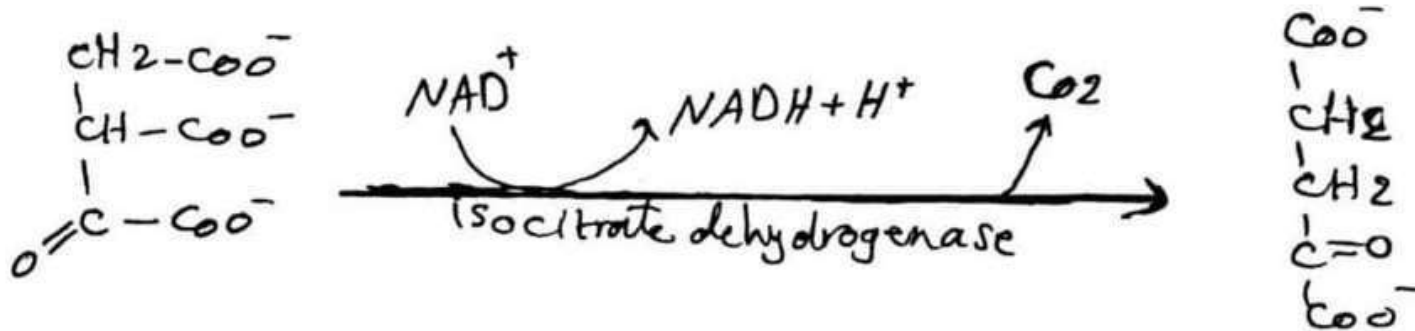
2nd step : **Formation of isocitrate**

Isomerization of citrate to isocitrate by the action of **Aconitase**



3rd step : **Formation of α -ketoglutarate**

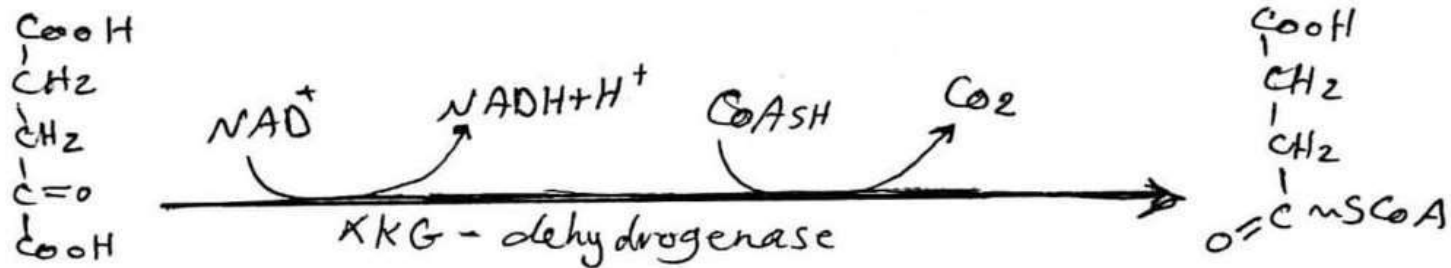
Oxidative decarboxylation of isocitrate to form α -ketoglutarate (α KG) catalyzed by **isocitrate dehydrogenase** in irreversible step .



NADH is generated in this step which is oxidized to 3ATP molecules in the electron transport chain . One molecule of CO_2 is liberated .

4th step : **Formation of succinyl CoA**

Oxidative decarboxylation of α KG to form succinyl CoA in irreversible step by the enzyme α KG dehydrogenase .

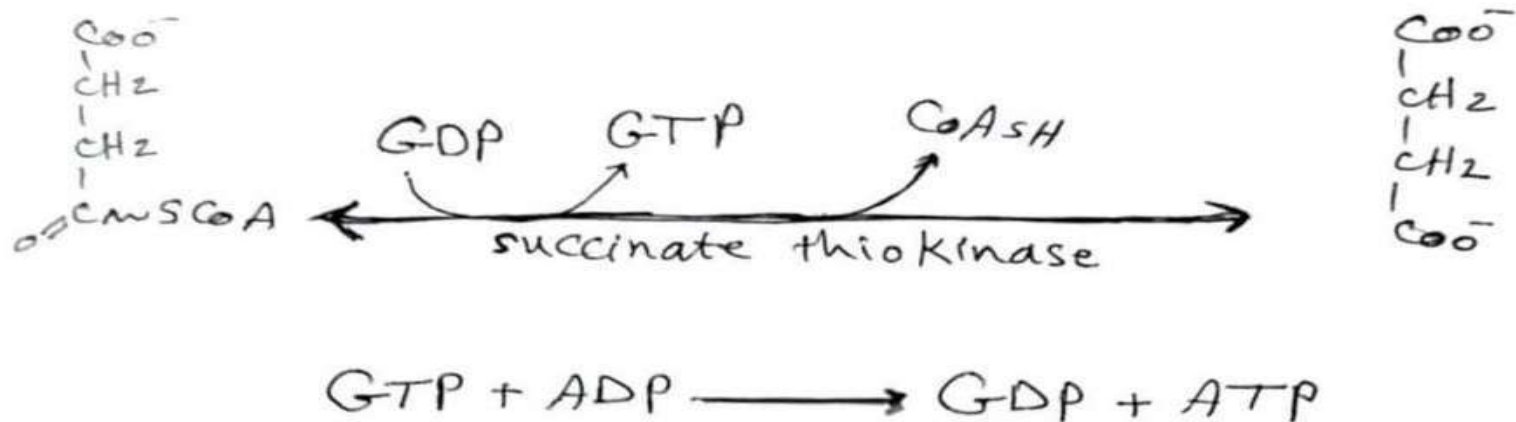


- NADH is generated and enters into electron transport chain to form 3 ATP molecules . Another molecule of CO_2 is removal .

- Succinyl CoA is another compound containing a high energy bond .
- The enzyme **α KG dehydrogenase** is a multi-enzyme complex consist of 3 enzymes and 5 coenzymes , this step is similar to the oxidation decarboxylation of pyruvate by the pyruvate dehydrogenase .
- **Note:** Acetyl CoA contains 2 carbon atoms , these atoms are now removed as CO₂ in step 3 and 4 .

5th step : **Formation of succinate**

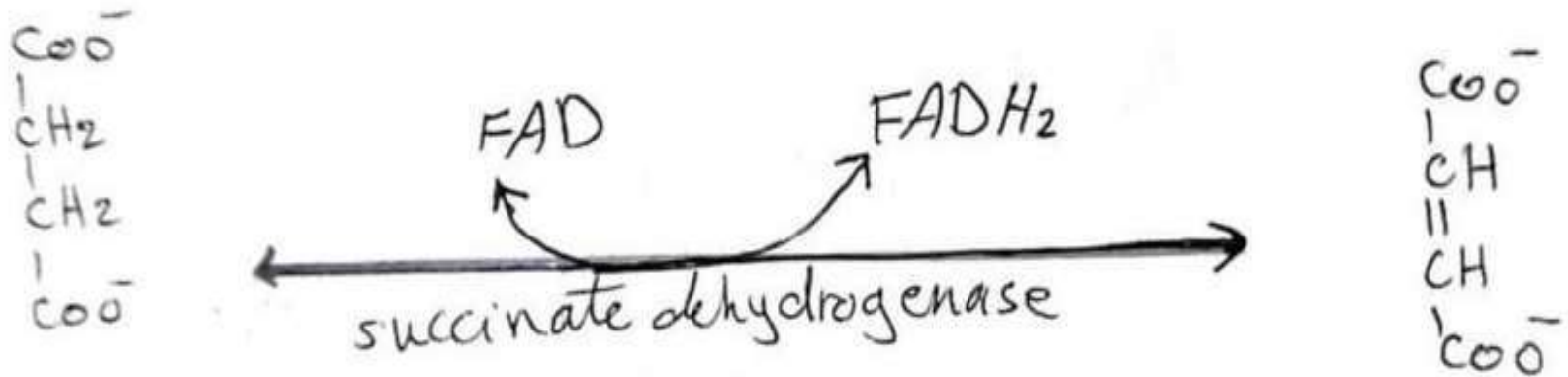
Conversion of succinyl CoA to succinate through the removal of CoASH by the enzyme **succinate thiokinase**.



A molecule of GTP is formed which is converted to ATP by reacting with ADP molecule.

6th step : **Formation of fumarate**

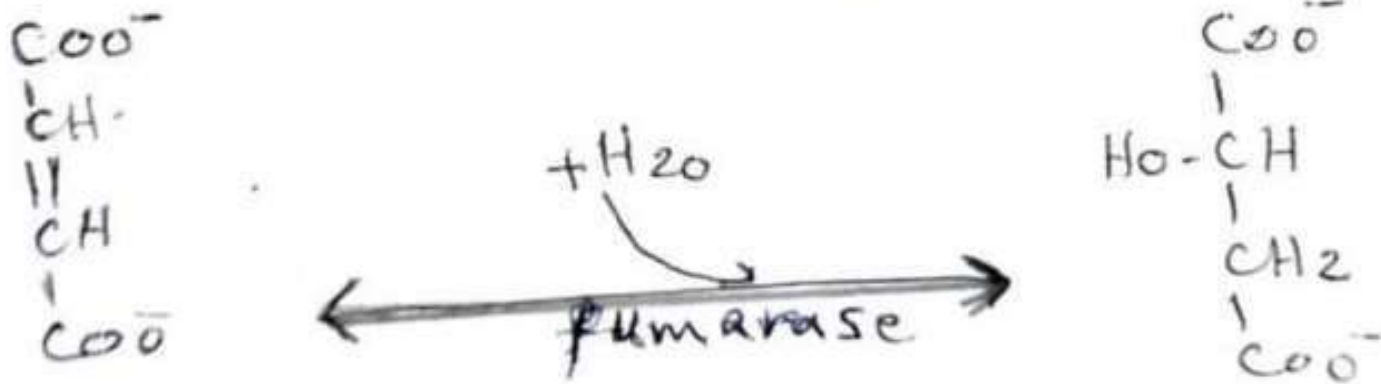
Oxidation (dehydrogenation) of succinate to fumarate by the flavoprotein **succinate dehydrogenase** . The hydrogen atoms are accepted by **FAD** .



The **FADH₂** then completes the electron transport chain to generate 2ATP molecules

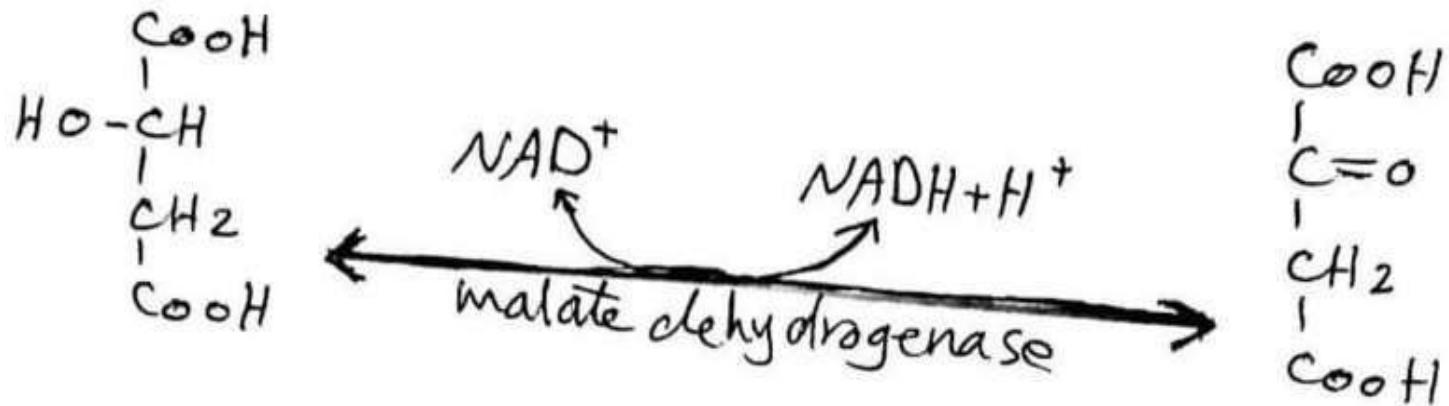
7th step : **Formation of malate**

Addition of H₂O molecule in reaction catalyzed by **fumarase** , formed malate.



8th step : **Regeneration of oxaloacetate**

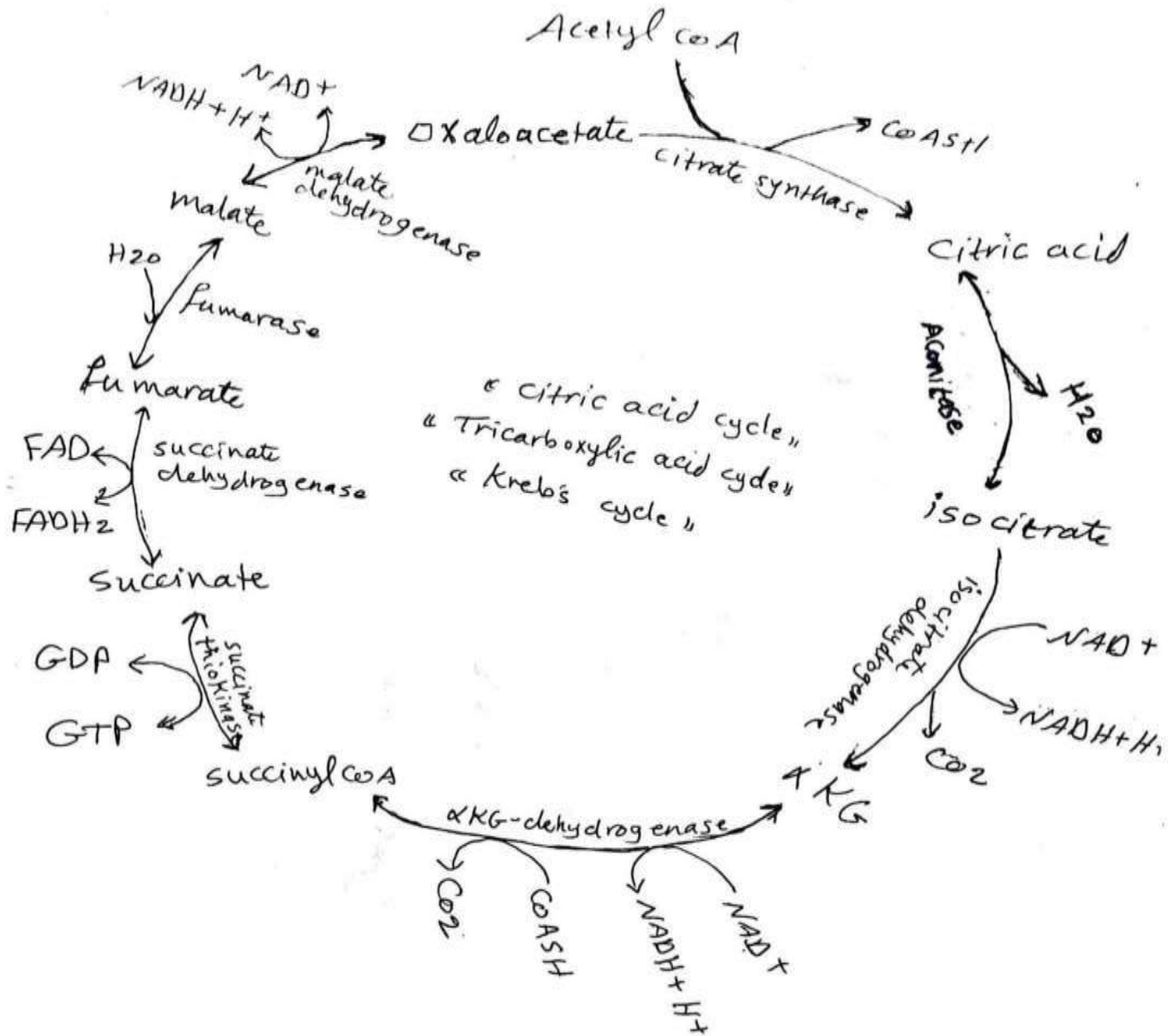
Finally , malate is oxidized to oxaloacetate by **malate dehydrogenase** . NADH generates and passes through the electron transport chain to form 3ATP molecules .



Oxaloacetate can further condense with another acetyl CoA molecule and the cycle continues .

Energy generated in Citric Acid Cycle

- 3 NADH molecules are generated to each AcetylCoA oxidized during one cycle , each of them will give 3 ATP molecules on oxidation by electron transport chain , so altogether will give 18 ATP molecules .
- 1 FADH₂ will generate 2 molecules of ATP in ETC , this will give 4 ATP .
- 1 GTP is formed by , this equivalent to one molecule of ATP .
- Hence , each turn of acetyl CoA , 12 ATP are produced .
- Oxidation of pyruvate to acetyl CoA yield 6 ATP molecules .
- This is added to ATP produced by the glycolysis to end up 38 ATP molecules produced by the metabolism of one molecule of glucose in aerobic condition .



Regulation of Citric Acid Cycle

ATP available

- The primary role of citric acid cycle is to providing ATP . the cycle is tightly coupled to the respiratory chain providing ATP . Thus, respiratory chain regulates this cycle .
- When the energy of the cell is low , the cycle operates at faster rate .
- Anaerobic conditions (hypoxia) will inhibit the electron transport chain , thus NADH and FADH_2 are accumulated which in turn will cause inhibition of citric acid cycle

Enzymes

The sites for regulation are the irreversible reactions which catalyzed by :

- Pyruvate dehydrogenase
- Citrate synthase
- Isocitrate dehydrogenase
- α KG dehydrogenase

They are allosteric enzymes , inhibited by ATP and NADH

Importance Of C .A .C and Acetyl CoA

- Citric acid cycle may be considered as the final common oxidative pathway for all food molecules .

Carbohydrates , lipids , and proteins are finally oxidized through this cycle .

- This cycle functions in both oxidative and synthetic processes

(1) Carbohydrates are metabolized to pyruvate through glycolysis , pyruvate then oxidized to Acetyl CoA , which enters to C .A .C .

(2) Fatty acids through β - Oxidation are broken down to Acetyl CoA which enters C.A.C .

(3) Amino acids after transamination (the first step in the catabolism of amino acids) are converted to keto acids (oxaloacetate , pyruvate , and α KG) these are intermediates of C.A.C .

(4) C.A.C also serves as a source of keto acids for the synthesis of amino acids (alanine from pyruvate) , (aspartate from oxaloacetate) , (glutamate from α KG).

(5) Acetyl CoA serves as precursor for the synthesis of lipids , such as :

- Cholesterol , which is a precursor of steroids hormone synthesis .
- Fatty acids . Ketone bodies .

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Carbohydrate Metabolism

Digestion and absorption

Glycolysis

Fate of pyruvate

Glycolysis in erythrocytes

Digestion and Absorption

- The carbohydrates in diet present as polysaccharides (starch and glycogen) , and to a minor extent as disaccharides (sucrose and lactose). They are hydrolyzed to a monosaccharides in gastrointestinal tract (GIT) .
- The digestion begins in the mouth by salivary α -amylase enzyme which hydrolyzes the α -1,4-glycosidic bonds of starch and glycogen randomly and produced small chain compounds (dextrins and branched or unbranched oligosaccharides) .
- These compounds are transferred into the small intestine where a further digestion is completed by the action of pancreatic α -amylase , which produced mainly maltose and isomaltose .

- The final digestion occurs in the small intestine by the action of intestinal enzymes (sucrase , maltase , isomaltase , and lactase) which are secreted by mucosal intestine cells , the disaccharides are hydrolyzed into corresponding monosaccharides .

- Maltose $\xrightarrow{\text{maltase}}$ 2 glucose

- Iso maltose $\xrightarrow{\text{iso maltase}}$ 2 glucose

- Sucrose $\xrightarrow{\text{sucrase (invertase)}}$ glucose + fructose

- Lactose $\xrightarrow{\text{lactase}}$ glucose + galactose

Lactose Intolerance :

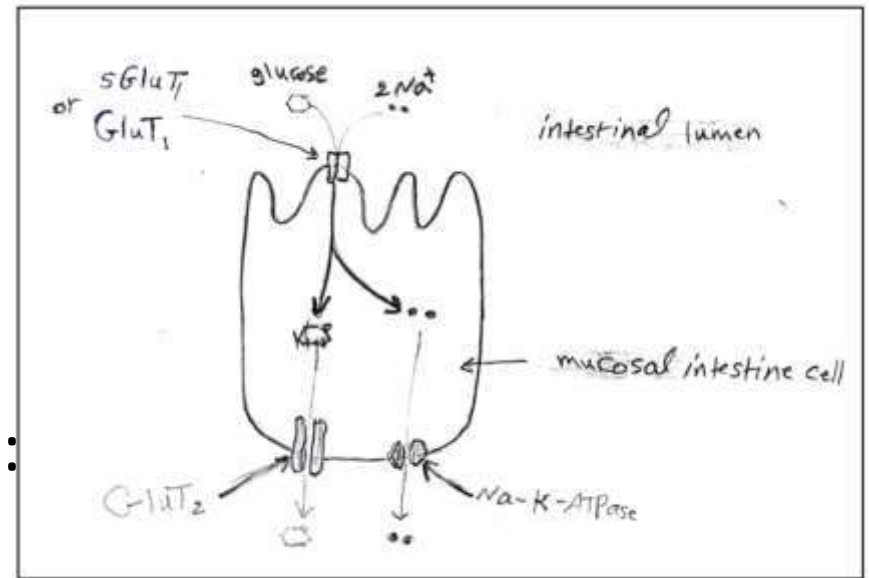
- In most people , lactase enzyme is lost as age advances (through adolescence) ,Leading to lactose intolerance .

In this condition , lactose remains and accumulates in the intestine and become a substrate for bacterial fermentation. This resulting in discomfort and diarrhea .

- Another reason for lactose intolerance may be sudden change into milk diet. Lactase is an inducible enzyme, if milk is withdrawn , the diarrhea will be limited .
- Curd is an effective treatment, because the curd contains bacteria (lactobacilli) which secretes lactase enzyme . Yeast also be used in treatment because lactase enzyme seen in yeast .

Absorption of Monosaccharides and Glucose Transporters

- Glucose and galactose are absorbed from intestinal lumen into mucosal intestine cells by active transport process together with sodium , they carried by the same transporter protein called **Sodium Glucose Transporter 1 (SGLT₁)** . It is an energy dependent process against concentration gradient . Fructose is absorbed by facilitated diffusion system .
- Mucosal intestine cells release the glucose into blood stream by facilitated diffusion by the transporter called **Glucose Transporter 2 (GLUT₂)** . This transporter is with concentration gradient , present in mucosal intestine cells , liver , kidney , and pancreas cells . It involves in the absorption of glucose to these cells . Galactose and fructose are readily converted in the liver to glucose .



Other glucose transporters are :

- **GLUT₃** is mainly found in brain , it has a high affinity for glucose
- **GLUT₄** is the major glucose transporter in skeletal muscle and adipose tissues . It is under the control of insulin (insulin activates the glucose uptake) . In diabetes , GLUT₄ is reduced leading to insulin resistance in muscle and adipose tissues .

Metabolism of Glucose

Glucose metabolism in the tissues are regulated according to the need of tissues to the energy . The following metabolic processes occur on the glucose inside the tissues :

- Glycolysis
- Citric acid cycle (Krebs cycle)
- Hexose Mono Phosphate Shunt
- Gluconeogenesis
- Glycogen break down (Glycogenolysis)
- Glycogen synthesis (Glycogenesis)

Glycolysis (EMBDEN - MEYERHOF Pathway)

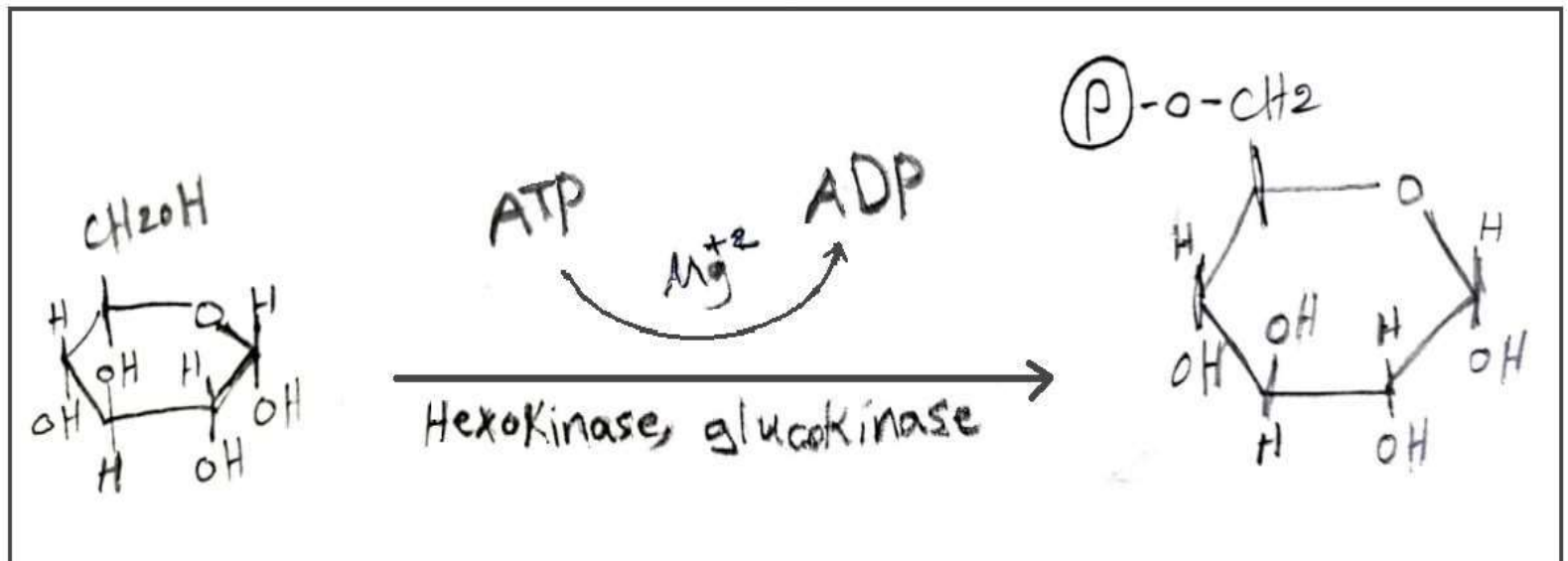
Definition :

Oxidation of glucose to pyruvate in aerobic condition , or to lactate under anaerobic conditions , with small quantity of energy produced per mole of glucose.

- Glycolysis is the only pathway that is taking place in all the tissues of the body to provide the energy .
- All enzymes of glycolysis are found in the cytoplasm , so the site of reaction steps take place in the cytoplasm .
- In heavy work , when muscle tissue lacks enough oxygen , anaerobic glycolysis forms the major source of energy for muscles . In eye cells , anaerobic glycolysis is the important pathway .
- Anaerobic glycolysis is the only source of energy in erythrocytes .
- The glucose is phosphorylated in all steps by binding the glucose with phosphate group .
- Most of the reactions of the glycolysis are reversible which are also used for gluconeogenesis pathway .

Steps of Glycolysis

Step 1 : Glucose is phosphorylated to glucose -6-P by the enzyme **hexokinase** or **glucokinase** , which split the ATP into ADP and the P_i is added to the glucose . Hexokinase is allosteric enzyme inhibited by its product glucose-6-P .

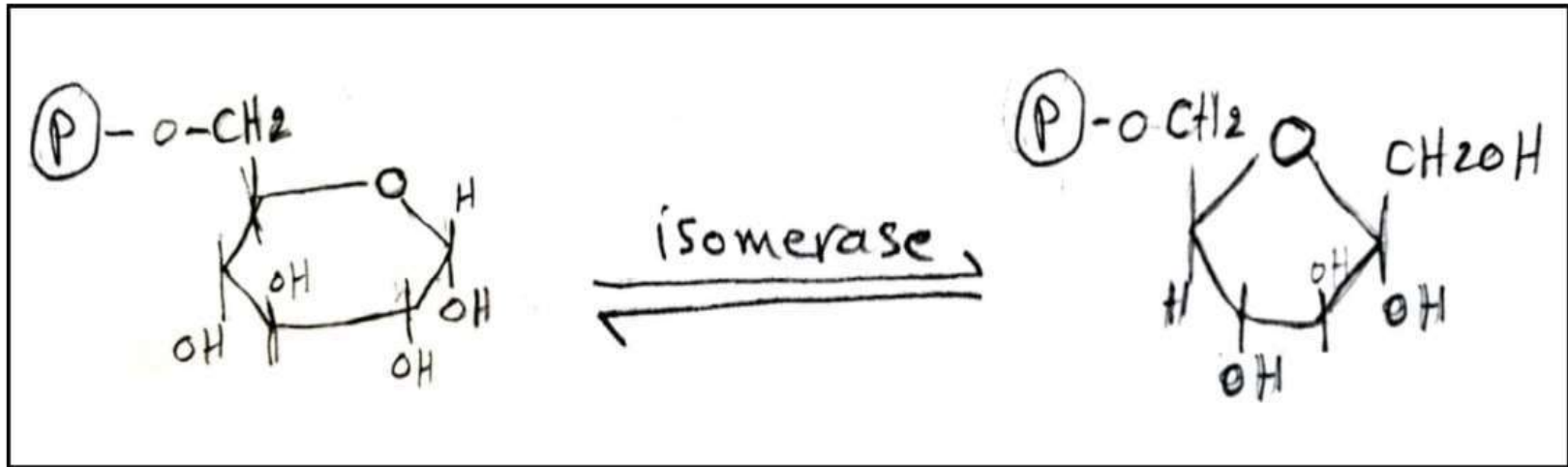


The 2 phosphorylated enzymes differ in the following :

- Hexokinase is found in all tissues , while glucokinase present in liver mainly .
- Hexokinase is non- specific enzyme , because it can phosphorylate any hexose sugar , glucose , fructose , galactose , and mannose . While glucokinase is highly specific enzyme and act only on glucose .
- Hexokinase is not induced by insulin , while glucokinase is under influence of insulin .
- Hexokinase acts when blood glucose level is low , and then the glucose utilized by all body cells . **Glucokinase acts only when blood glucose level is more than 100 mg / dL , then glucose is taken up by the liver cells for glycogen synthesis .**

Step 2 :

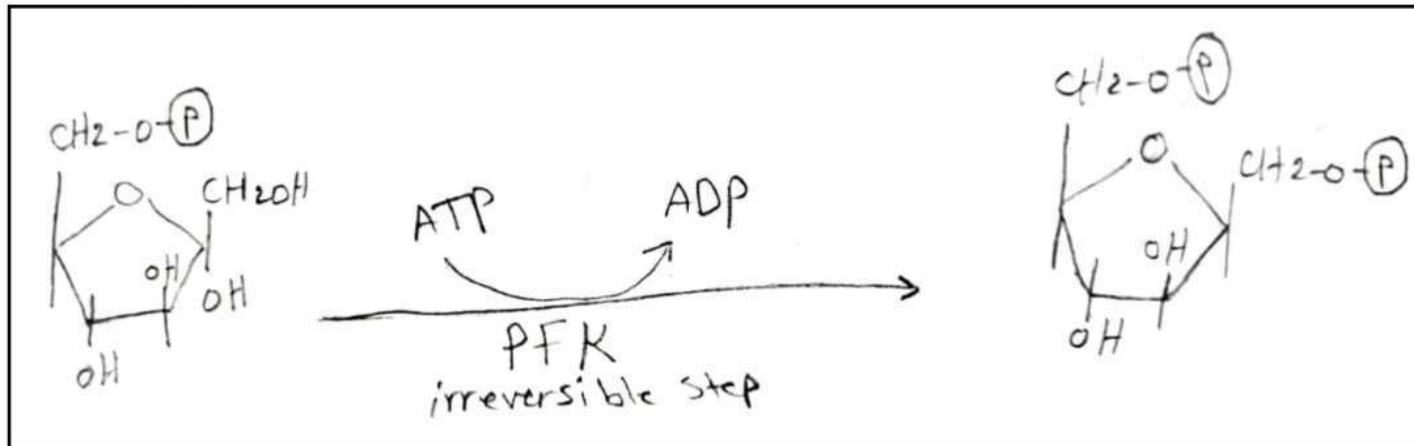
Isomerization of glucose-6-P to fructose-6-P by the action of **isomerase** . This is a reversible step .



Step 3 :

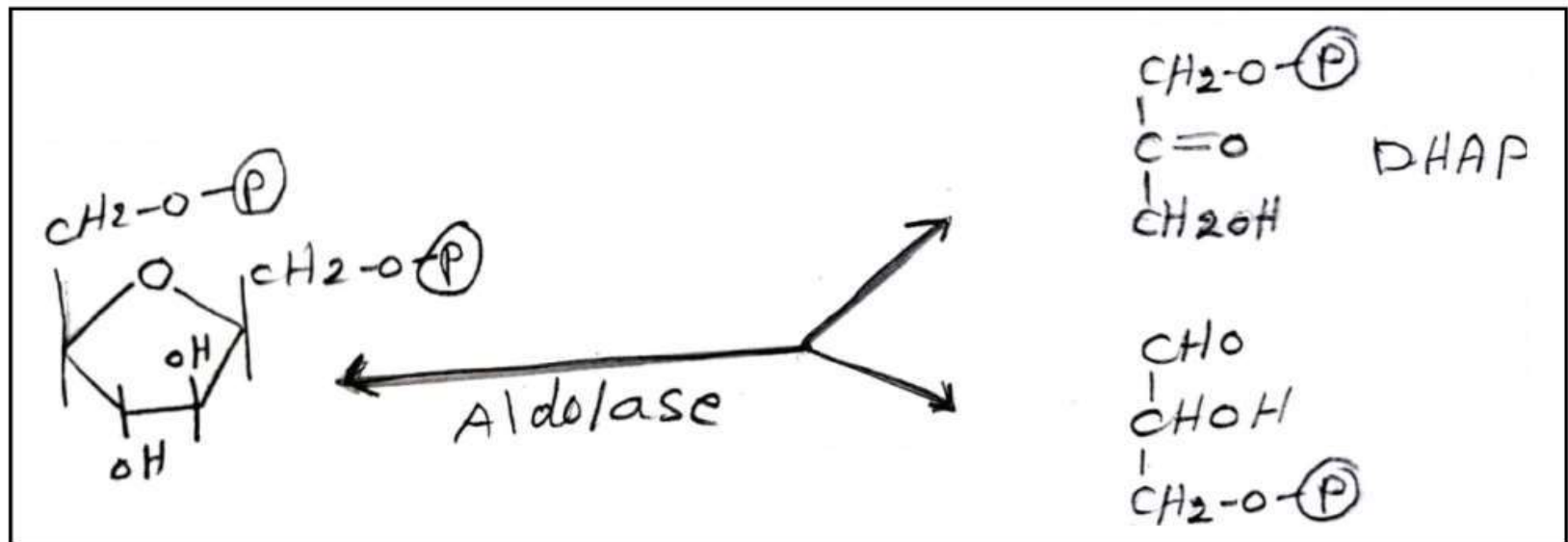
Phosphorylation of fructose-6-P into fructose-1,6-bisphosphate by enzyme called **phosphofructo kinase (PFK)** which need one molecule of ATP hydrolyzed to ADP .

This is an irreversible step , and it is the rate limiting reaction in glycolysis . PFK is an allosteric enzyme , it is an important key enzyme of this pathway.

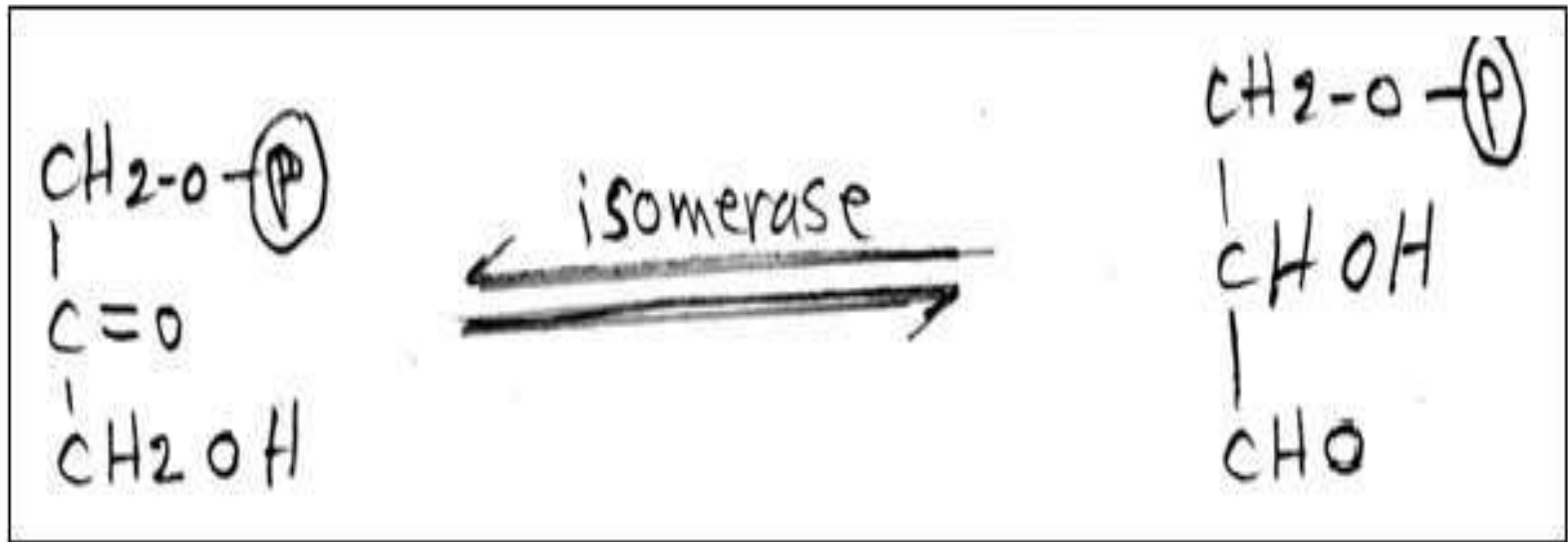


Step 4 :

Splitting of fructose-1,6-bisphosphate into two compounds (each with 3 carbon atom) , glyceraldehyde-3-P and dihydroxy acetone phosphate (DHAP) . This reaction is reversible and occur by **aldolase** enzyme .



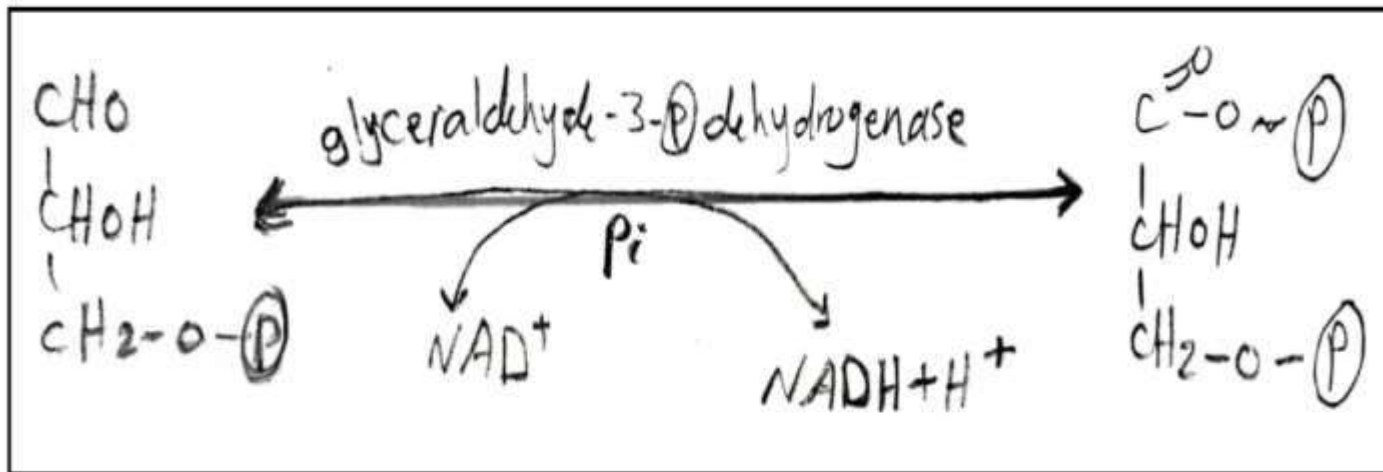
- **DHAP** is isomerized to **glyceraldehyde-3-P** by the isomerase enzyme , thus the net result is that glucose converted into 2 molecules of **glyceraldehyde-3-P** .



Step 5 :

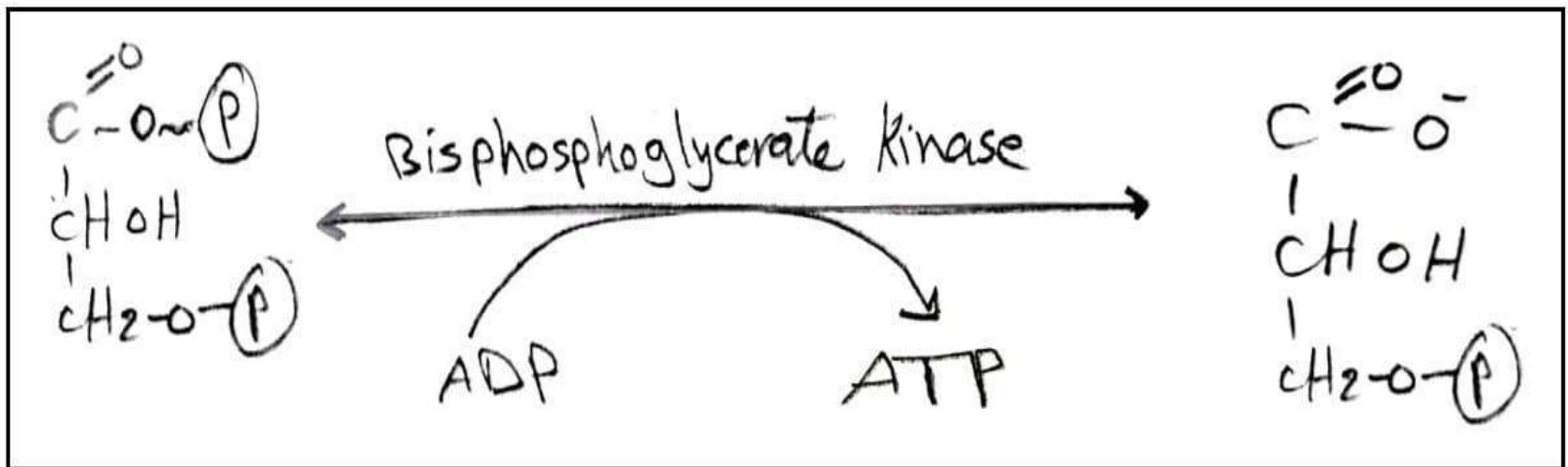
Oxidation and phosphorylation of glyceraldehyde-3-P to 1,3-bisphosphoglycerate (1,3-BPG) with the help of NAD^+ by the action of **glyceraldehyde-3-P dehydrogenase** enzyme .

This reaction is reversible , and the product contains a high energy phosphate bond . During this reaction , NAD^+ is reduced to NADH .



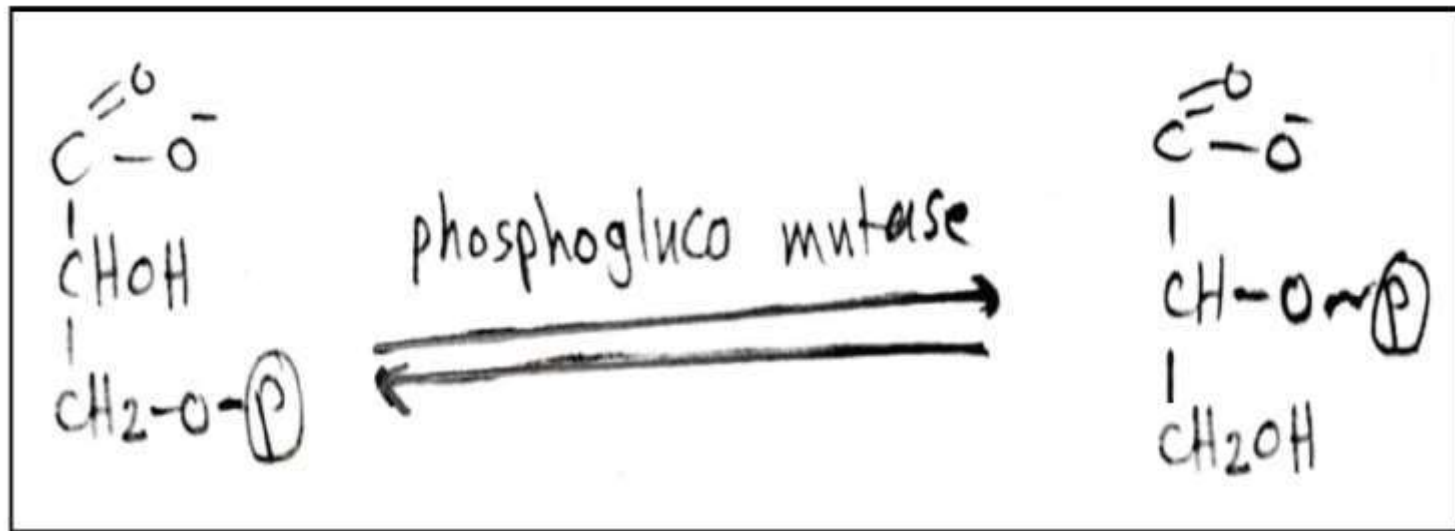
Step 6 :

The phosphate is transferred from 1,3-BPG into ADP , forming ATP and 3-phosphoglycerate with the help of **bisphosphoglycerate kinase** .



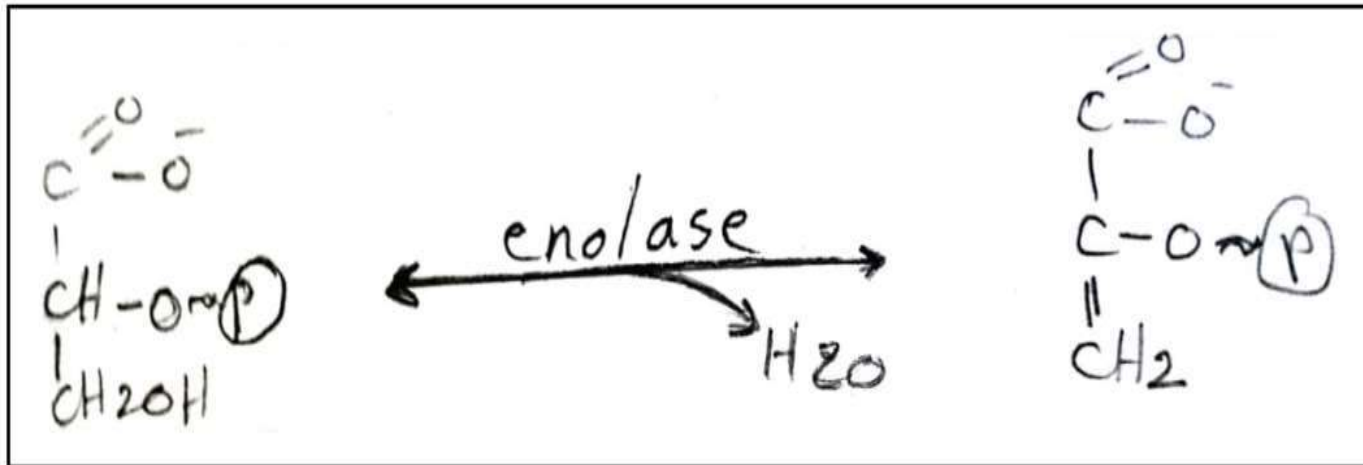
Step 7 :

3-phosphoglycerate is isomerized to 2-phosphoglycerate by shifting the phosphate group from 3rd to 2nd carbon atom . The enzyme is **phosphoglucomutase** , and this a reversible reaction .



Step 8 :

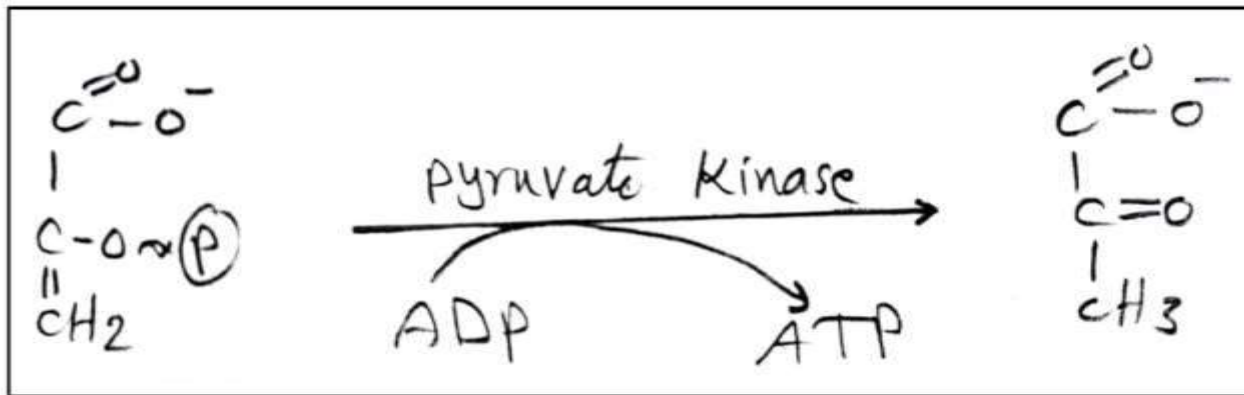
2-phosphoglycerate is converted to phosphoenolpyruvate (PEP) by the enolase enzyme . A high energy phosphate bond is produced , and the reaction is reversible .



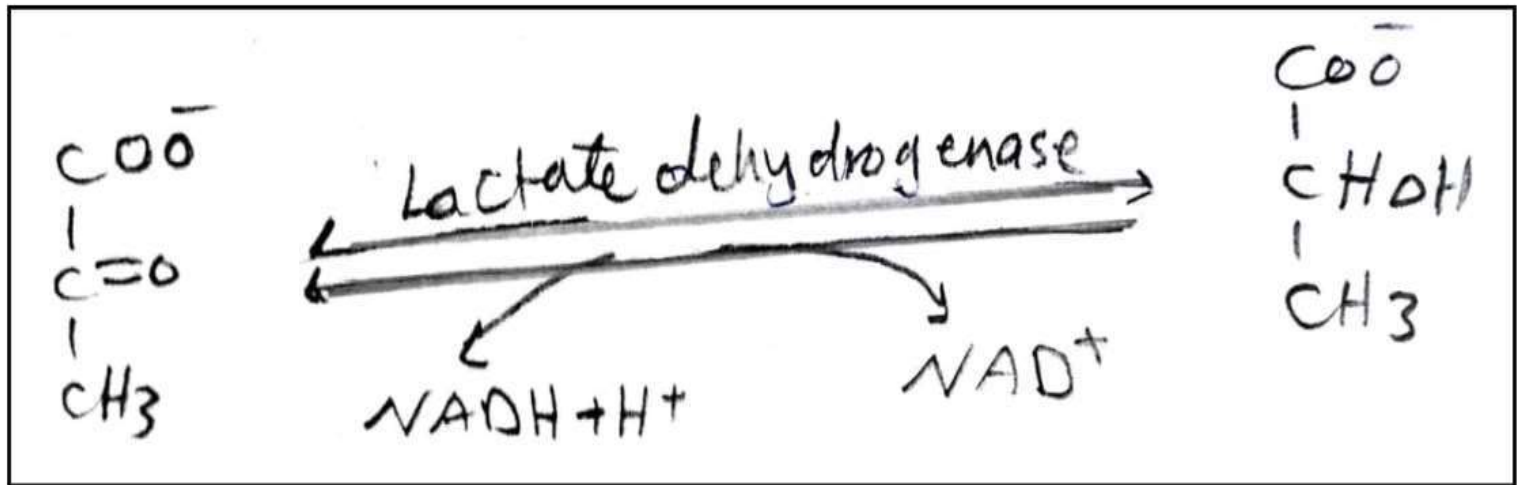
Step 9 :

PEP is dephosphorylated to pyruvate by **pyruvate kinase** enzyme . One mole of ATP is generated .

The pyruvate kinase is a key enzyme of glycolysis and this step is irreversible .



Step 10 : In anaerobic condition , pyruvate is reduced to lactate by the enzyme **Lactate dehydrogenase (LDH)** in a reversible reaction .



Steps 5 and 10 are coupled :

- In the 5th step , for each molecule of glucose entering in glycolysis , 2 molecules of NAD^+ are reduced to NADH .
- The NADH is to be reconverted to NAD^+ , this can be done in the cytoplasm during exercise where is lack of oxygen . Therefore , the cell has to couple 5 to 10 reactions in which NAD^+ is regenerated by reducing the pyruvate to lactate .

Glyceraldehyde-3-P

dehydro-
genase
step (5)

NAD^+

NADH

1,3-bisphosphoglycerate

NADH

NAD^+

LDH

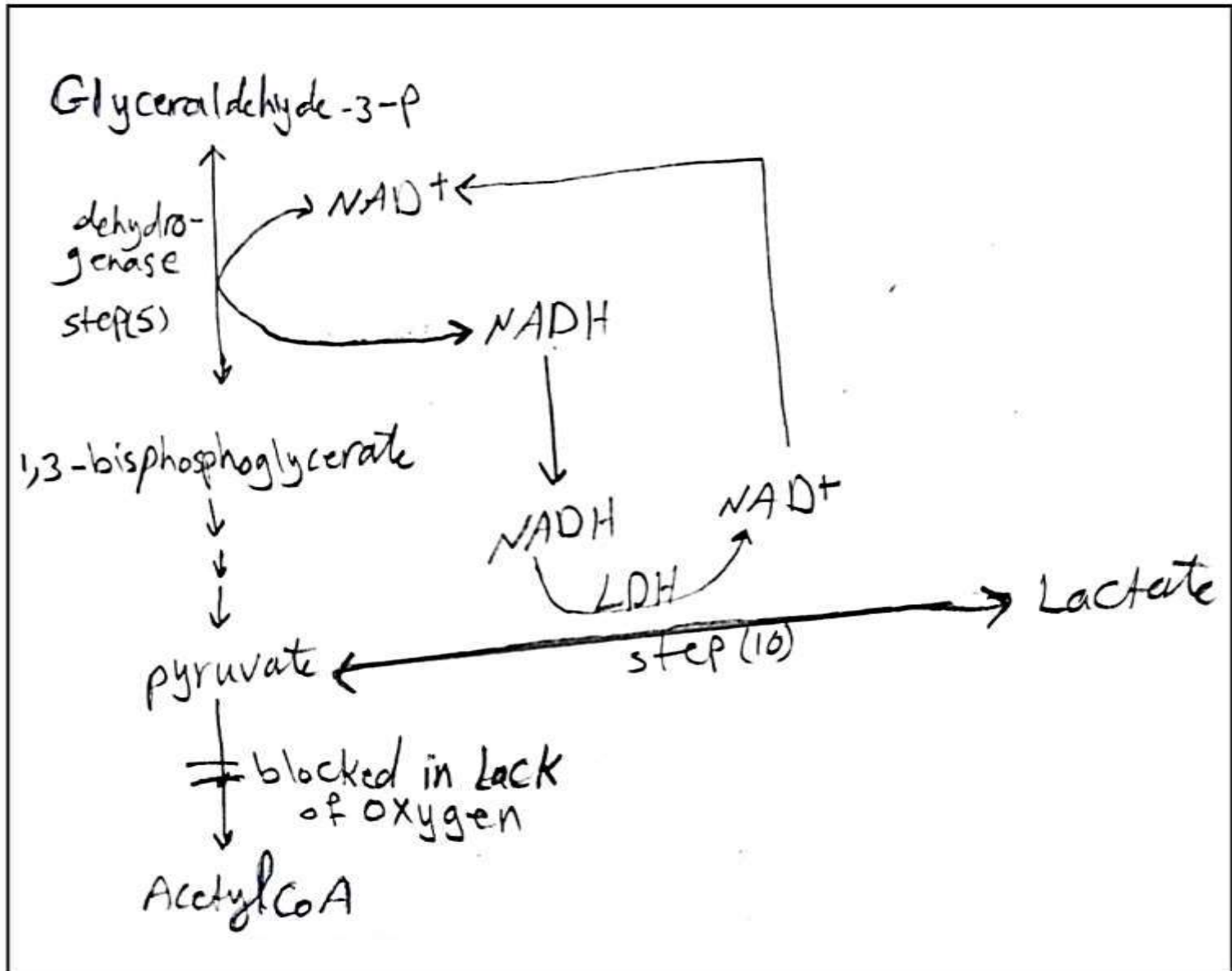
pyruvate

step (10)

Lactate

blocked in lack
of oxygen

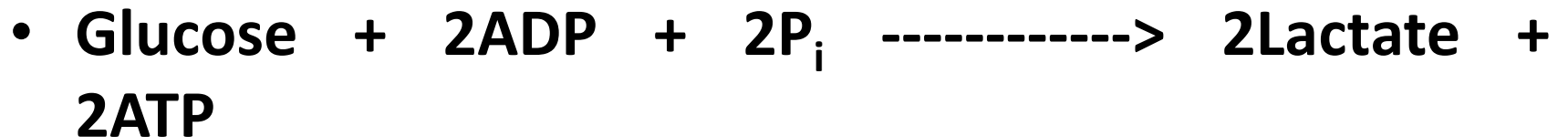
AcetylCoA



Energy yield from glycolysis :

- During anaerobic condition (oxygen deficiency) when one molecule of glucose is converted to 2 molecules of lactate , the net yield is 2 ATP molecules after the lost of 2ATP molecules in steps 1 and 3 .

The overall reaction is :



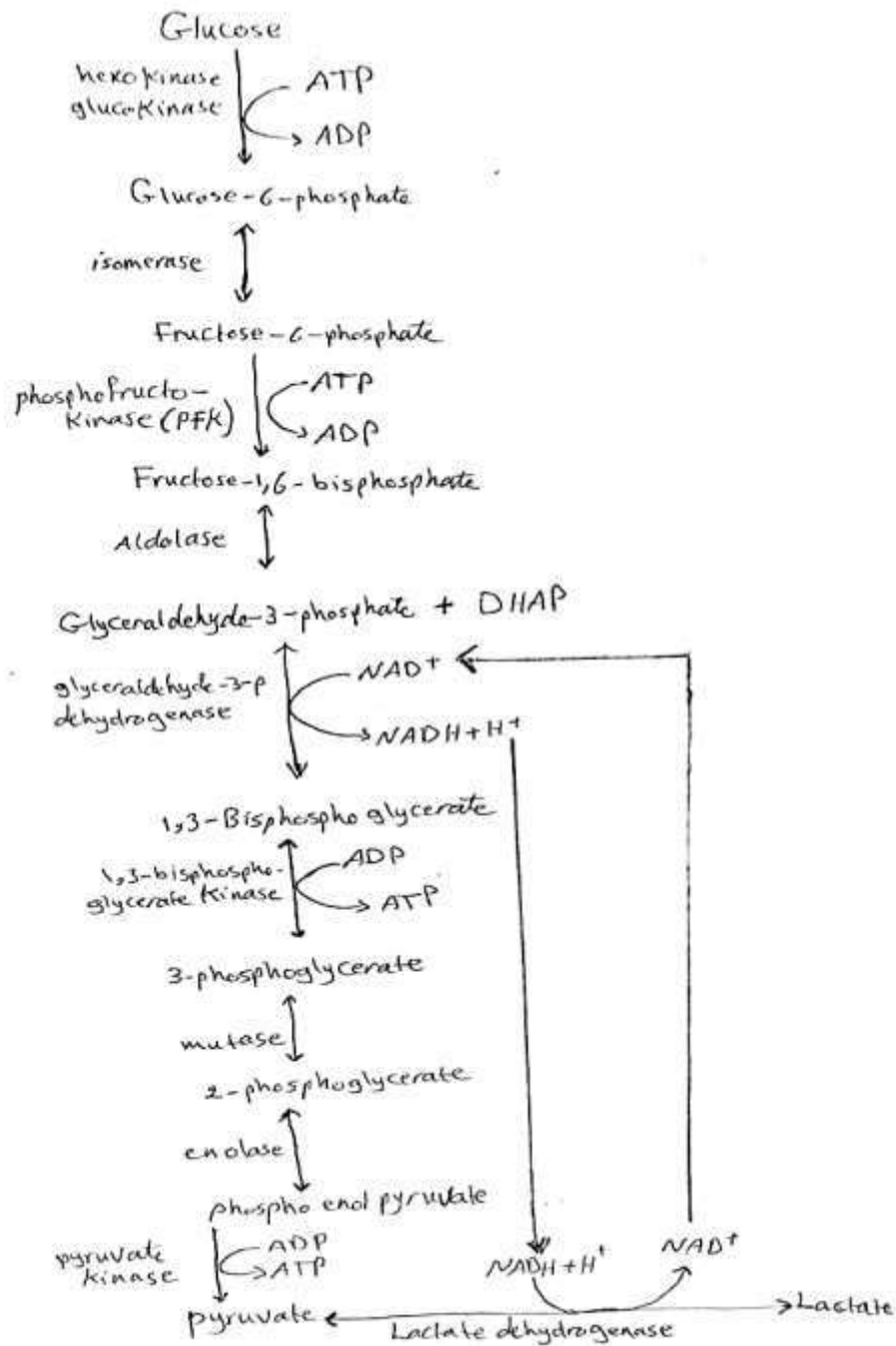
- In aerobic condition (oxygen is in plenty) , the 2 NADH generated in step 5 will enter the mitochondrial electron transport by the shuttle and each NADH will provides 3ATP molecules , so the net gain of energy is 8ATP .



Regulation of Glycolysis :

Glycolysis is regulated by the three irreversible reactions which catalyzed by :

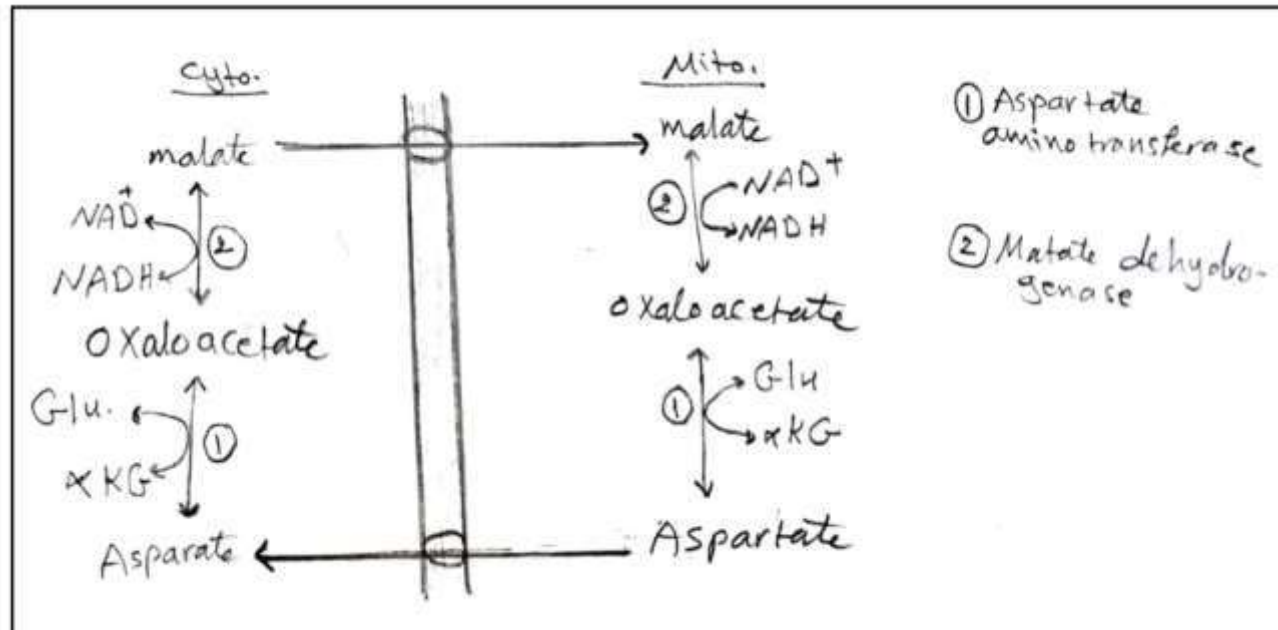
- Hexokinase and glucokinase
- Phosphofructo kinase
- Pyruvate kinase
- Insulin hormone increases the activity of above enzymes , while glucagon inhibits them .



Entry of cytoplasmic NADH to the mitochondria

Malate shuttle

- Operates mainly in liver, kidney, and heart, by the help of enzymes **malate dehydrogenase** and **aspartate aminotransferase**. Each molecule of NADH enters the mitochondria, 3ATP molecules are generated.



Fate of Pyruvates

(1) Under hypoxic conditions as in heavy exercise when skeletal muscle lacks enough oxygen, and in RBCs which have no mitochondria, pyruvate cannot oxidize, and anaerobic glycolysis represents the major source of energy. 2 ATP molecules and lactate are the end products.

(2) Under aerobic condition, pyruvate is converted to Acetyl CoA which enters the Krebs cycle to be oxidized to H_2O and CO_2 and ATP is generated.

Oxidation of pyruvate into Acetyl CoA :

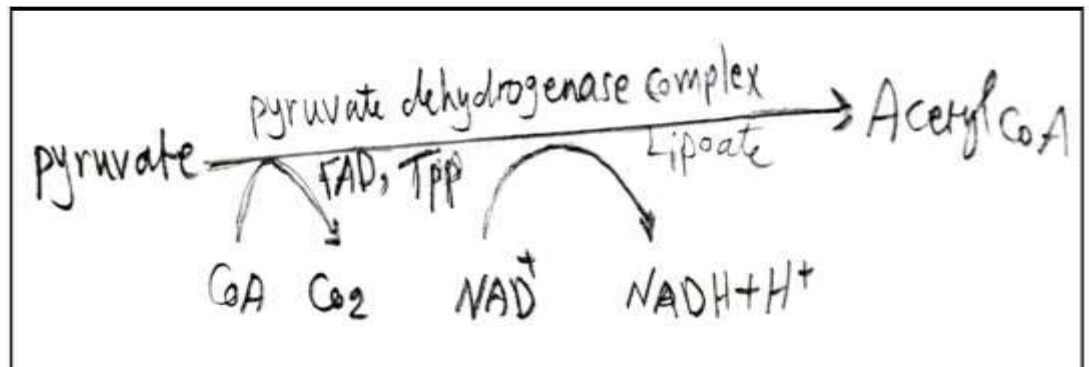
- Glycolysis is taking place in cytoplasm , so pyruvate is transported into mitochondria and undergoes **oxidation decarboxylation** to Acetyl CoA
- This reaction is catalyzed by different enzymes and coenzymes working as a multienzyme complex called **pyruvate dehydrogenase complex** .

The enzymes included in this multienzyme complex are :

- Pyruvate decarboxylase
- Dihydro lipoyl transacetylase
- Dihydro lipoyl dehydrogenase

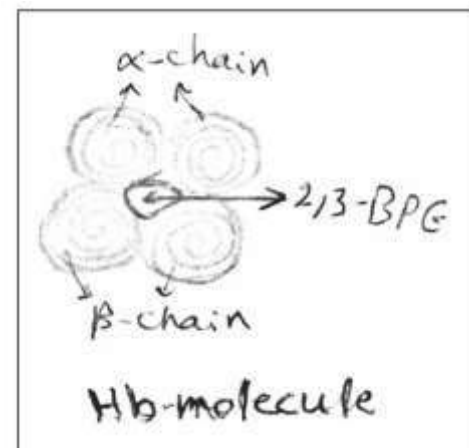
The coenzymes needed are :

- Vitamin B₁ (Thiamine pyrophosphate)
- Vitamin B₂ (FAD)
- Vitamin B₃ (NAD⁺)
- CoA
- Lipoic acid



Glycolysis in Erythrocytes

- In RBC_s , step 6 of glycolysis is bypassed . 1,3-BPG is converted to 2,3-BPG by the enzyme BPG-mutase . Then BPG-phosphatase enzyme removes the phosphate group to form 3-phosphoglycerate .
- In this pathway , no ATP molecules is generated , this may be of advantage since it would allow the glycolysis to proceed when the need for ATP is minimal .



There are high concentration of 2,3-BPG in erythrocytes , it found in the same concentration of Hb .

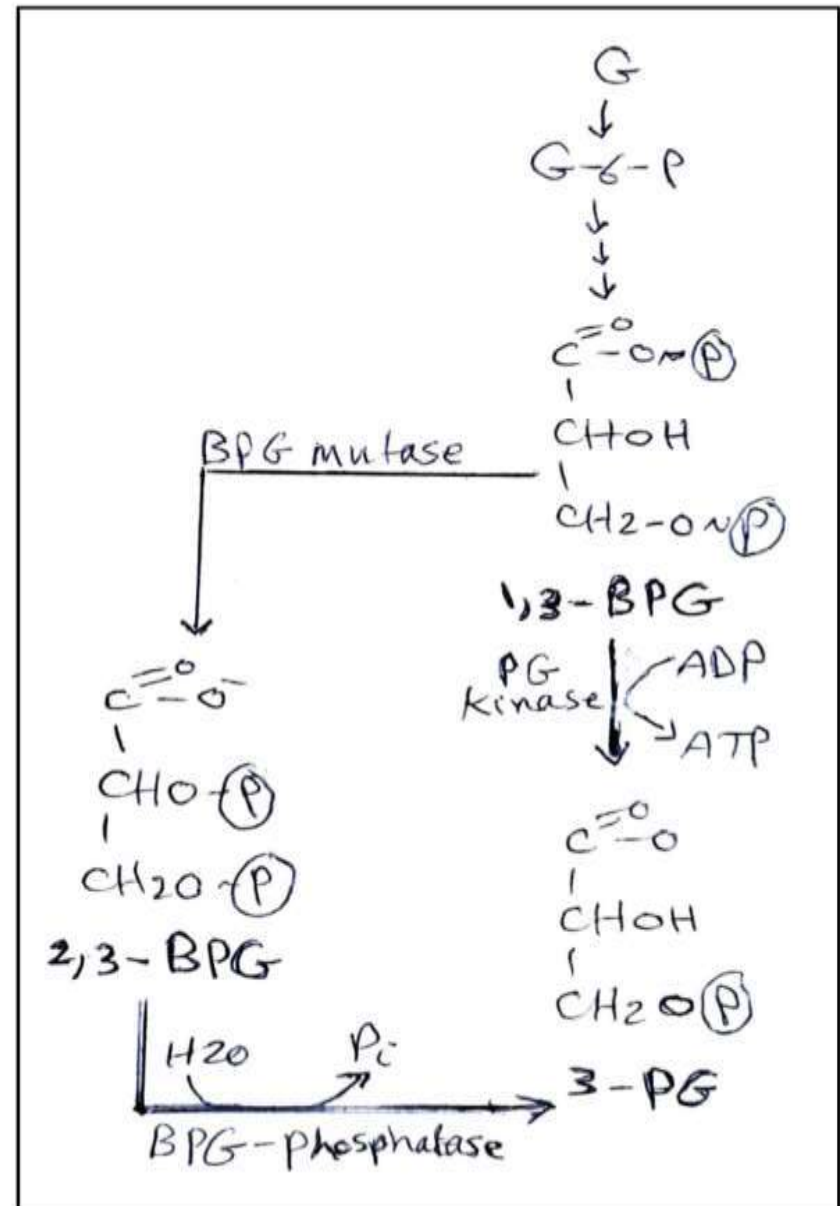
2,3-BPG is responsible for a high efficiency of oxygen transport that occur in Hb molecules .

2,3-BPG binds with great affinity to the Hb found in tissues (de-oxygenated Hb) than to Hb found in lungs (oxygenated Hb) .

Pure Hb releases only 8% of O_2 to the tissues , while Hb with 2,3-BPG allows it to release 66% of O_2 to the tissues .

In lungs , 2,3-BPG has a lower affinity toward Hb , this help the Hb to saturate itself with O_2 in the lungs and hold the O_2 until it reaches the tissues which have a lower O_2 concentration . Thus , 2,3-BPG helps in the regulation of O_2 carrying in Hb .

The effect of , 2,3-BPG is shown between fetal Hb and maternal Hb . Maternal Hb is able to bind 2,3-BPG better than fetal Hb , therefore the fetal Hb has a higher affinity toward O_2 , this help the fetus to get more O_2 from the mothers blood stream .



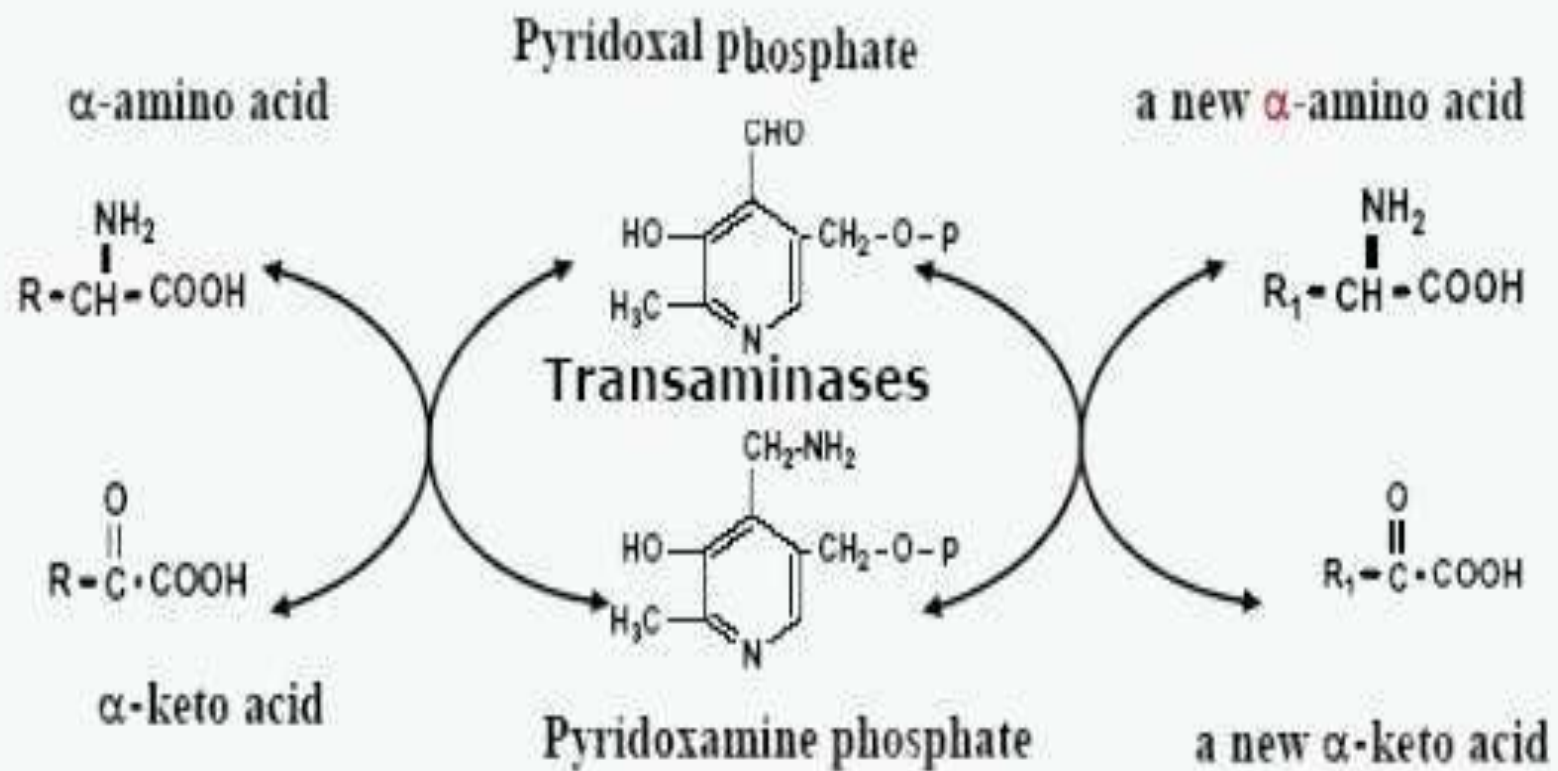
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Catabolism of Amino Acids

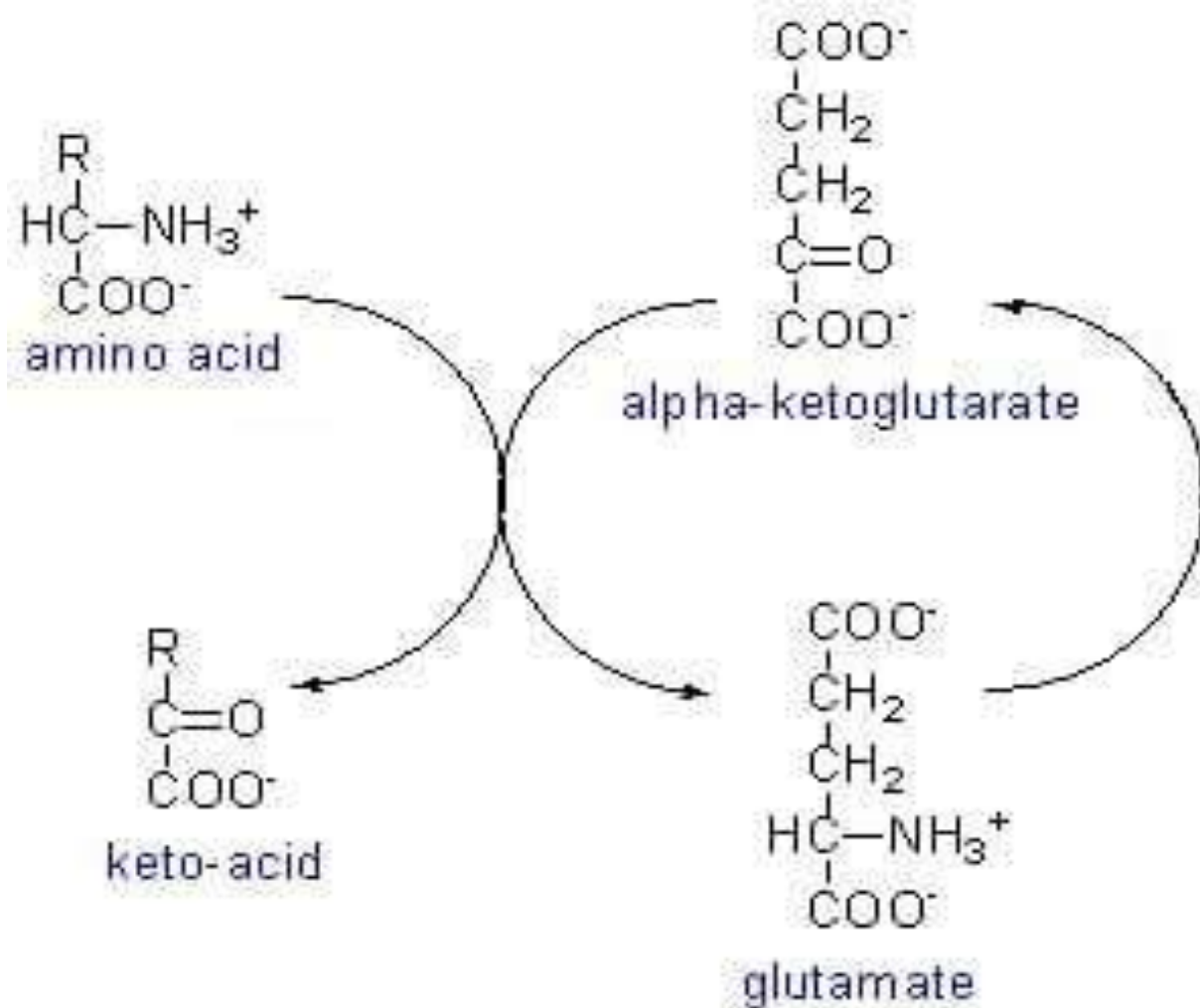
- Source of amino acids (A.A_s) in the body are derived either from the breakdown of dietary protein (exogenous source) , or from endogenous source from the breakdown of tissue proteins especially muscle proteins . About 1- 2% of total body proteins are hydrolyzed to amino acids daily .
- About 80% of the amino acids in the cell are used for proteins synthesis , other part used for synthesis of biological compounds needed by the cell .
- Excess amino acids are not stored , but it catabolized for energy supply .
- **Catabolic reactions of amino acids include Transamination and Oxidative deamination . The end product is ammonia formation (NH₃) which is highly toxic to the brain , so it converted to nontoxic substance called urea which is less toxic and easily removed by the kidney .**

Transamination

- Define as the transfer of **α -amino group** from one **α - A.A** to **α - keto acid** , forming a new **α - A.A** and a new **α - keto acid** .
- Transamination takes place in the cytoplasm of all the cells of body . It is a reversible process and catalyzed by a group of enzymes called transaminases or called aminotransferases which require pyridoxal phosphate (Vit B₆) as a coenzyme which act a carrier for NH₂ group.



- The net product of Transamination process is glutamic acid formation , because at the end of the Transamination , all NH_2 groups are transferred to α KG forming glutamic acid by the help of glutamate transaminase enzyme .
- This is very important because the glutamate is the only amino acid that undergoes oxidative deamination in the mammalian tissues .



giutamate transaminase

Biological Important of Transamination :

The keto acids resulting from transamination undergo oxidation in citric acid cycle , or it enter ketone bodies synthesis to provide the energy . Therefore , A.A_s are classified according to keto acids formed into :

Glucogenic amino acids : Which its catabolism produce pyruvate , α KG , oxaloacetate , succinyl CoA , and these are intermediates for citric acid cycle.

Ketogenic amino acids : Which its catabolism produce acetyl CoA and acetoacetate which are used in ketone bodies synthesis .

Clinical Value Of Specific Transaminases

GOT (AST) : Glutamate Oxaloacetate Transaminase

(Aspartate Transaminase)



GPT (ALT) : Glutamate Pyruvate Transaminase

(Alanine Transaminase)



Liver and heart tissues are rich in both GOT and GPT . Both enzymes levels are increased in liver diseases , but ALT increases more than AST .

- Normal serum level of ALT is 13 – 35 U/L for male , and 10 – 30 U/L for female . Very high values (300 – 1000 U/L) are seen in acute hepatitis . Moderate increase (50 – 100U/L) may be seen in chronic liver diseases such as cirrhosis and hepatitis C .
- **ALT which is only slightly elevated in heart diseases , is more specific indicator of liver damage .**
- Normal serum level of AST ranges from 8 to 20 U/L . The level is significantly elevated (10 – 100 times the normal range) in myocardial infraction . So , AST test is used as a marker of heart diseases .

Troponin test

- Troponin test is the most effective for diagnosis the heart diseases than AST test .
- Troponin refers to a group of proteins (Troponin T , Troponin I , Troponin C) found in muscles , regulate the contraction of heart and skeletal muscles.
- Normal blood level is 0 – 0.4 ng/ ml . High level of troponin in blood indicate there is a damage in heart muscle , this is mean that the person has a heart attack .

Oxidative Deamination

- It is the second process of amino acids metabolism which follows transamination .
- Transamination process yields glutamic acid , this glutamic acid is transported to the liver and undergoes oxidative deamination in the mitochondria . So glutamic acid acting as a carrier of NH_2 groups of amino acids .
- Oxidative deamination mean the liberation of ammonia (NH_3) from glutamic acid coupled with oxidation .This is done by **L- glutamate dehydrogenase** enzyme which is present only in the matrix of liver cells and responsible for the elimination of most NH_3 formed in the tissues .

Any amino acid Alpha keto glutarate

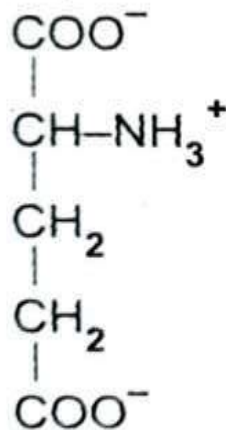
Transamination **in all tissues**

Corresponding
keto acid

Glutamate

Transport from tissues to liver

**Oxidative
De-amination in liver**

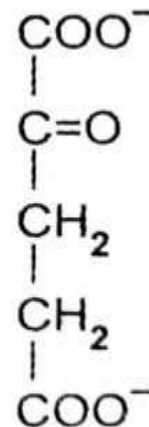


Glutamate

**Glutamate
dehydrogenase**
+H₂O

NAD⁺

NADH+H

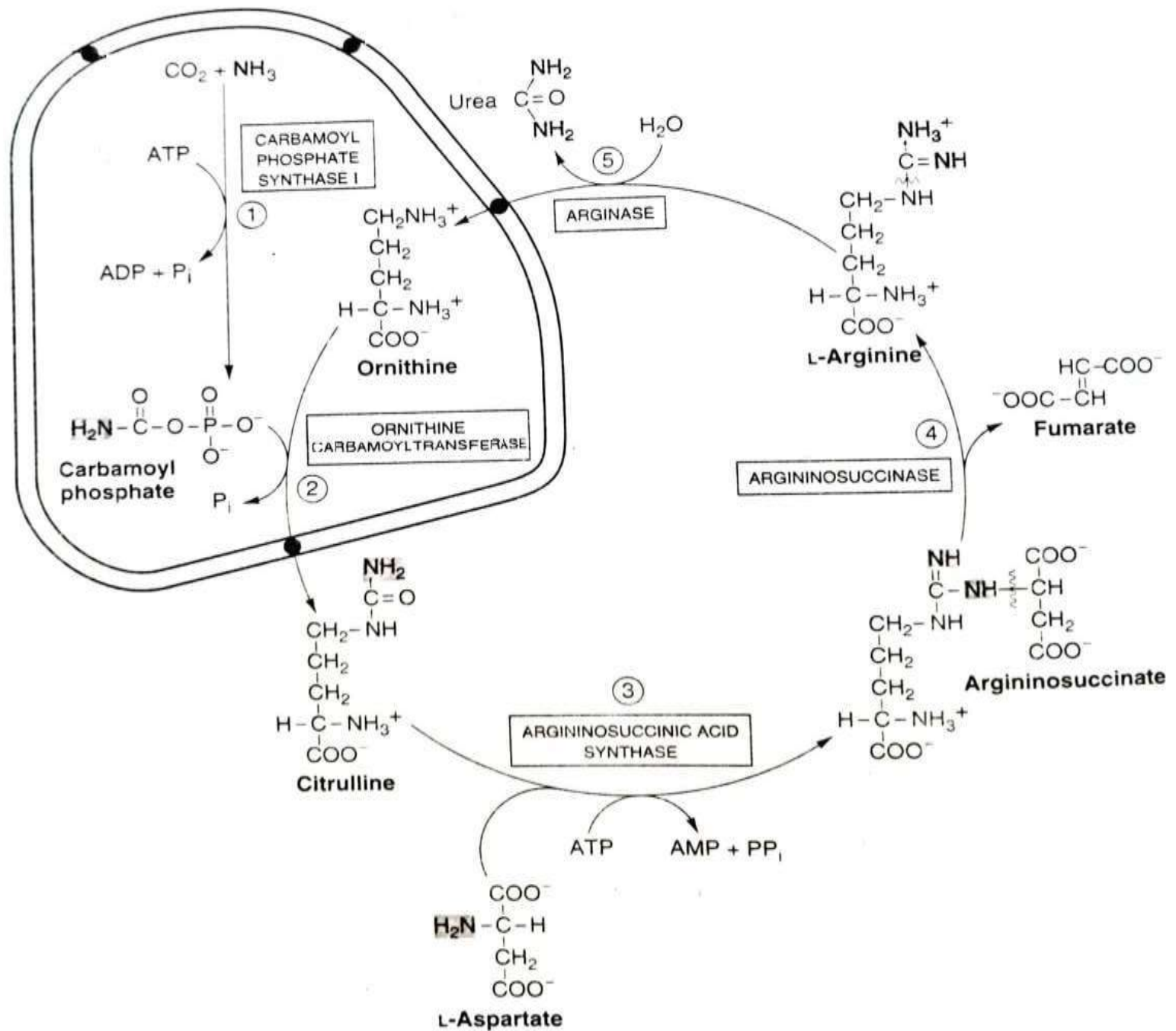


Alpha keto glutarate

+ NH₃

Urea Cycle

- It is the end process in amino acids catabolism . The NH_3 from all the body transport to the liver in form of glutamic acid , it is then enter urea cycle and then excreted as urea through the urine .
- Urea cycle needed for removal about 90 % of ammonia to the urea which is less toxic and can be excreted by normal kidney .
- Normal urea level in plasma is 20 – 40 mg/dL . Blood urea level is taken as indicator for renal function , increase when renal function is inadequate .
- Enzymes of urea cycle are distributed between Mitochondria and cytosol of the liver cells . The first 2 enzymes are located in mitochondria , whereas the 3 other enzymes are in the cytosol .
- Urea synthesis needs 4 moles of ATP .



Urea Cycle Disorder

- Urea cycle disorders are inborn error diseases caused by the deficiency of any enzymes of urea cycle which result in the increased of ammonia level in the blood(hyperammonemia)
- If the metabolic disorder occurs at enzyme 1 (carbamoyl phosphate synthetase) or enzyme 2 (ornithine carbamoyl transferase), the condition is more severe since the ammonia itself accumulates .
- Deficiency of later enzymes (enzymes catalyzed steps 3, 4, 5) result in the accumulation of other intermediates (citrulline , Argininosuccinate or Arginine) which are less toxic , and hence symptoms are less .
- Common symptoms to all urea cycle disorders include vomiting , lethargy , irritability , and mental retardation .
- Infants appear normal at birth , but within days the symptoms increased .

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Cholesterol Metabolism

Dietary cholesterol :

- Dietary cholesterol is found in 2 forms , free and esterified cholesterol
- Cholesterol ester found in the diet , is hydrolyzed to free cholesterol by cholesterol esterase .
- The free cholesterol is bound into bile salts micelles and absorbed to mucosal intestine cells together with the dietary free cholesterol and other lipids .
- About 30 – 60 % of dietary cholesterol is absorbed daily .
- Inside mucosal cells , free cholesterol is re – esterified by the action of Acyl Cholesterol Acyl Transferase (ACAT) enzyme , and bind with chylomicrons to extrahepatic tissues , mainly muscle and adipose tissue .
- Chylomicron is synthesized in mucosal intestine cells , and the main lipid in chylomicron is dietary T.G and then the cholesterol .
- In muscle and adipose tissue , Lipoprotein Lipase (LPL) enzyme hydrolyze the T.G from chylomicron , and obtain chylomicron remnant .
- Chylomicron remnant return to the liver and the dietary cholesterol is taken up by the liver .

Synthesized cholesterol :

- Cholesterol is synthesized in many tissues (endogenous cholesterol) , the liver is the main site of cholesterol synthesis . This cholesterol is transported to the extrahepatic tissues mainly muscle and adipose tissue binding with VLDL .
- VLDL synthesized in the liver , and the main lipid in VLDL is synthesized T.G and synthesized cholesterol .
- Hydrolysis of T.G from VLDL by muscle and adipose tissues obtained LDL which contain a higher amount of cholesterol.
- LDL is the important carrier of free cholesterol to the all tissues (liver and extrahepatic tissues)
- Cholesterol enters the tissues by the help of LDL – receptors .

Function of cholesterol in the tissues :

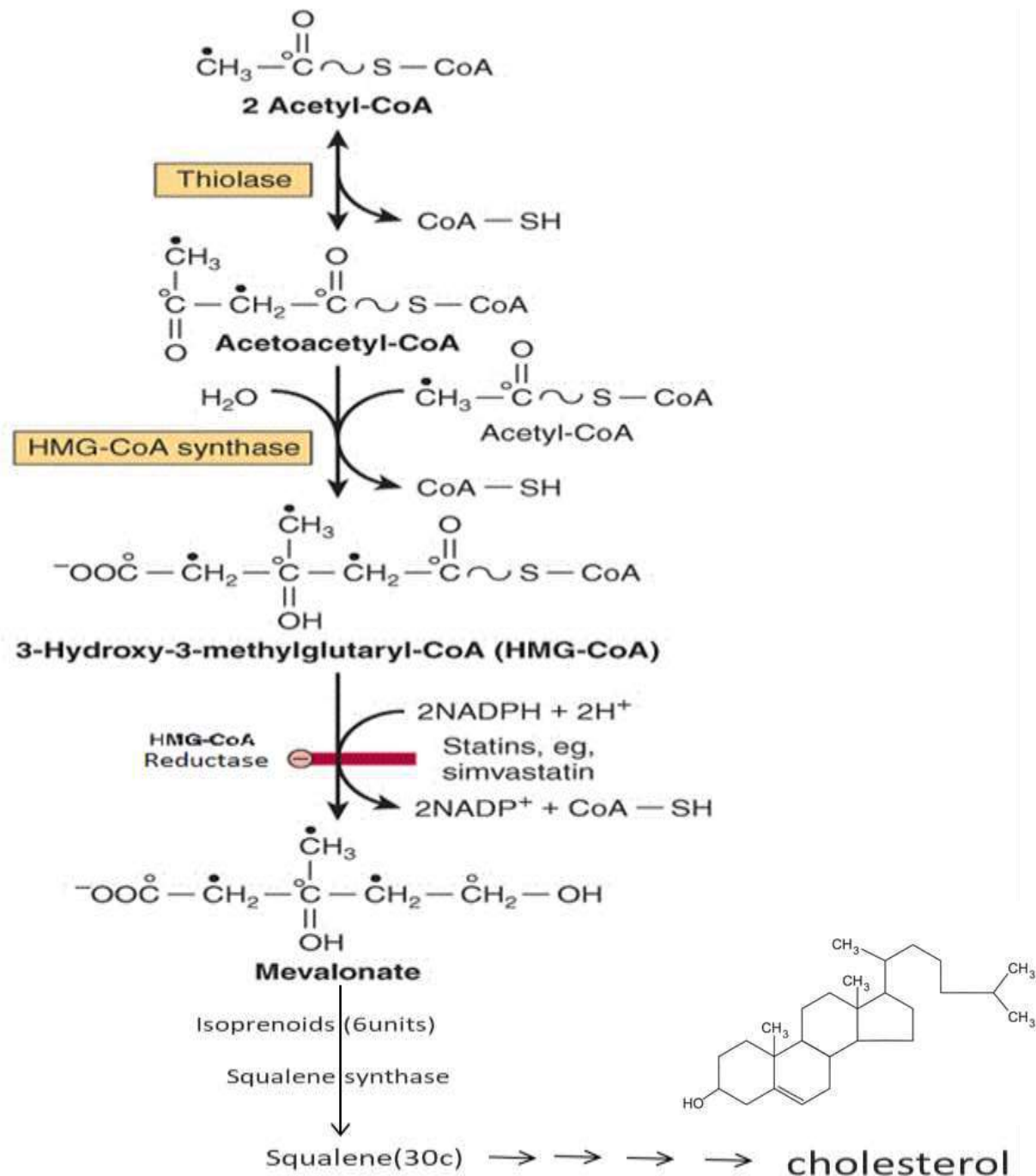
- Bound into the cell membrane
- Used for bile acids synthesis
- Used for steroid hormones synthesis
- Used for vitamin D synthesis

Biosynthesis of cholesterol

- Synthesis of cholesterol occur in the cytoplasm of many tissues , mainly in the liver :
- Condensation of 2 molecules of acetyl CoA by the help of cytoplasmic Thiolase enzyme forming acetoacetyl CoA .
- A third molecule of acetyl CoA is added by the help of HMG CoA synthase enzyme forming HMG CoA .
- Reduction of HMG CoA to mevalonic acid , this step catalyzed by HMG CoA reductase and 2 molecules of NADPH. It is a rate limiting step and HMG CoA reductase enzyme is the limiting enzyme of cholesterol synthesis .

Statin drugs inhibit HMG-CoA reductase enzyme, so **statin drugs** inhibit cholesterol synthesis and decrease the cholesterol level in blood .

- Six number of isoprenoids units (5 carbon atom each unit) are condensed with mevalonate to form squalene (30C) through a series of reactions requiring ATP molecules .
- By several steps including oxidation , cyclation , reduction , and elimination , the squalene converted into free cholesterol .



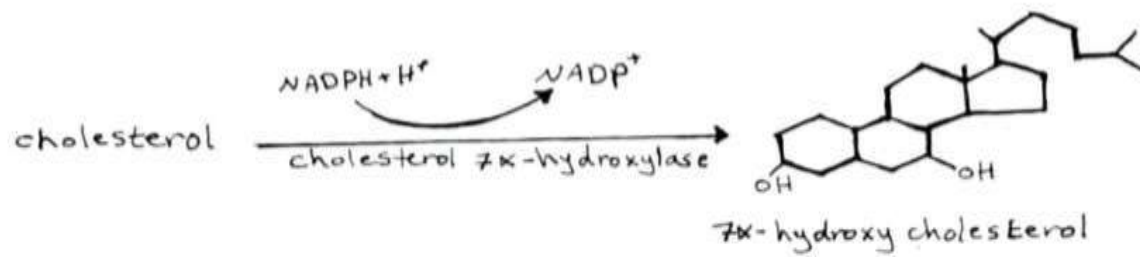
Catabolism of Cholesterol

- Cholesterol is removed from the tissues in form of cholesterol ester by HDL.
- HDL is a type of lipoprotein transport the esterified cholesterol from the all tissues to the liver where it is eliminated from the body.
- The ring structure of cholesterol cannot be metabolized to CO_2 and H_2O in human . Therefore , the cholesterol is eliminated from the body either unchanged or after conversion to bile acids .

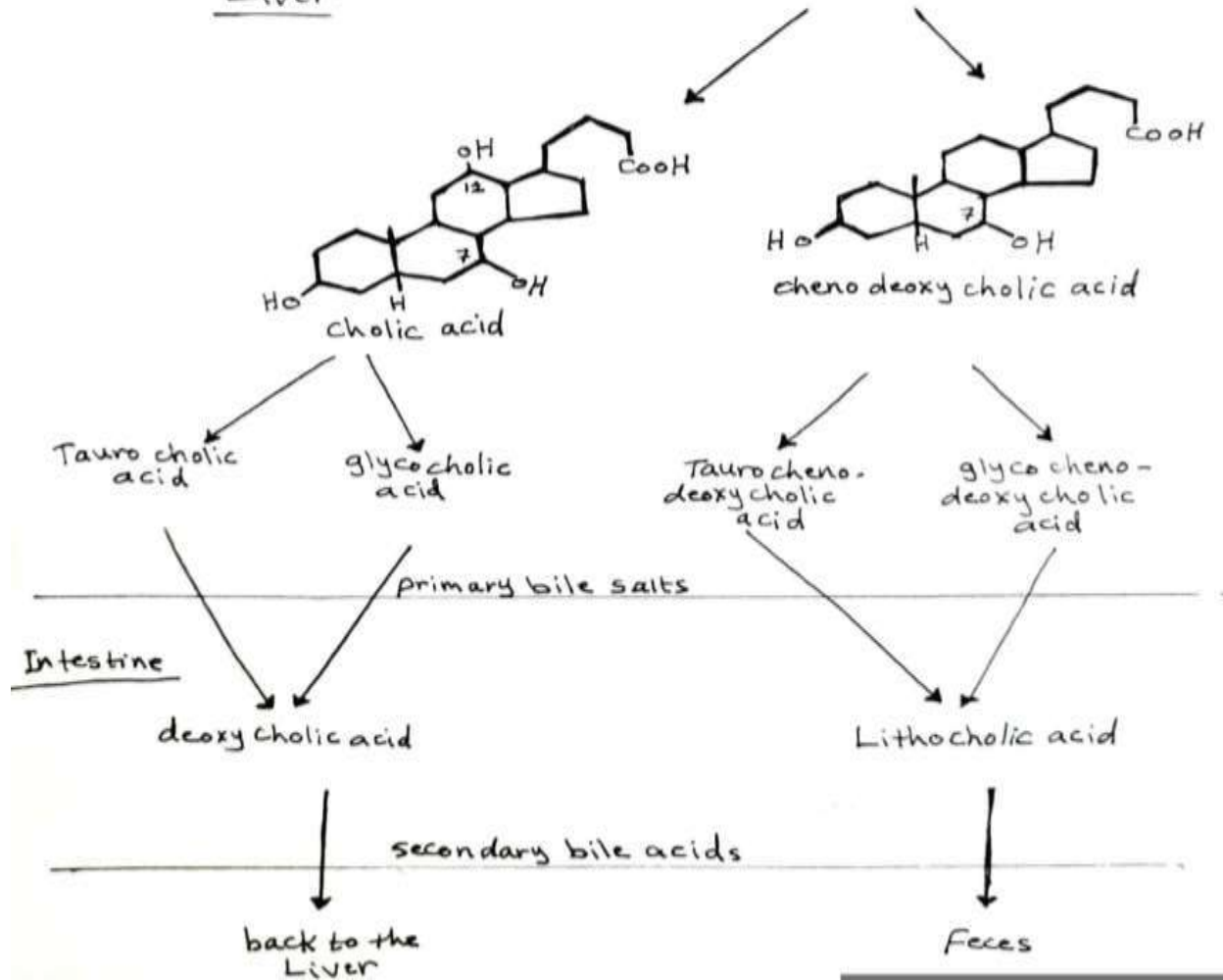
Synthesis of Bile Acids :

- Bile acids are synthesized in the liver , and stored in the gallbladder , and transported to small intestine to participate in the digestion and absorption of lipids .
- Hydroxylation of cholesterol at the 7 position by 7 α - hydroxylase enzyme .
- The double bond at C₅ – C₆ is reduced .
- The hydrocarbon chain at C₁₇ is shortened , and a carboxyl group is added at the end of the chain .
- The most common resulting compounds are Cholic acid and Chenodeoxy cholic acid . These 2 compounds called primary bile acids .
- Conjugation of bile acids with the amino acids **glycine** and **taurine** to form primary bile salts including : glycocholic acid , taurocholic acid , glycochenodeoxy cholic acid , taurochenodeoxy cholic acid.

- Bile salts are more effective than bile acids because of their higher amphoteric nature . Therefore , only the **bile salts** are found in the bile .
- Primary bile salts enter the small intestine . The bacteria in the intestine can remove the glycine and taurine regenerating bile acids .
- They can also convert some of the primary bile acids into secondary bile acids by removing the OH group , producing deoxycholic acid from cholic acid and Lithocholic acid from chenodeoxy cholic acid .
- More than 90% of bile acids (except Lithocholic acid) are return to the liver by the portal vein and re-used , this called Enterohepatic Circulation .
- Lithocholic acid because of its insolubility , it is eliminated in feces



Liver



Bile Salts Deficiency : Cholelithiasis

Cholelithiasis mean the precipitation of cholesterol in the gallbladder leading to cholesterol gallstone disease.

This disorder is typically caused by a decrease of bile acids in the bile , which may result from :

- Malabsorption of bile acids from the intestine
- Obstruction of biliary tract
- Severe hepatic dysfunction leading to decreased synthesis of bile acids
- Cholelithiasis also may result from increased biliary cholesterol excretion , as seen with the use of fibrate drugs (drug used to reduce the T.G level in blood)

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Metabolism of Proteins

Digestion and Absorption of Proteins

Proteases or **proteolytic enzymes** , this is a general name for all enzymes that hydrolyzed the protein molecules .

(1) Gastric digestion of proteins :

- Digestion of proteins begins in stomach by the action of **pepsin** . This enzyme is synthesized in stomach cells as inactive called **pepsinogen** .
- The conversion of pepsinogen to pepsin occur by the removal of 44 amino acids .
- Pepsin acts in acidic medium ($\text{pH} = 1.5 - 2.5$) formed by HCl acid .
- Pepsin hydrolyzes the peptide bonds between phenyl alanine , tyrosine , tryptophane , and methionine . The protein molecule is broken into **small peptides** .

(2) Pancreatic digestion of proteins :

The next step of digestion occur in the intestine by the action of pancreatic enzymes :

- **Trypsin**
- **Chemotrypsin**
- **Elastase**

- **Carboxypeptidase**

The end product of pancreatic enzymes are **tripeptides** and **dipeptides**.

(3) Intestinal digestion of proteins :

the dipeptides and tripeptides are completely digested to free amino acids by **aminopeptidase** , enzyme in the mucosal surface of small intestine .

di- and tripeptides --- aminopeptidase-----→ Amino Acids

this is the dietary source

(exogenous source)

Absorption of Amino Acids

The absorption of amino acids occur by the active transport which is an energy required process , then the amino acids are transported to the different tissues.

Endogenous source : Amino acids come from the breakdown of tissue proteins especially muscle proteins (about 1-2% of tissues proteins are hydrolyzed to amino acids daily).

Inside the tissues, amino acids can be used in different processes such as:

(1) About 80% of amino acids are used for synthesis of new proteins which included :

- Tissue proteins
- Hemoglobin
- Plasma proteins
- All enzymes (tissues and plasma enzymes)
- Some hormones (pituitary , thyroid and parathyroid , pancreatic and gastrointestinal hormones)

(2) Other part used for synthesis of biological compounds needed by the cell called non-protein nitrogenous compounds , for example :

- Amino sugars
- Bases of nucleic acids
- Neurotransmitters such as serotonin and acetyl choline
- Heme which are important in hemoglobin structure

(3) Excess amino acids that are not needed for synthesis , undergo catabolic reactions and used for energy supply , this is because amino acids cannot be stored .

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Body Stores Of Fat

- Fatty acids are stored in adipose tissue in the form of T.G .
- T.G is the major energy reserve of the body.
- Liver and adipose tissue are the main sites of T.G synthesis .
- T.G in adipose tissue is used for energy storage , whereas in liver , the T.G is transported to extra hepatic tissue by VLDL .

(a) Adipose tissue in well – fed condition:

Under well – fed condition , the synthesis of T.G in adipose tissue is increased.

The dietary T.G transported by chylomicrons , and the endogenous T.G by VLDL ,

Both are hydrolyzed by lipoprotein lipase (LPL) enzyme present on the capillary wall of adipose tissue .

Glucose and insulin levels are increased . Insulin increase the uptake of glucose to adipose tissue , stimulate glycolysis , and stimulate hexose mono phosphate shunt (HMPS) . This effect of insulin increase the T.G synthesis .
How ?

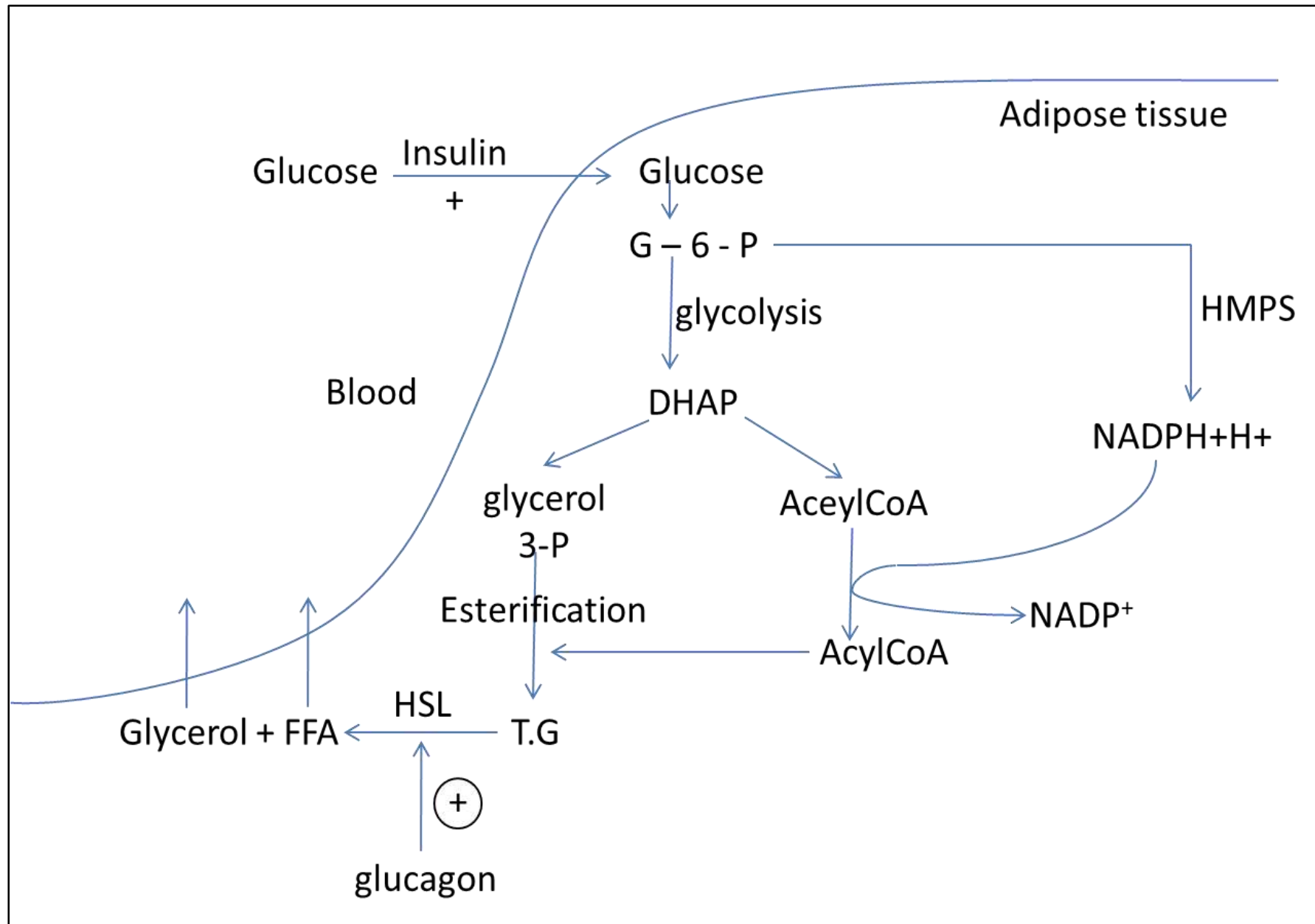
Insulin also inhibits hormone sensitive lipase (HSL) enzyme , so the breakdown of T.G is decreased .

(b) Adipose tissue in fasting condition:

Under conditions of fasting , T.G in adipose tissue is hydrolyzed by the effect of glucagon and adrenaline .

Glucagon and adrenalin activate the HSL enzyme , found in adipose tissue cells which hydrolyze the T.G and liberate FFA_s .

These fatty acids are taken up by peripheral tissues especially the liver and used as a fuel .



Fatty Liver

The fat (T.G) in normal liver is about 5% of its weight .

When this ratio reaches about 25 - 30 % , this case called fatty liver .

So , fatty liver refers to the deposition of excess T.G in the liver cells .

If this case is prolonged , the liver cells become fibrotic , cirrhosis , and impaired the liver functions .

Causes of fatty liver

- Increase the level of plasma FFA_s resulting from the mobilization from adipose tissue . This seen in diabetes mellitus .
- Excess carbohydrates or fats are deposited as fats , hence obesity may be related to fatty liver
- Toxic injury to the liver due to the poisoning by compounds like carbon tetrachloride (CCL₄) , arsenic , lead , chloroform , leading to decrease production of VLDL , which leads to accumulation of T.G in liver .
- Alcoholism .

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Gluconeogenesis

Definition :

Formation of glucose from non-carbohydrate sources , these include :

Alanine , lactate , glycerol , and propionyl CoA .

Gluconeogenesis occur mainly in liver ,

Liver is the main tissue for Gluconeogenesis because G-6-phosphatase enzyme present mainly in liver , so liver plays the major role in maintaining the blood glucose level .

During starvation , the stored glycogen is depleted within 12 – 18 hours of fasting . The Gluconeogenesis is increased and protein catabolism increased to provides the substrates for Gluconeogenesis .

The pathway is partly occur in mitochondria and partly in cytoplasm.

Gluconeogenesis involved the reversible steps of glycolysis , but it is not a reversal of glycolysis .

The key enzymes of gluconeogenesis are :

Pyruvate carboxylase

PEP-Carboxy kinase

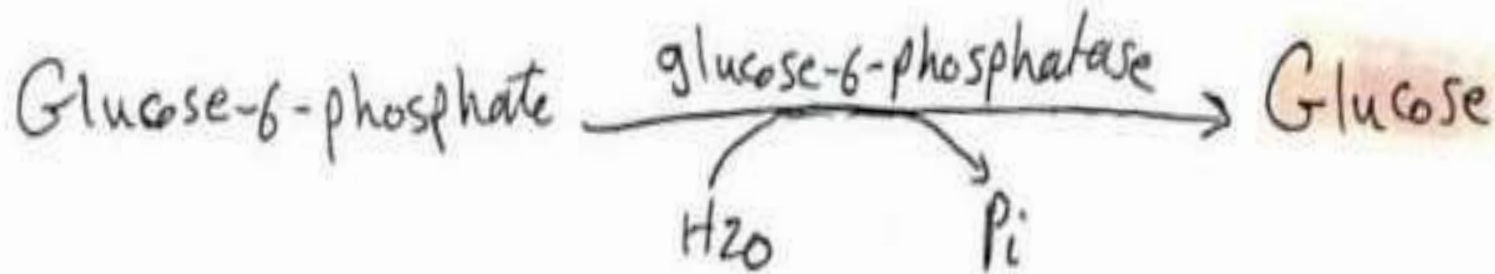
F-1,6-bisphosphatase

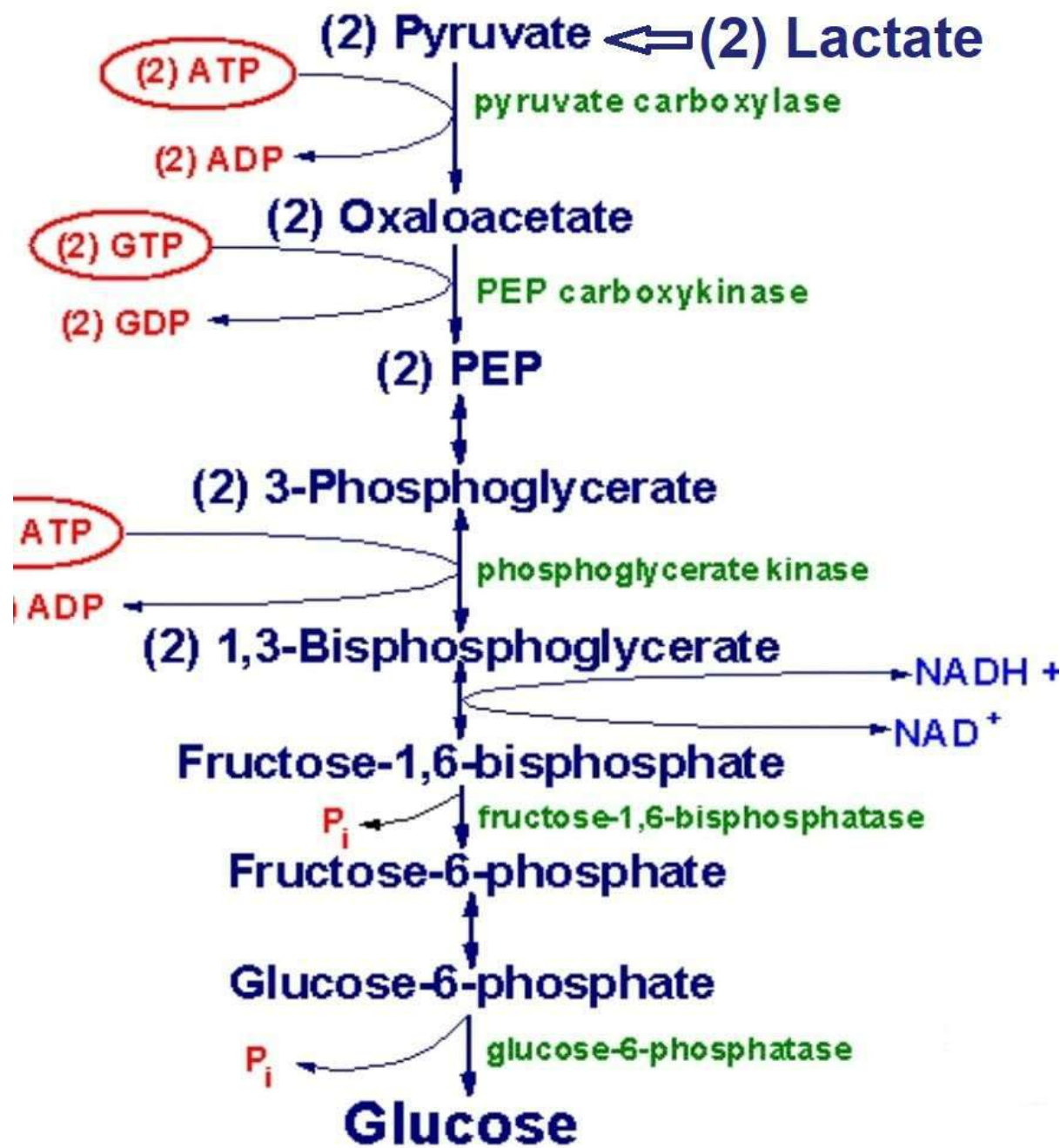
G-6-phosphatase

Glucose-6-phosphatase :

G-6-P is hydrolyzed to free glucose by glucose-6-phosphatase .

This enzyme is active in liver , but it absent in muscle and adipose tissue .





Importance of Gluconeogenesis

Gluconeogenesis is necessary for the body to :

- Maintain basal level of glucose to brain and RBCs when carbohydrates is not available in sufficient amount from diet .

Failure of Gluconeogenesis usually causes hypoglycemia , which leads to brain dysfunction that result in coma and death .

- Clear the blood from lactate produced by muscle and RBCs. Lactate from muscle enter the blood , and then reach the liver. In the liver , lactate is oxidized to pyruvate and channeled to gluconeogenesis . The generated glucose can enter into blood

Gluconeogenesis is regulated by hormones :

- Glucagon activates the gluconeogenesis by activation the key enzymes .

At the same time , glucagon inhibits the key enzymes of glycolysis pathway .

- Insulin inhibits the gluconeogenesis by inhibition the key enzymes.

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Glycogen Metabolism

Function of Glycogen

- Glycogen is the storage form of carbohydrates in the human body . The major sites of glycogen storage are liver and muscle .
- The major function of liver glycogen is to provide glucose during fasting . The glycogen content of liver (10 g / 100 g tissue) is more than in the skeletal muscle (1-2 g / 100 g tissue) . But the total amount of muscle glycogen is more than liver glycogen because of the larger muscle mass .
- After taking food rich in carbohydrates , blood glucose tends to rise and glycogen synthesis occur in liver and muscle .
- About 5 hours after taking food , the blood glucose tends to fall , liver glycogen hydrolyzed into glucose to maintain the blood glucose level , and to supply energy needed for brain .
- After about 12-18 hour of fasting ,most liver glycogen is finished so the energy is supplied by gluconeogenesis and fatty acid oxidation .

The function of muscle glycogen is to act as a source of fuel for muscle contraction .

Note : All enzymes related to glycogen metabolism are located in cytoplasm.

Catabolism of Glycogen (Glycogenolysis)

Definition : Break down of glycogen in liver and muscle . Glucose is the main end product in liver, while lactate is the main product in muscle.

Glycogenolysis needed when there is a need of energy for the body , like fasting or starvation or during muscle exercises .

(1) Glycogen phosphorylase :

The first step of glycogenolysis begins with highly specific enzyme called glycogen phosphorylase . This enzyme is found in both active and inactive forms

The active form is called phosphorylase a

The inactive form is called phosphorylase b

The active phosphorylase removes the glucose from all the branches of glycogen as a glucose-1-P . This enzyme hydrolyzes the α -1,4-glycosidic linkages and releases glucose units one at a time till it reaches 4 glucose units away from a branch point .

(2) Transferase :

A block of 3 glucose units are transferred from the branching point to another branch . This enzyme is α -1,4 \rightarrow α -1,4-glycan transferase .

(3) Debranching enzyme :

Now the branch point is free , so the α -1,6-glucosidase (debranching enzyme) can removing the glucose unit held in α -1,6-linkage at the branch point , converting the branch point to a linear one . This glucose unit released as free glucose .

With the removal of the branch point , further action of phosphorylase enzyme which leads to the complete breakdown of glycogen .

(4) Phosphogluco mutase :

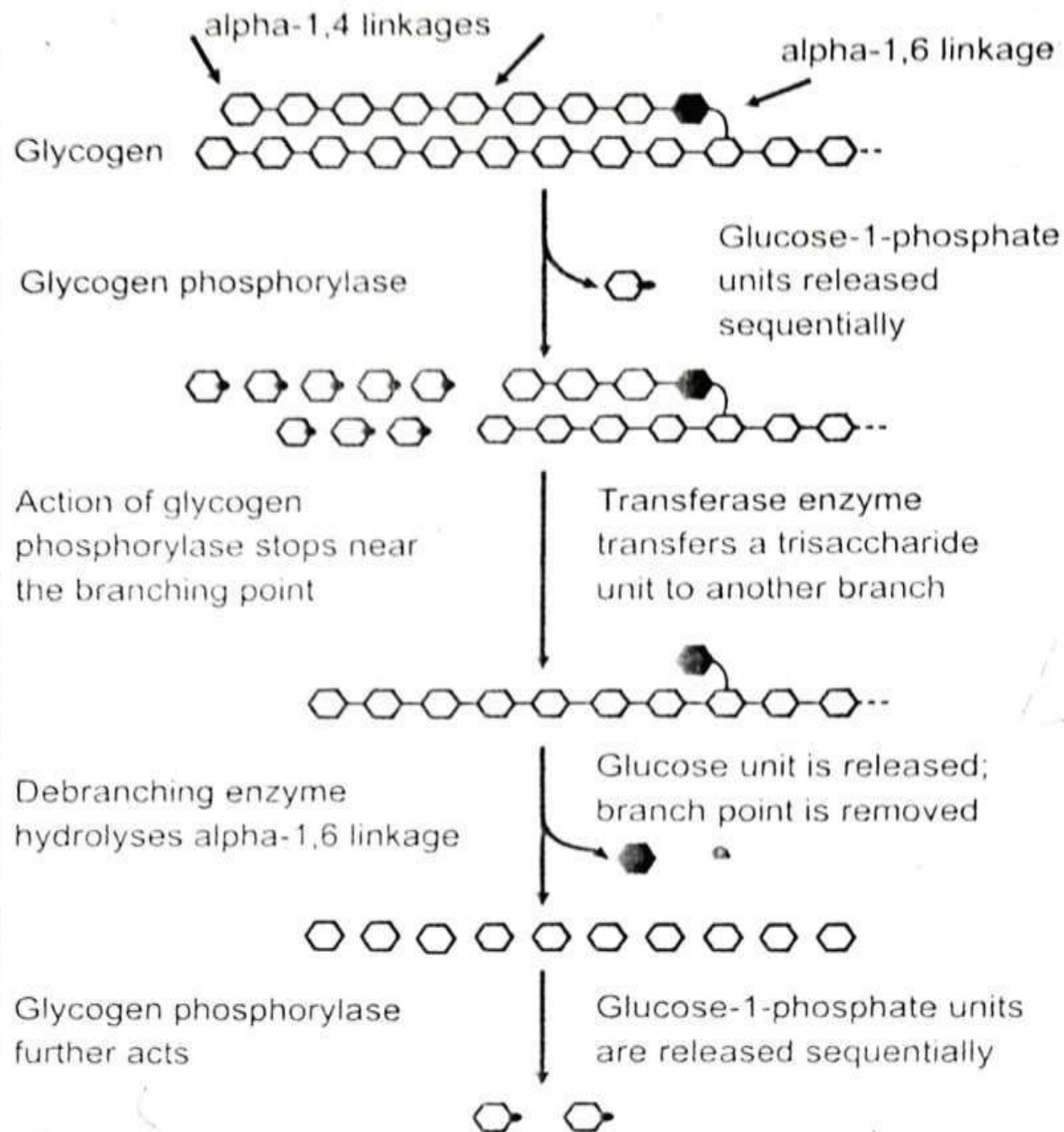
Glucose-1-P is converted to glucose-6-P by phosphogluco mutase .

(5) Glucose-6-phosphatase in liver :

Hepatic glucose-6-phosphatase hydrolyzes glucose-6-P to free glucose . The free glucose releases to the blood stream .

Muscle lacks Glucose-6-phosphatase :

Glucose-6-phosphatase found only in liver , but it absent in the skeletal muscle , so muscle will not release the glucose to blood stream , but instead glucose-6-P undergoes glycolysis to produce ATP for muscle contraction



Glycogen synthesis (Glycogenesis)

The glycogen is synthesized from glucose , this is occur mainly in liver and skeletal muscle . The pathway of glycogen synthesis is completely different from the pathway of glycogen break down .

The steps are :

- Glucose-6-P converted to glucose-1-P by the action of **isomerase**, a reaction which is reversible .



- UDP-glucose formation from glucose-1-P and uridine triphosphate (UTP) by the enzyme glucose-1-P uridine transferase



(3)The glucose part from UDP-glucose is transferred to a glycogen primer molecule which is made up of a protein-carbohydrate complex . This step is catalyzed by **glycogen synthase enzyme** through the formation of glycosidic bond at α -1,4-linkage .

Glycogen primer (n) + UDP-glucose ----->

glycogen(n+1) + UDP

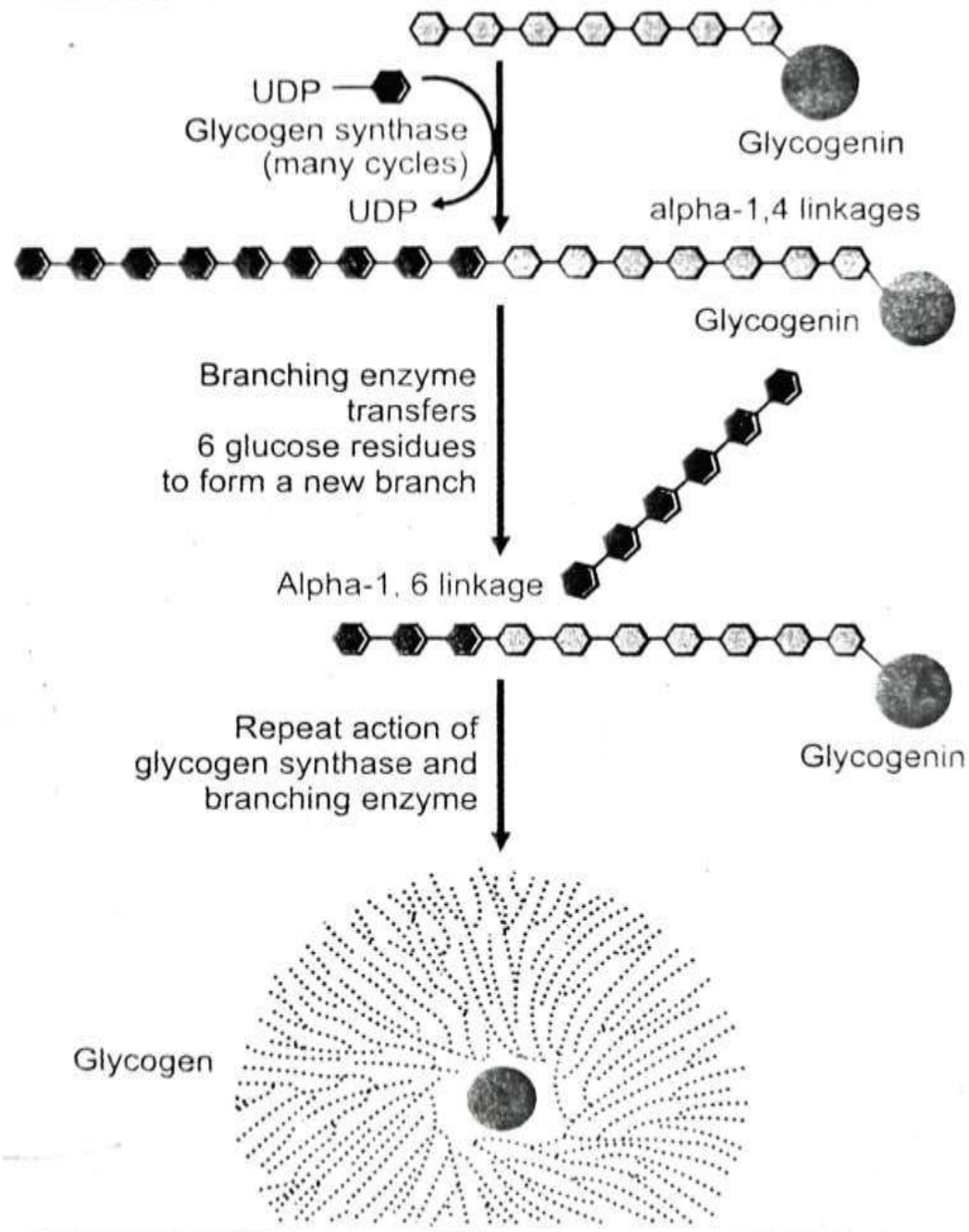
In the next step , the activated glucose unit will continue added to the outer end of the glycogen primer to form an α -1,4-linkage until 11-12 glucose molecules are attached together in the chain .

Branching enzyme

Glycogen synthase will inhibit and the branching enzyme will activate . This enzyme will build up the branching site of the chain. i.e it forms the α -1,6-glucosidic bond .

This enzyme will transfer a part of 6-8 glucose units from this chain to another site on the growing molecule .

At this level of building , the **branching enzyme** is inhibited and the **glycogen synthase** is again activated to add glucose molecules in α -1,4-linkage , and this will continue until the new α -1,4-chain reach maximum up to 14 glucose molecules . Then **branching enzyme** is activated again and **glycogen synthase** inactivated , and this will continue until the whole glycogen structure is formed .



Regulation of glycogen metabolism

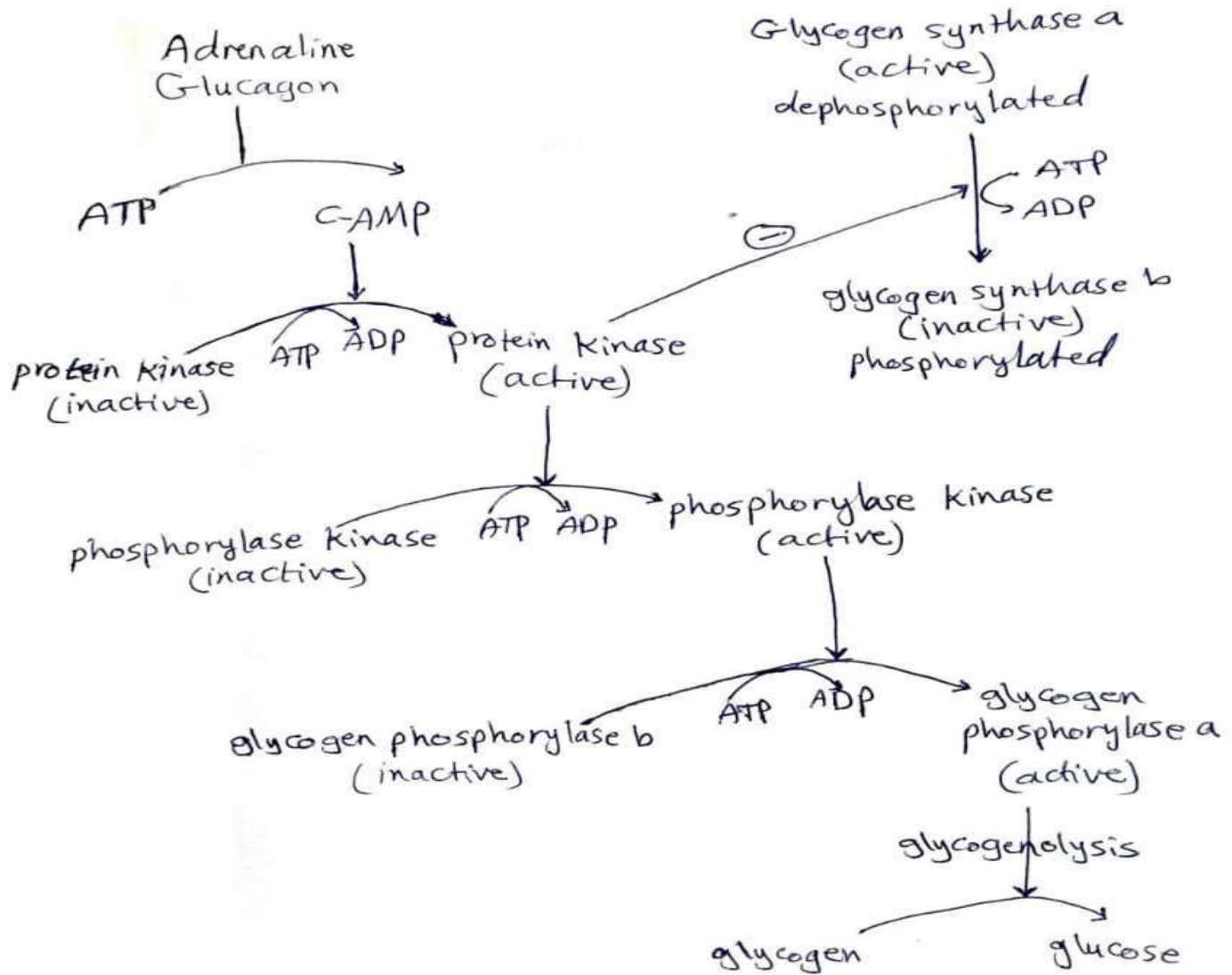
The principle enzymes controlling glycogen metabolism are glycogen synthase and glycogen phosphorylase . They are regulated by allosteric mechanism and by covalent modification mechanism .

Covalent Modification (Phosphorylation and dephosphorylation)

Phosphorylase enzyme becomes active (Phosphorylase a) on Phosphorylation , the inactive form (Phosphorylase b) is dephosphorylated .

Phosphorylation process decreases the activity of glycogen synthase , but dephosphorylation increases it .

- The covalent modification of glycogen Phosphorylase and glycogen synthase is controlled by the hormones .
- Adrenaline and glucagon can activate liver glycogen Phosphorylase , but glucagon has no effect on muscle glycogen Phosphorylase because receptors for glucagon is not present in muscle .
- Glycogen synthase activity is increased by insulin hormone and inhibited by adrenaline .
- These hormones act through the second messenger cyclic-AMP .
- Adrenaline and glucagon increased the level of C-AMP in the cells , which activates the glycogenolysis through cascade reactions .



[Cascade reactions for
glycogen break down]

Glycogen Storage Diseases

These are inborn errors of metabolism .

Glycogen storage disease type (1) :

- It is also called **Von Gierke's disease** . It is the most common type of glycogen diseases .
- Incidence is 1 in 100,000 live births . Salient features of the disease are :
- G-6-phosphatase enzyme is deficient .
- G-6-P is accumulated , so it channeled to HMPS and producing more ribose and then more nucleotides .
- Purins are then catabolized to uric acid leading to hyperuricemia .

- Fasting hypoglycemia that does not respond to stimulation by adrenaline .
- Hyperlipidemia , lactic acidosis , and ketosis .
- Glycogen accumulates in liver leading to liver enlargement , which may lead to cirrhosis
- Children usually die in early childhood

Note : Other types of glycogen storage diseases are very rare , incidence being 1 in million births .

Regulation of Blood Glucose

- The concentration of blood glucose during fast state is between 70 – 110 mg / dL.
- Fasting state means , glucose is estimated after overnight fast , i.e 8 hour after the food intake .

Auto-regulation of blood glucose :

- Following a good meal of carbohydrates , glucose is absorbed from intestine and enters the blood , the blood glucose rises to 120 – 140 mg /dL . The rise in blood glucose level will stimulate the secretion of insulin which lead to the taken up of glucose and utilizing it by most tissues .

Fasting State Regulation:

About 8-10 hours after a meal , blood glucose level normally falls to fasting levels . In this case , the secretion of insulin will decrease , and the secretion of glucagon and adrenaline will increase , and the liver will supply the blood with glucose by the breakdown of glycogen (**hepatic glycogenolysis**) , and by **gluconeogenesis** .

The plasma glucose level at these normal ranges depends on the balance between two processes :

Rate of glucose entering the blood

Rate of glucose removing from the blood

Factors leading to entry of glucose into the blood are :

- Absorption from intestine
- Glycogenolysis in liver
- Gluconeogenesis

Factors leading to removal of glucose from blood are :

- Glycolysis and krebs cycle in tissues
- Glycogenesis in liver and muscle
- Synthesis of T.G in adipose tissues
- Excretion in urine

Insulin hormone

- Insulin is the only hypoglycemic hormone , where the rest hormones are hyperglycemic in nature .
- Insulin is a protein consist of 51 amino acid , secreted by β -cells of the islets of Langerhans in the pancreas
- Insulin has anabolic action , activates the synthesis of glycogen , T.G , and proteins .

Metabolic Effects of Insulin on Carbohydrates :

- Increase the uptake of glucose by the cells .
- Stimulate the utilization of glucose through glycolysis and krebs cycle .
- Stimulate the glycogen synthesis (glycogenesis) in liver and muscle
- Inhibit the breakdown of glycogen (glycogenolysis) in liver by inhibiting C-AMP
- Inhibit gluconeogenesis .

Metabolic Effects of Insulin on Lipids :

- Inhibits the breakdown of T.G , by inhibiting the **hormone sensitive lipase (LPL)** enzyme in adipose tissues .
- Stimulate the synthesis of T.G in adipose tissues

Metabolic Effects of Insulin on Proteins :

- Insulin stimulate the synthesis of proteins in most tissues by stimulating the uptake of amino acids by the tissues . So insulin reduces the amino acids needed for the gluconeogenesis .

Diabetes Mellitus (DM)

It is a metabolic disease characterized by chronic hyperglycemia and abnormal metabolism of carbohydrates and lipids . This is due to absolute or relative insulin deficiency .

It is defined as the concentration of blood glucose in fasting state is greater than 140mg /dL .

Classification

Diabetic patients can be classified into 2 types according to the need for insulin :

***Type 1:* Insulin Dependent Diabetes Mellitus(1DDM)**

***Type2:* Non-Insulin Dependent Diabetes Mellitus (N1DDM)**

Type 1 : Insulin Dependent Diabetes Mellitus (IDDM)

- About 5% of total diabetic patients are of type 1 .
- It is due to decreased in insulin production , so the insulin level in blood is absent or very low .
- Type 1 appears in the early years of life , during the first or second decade of life .
- It is autoimmune disease usually stimulate by viral infection which has destroyed the β -cells of pancreas and therefore decreasing the production of insulin .
- The patients of this type are dependent on insulin injections which is necessary for treatment .

Type 2 : Non-Insulin Dependent Diabetes Mellitus (NIDDM)

- It is inherited hyperglycemia , caused by impaired the function of β -cells of pancreas , and hence fails to produce enough insulin .
- In some cases , it is due to insulin resistance , i.e. the patient have a normal level of insulin in blood , but it is not utilized due to decrease in the number of insulin receptors .
- This type is mainly occur in obese , and it seen in middle aged (above 45 year) .
- It is the common type , and about 95% of the diabetic patients are belonged it .
- Oral hypoglycemic drugs are mainly used in treatment type 2 . Insulin injections also used when oral drugs are not sufficient .

Chronic Complication of DM

- ***Vascular diseases*** : It is the common complication of diabetes which result in atherosclerosis or myocardial infraction .
- ***Complication in eye*** : Which include formation of cataract of lens and retinal diseases .
- ***Peripheral Neuropathy*** : Which result in risk of foot ulcers and gangrene .
- ***Nephropathy*** : Excretion of more than 300 mg /day of glucose in urine , indicates the presence of nephropathy , which can lead to renal damage .

Glycated Hemoglobin (HbA₁C)

- When blood glucose increase (hyperglycemia) , it will binding to proteins in a process called **glycation** .
- When glycation occur in tissues , it will lead to complication of diabetes mellitus , like Neuropathy , Nephropathy , and retinal diseases .
- When glycation occur in blood (hemoglobin) , it will form glycated hemoglobin which called **HbA₁C** .
- In HbA₁C , the glucose is attached to the valine of β -chain of hemoglobin.
- Measurement of HbA₁C is important for :

(1) *Diagnosis of diabetes mellitus*

(2) *Follow up of diabetic patients*

- The value of HbA₁C gives the actual mean of glucose level over 10-12 weeks ago.
- It is the best index to control the blood glucose level for long time.
- The binding of glucose with Hb is irreversible , so the glucose is not removed from Hb , and it remains inside the RBC through the life span of RBC (120 day).
- The measurement should be done every 3 months in all diabetic patients.

Values of HbA₁C :

- Normal range of HbA₁C is between (4% - 5.6%) or (68 – 110) mg / dL
- HbA₁C level between(5.7% - 6.4%) or (111 – 145)mg / dL mean there is a higher chance for getting diabetes .
- HbA₁C level of (6.5%) or higher , mean the person have diabetes .

Prof . Dr . Samera Alkatib

Glycolysis

Glycolysis (EMBDEN - MEYERHOF Pathway)

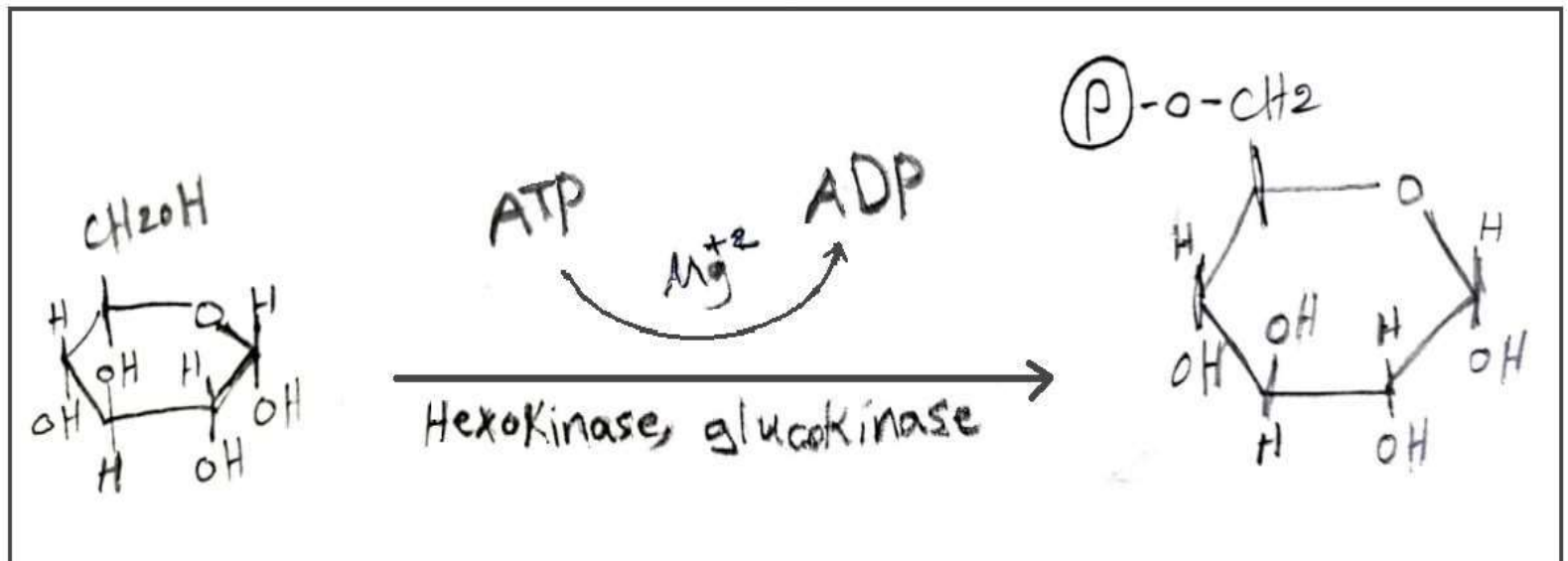
Definition :

Oxidation of glucose to pyruvate in aerobic condition , or to lactate under anaerobic conditions , with small quantity of energy produced per mole of glucose.

- Glycolysis is the only pathway that is taking place in all the tissues of the body to provide the energy .
- All enzymes of glycolysis are found in the cytoplasm , so the site of reaction steps take place in the cytoplasm .
- In heavy work , when muscle tissue lacks enough oxygen , anaerobic glycolysis forms the major source of energy for muscles . In eye cells , anaerobic glycolysis is the important pathway .
- Anaerobic glycolysis is the only source of energy in erythrocytes .
- The glucose is phosphorylated in all steps by binding the glucose with phosphate group .
- Most of the reactions of the glycolysis are reversible which are also used for gluconeogenesis pathway .

Steps of Glycolysis

Step 1 : Glucose is phosphorylated to glucose -6-P by the enzyme **hexokinase** or **glucokinase** , which split the ATP into ADP and the P_i is added to the glucose . Hexokinase is allosteric enzyme inhibited by its product glucose-6-P .

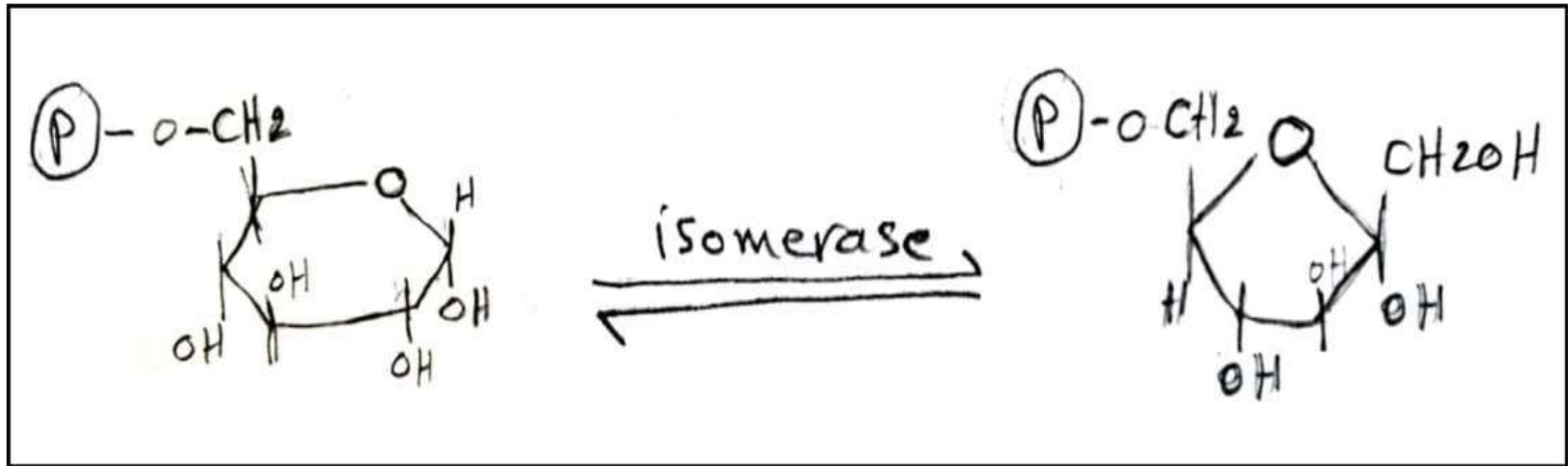


The 2 phosphorylated enzymes differ in the following :

- Hexokinase is found in all tissues , while glucokinase present in liver mainly .
- Hexokinase is non- specific enzyme , because it can phosphorylate any hexose sugar , glucose , fructose , galactose , and mannose . While glucokinase is highly specific enzyme and act only on glucose .
- Hexokinase is not induced by insulin , while glucokinase is under influence of insulin .
- Hexokinase acts when blood glucose level is low , and then the glucose utilized by all body cells . **Glucokinase acts only when blood glucose level is more than 100 mg / dL , then glucose is taken up by the liver cells for glycogen synthesis .**

Step 2 :

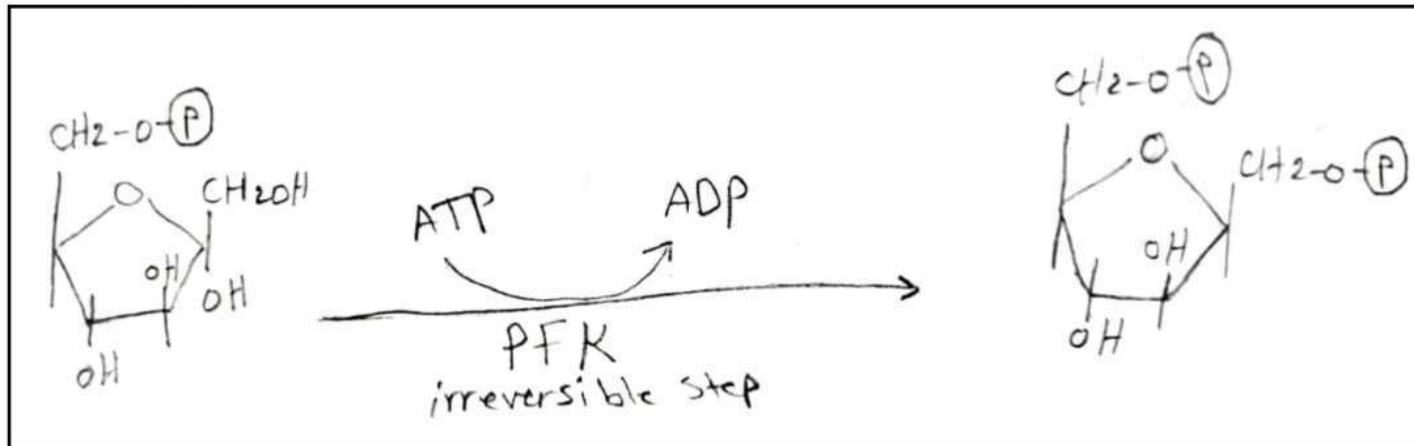
Isomerization of glucose-6-P to fructose-6-P by the action of **isomerase** . This is a reversible step .



Step 3 :

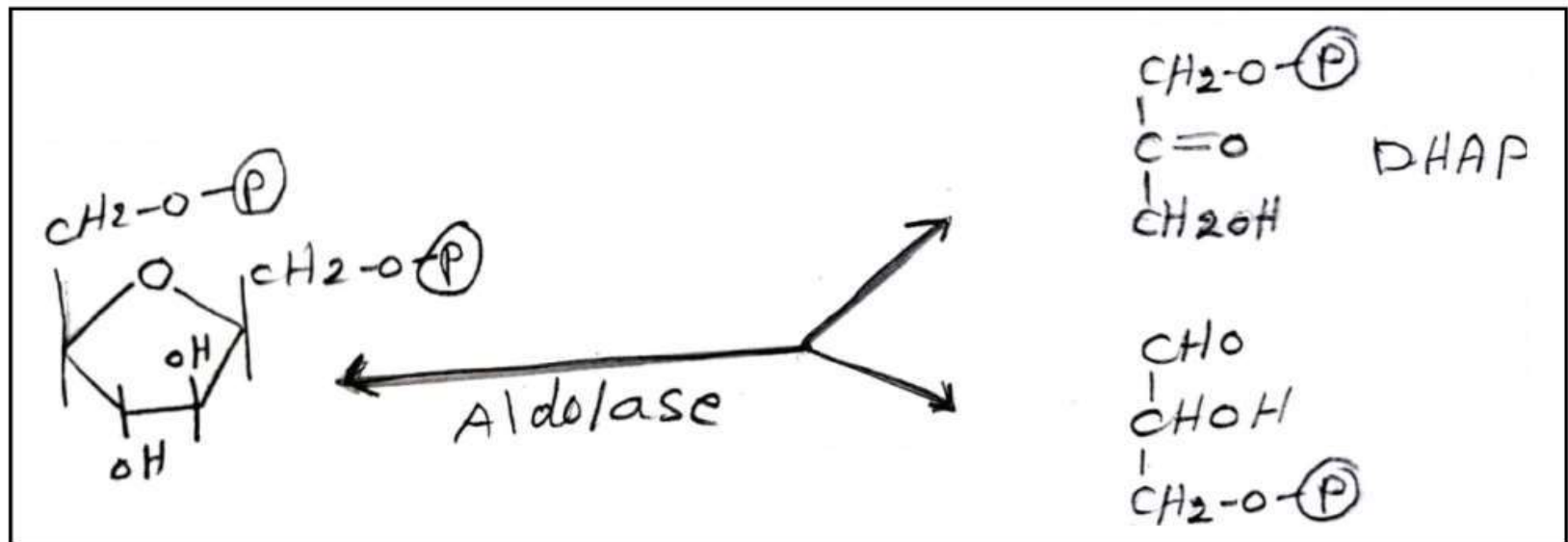
Phosphorylation of fructose-6-P into fructose-1,6-bisphosphate by enzyme called **phosphofructo kinase (PFK)** which need one molecule of ATP hydrolyzed to ADP .

This is an irreversible step , and it is the rate limiting reaction in glycolysis . PFK is an allosteric enzyme , it is an important key enzyme of this pathway.

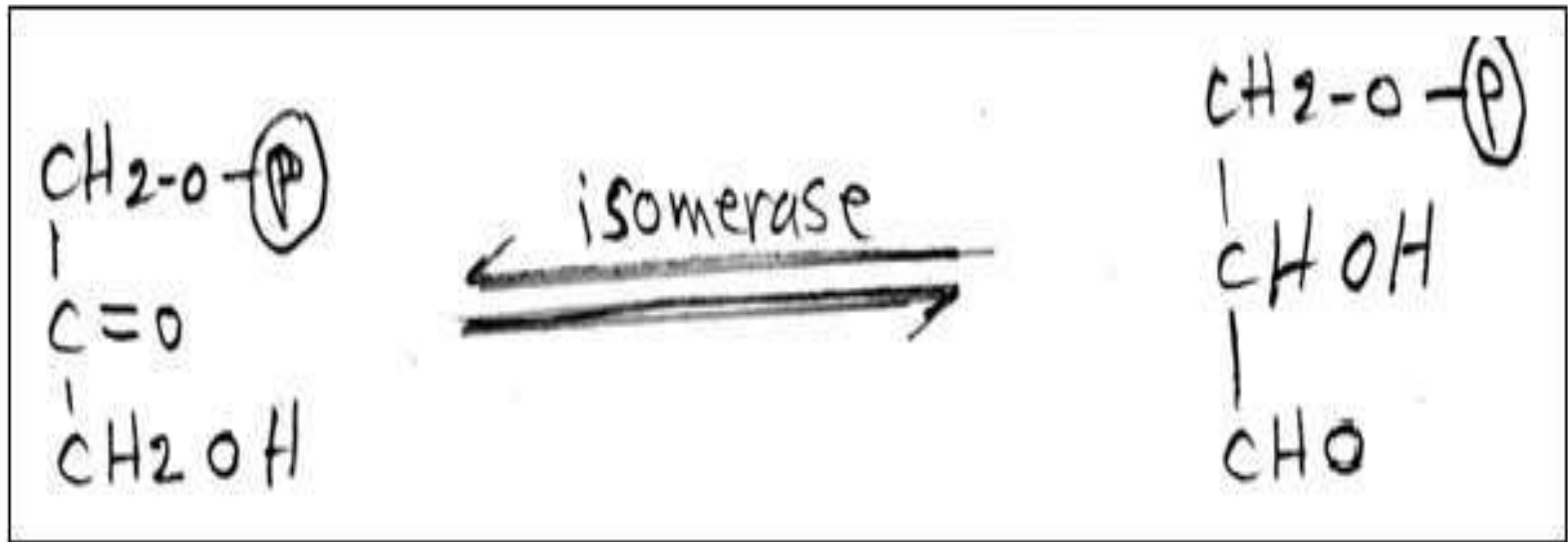


Step 4 :

Splitting of fructose-1,6-bisphosphate into two compounds (each with 3 carbon atom) , glyceraldehyde-3-P and dihydroxy acetone phosphate (DHAP) . This reaction is reversible and occur by **aldolase** enzyme .



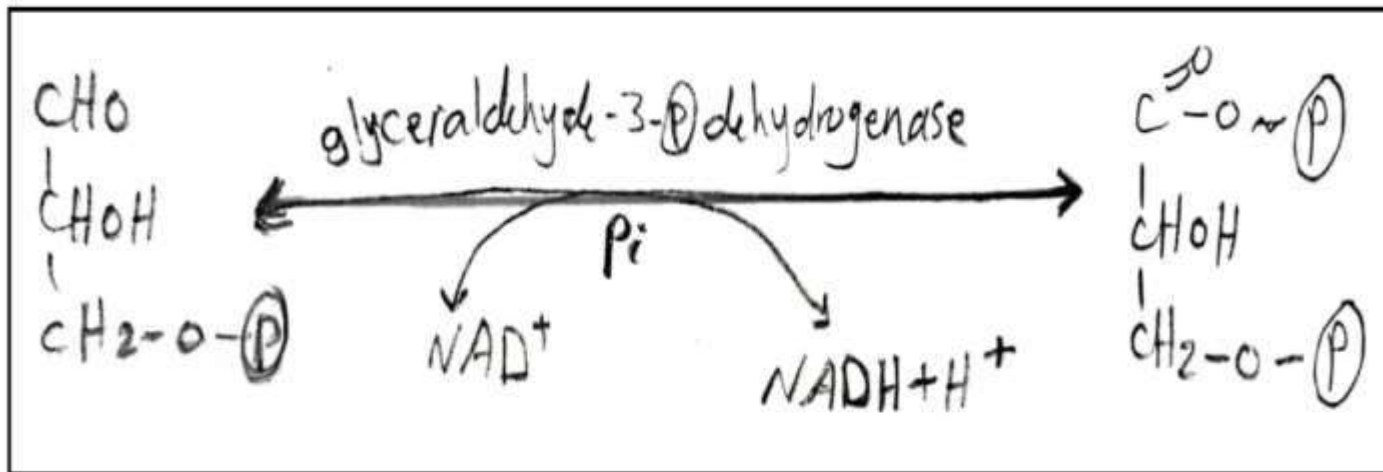
- **DHAP** is isomerized to **glyceraldehyde-3-P** by the isomerase enzyme , thus the net result is that glucose converted into 2 molecules of **glyceraldehyde-3-P** .



Step 5 :

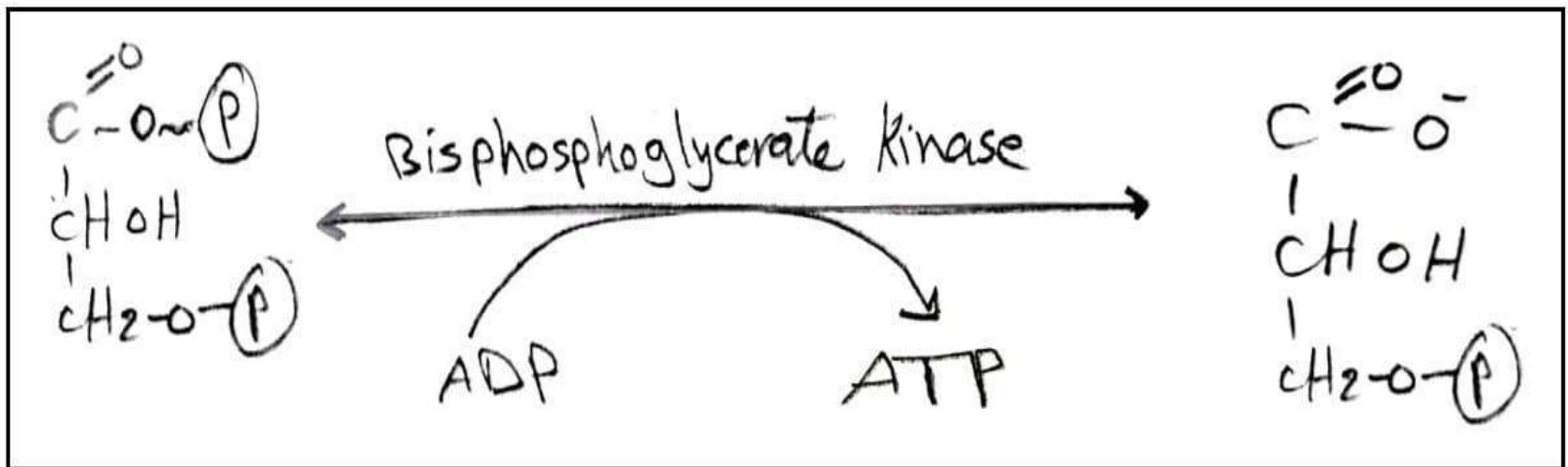
Oxidation and phosphorylation of glyceraldehyde-3-P to 1,3-bisphosphoglycerate (1,3-BPG) with the help of NAD^+ by the action of **glyceraldehyde-3-P dehydrogenase** enzyme .

This reaction is reversible , and the product contains a high energy phosphate bond . During this reaction , NAD^+ is reduced to NADH .



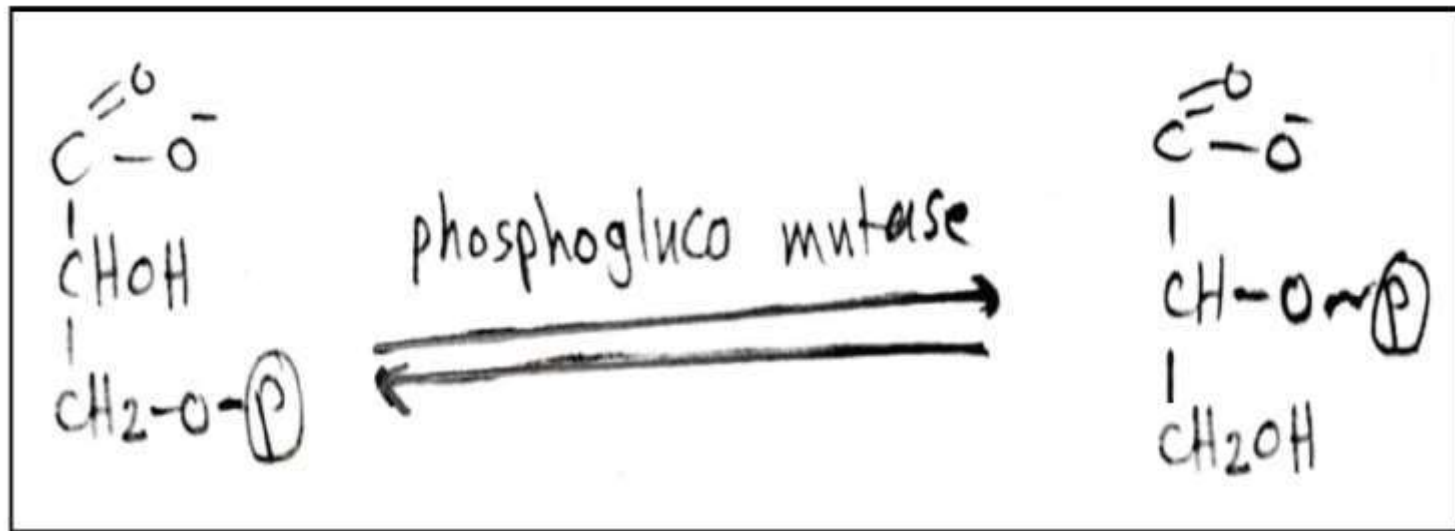
Step 6 :

The phosphate is transferred from 1,3-BPG into ADP , forming ATP and 3-phosphoglycerate with the help of **bisphosphoglycerate kinase** .



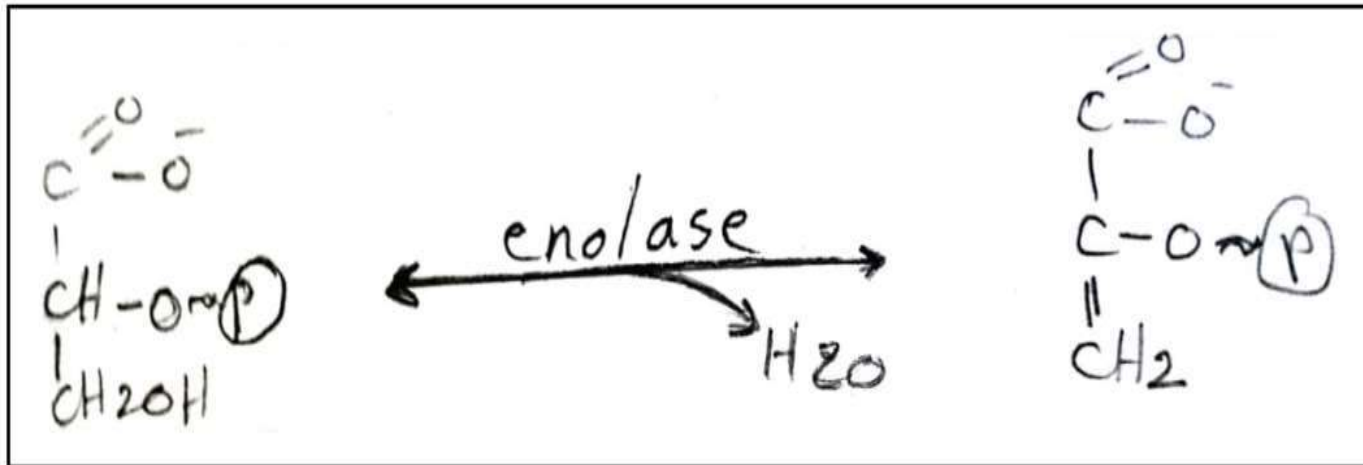
Step 7 :

3-phosphoglycerate is isomerized to 2-phosphoglycerate by shifting the phosphate group from 3rd to 2nd carbon atom . The enzyme is **phosphoglucomutase** , and this a reversible reaction .



Step 8 :

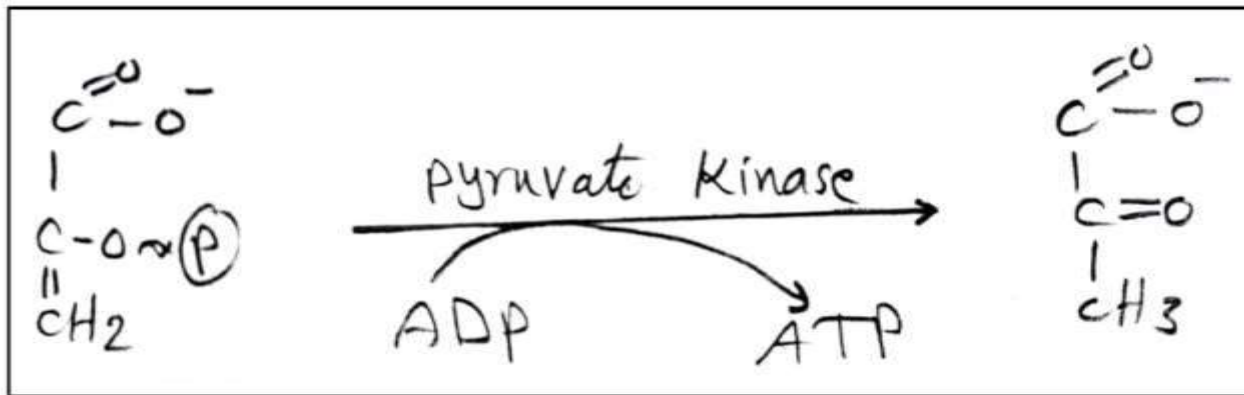
2-phosphoglycerate is converted to phosphoenolpyruvate (PEP) by the enolase enzyme . A high energy phosphate bond is produced , and the reaction is reversible .



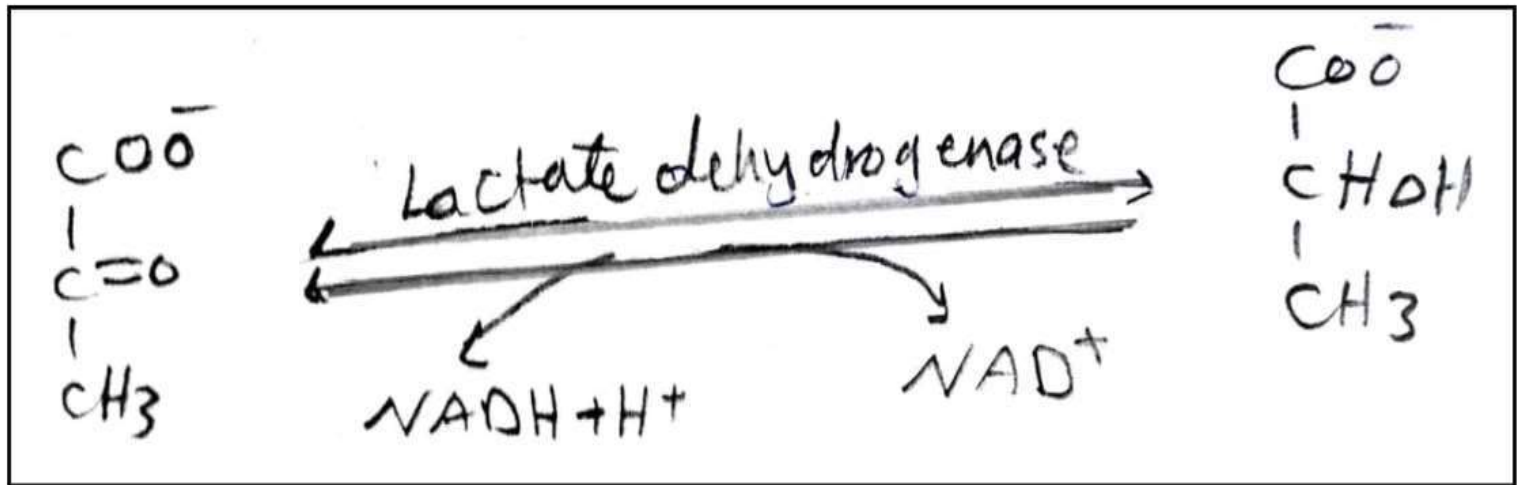
Step 9 :

PEP is dephosphorylated to pyruvate by **pyruvate kinase** enzyme . One mole of ATP is generated .

The pyruvate kinase is a key enzyme of glycolysis and this step is irreversible .



Step 10 : In anaerobic condition , pyruvate is reduced to lactate by the enzyme **Lactate dehydrogenase (LDH)** in a reversible reaction .



Steps 5 and 10 are coupled :

- In the 5th step , for each molecule of glucose entering in glycolysis , 2 molecules of NAD^+ are reduced to NADH .
- The NADH is to be reconverted to NAD^+ , this can be done in the cytoplasm during exercise where is lack of oxygen . Therefore , the cell has to couple 5 to 10 reactions in which NAD^+ is regenerated by reducing the pyruvate to lactate .

Glyceraldehyde-3-P

dehydro-
genase
step (5)

NAD^+

NADH

1,3-bisphosphoglycerate

NADH

NAD^+

LDH

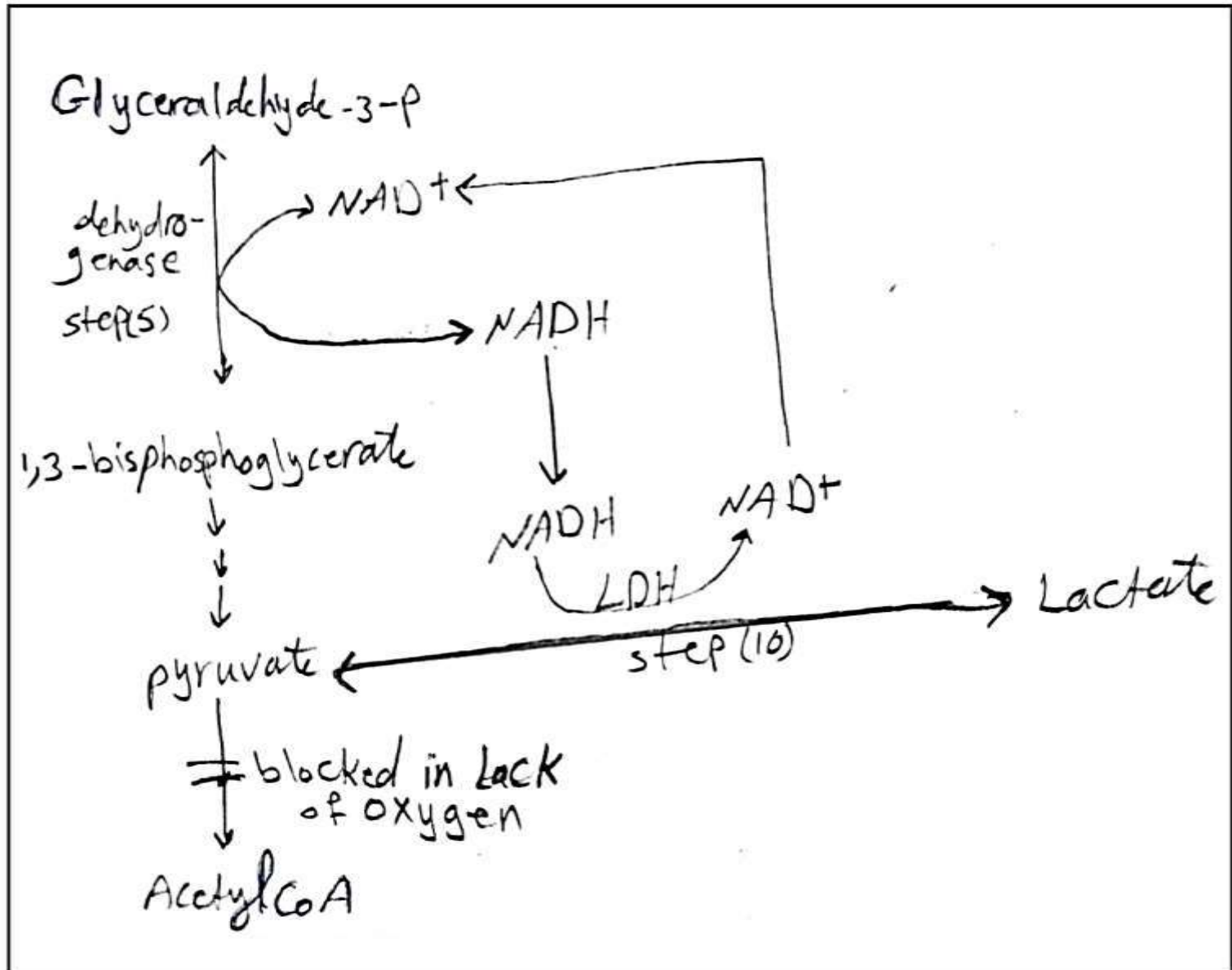
pyruvate

step (10)

Lactate

blocked in lack
of oxygen

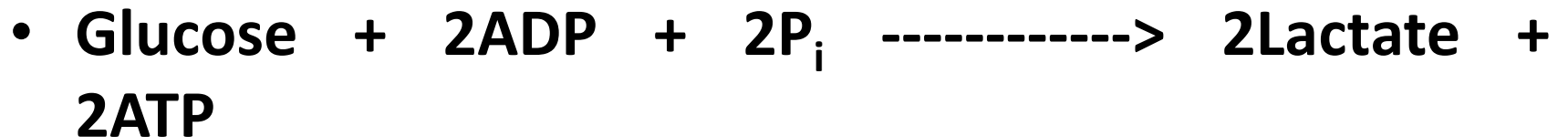
AcetylCoA



Energy yield from glycolysis :

- During anaerobic condition (oxygen deficiency) when one molecule of glucose is converted to 2 molecules of lactate , the net yield is 2 ATP molecules after the lost of 2ATP molecules in steps 1 and 3 .

The overall reaction is :



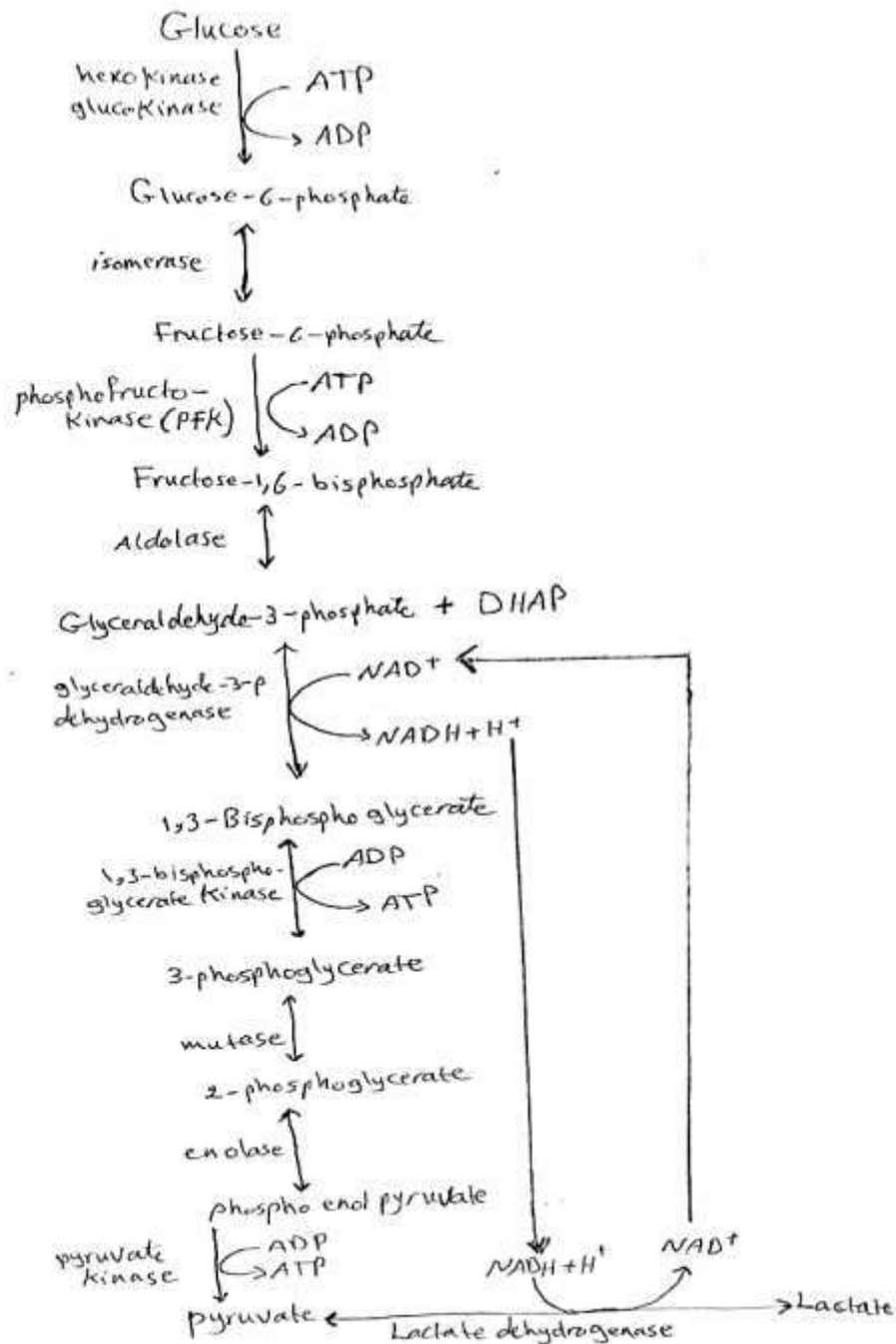
- In aerobic condition (oxygen is in plenty) , the 2 NADH generated in step 5 will enter the mitochondrial electron transport by the shuttle and each NADH will provides 3ATP molecules , so the net gain of energy is 8ATP .



Regulation of Glycolysis :

Glycolysis is regulated by the three irreversible reactions which catalyzed by :

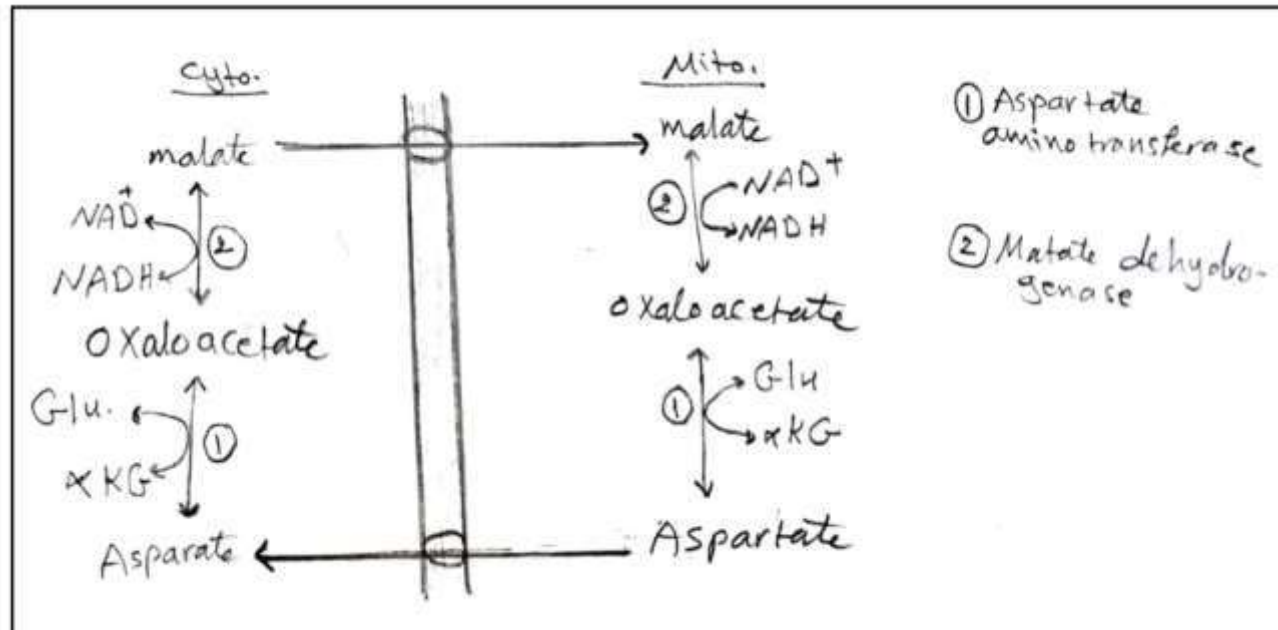
- Hexokinase and glucokinase
- Phosphofructo kinase
- Pyruvate kinase
- Insulin hormone increases the activity of above enzymes , while glucagon inhibits them .



Entry of cytoplasmic NADH to the mitochondria

Malate shuttle

- Operates mainly in liver, kidney, and heart, by the help of enzymes **malate dehydrogenase** and **aspartate aminotransferase**. Each molecule of NADH enters the mitochondria, 3ATP molecules are generated.



Fate of Pyruvates

(1) Under hypoxic conditions as in heavy exercise when skeletal muscle lacks enough oxygen, and in RBCs which have no mitochondria, pyruvate cannot oxidize, and anaerobic glycolysis represents the major source of energy. 2 ATP molecules and lactate are the end products.

(2) Under aerobic condition, pyruvate is converted to Acetyl CoA which enters the Krebs cycle to be oxidized to H_2O and CO_2 and ATP is generated.

Oxidation of pyruvate into Acetyl CoA :

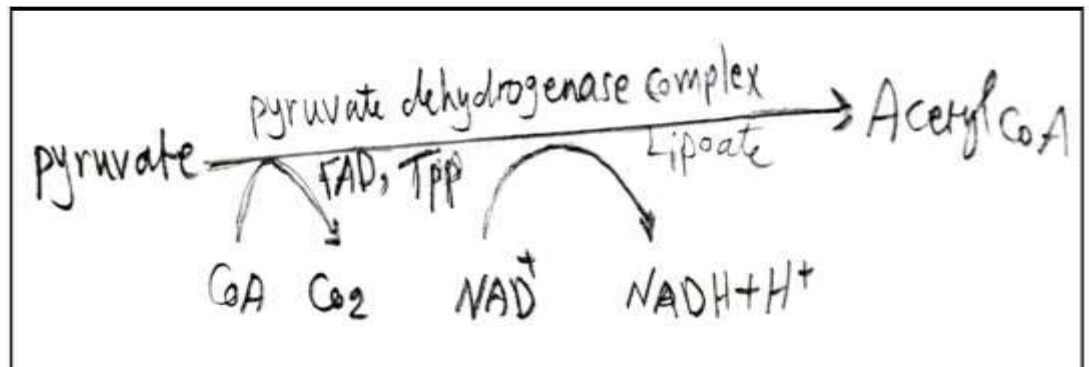
- Glycolysis is taking place in cytoplasm , so pyruvate is transported into mitochondria and undergoes **oxidation decarboxylation** to Acetyl CoA
- This reaction is catalyzed by different enzymes and coenzymes working as a multienzyme complex called **pyruvate dehydrogenase complex** .

The enzymes included in this multienzyme complex are :

- Pyruvate decarboxylase
- Dihydro lipoyl transacetylase
- Dihydro lipoyl dehydrogenase

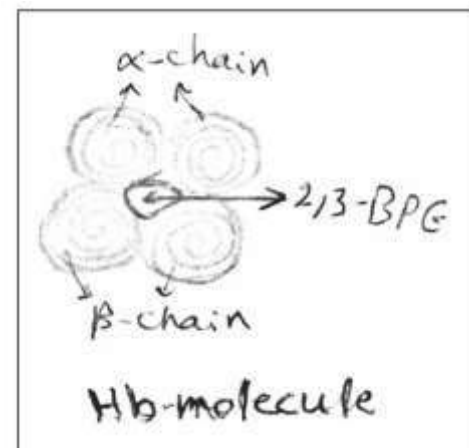
The coenzymes needed are :

- Vitamin B₁ (Thiamine pyrophosphate)
- Vitamin B₂ (FAD)
- Vitamin B₃ (NAD⁺)
- CoA
- Lipoic acid



Glycolysis in Erythrocytes

- In RBC_s , step 6 of glycolysis is bypassed . 1,3-BPG is converted to 2,3-BPG by the enzyme BPG-mutase . Then BPG-phosphatase enzyme removes the phosphate group to form 3-phosphoglycerate .
- In this pathway , no ATP molecules is generated , this may be of advantage since it would allow the glycolysis to proceed when the need for ATP is minimal .



There are high concentration of 2,3-BPG in erythrocytes , it found in the same concentration of Hb .

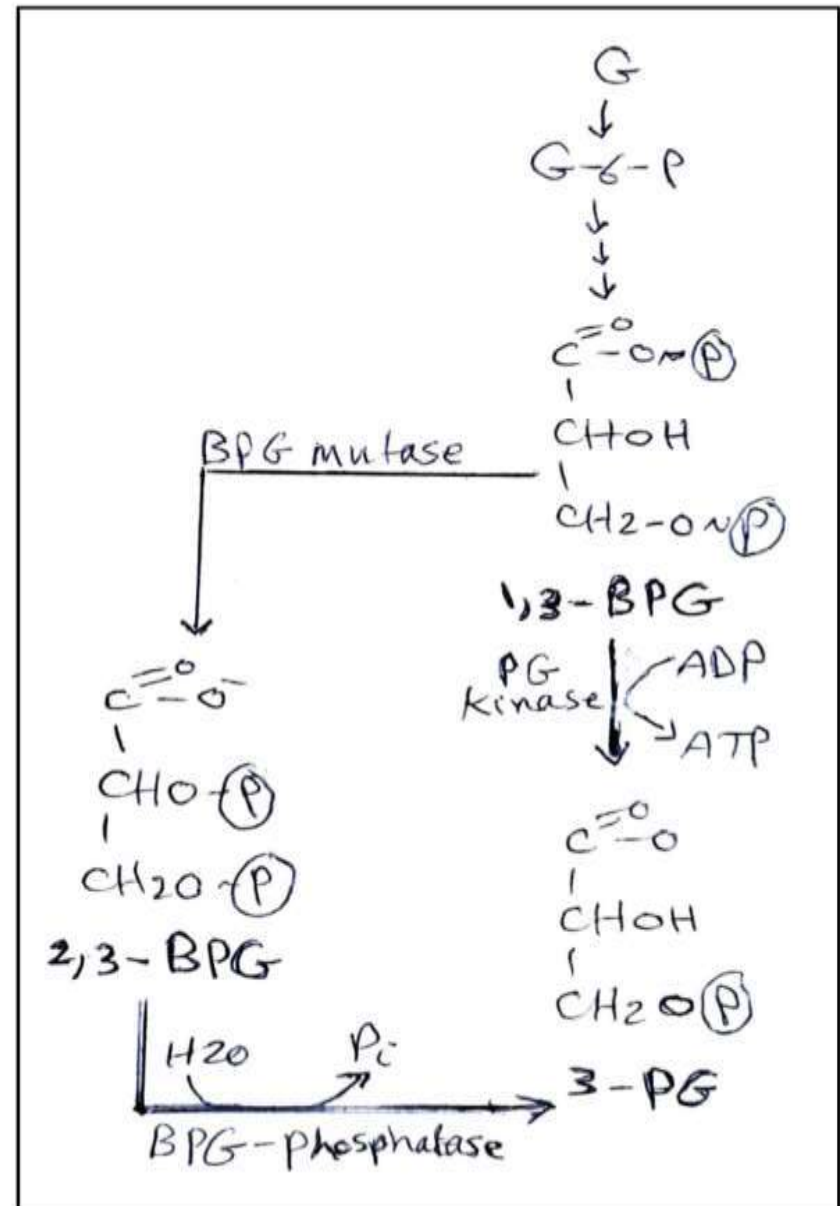
2,3-BPG is responsible for a high efficiency of oxygen transport that occur in Hb molecules .

2,3-BPG binds with great affinity to the Hb found in tissues (de-oxygenated Hb) than to Hb found in lungs (oxygenated Hb) .

Pure Hb releases only 8% of O_2 to the tissues , while Hb with 2,3-BPG allows it to release 66% of O_2 to the tissues .

In lungs , 2,3-BPG has a lower affinity toward Hb , this help the Hb to saturate itself with O_2 in the lungs and hold the O_2 until it reaches the tissues which have a lower O_2 concentration . Thus , 2,3-BPG helps in the regulation of O_2 carrying in Hb .

The effect of , 2,3-BPG is shown between fetal Hb and maternal Hb . Maternal Hb is able to bind 2,3-BPG better than fetal Hb , therefore the fetal Hb has a higher affinity toward O_2 , this help the fetus to get more O_2 from the mothers blood stream .



Prof . Dr . Samera Alkatib

Hexose MonoPhosphate Shunt(HMPS)

Pentose Phosphate Pathway

Phosphogluconate Pathway

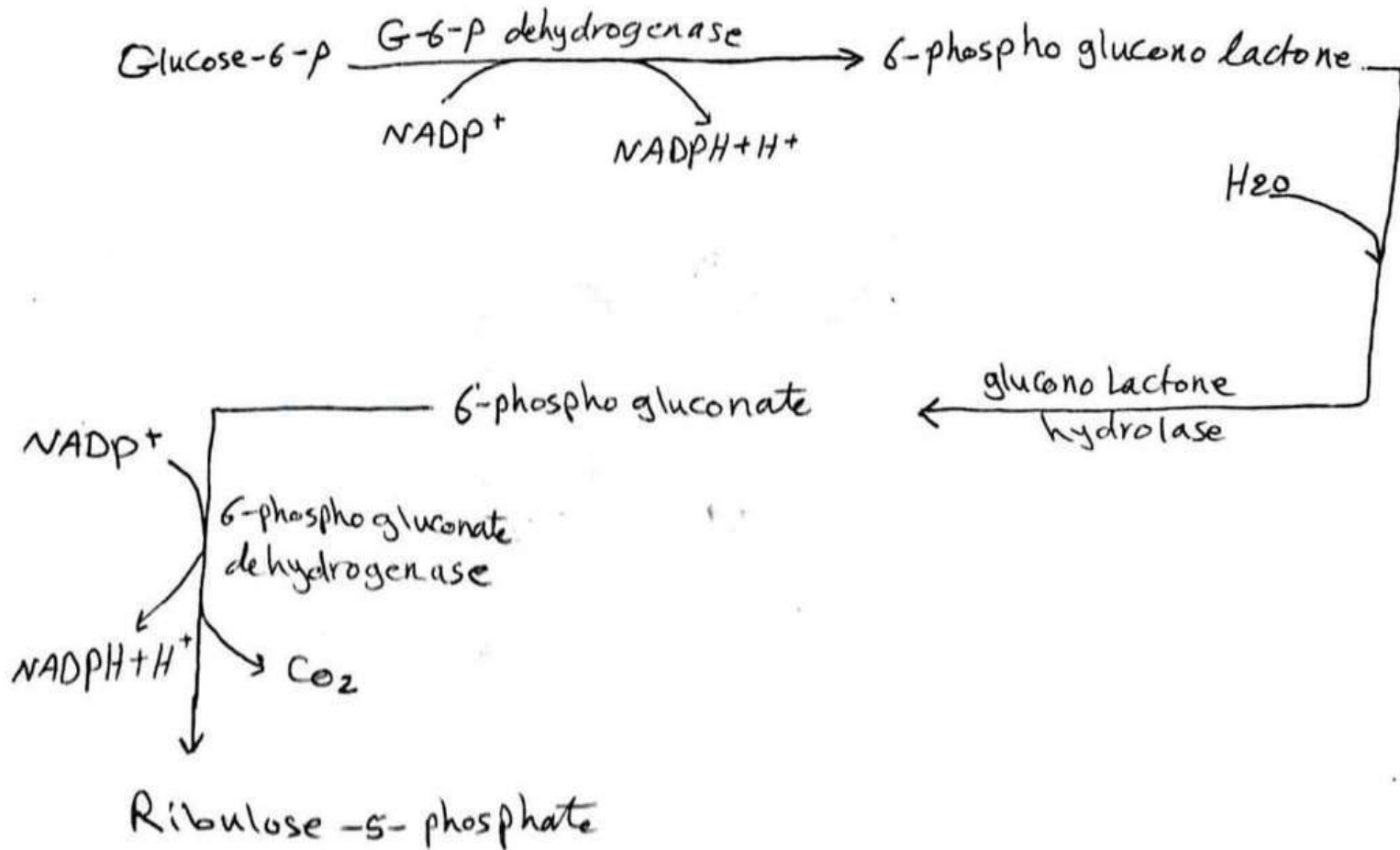
- Another pathway for glucose metabolism . Instead of glucose going through the glycolysis , it shunts through this pathway which also occur in the cytoplasm .
- About 10% of glucose molecules per day are entering in this pathway . Liver and RBC cells are used about 30% of glucose by this pathway .

The major purpose of HMPS :

ATP molecules are neither needed nor produced by the HMPS , so the cells do not use this pathway for energy production , but for :

- Generation of NADPH
- Generation of pentose phosphate sugar

The sequence of the reactions are divided into 2 phases , oxidative non-reversible phase , and non-oxidative reversible phase



« oxidative steps of HMPs »

Non-Oxidative reversible reactions :

- Ribulose-5-P is a substrate for 2 enzymes , **isomerase** which converts ribulose-5-P into ribose-5-P (the precursor of nucleic acids synthesis) , and **epimerase** which form xylulose-5-P .
- **Transketolase** enzyme catalyze the formation of glyceraldehyde-3-P from xylulose-5-P and ribose-5-P , then **Transaldolase** forms fructose-6-P .
- This interconversion between triose , pentose , and hexose , need transketolase and and transaldolase enzymes . Transketolase need vitamin B₁ as a coenzyme .

THE PENTOSE PHOSPHATE PATHWAY & OTHER PATHWAYS OF HEXOSE METABOLISM

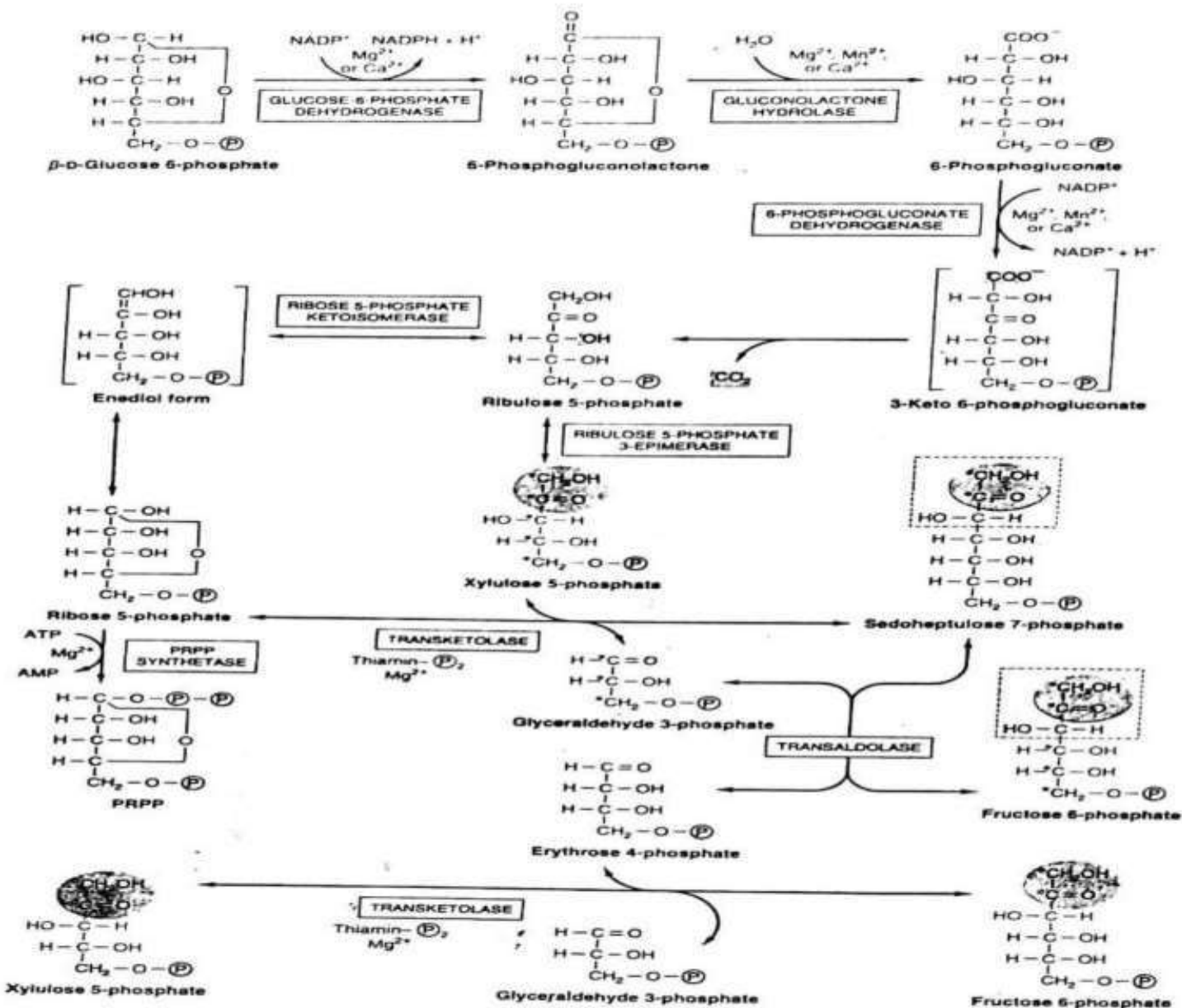


Figure 20-2. The pentose phosphate pathway. (●, — PO_3^{2-} ; PRPP, 5-phosphoribosyl 1-pyrophosphate.)

Significance of HMPS :

(1) The oxidative phase is active in the tissues which synthesized the lipid (liver, adipose tissues , adrenal cortex , mammary gland , testes and ovaries , RBC_s , lens of eyes) , These organs require NADPH for lipid and steroid synthesis .

Non- oxidative phase is present in all tissues , because ribose is not circulates in the blood stream , so tissues must synthesize the ribose needed for nucleic acids synthesis . The source of this synthesis is HMPS.

Note : HMPS is inactive in muscle , because muscle has low activity of **glucose-6-P dehydrogenase** .

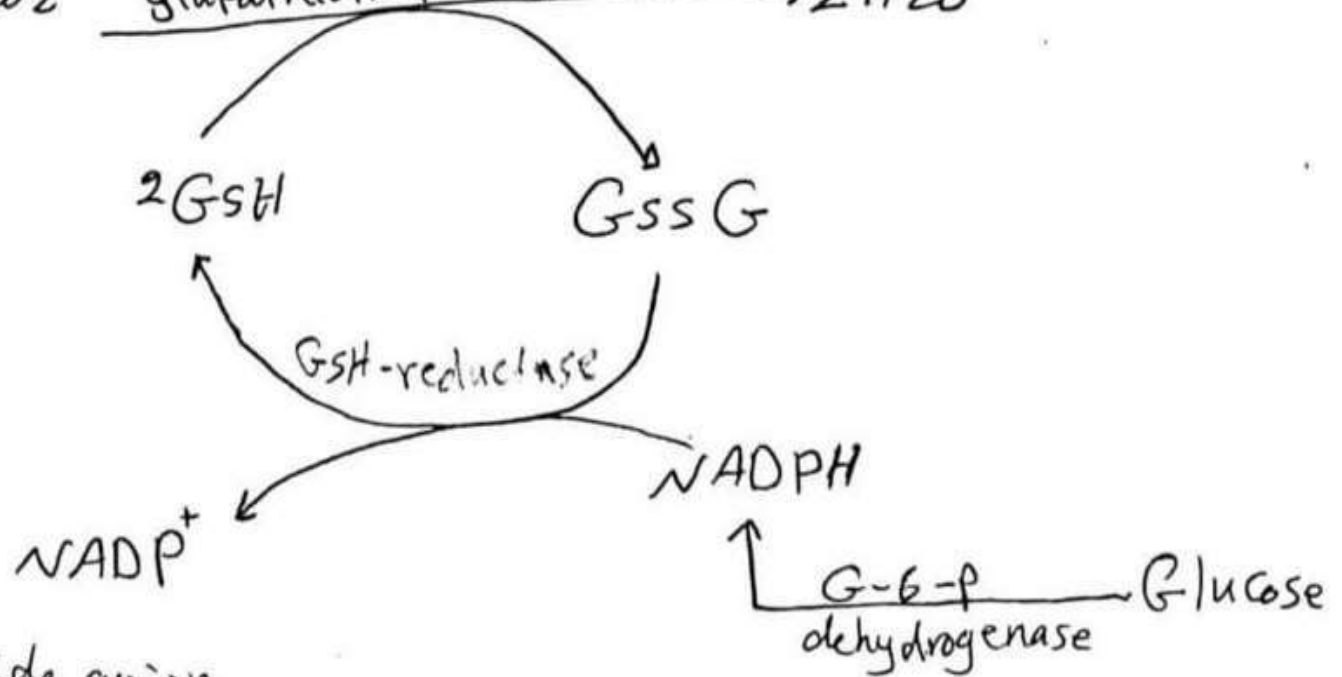
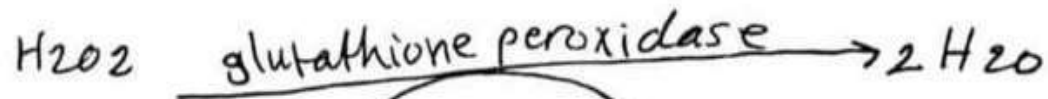
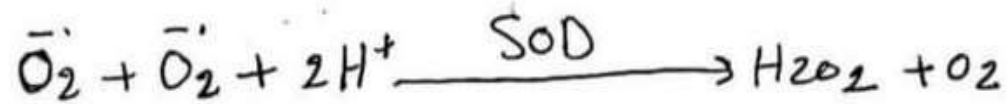
Nevertheless , it can synthesize the ribose-5-P by non-oxidative phase using fructose-6-P .

(2) Free radical scavenging

- Super oxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are free radicals formed by normal metabolism , they have highly oxidative damage to the cell membranes especially the lipid part of the membrane . Free radicals destroy DNA , proteins , fatty acids , and all biomolecules , in turn the cell is destroyed .

The antioxidant system of the body include :

- Enzymes system which include superoxide dismutase (SOD) , glutathione peroxidase , glutathione reductase , catalase .
- Vitamins which include A , E , and vitamin C .
- Reduced glutathione (GSH).
- Metals such as selenium (Se) .

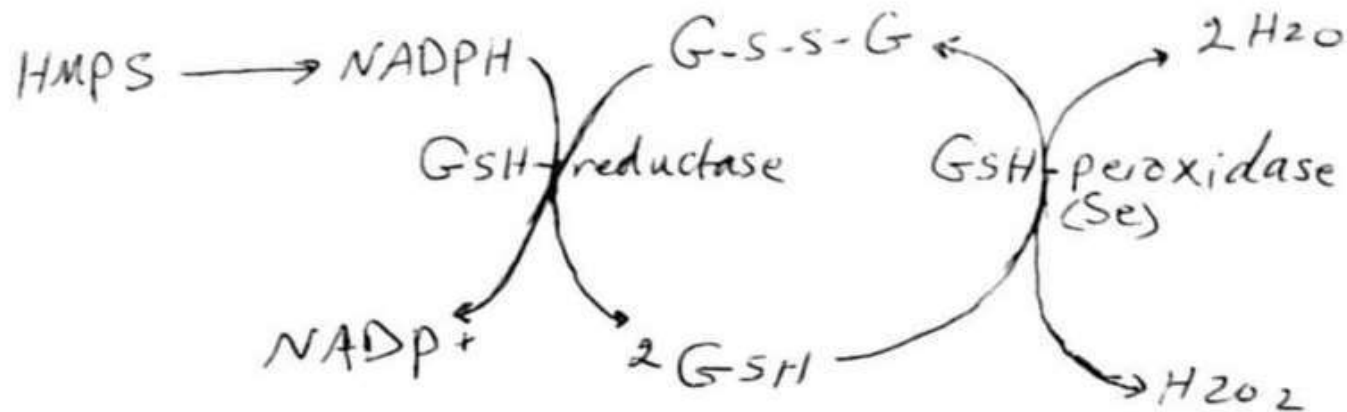


\bar{O}_2 : superoxide anion

H_2O_2 : hydrogen peroxide

(3)HMPS in RBC_s

NADPH is required for erythrocytes to keep the glutathione in reduced form (GSH) which removes H_2O_2 formed within RBC_s.



This reaction is important because the accumulation of H_2O_2 decrease the life span of RBC .

(4) **Lens of eye**

Maximum concentration of NADPH is seen in lens of eye . NADPH is required for maintaining the transparency of the lens .

Favism :

It is an inherited disease , due to the deficiency of **glucose-6-P dehydrogenase** , a key enzyme of HMPS .

The deficiency of enzyme is not absolute , but it is partial deficiency . Intake of certain food like fava beans , or certain drugs like antimalarial drugs (e.g. chloroquines) lead to more inhibition of this enzyme . This lead the formation of NADPH is low , which is important for the reducing form of glutathione .

Unavailability of NADPH result in high decrease in GSH amount , so the free oxygen radicals which formed by normal metabolism , lead to oxidative damage of the cell wall , and in the case of the favism it lead to destruction of RBC wall leading to hemolysis , and then to anemia called (hemolytic anemia).

This state can be corrected by need blood transfusion to the patients .

And the drug intake (chloroquine) must be stopped . And this type of persons have to be prevented from further intake of fava beans to prevent further attack of favism .

Fatty Acids Oxidation (Lipolysis)

Prof .Dr . Samera Alkatib

- Fatty acids (FA_s) exist in the body as free fatty acids (FFA_s) . Also found in the tissues as triglycerides (T.G) .
- FA_s are stored in adipose tissues in form of T.G and serve as the major energy source of the body .
- In fasting , FFA_s are transported from adipose tissues to the blood binding with albumin to be oxidized by many tissues for providing energy .
- Low levels of FFA_s occur in tissues , but the most amounts can be found in the plasma binding with albumin .
- The yield from the complete oxidation of FA to CO_2 and H_2O is 9Kcal /g fat , as compared to 4Kcal /g carbohydrate or protein oxidation .

β – Oxidation

The process at which the fatty acid oxidized to Acetyl CoA in many tissues , and produce the energy in form of NADH and FADH_2 which pass in the respiratory chain and generate ATP molecules .

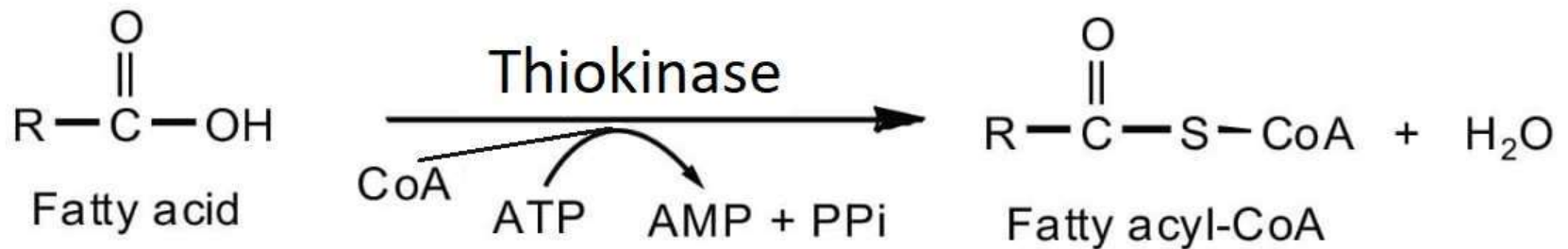
B – Oxidation takes place in the matrix of the mitochondria because the enzymes **Acyl CoA dehydrogenase** and **β -Hydroxy acyl CoA dehydrogenase** are a member of respiratory chain .

The name derived from the oxidation of β – carbon atom with removal of 2 carbon atoms each time as Acetyl CoA . This Acetyl CoA is oxidized in krebs cycle and generate further ATP molecules .

Steps of β – Oxidation :

Activation of FA :

Fatty acids found in the cytosol , and it must be activated to Acyl CoA and then penetrate to mitochondria . The activation of fatty acids takes place in cytosol by the help of thiokinase enzyme , an enzyme of outer mitochondrial membrane .



- Because β – Oxidation occur in the matrix , the Acyl group must be transported across the inner membrane of mitochondria ,

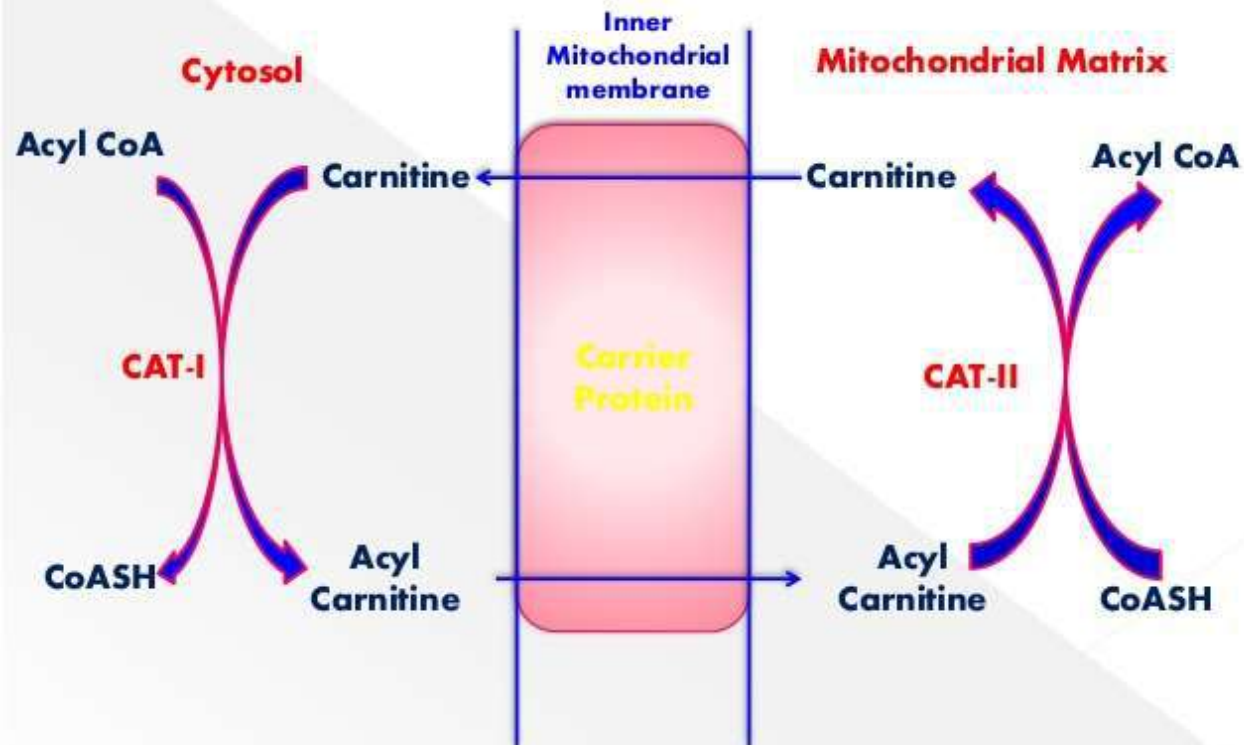
therefore the specialized carrier called carnitine , transports the acyl group from cytosol into the matrix , and the process is called carnitine shuttle or(carnitine transport system) .

Carnitine Shuttle :

1 – The acyl group binds with carnitine by carnitine acyl transferase (CAT 1) an enzyme found in outer mitochondrial membrane , forming acyl carnitine.

2 – Acyl carnitine is transported into the matrix by carnitine acyl transferase 11 (CAT 11) , an enzyme found in inner mitochondrial membrane , which catalyze the transfer of acyl group from carnitine to the CoA and release free carnitine

Carnitine transport system



β – Oxidation reactions :

1 – Oxidation of Acyl CoA by FAD – dependent dehydrogenase forming α , β – unsaturated acyl CoA and FADH_2 . FADH_2 pass in respiratory chain and oxidized to 2ATP molecules .

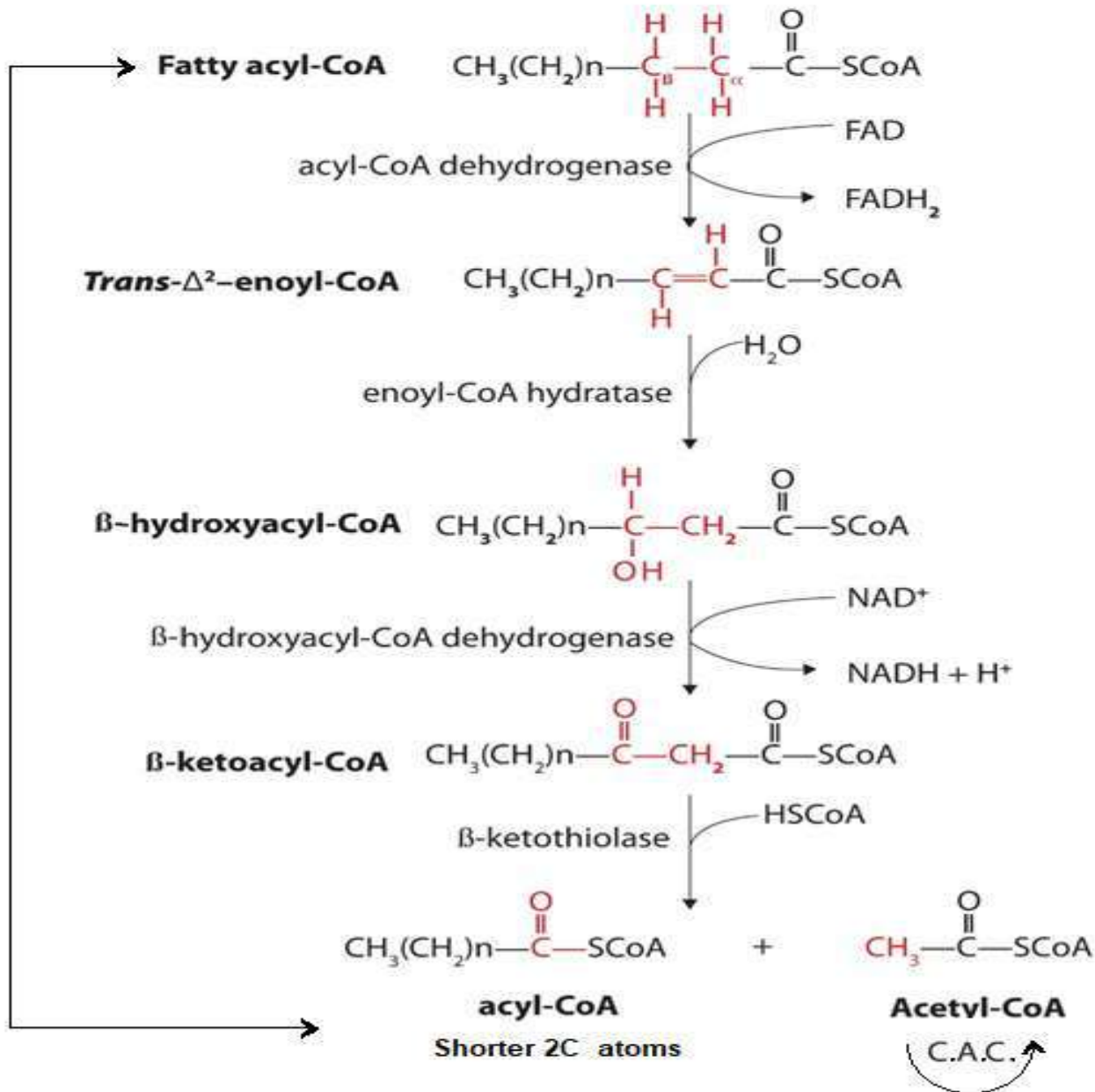
2 – Hydration by enoyl CoA hydratase forming β – hydroxyl acyl CoA .

3 – Oxidation by NAD^+ - linked dehydrogenase forming β – keto acyl CoA and NADH which oxidized in respiratory chain and yield 3ATP molecules.

4 – Splitting off a molecule of Acetyl CoA and leaving fatty acid with 2 carbon atoms less .

Acetyl CoA enter the krebs cycle and completely oxidized to CO_2 and H_2O forming 12 ATP molecules .

The remaining Acyl CoA is repeated these 4 steps until the fatty acid is completely oxidized .



Energetics of β – Oxidation

The complete oxidation of palmitic acid (16C) in β – oxidation needs 7 cycles . Each cycle produces 1FADH₂ , 1NADH , 1Acetyl CoA

$$2 \times 7 + 3 \times 7 + 12 \times 8 = 131 \text{ ATP}$$

The activation of fatty acid in cytosol utilized 2ATP , so the net energy yield is 129 ATP .

Metabolism Of Lipids

Prof . Dr . Samera Alkatib

Digestion and Absorption

Source of lipids in the body:

Exogenous lipids : The dietary lipids.

Endogenous Lipids : The lipids synthesized in the liver.

Dietary Lipids

The lipids in the diet are usually a mixture of Triglycerides (T.G) , free and esterified cholesterol , phospholipids , and free fatty acids (FFA).

T.G is the major lipid in the diet and in the body.

Digestion in stomach

The digestion of lipids begins in stomach by **stomach lipase** which acts on short chain T.G .

Short chain T.G are present in milk and butter , so the digestion in stomach is observed to be more significant in the newborn infants .

The main products of stomach lipase are free fatty acids and diglyceride.

Digestion in Intestine

Enzymes in the intestine that hydrolyze the lipids are :

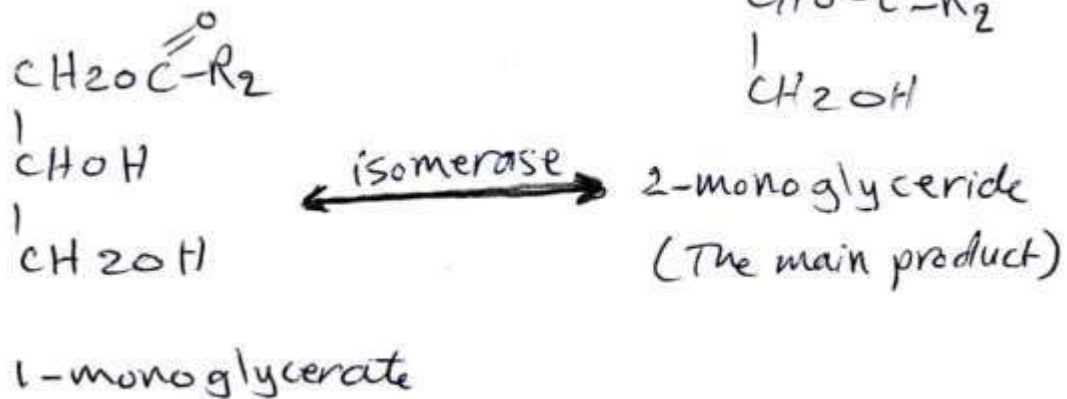
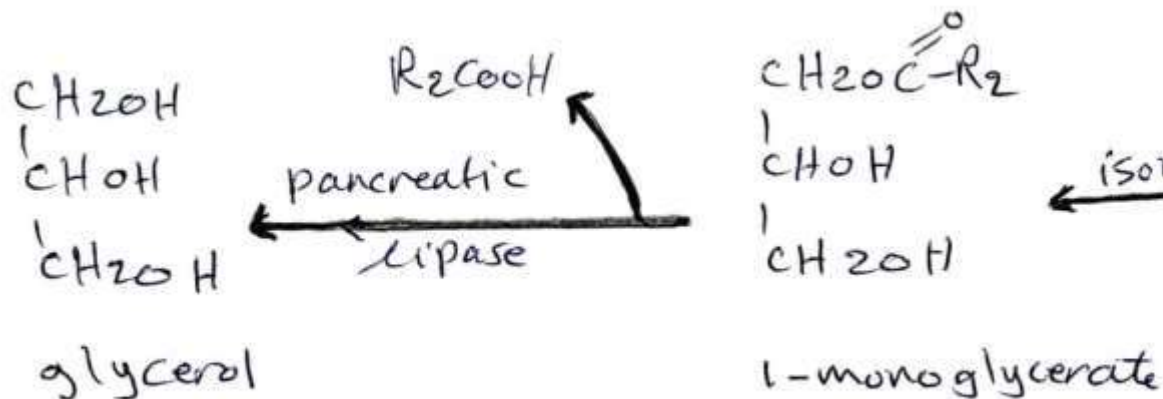
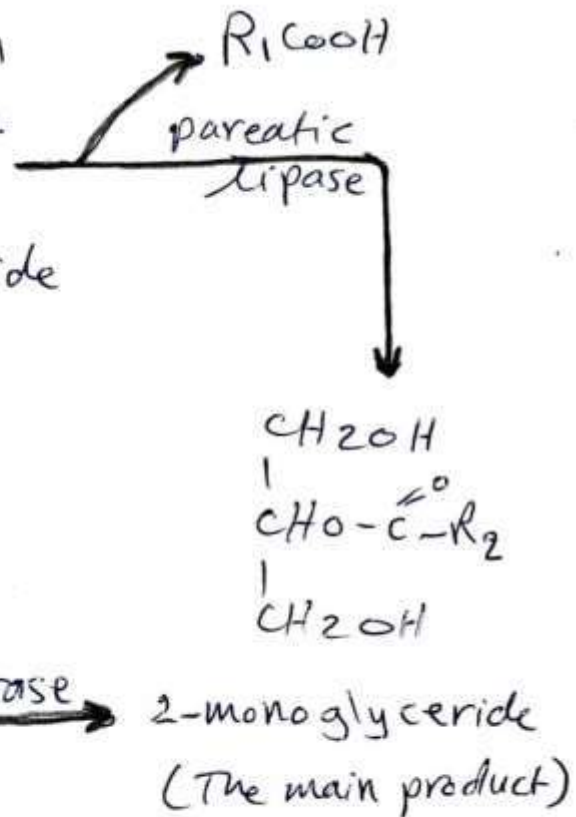
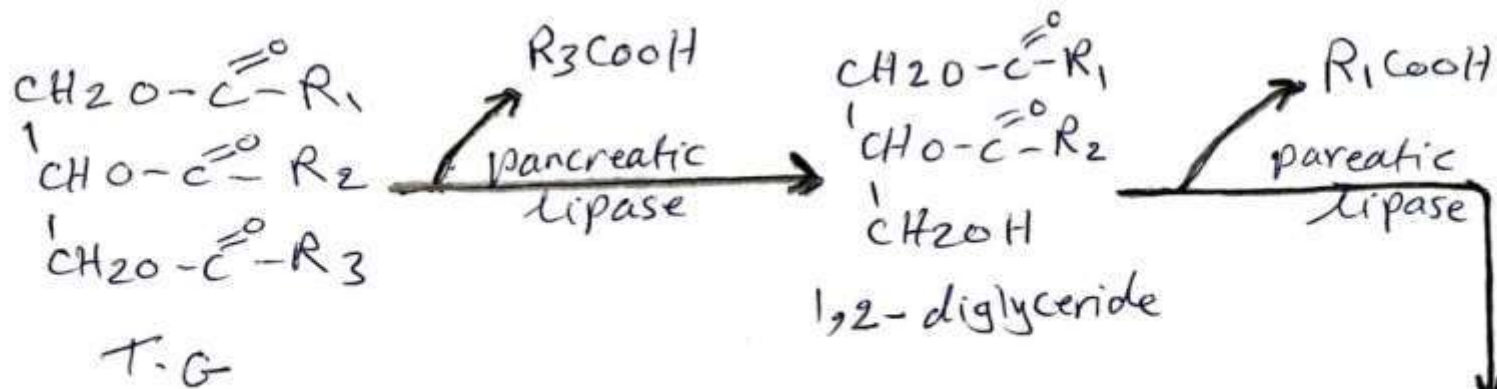
- Pancreatic Lipase
- Cholesterol esterase
- Phospholipase A₂

Pancreatic lipase: hydrolyze the fatty acids at the first and third carbon atoms of triglyceride , forming 2-monoglyceride and FFA_s .

Then **isomerase** enzyme shifts the fatty acid from carbon 2 to the carbon 1 which is then hydrolyzed by the pancreatic lipase forming glycerol and FFA .

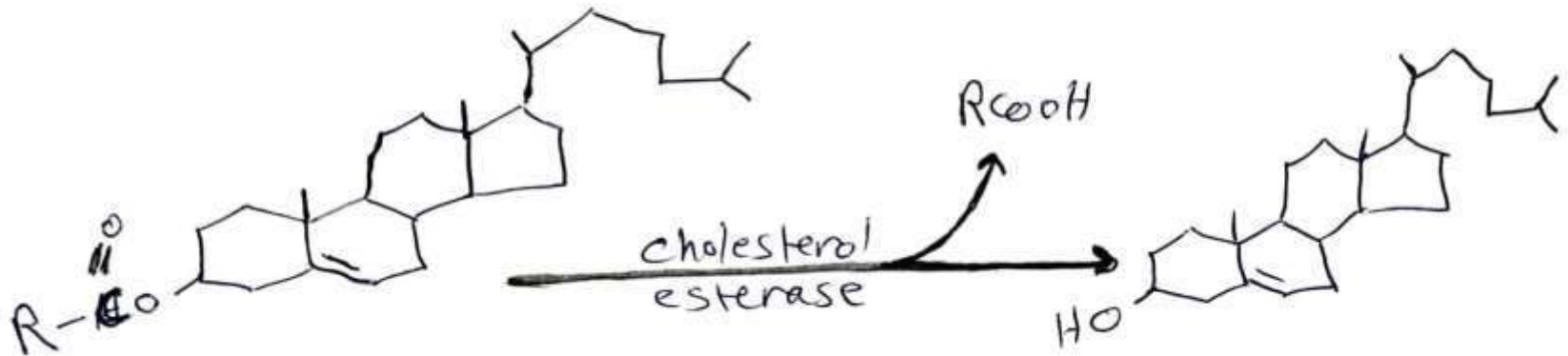
The end products of pancreatic lipase are :

2- monoglyceride(78%) , 1-monoglyceride(6%) , glycerol and fatty acids(14%)

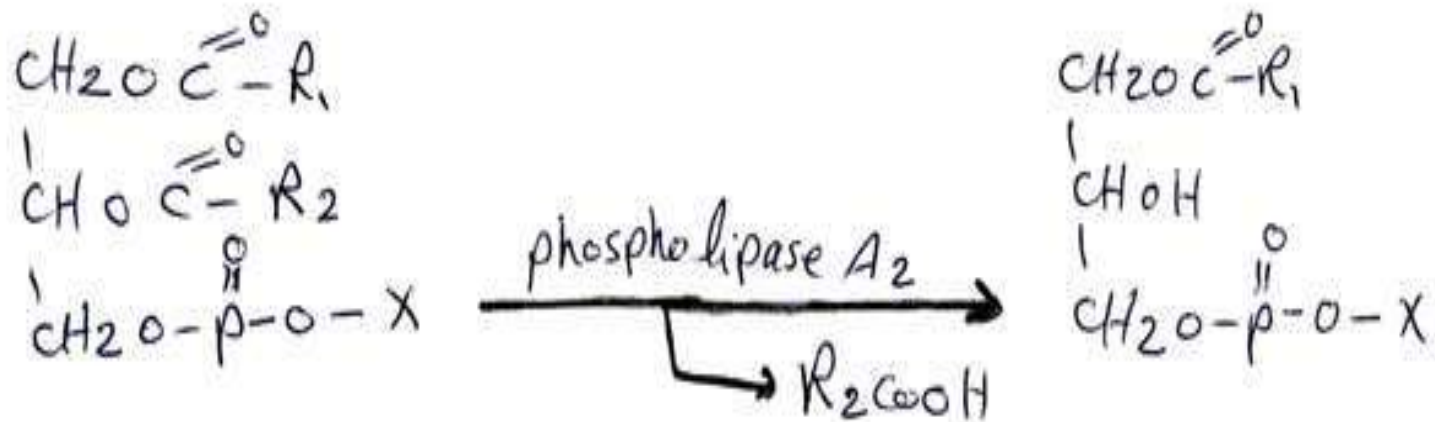


Cholesterol esterase : Most dietary cholesterol is present in form of free cholesterol
10 – 15% present in form of esterified cholesterol .

Cholesterol esterase hydrolyze the esterified cholesterol into free cholesterol and fatty acid .



Phospholipase A₂ : Hydrolyzes the fatty acid at carbon 2 of phospholipid forming lysophospholipid and fatty acid



Bile Salts :

Bile salts are important for digestion and absorption of lipids. Bile salts lower the surface tension of water and emulsify the fat molecules (remove the other molecules , proteins and carbohydrates from the fat molecules) .

This will help the lipase enzymes to hydrolyze the lipids .

The products of digestion form a **micelles** with bile salts , this help the absorption of lipids into mucosal intestine cells .

In Mucosal Intestine Cells :

- Resynthesis of T.G
- Resynthesis of phospholipids
- Resynthesis of esterified cholesterol

Transport of Dietary Lipids

Inside the mucosal intestine cells , the T.G , esterified cholesterol , phospholipids , and together with long chain fatty acids , are bound into **chylomicrons** and transported into lymph circulation and then to the blood and they are taken up by extra hepatic tissues , mainly muscle and adipose tissues .

Chylomicrons : Lipoprotein synthesized in mucosal intestine cells transport the dietary lipids(mainly T.G) into muscle and adipose tissues .

In Muscle and Adipose Tissues :

Chylomicrons are taken up by muscle and adipose tissues , the T.G is hydrolyzed by the enzyme **Lipoprotein Lipase (LPL)** into 3FFA and glycerol .

Lipoprotein Lipase(LPL) :

Enzyme located in the walls of blood capillaries of muscle and adipose tissues . Its hydrolyzes the T.G in chylomicrons and VLDL into glycerol and FFA_s . It activates by insulin hormone .



FFA_s either oxidized in β -oxidation to get energy , or they are stored in adipose tissue as T.G .

During fasting , this stored T.G are hydrolyzed by **hormone sensitive Lipase (HSL)** to produce glycerol and 3FFA_s which transported in the blood to the tissues needed for energy



Endogenous Lipids

These are lipids synthesized in the liver

(T.G, cholesterol, and phospholipids) . They are transported by VLDL (very low density lipoprotein) to extrahepatic tissues ,mainly muscle and adipose tissues .

T.G in VLDL is hydrolyzed by LPL enzyme in the same way of dietary T.G .

Very Low Density Lipoprotein(VLDL):

It is a lipoprotein synthesized in the liver , transport the endogenous lipids(mainly T.G) from liver to muscle and adipose tissues.

Regulation of β – Oxidation

- The availability of FFA_s increase the oxidation .
- The level of FFA_s is controlled by glucagon : insulin ratio . Glucagon increase the FFA level by activating the hydrolysis of T.G in adipose tissue , while insulin has the opposite effect .
- CAT 1 enzyme regulates the entry of FA into mitochondria . Malonyl CoA inhibits CAT 1 activity . Thus during synthesis of FA , the β – oxidation is inhibited .

Metabolism of Nucleotides

Prof.Dr. Samera Alkatib

Metabolism of Purines and Pyrimidines

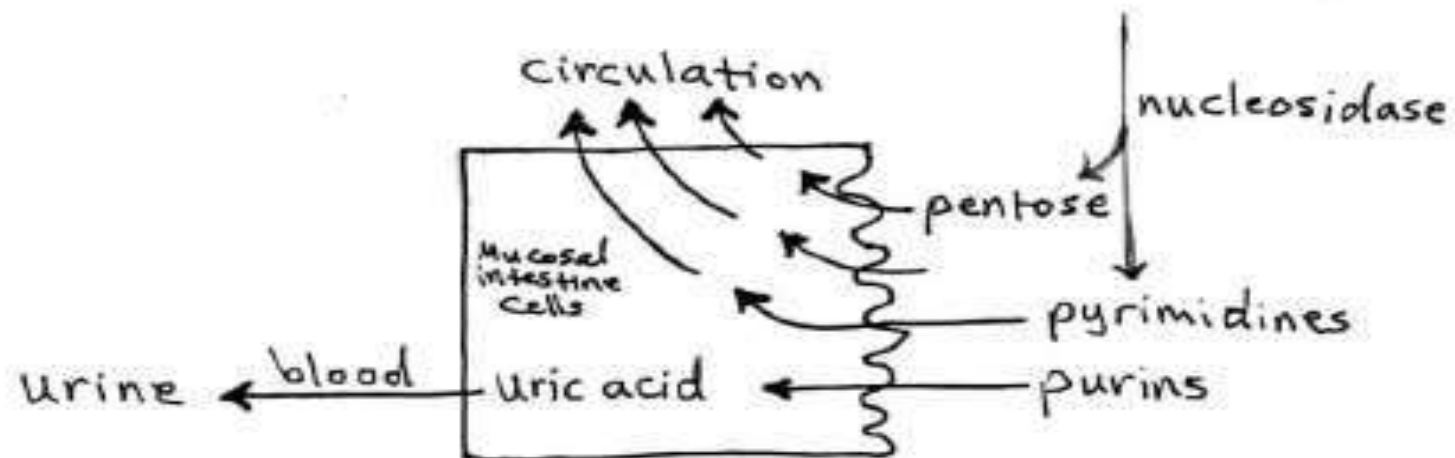
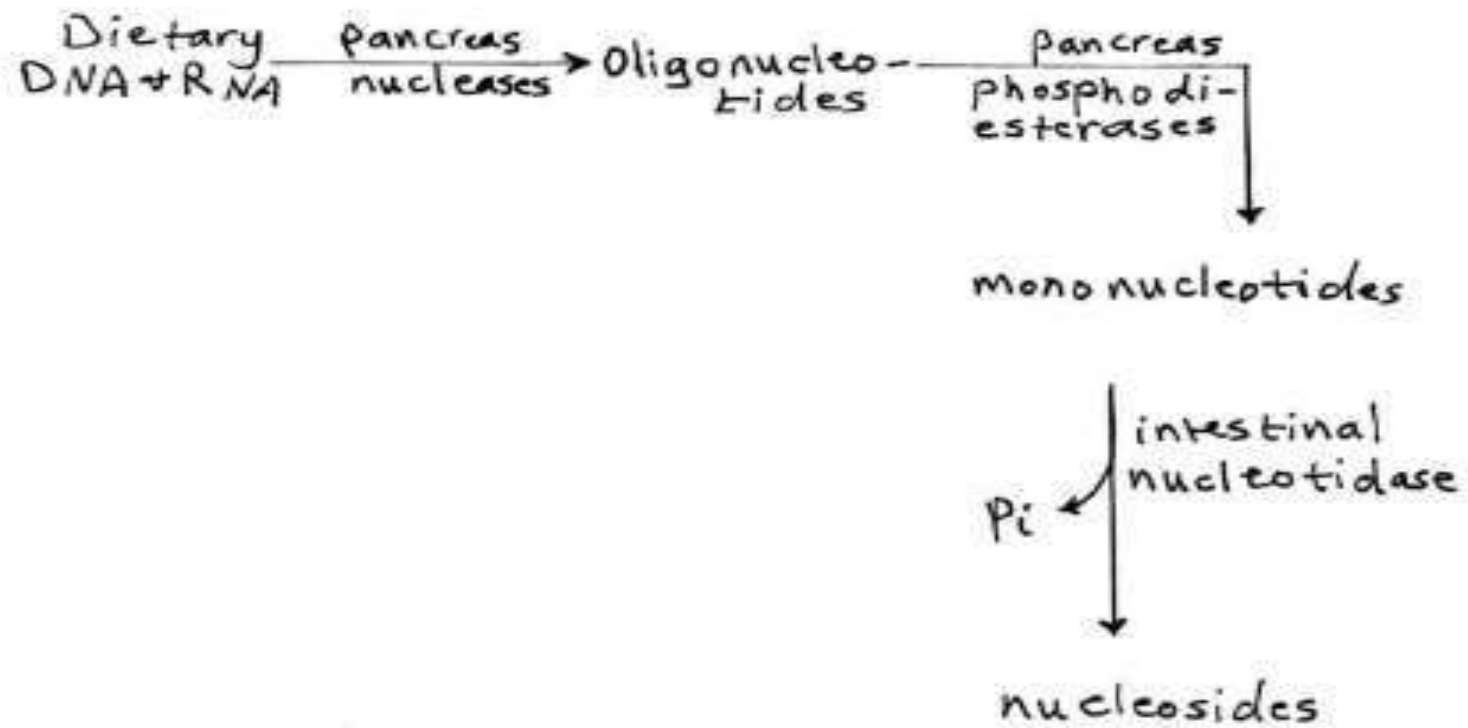
Digestion and absorption of dietary nucleic acids:

Hydrolysis of dietary nucleic acids DNA and RNA occurs in the small intestine by a family of pancreatic enzymes called ribonucleases and deoxyribonucleases . These enzymes hydrolyze RNA and DNA into oligonucleotides .

Then further hydrolysis occurs by pancreatic phosphodiesterases producing a mono-nucleotides .

A family of nucleotidases remove the phosphate groups and releasing the nucleosides, which are further hydrolyzed to free bases by nucleosidases .

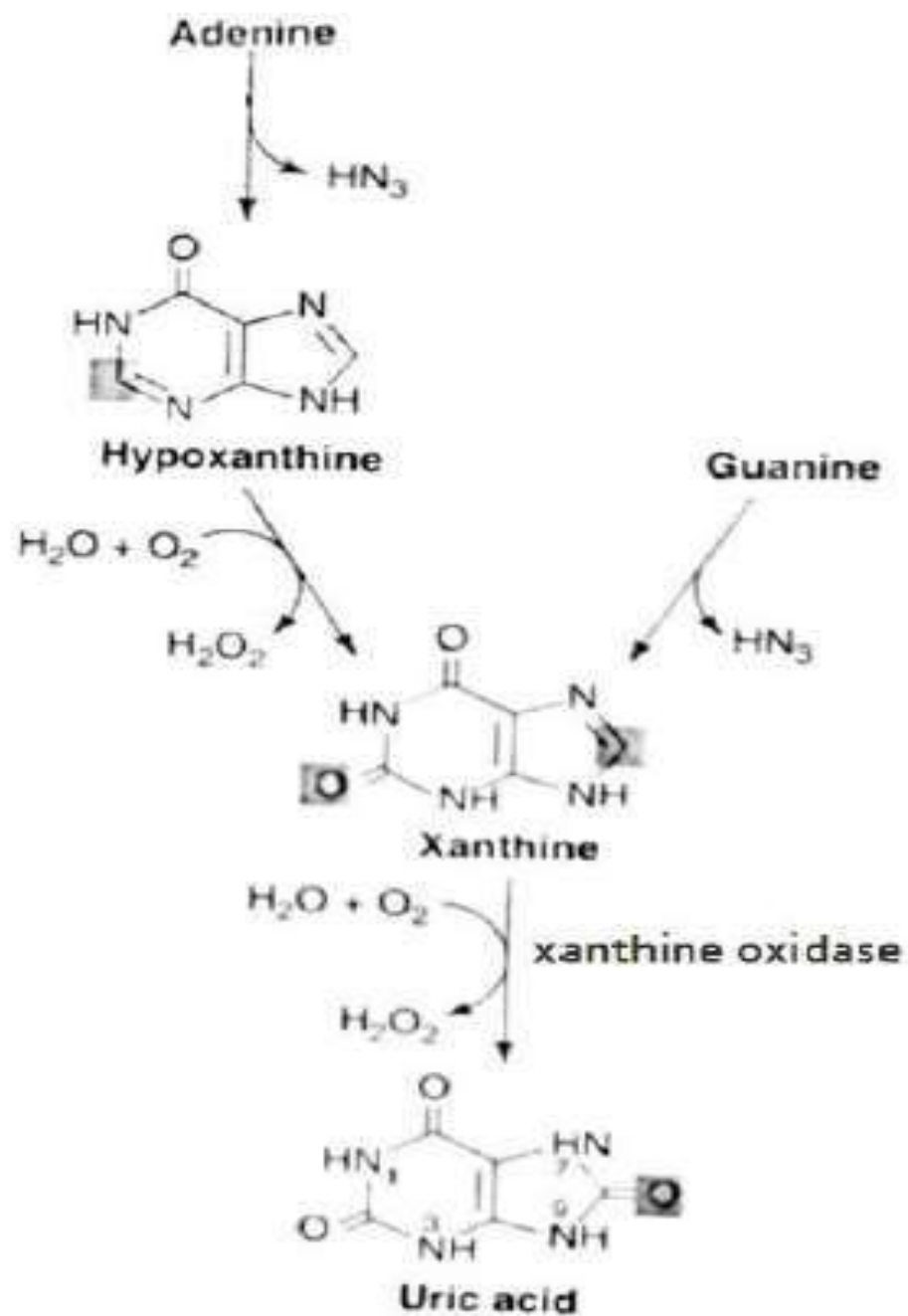
Dietary purins and pyrimidines are not used to a large extent for the synthesis of nucleic acids in the tissues , but instead , the dietary purins are converted to uric acid by intestinal mucosal cells . Most of uric acid enters the blood and excreted in the urine .



Catabolism of Purins :

- Adenine is deaminated by adenase to form hypoxanthine.
- Guanine is deaminated by guanase to form xanthine.
- Hypoxanthine is oxidized by xanthine oxidase to xanthine
- Xanthine is further oxidized by xanthine oxidase to uric acid.

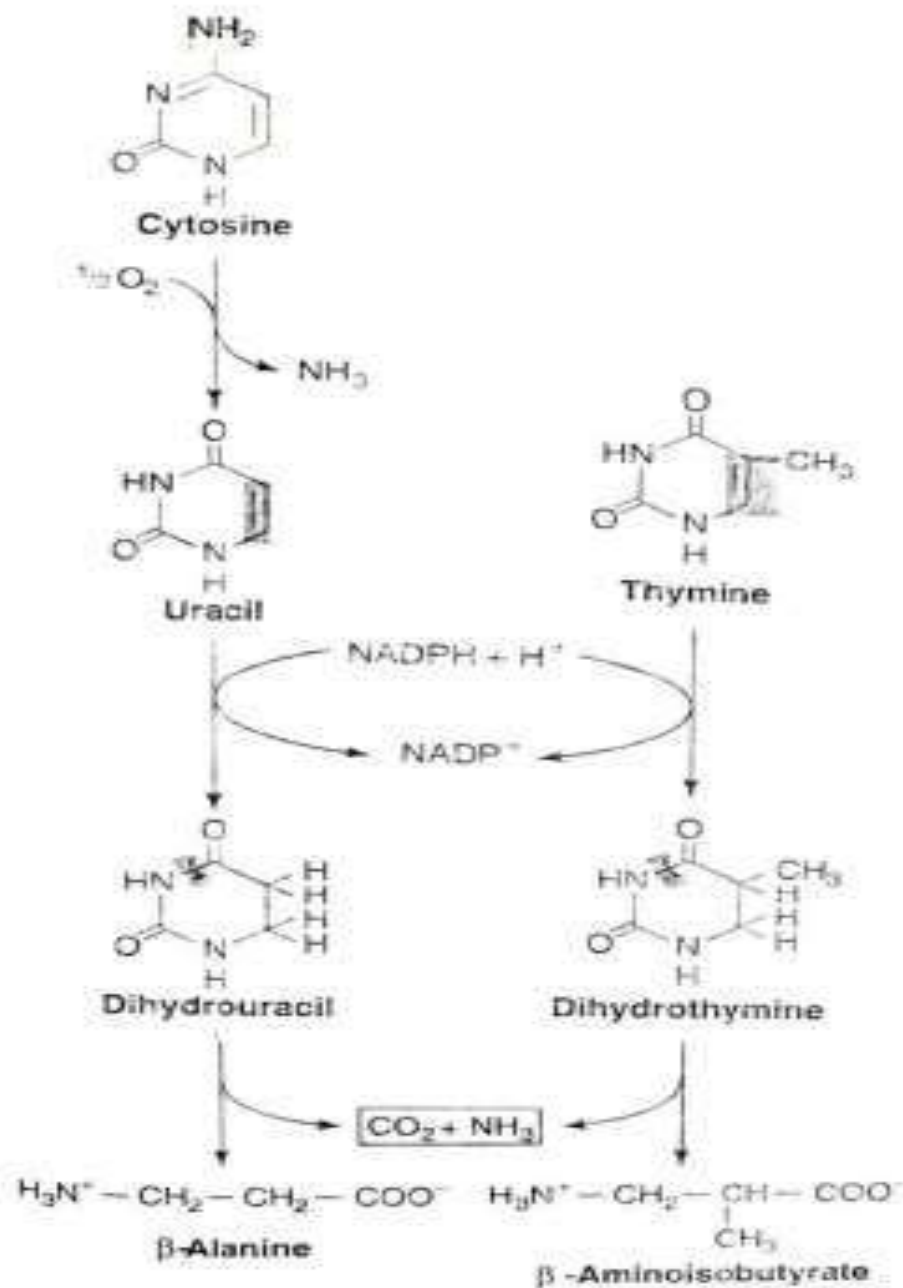
- Uric acid is the final product of human purine degradation, which is excreted in the urine.



Catabolism of pyrimidine:

Unlike the purine ring, pyrimidine ring is opened and degraded to highly soluble products which include:

- B- alanine
- B- amino isobutyric acid
- NH_3
- CO_2



Gout:

A disease associated with purine degradation . Gout is a disorder characterized by high levels of uric acid in the blood (hyperuricemia) as a result of either overproduction or less excretion of uric acid .

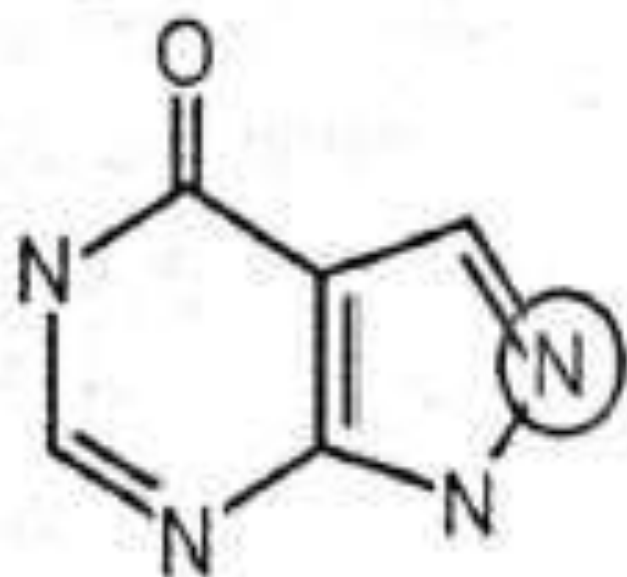
Hyperuricemia leads to the deposition of monosodium urate crystals in the joints and inflammation , causing acute and then chronic **gouty arthritis** .

Nodal masses of monosodium urate crystals may be deposited in the soft tissues (at the surface of joints , skin , cartilage , or tendons) resulting in chronic **tophi gout** .Patients with gout are easily affected by the development of **urate renal stones** .

Diagnosis of gout requires examination of synovial fluid from an affected joint using polarized light microscopy to assure the presence of needle-shaped monosodium urate crystals.

The major common cause of gout is from the under excretion of uric acid . Under excretion can be due to inherent excretory defects , or disease that affect how the kidney handles urates , for example lactic acidosis.

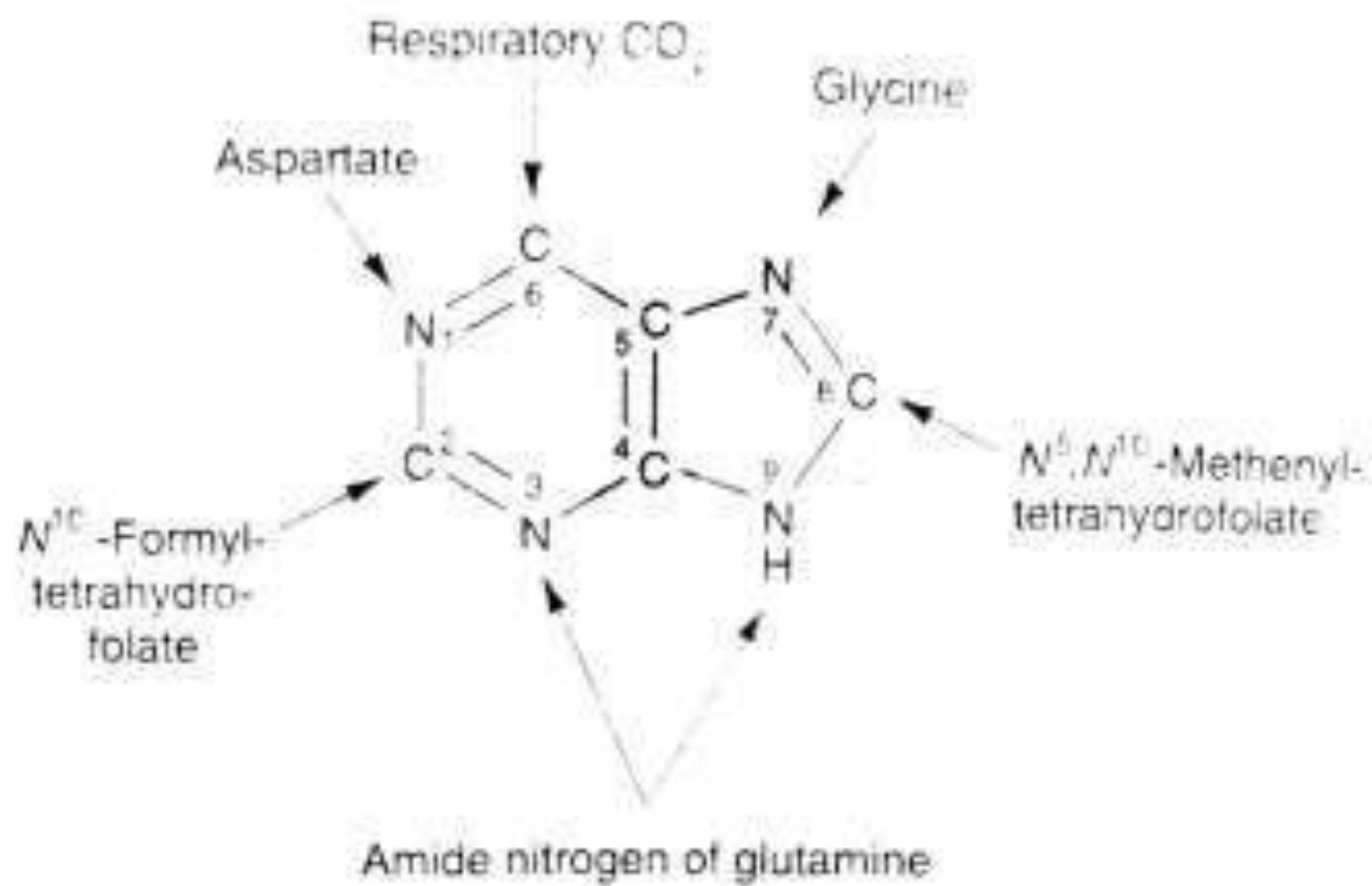
Treatment : Allopurinol , an inhibitor of uric acid synthesis . Allopurinol is converted in the body to oxypurinol which inhibits xanthine oxidase , resulting in accumulation of hypoxanthine and xanthine , which are more soluble than uric acid .



Allopurinol

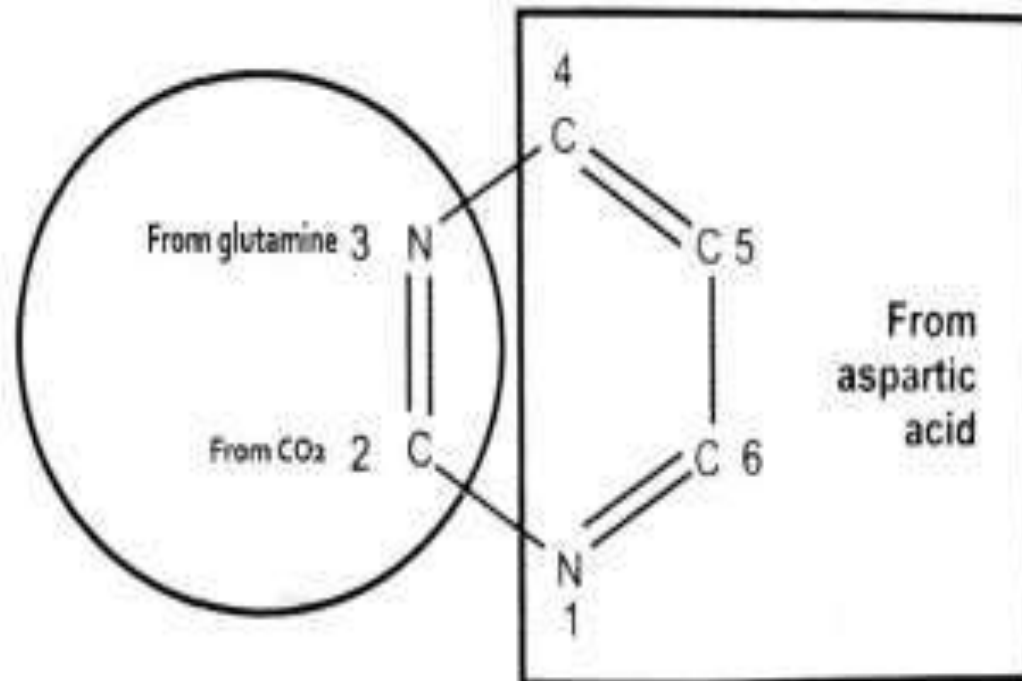
Synthesis of Purines

The atoms of the purine ring are provided by a number of compounds including amino acids (aspartic acid , glycine , and glutamine) , CO_2 , and N^{10} - formyl tetrahydrofolic acid (N^{10} - THFA) .



Pyrimidine synthesis :

The source of the atoms in the pyrimidine ring are glutamine , aspartic acid , and CO_2 .



Prof.Dr.Samera Alkatib

Nucleotides

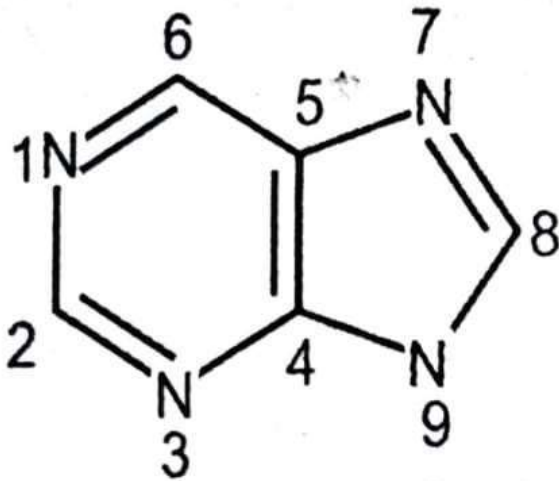
- Nucleotides are essential for the cells , without them , neither DNA nor RNA can be synthesized and therefore , proteins cannot be synthesized .
- Nucleotides are structural components of several coenzymes , such as FAD, NAD^+ , NADP^+ , and Coenzyme A .
- Nucleotides act as a carriers of activated molecules in the synthesis of some carbohydrates , lipids , and proteins . e.g. UTP and GTP .
- Cyclic nucleotides such as C-AMP and C-GMP act as second messengers in the cell.

Nucleotide Structure

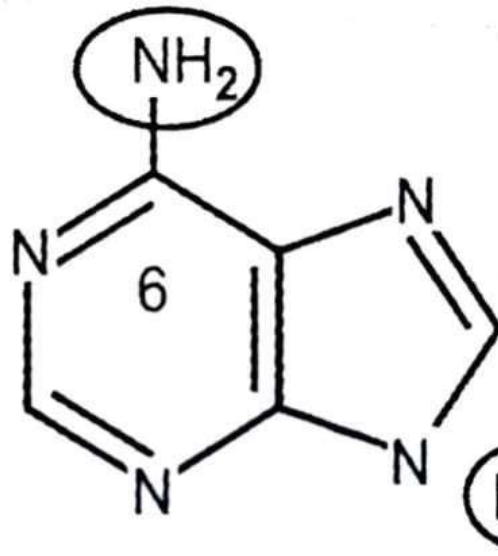
Nucleotides are composed of :

- Nitrogen base (purine or pyrimidine)
- Pentose monosaccharide (ribose or deoxyribose)
- Phosphate groups (one , two , or three)

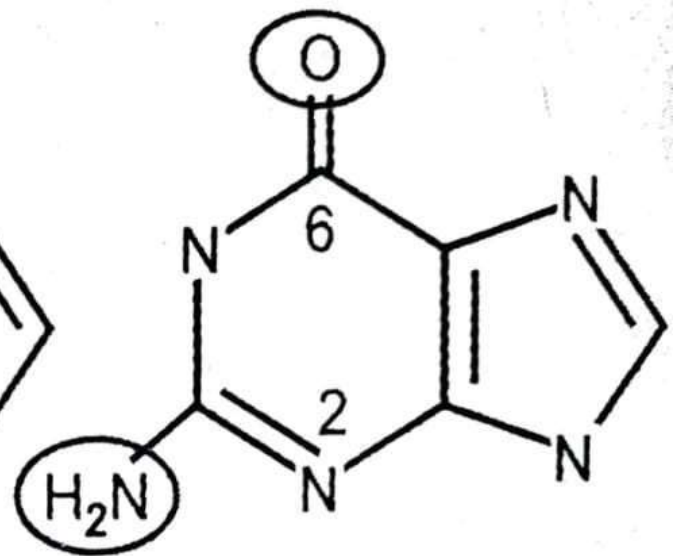
Nitrogen Bases structure:



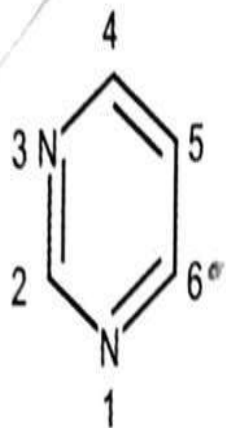
PURINE RING



ADENINE

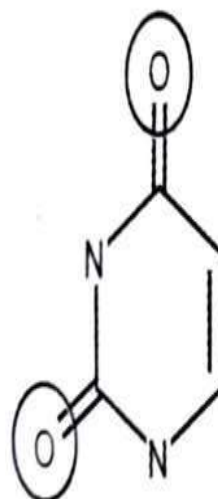
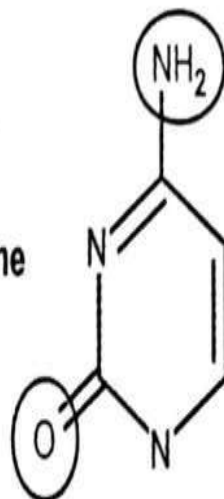


GUANINE

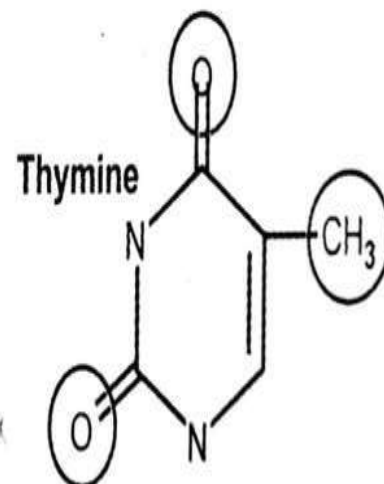


Pyrimidine

Cytosine



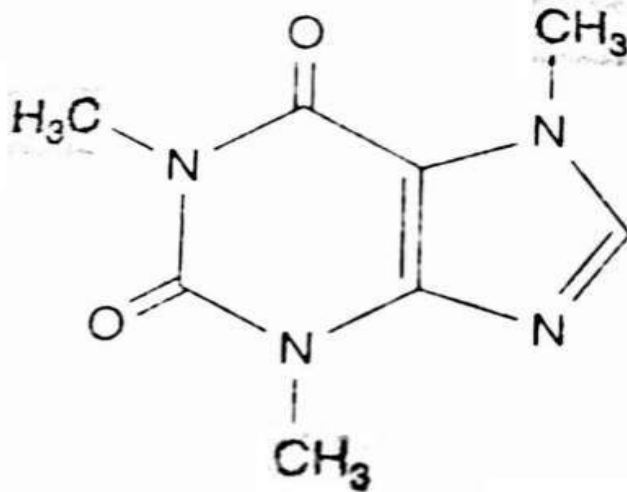
Uracil



Thymine

- Unusual bases are naturally found in some species of plants or some microorganism .

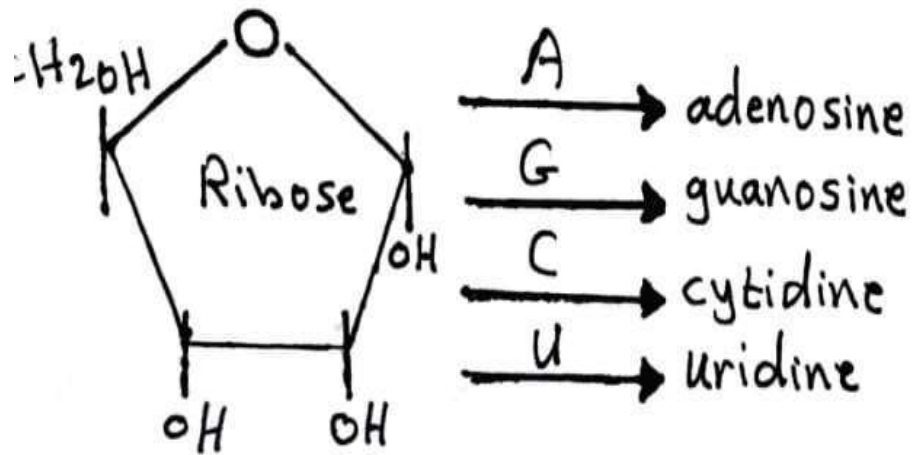
For example Caffeine (1,3,7- trimethyl xanthine) in coffee and tea.



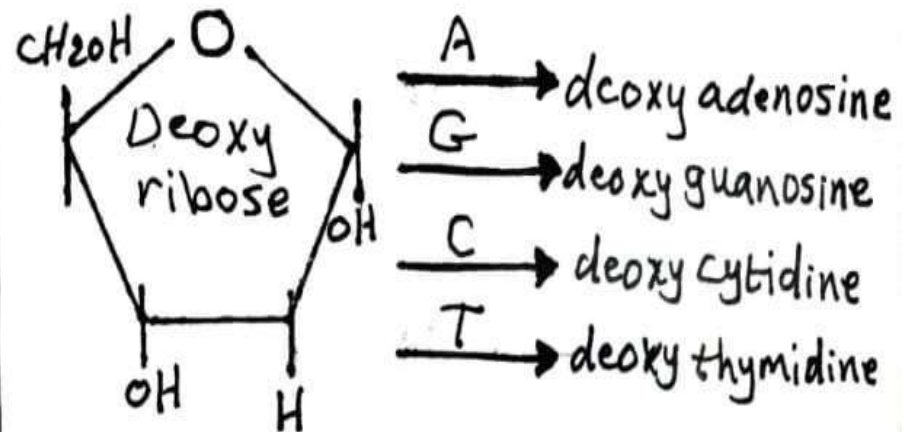
Caffeine structure

Nucleosides

- The addition of pentose sugar to the nitrogen base , forms a nucleoside .
- If the sugar is ribose , the product is ribonucleoside.
- If the sugar is deoxyribose , the product is deoxyribonucleoside .



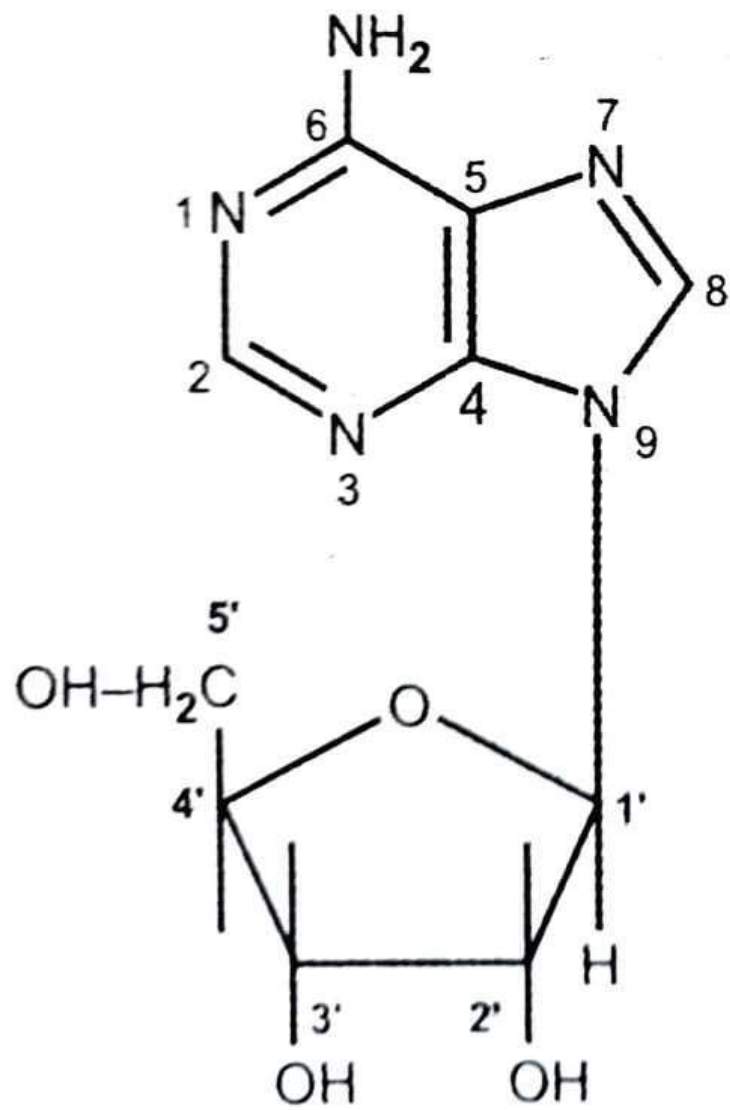
Ribonucleosides



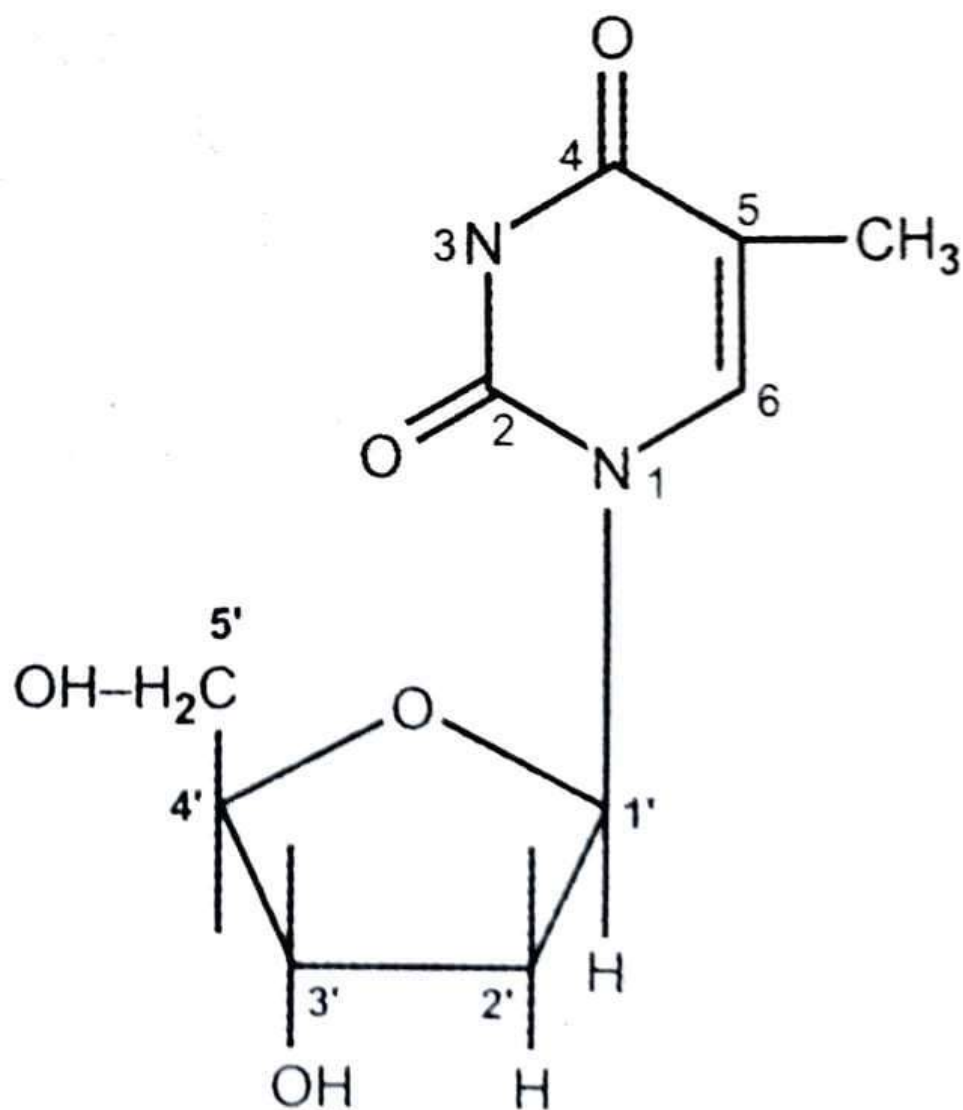
Deoxyribonucleosides

- The nitrogen bases : Adenine (A) , Guanine (G) , Cytosine (C) , and Thymine (T) are structural components of DNA molecules .
- but Uracil (U) are replaced by Thymine in RNA molecules .
- The carbon and nitrogen atoms in the rings of the base and of the sugar , are numbered separately ,
- the carbons in the sugar are numbered 1̂ to 5′

Adenosine

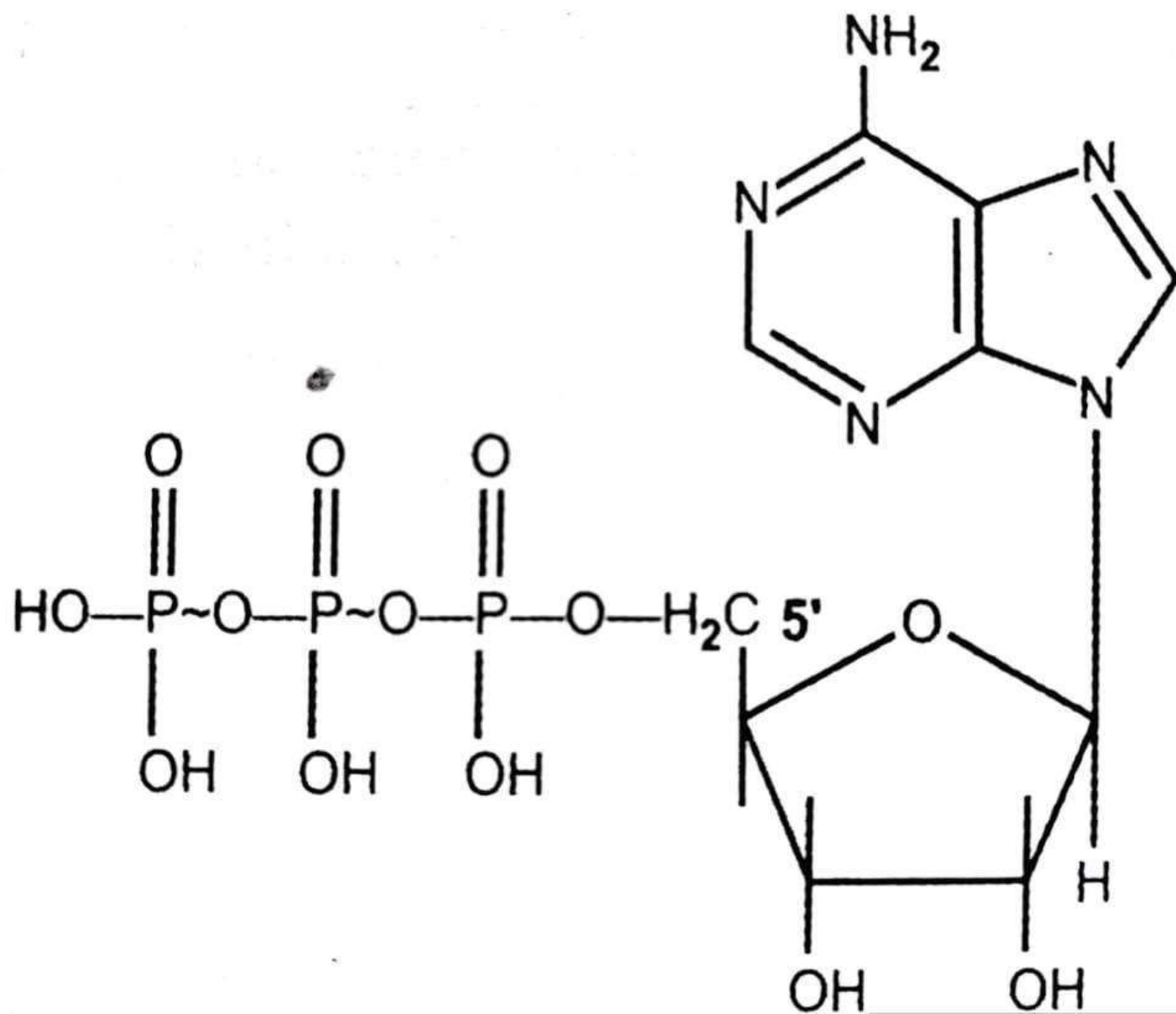


Deoxy thymidine



Nucleotides

- Addition of one or more phosphate group to the nucleoside , forms a nucleotide .
- The first phosphate group is attached to 5'-OH of the pentose sugar , and the structure is called nucleoside monophosphate .
- For example adenosine monophosphate (AMP) , deoxyadenosine monophosphate (d AMP).
- If a second or third phosphate added to the nucleoside , the result is nucleoside diphosphate
(e.g adenosine diphosphate) (ADP)
or nucleoside triphosphate (e.g adenosine triphosphate)
(ATP)



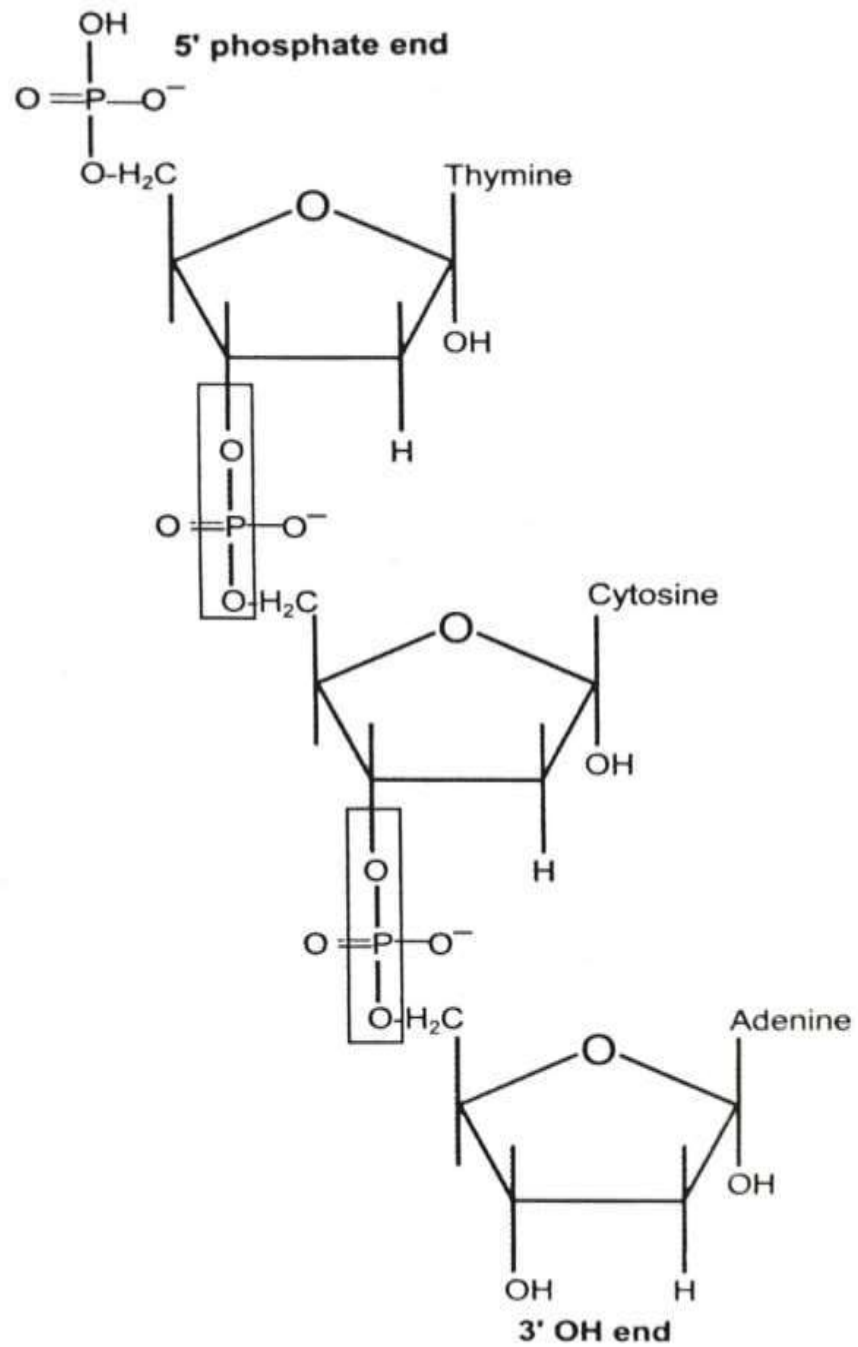
Deoxyribo Nucleic Acid (DNA)

DNA is a polymer of purine and pyrimidine nucleotides linked by phosphodiester bonds .

The molecule is formed by a combination of base + sugar + phosphate group .

The 3'- hydroxyl of one sugar is combined to the 5'-hydroxyl of another sugar through a phosphate group .

In the DNA , the base sequence is very important because the genetic information is coded in the specific sequence of bases ,
if the base is altered , the information is also altered .



RiboNucleic Acid (RNA)

- Also a polymer of purine and pyrimidine nucleotides linked by phosphodiester bonds ,
- but ***RNA differs from DNA*** in :
- Mainly seen in cytoplasm , but DNA inside the nucleus .
- Consist of single stranded , but DNA consist of double stranded .
- Sugar in RNA is ribose , sugar in DNA is deoxyribose .

Prof. Dr. Samera Alkatib

Plasma Proteins

Total blood volume is about 4.5 – 5 liters in adult human .

If the blood mixed with anti-coagulant and centrifuged , the cell components (**RBC and WBC**) are precipitated , and the supernatant (filtrate) is called **plasma**.

If the blood taken up without anti-coagulant , and allowed to clot , after about 2 hours the filtrate is separated which is called **serum** . The precipitate include **RBC , WBC , blood clotting factors**.

The serum consist of water and dissolved solutes . The major solutes in plasma are :

Proteins (about 70% of total solutes)

Small molecules (20%) such as glucose , amino acids , lipids , and other metabolites .

Ions (10%) such as Na^+ , K^+ , Mg^{+2} , Ca^{+2} , Cl^- , HCO_3^- , I^- .

The level of these solutes reflect the functions of the tissues .

Almost all plasma proteins are synthesized in the liver except immunoglobulins .

More than 500 proteins were found in blood , several of them have well known functions.

Electrophoresis

In clinical laboratory , electrophoresis is an instrument used for separation of serum proteins

Electrophoresis refers to the movement of charged molecules when it applied to an electric field .

Plasma proteins are separated into **5 bands or groups :**

Albumin

α_1 - Globulin

α_2 - Globulin

β – Globulin

γ – Globulin

Group 1 : Albumin

Functions :

Maintain about 80% of the osmotic pressure (keep the fluids in the blood , so it does not move into the tissues)

Transport various substances in the blood such as bilirubin , fatty acids , ions , and some drugs .

Hypo-albuminemia:

Decreases the level of albumin in blood . this leads to edema in the tissues .

Causes of hyopalbuminemia :

- ***Cirrhosis of liver:*** synthesis of albumin is decreased
- ***Nephrotic syndrome:*** a large amount (a few grams) of albumin is lost in urine
- ***Malnutrition:*** the availability of amino acids is reduced , so the albumin synthesis is affected
- ***Protein losing entropathy:*** large amount of albumin is lost from intestinal tract

Group (2) : **α_1 globulin**

This group includes:

Antitrypsin

Antichymotrypsin

Fetoprotein

Acid glycoprotein

Vitamin D binding protein (Gc globulin)

Antitrypsin:

Also called protease inhibitor . it inhibits trypsin , chymotrypsin , elastase and other proteases , so it prevent the hydrolysis of structural proteins .

Fetoprotein :

The major plasma protein found in the fetus .

It is synthesized in the liver of fetus.

Its level is usually high when a baby is born , then decreased to a very low levels at the first age of the life

Normal range of fetoprotein in healthy adults and children is under 10 ng/ ml

At birth , the normal infants have fetoprotein level 4 times above normal range.

Function:

Fetoprotein protects the fetus from the immunolytic attack by its mother.

Fetoprotein transports the compounds to the fetus such as steroids.

Group (3): **α_2 globulin**

This group includes:

α_2 -macroglobulin
ceruloplasmin
Haptoglobin

ceruloplasmin

consist of a single polypeptide chain with a MWt = 123KD
it is synthesized in the liver , and then secreted into the plasma , and bound with copper

each molecule of ceruloplasmin contains 6-8 atoms of copper in form of cupric (Cu^{+2}) and cuprous (Cu^{+1})

- ceruloplasmin stores and carries the copper around the body to the tissues that need it.
- Ceruloplasmin eliminates the excess copper from the body into the bile where it excreted as a waste product.
- Ceruloplasmin is helped in the iron metabolism, it called **ferroxidase** where it catalyzes the oxidation of dietary ferrous ion (Fe^{+2}) to ferric ion (Fe^{+3}) in mucosal intestine cells which then binds to ferritin ,
- so ceruloplasmin reduces the level of free ferrous ions and hence reduce the free radical reactions come by the small amount of Fe^{+2} in the blood.
- So ceruloplasmin is an important antioxidant in plasma.

Wilson disease:

- An inherited disorder , incidence is 1 in 50000
- Characterized by stored excess copper in the tissues especially liver and brain , causing liver cirrhosis and neurological symptoms.
- Wilson disease is caused by mutation in gene responsible for the synthesis of ceruloplasmin ,
- so excess copper is not removed and accumulates at toxic levels that can damage the tissues

Haptoglobin

- Protein used to clear the blood from free hemoglobin (Hb) found outside the RBC .
- Most Hb is located inside RBC , but small amounts are circulate free in the blood.
- Haptoglobin binds to free Hb and formed a complex called haptoglobin-Hb. this complex is removed from the circulation within minutes of its formation into the liver and spleen for storage.
- So haptoglobin prevents the loss of free Hb and its iron by the kidney.

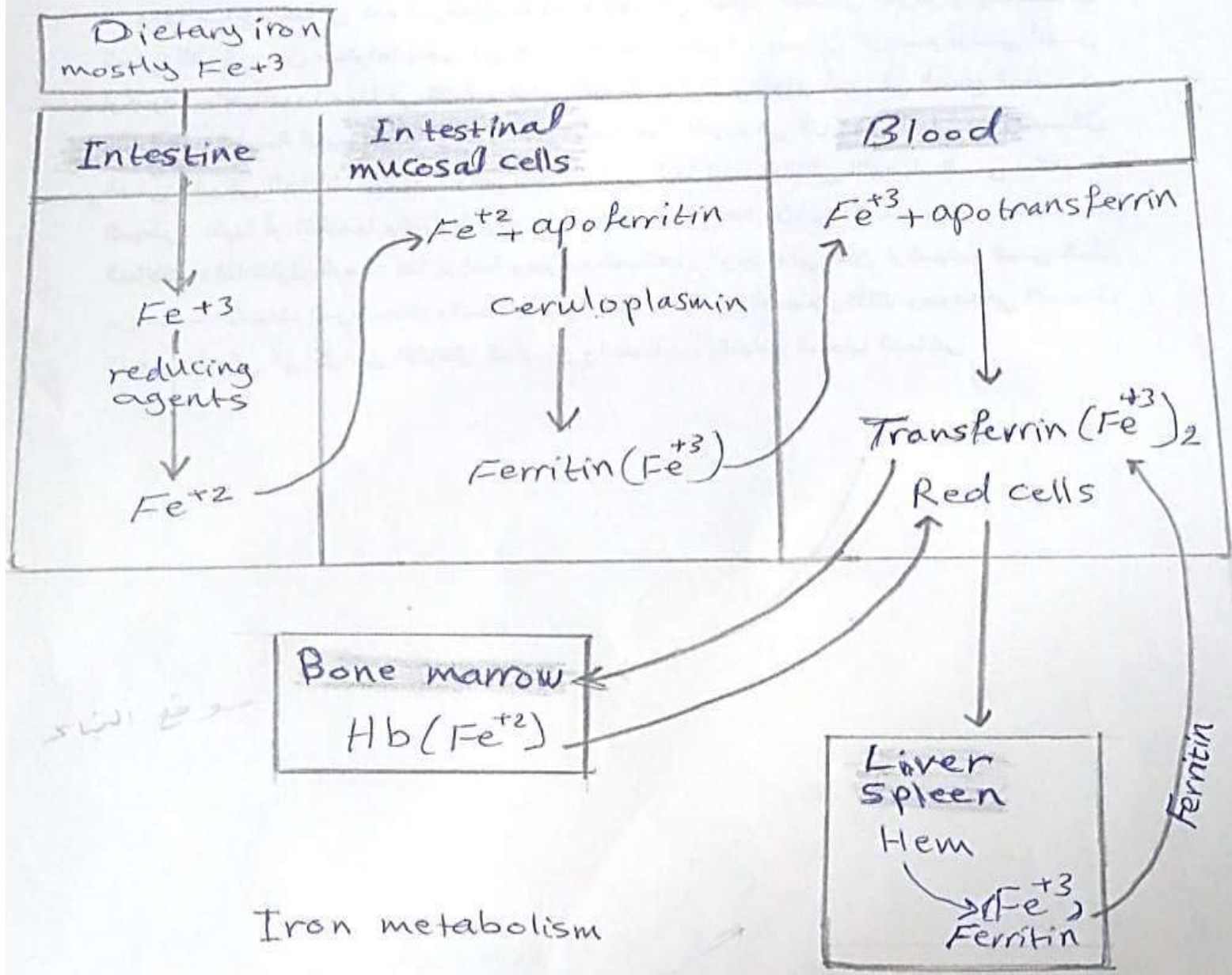
Group (4): β - globulin

This group includes :

- Transferrin
- Hemopexin
- Lipoproteins
- Macroglobulin
- Complements
- Fibrinogen
- C- reactive protein

Transferrin

- Play a central role in iron metabolism . it responsible for the distribution of ferric ion (Fe^{+3}).
- Each molecule of transferrin binds with 2 ions of Fe^{+3}
- Transferrin transports Fe^{+3} through the blood into its storage sites liver and spleen . Fe^{+3} bound with apoferritin and stored as ferritin.
- Transferrin transports Fe^{+3} to the bone marrow cells which synthesized the Hb and other heme containing compounds.
- Therefore transferrin is an essential marker for body iron status.



Hemopexin

- Hemopexin removes the free heme from the circulation.
- The free Hb circulating in the blood , will soon breakdown into free heme and globin .
- The free heme binds with hemopexin forming a complex called heme-hemopexin.
- This complex is eliminated from the circulation by the liver to be destroyed.
- So, hemopexin prevents the loss of iron , and acts as a second line of defense against oxidative damage by Hb .

Complements

Complements is a multiproteins called $C_{1q} - C_9$, circulating in the blood , and activate the immune system to clear pathogens (bacteria , virus , fungi ,-- --)

Complements is a part of immune system , consist of series proteins bind together at the surface of Ab – Ag complex during inflammation in order to eliminate and lysis the pathogens.

C- Reactive protein (CRP)

C- reactive protein synthesized in liver and sent into blood in response of inflammation.

CRP is one of acute phase proteins that increase in response to a wide range of acute and chronic inflammatory conditions such as bacterial , viral , fungal infection , and other inflammatory diseases.

CRP binds on the surface of pathogens , and this will activate the complements system.

Fibrinogen

Factor 1 , synthesized in liver and circulates in the blood

During injury ,
fibrinogen is converted from soluble protein into insoluble protein called fibrin. This is done by thrombin enzyme (factor 11)

Prothrombin

a single polypeptide chain with Mwt 69000 dalton ,
converted to thrombin by the removal of peptide chain of 35000 dalton

Coagulation process :

- When blood vessel is broken , platelets are aggregate , thrombin molecules bound with platelets by thrombin receptors on their surface .
- the result is conversion the soluble fibrinogen into insoluble fibrin and block blood vessel to stop bleeding by forming a clot .
- Coagulation process requires vitamin K , Ca^{+2} , phospholipids , and clotting factors (V11 , 1X , X , X1 , X11) for activation.

Acute phase proteins:

They are proteins which may increase 50 – 1000 fold in blood in response to a various acute and chronic inflammatory conditions such as bacterial , viral , or fungal , and cancers

Important acute phase proteins are :

C- reactive protein

Ceruloplasmin

Fibrinogen

Antitrypsin

macroglobulin

Group 5 : γ – globulin

also called Immunoglobulins (Ig) refers to the status of this globulin.

Also called antibodies (Ab) refers to the function of the globulin.

They are proteins synthesized in B- lymphocytes (which derived from bone marrow) and secreted into blood in response to exposure to pathogens called (antigens) such as pathogenic bacteria and viruses.

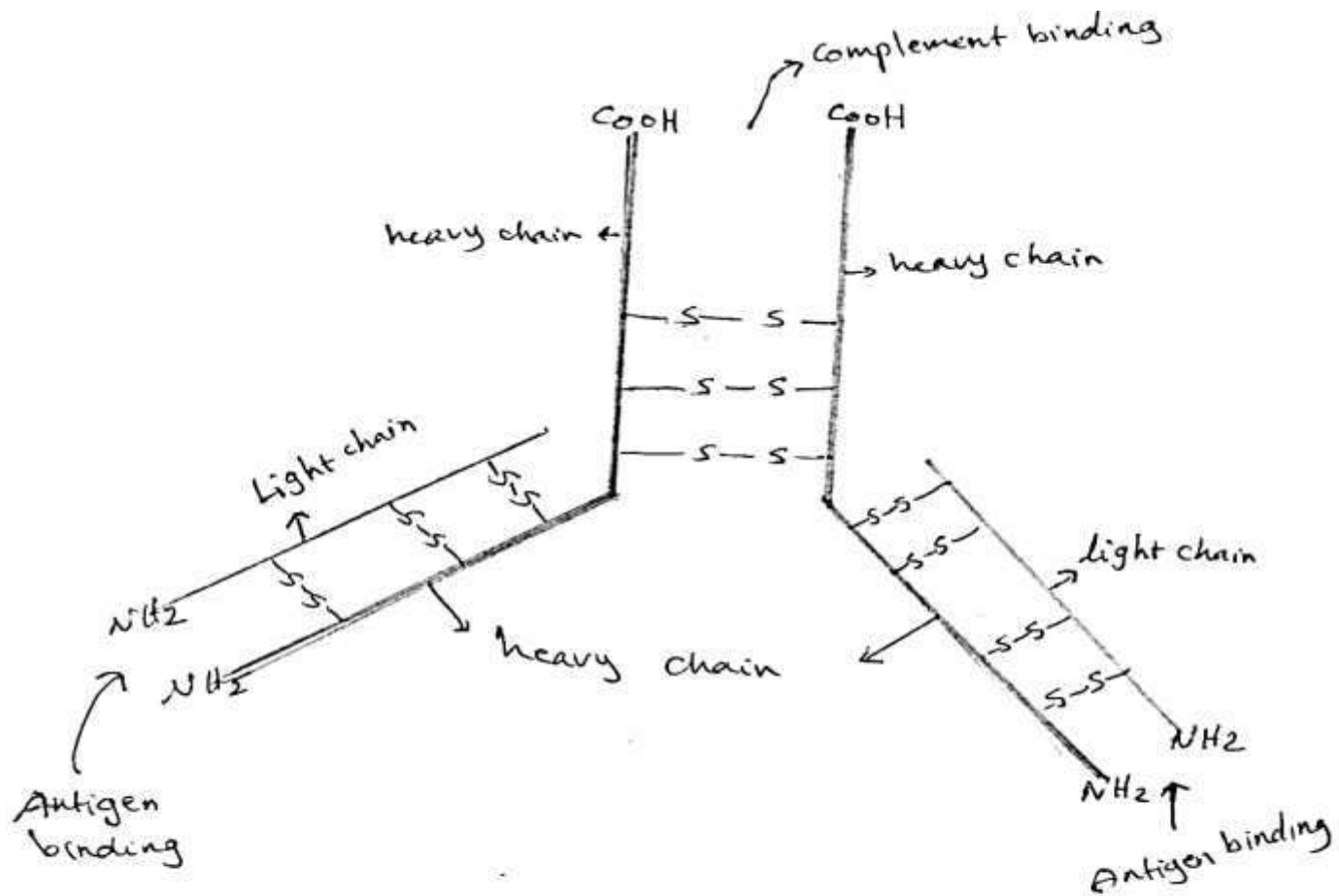
Structure of immunoglobulins:

- The structure of Ig molecule is made up of four polypeptide chains ,

2 heavy (H) chains and 2 light (L) chains, combined together through disulfide bonds (s – s) , forming Y shaped structure .Each Y shape is called monomer.

- H chains are composed of 440 amino acids with molecular wt 53000 – 75000 D
- L chains made up of 214 amino acids with Mwt 23000 D
- L chains are either kappa (K) or lambda ()

Usually the Ig molecule consist of either 2K or 2 , but never the mixture of them.



“ Immunoglobulin structure ”

NH₂ : amino terminal end . site of Antigen binding
 COOH : Carboxyl terminal end . site of Complement binding

Classes of Ig

There are 5 classes of Ig , depending on the type of heavy chain:

IgG has gamma heavy chain

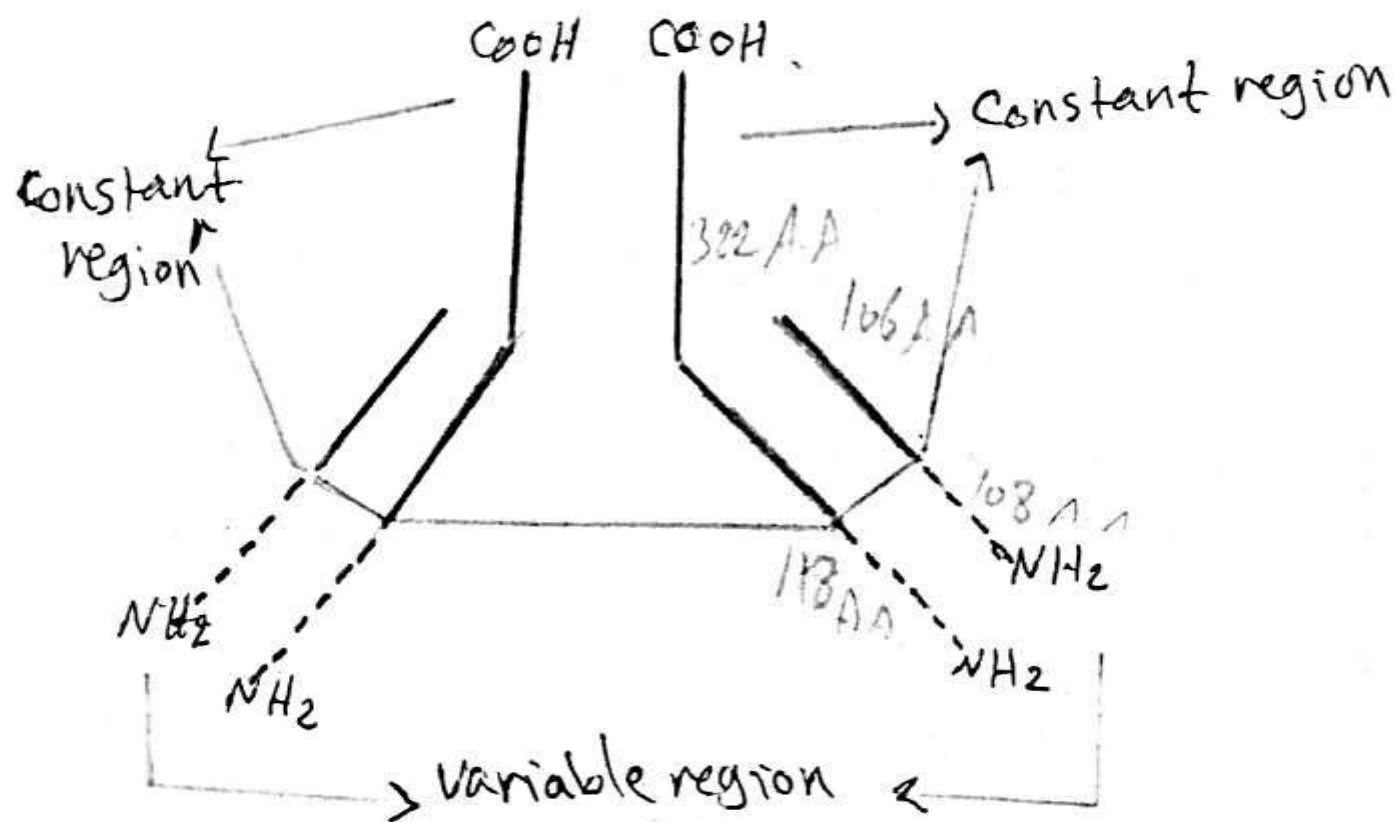
IgM has μ heavy chain

IgA has alpha heavy chain

IgD has delta heavy chain

IgE has epsilon ϵ heavy chain

Differences in heavy chain lead to a different functions of these Ig molecules.



Variable and constant regions :

Both the heavy and light chains are divided into 2 regions : variable (V) , and constant (C) .

Constant region includes half ($1/2$) light chain , and three quarter ($3/4$) heavy chain toward the carboxyl terminal .

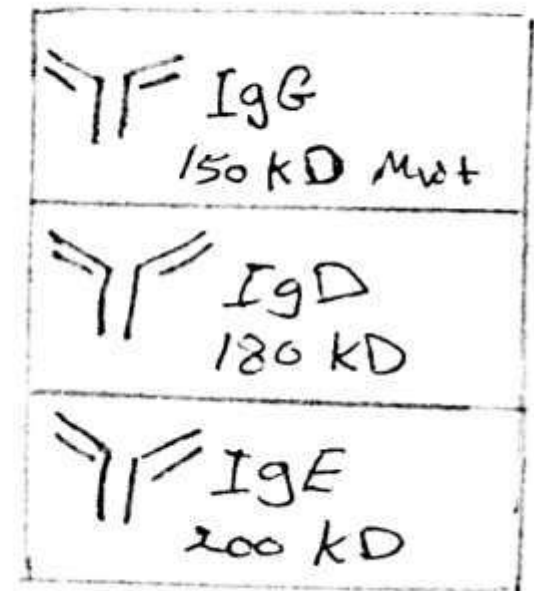
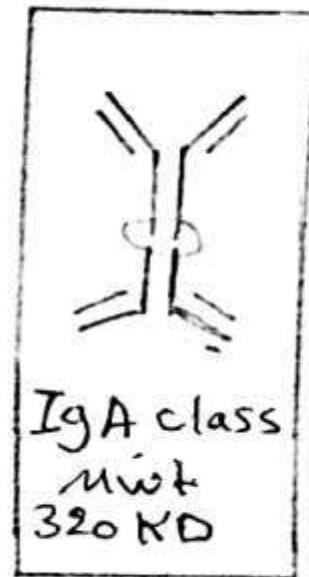
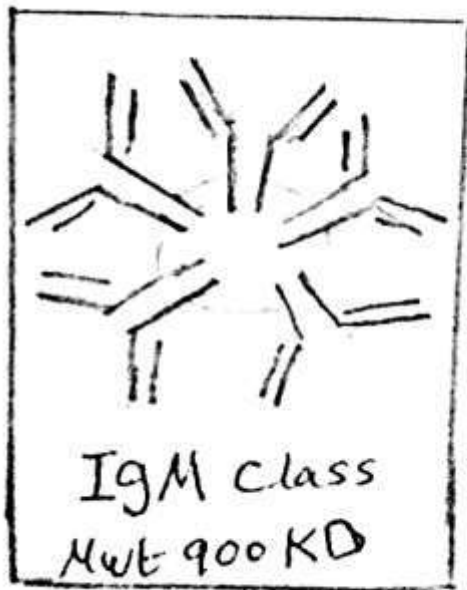
Constant region mean that all types of immunoglobulins have the same amino acids .

Constant region is a site of complements binding

Variable region includes the half ($1/2$) light chain and one quarter ($1/4$) heavy chain toward the amino terminal .

Variable region is a site of antigen binding . Each Ig molecule contains 2 antigen binding sites.

Immunoglobulin classes differ in number of Y units (monomers) which join together forming the complete protein molecule. For example , in human , the IgM have 5Y units , so containing a total of 10 light chains , 10 heavy chains , and 10 antigen binding sites .



Porphyrins and Hemoglobin

Part 2

Prof.Dr. Samera Alkatib

Abnormal Hemoglobins

There are more than 300 types of abnormal hemoglobin . The most common are :

1- Hemoglobin S (HbS) Sickle Cell disease

Normally there is 2α and 2β globin chains , but glutamine is replaced by valine at position 6 of β chain. This causing sickling RBC_s , tissue hypoxia , and leading to hemolytic anemia . The RBC_s life span is 30 days instead of 120 days .

2- α -Thalassemia :

Or called mediteranian mild anemia , also called Thalassemia minor .

In this condition the Hb contains 4 β – and there is a defective in the synthesis of α – chains

In the normal condition , each person has 4 genes that responsible for the synthesis of α -globin chains .

In α - thalassemia , there is a mutation or absent of one or more of these genes . This leads to reduction in the hemoglobin synthesis which prevents enough oxygen from reaching the tissues . The red blood cells are destroyed at a faster rate leading to mild or severe anemia , which can cause pale skin , weakness , fatigue , and other complications .

3- β – Thalassemia :

Or Thalassemia major . It is common in our country “ Iraq “ . There are 2 specific genes responsible for the synthesis of beta - globin chains . Beta type characterized by the decrease or absence of synthesis of β - chains leading to synthesis of abnormal hemoglobin.

Severe anemia occur , liver and spleen enlargement , pale skin , and weak bones . These people will need repeated blood transfusions for treatment .

4- Methemoglobinemia :

In this case , histidine is replaced by tyrosine , so protection of iron in ferrous state will be lost , leading to oxidation of iron to ferric state and Hb will lose ability to transport oxygen .

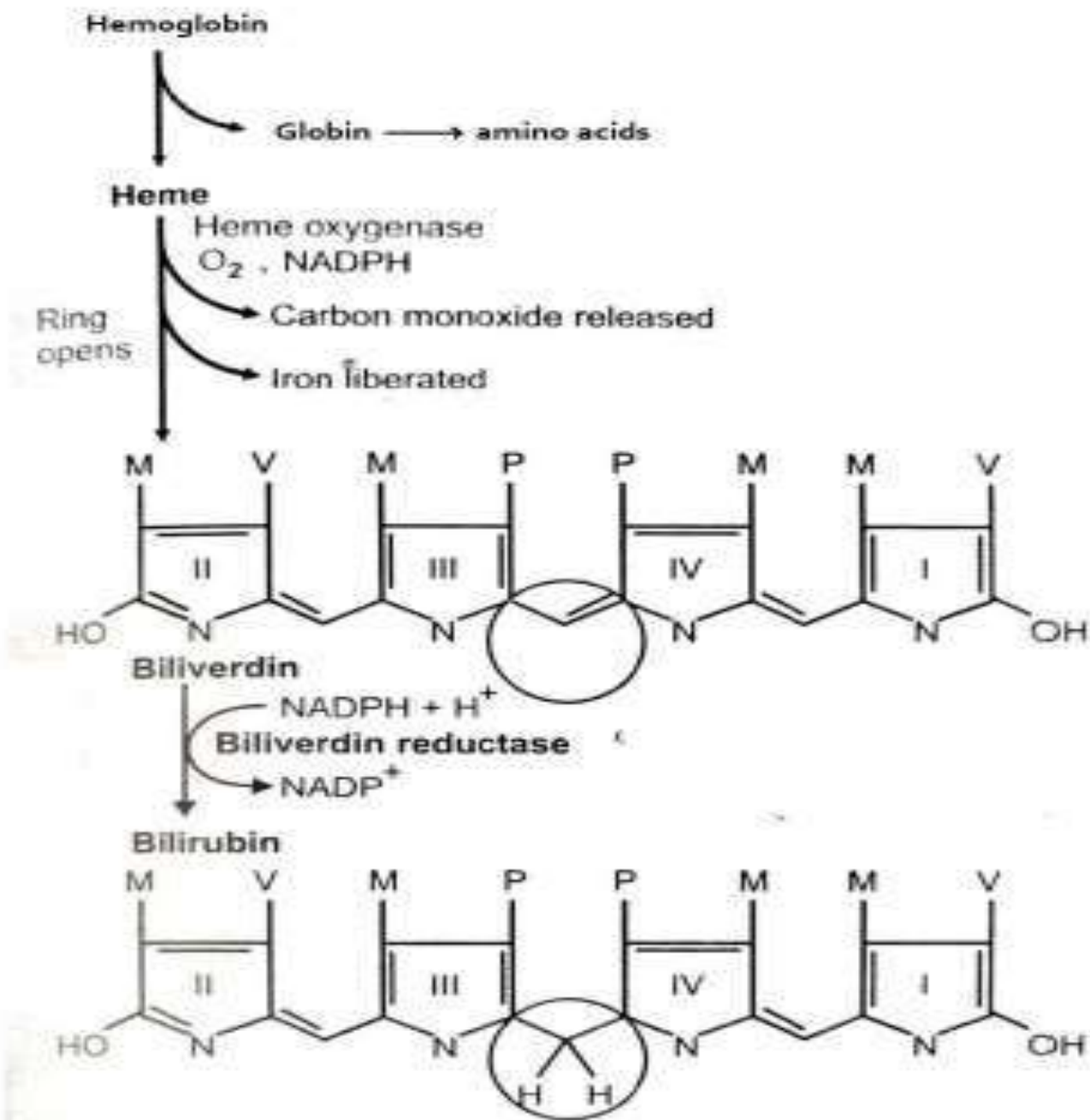
Various forms of hemoglobin :

- 1- Oxyhemoglobin (HbO_2) : Or fully oxygenated hemoglobin , iron in ferrous state (Fe^{+2}) .
- 2- Deoxy hemoglobin : Or reduced hemoglobin , it is Hb not combined with O_2 , also iron in reduced form (Fe^{+2}) .
- 3- Carboxy hemoglobin : It is Hb combined with CO_2 , the iron is in the ferrous state .
- 4- Methemoglobin : Iron is in the ferric state " Fe^{+3} ". It is either inborn abnormal Hb or due to presence of oxidizing agents like potassium ferricyanide .
- 5- Glycosylated hemoglobin (HbA_1C) : Glucose in RBC binds with hemoglobin forming glycosylated Hb . Normally , about 5% of glycosylated Hb found in blood . It reflects the concentration of blood glucose for 3 months ago and its measurement is important to follow up the diabetic patients .

Catabolism of Heme and Generation of Bilirubin

- The end product of Hb catabolism is a yellow pigments called bilirubin .
- Bilirubin has no function in the body, and excreted in the bile.
- When Hb is catabolized , globin chains are separated and hydrolyzed to its amino acids which are reused
- Iron liberated from heme and oxidized to ferric ion and taken up by transferrin .

- The porphyrin ring is broken down in the reticuloendothelial cells of liver , spleen , and bone marrow to a biliverdin which is green in color . Biliverdin is reduced to unconjugated bilirubin , a yellow pigments called indirect bilirubin .
- About 6 gm of Hb is catabolized daily , which form about 250 mg of bilirubin . Another 50mg of bilirubin is formed from myoglobin and other heme containing proteins .



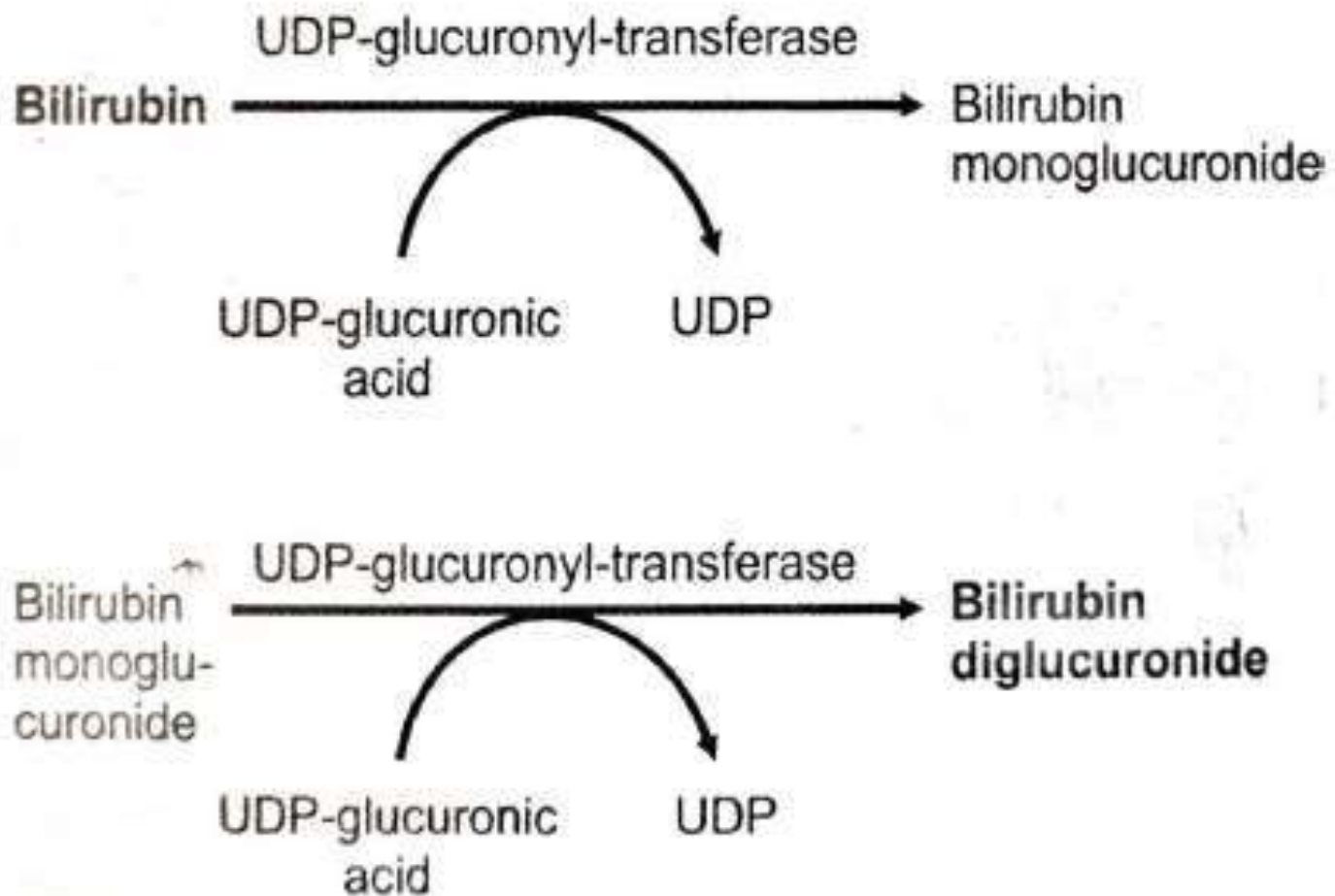
Liver takes up the bilirubin

Unconjugated Bilirubin formed in the reticuloendothelial cells is insoluble in water , so it transported in plasma bound with albumin . One molecule of albumin can bind 2 molecules of bilirubin .

When the albumin – bilirubin complex reach the surface of liver , bilirubin is taken up .

Conjugation in Liver

Inside the liver, bilirubin is conjugated with two molecules of glucuronic acid, to make it water soluble and it is called direct bilirubin.



Fate of conjugated Bilirubin is secreted into bile

The water soluble conjugated (direct) bilirubin is excreted into the bile

Conjugated Bilirubin Reduced by Intestinal Bacteria

The conjugated bilirubin reaches the large intestine . Intestinal bacteria deconjugate the conjugated bilirubin forming free bilirubin , which reduced by the bacteria to a colorless compounds called **urobilinogens** .

Enterohepatic Circulation

Small amount (about 20%) of urobilinogens is reabsorbed from intestine and returned to the liver by portal vein , and is again re-excreted through the liver (enterohepatic circulation) .

Final Excretion

Normally , most of the colorless Urobilinogens formed in the colon are oxidized to colored compounds called **urobilins** which are then excreted in the feces .

Darking of feces on standing in air , is due to oxidation of residual urobilinogens to urobilins .

Plasma Bilirubin

Normal plasma bilirubin level ranges from 0.2 – 0.8 mg / dL , while conjugated bilirubin is only 0 – 0.2 mg/ dL .

Jaundice

Jaundice means that there is excess amount of bilirubin in the blood , this leads to a yellow discoloration of the skin and eyes . Jaundice is not clinically visible until the bilirubin level reaches 2 – 3 mg/ dL .

Elevated Amounts of Unconjugated Bilirubin in Blood

Occur in number of Conditions : Some examples :

Neonatal “Physiologic Jaundice”

Jaundice is common in newborn babies because babies have a high number of red blood cells in their blood and have to broken down .

The liver of newborn baby is not completely developed , so it less effective at processing the bilirubin and removing it from the blood .

Normal indirect bilirubin would be under 5.2 mg/dL within the first 24 hours of birth .

Most jaundice goes away by 2 weeks . Phototherapy sometimes used to treat newborn jaundice through process called photo – oxidation . Photo – oxidation adds oxygen to the bilirubin , so it dissolves easily in water and easily removed from the blood .

Gilbert Disease

It is inherited caused by the defect in the uptake of bilirubin by the liver. Patient is asymptomatic (with no symptoms) except for the presence of mild jaundice , so the condition is harmless .

Toxic Hyperbilirubinemia

Unconjugated hyperbilirubinemia can result from liver cells dysfunction which caused by chloroform , carbon tetrachloride , hepatitis virus , cirrhosis , and mushroom poisoning . These disorders are due to hepatic cell damage , which impairs the conjugation process .

Obstructive Jaundice

Results from blockage of the hepatic or bile ducts , most often due to gallstone or to cancer of the pancreas . Because of the obstruction , conjugated bilirubin cannot be excreted , it thus go again into hepatic veins and appears in the blood and urine

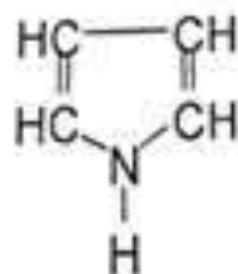
Porphyrins and Hemoglobin

Lecture 7

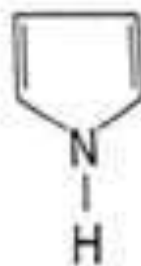
Prof.Dr. Samera Alkatib

Porphyrins and Hemoglobin

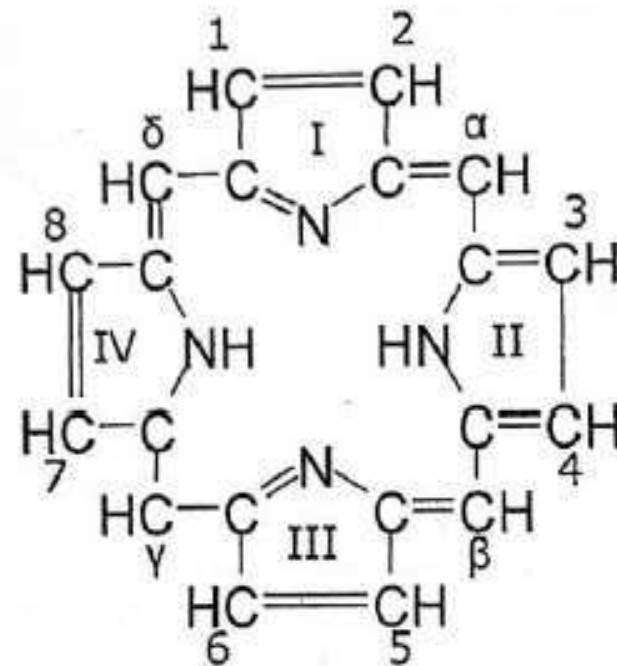
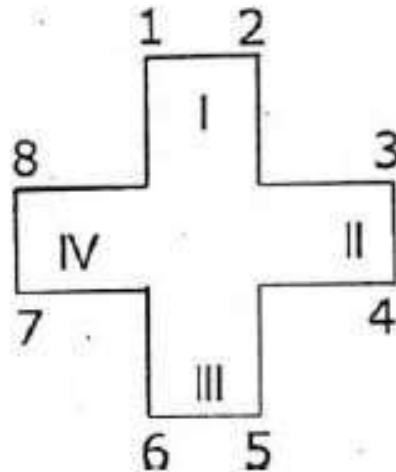
- Porphyrins are cyclic compounds consist of four pyrrole rings which are linked together by methylene bridges , and usually bind with metals at the center to form biologically important compounds .



pyrrole



- If the porphyrin combines with iron (Fe^{+2}), the molecule is called "heme", but if the metal is (Mg^{+2}), the compound will be chlorophyll, the green pigment in the plants.
- The pyrrole rings are named as I, II, III, IV and the bridges are named as α , β , γ , and δ . The site of substitutions are named 1 to 8.



- In nature , these porphyrins are conjugated to proteins and forming biological important compounds such as hemoproteins .

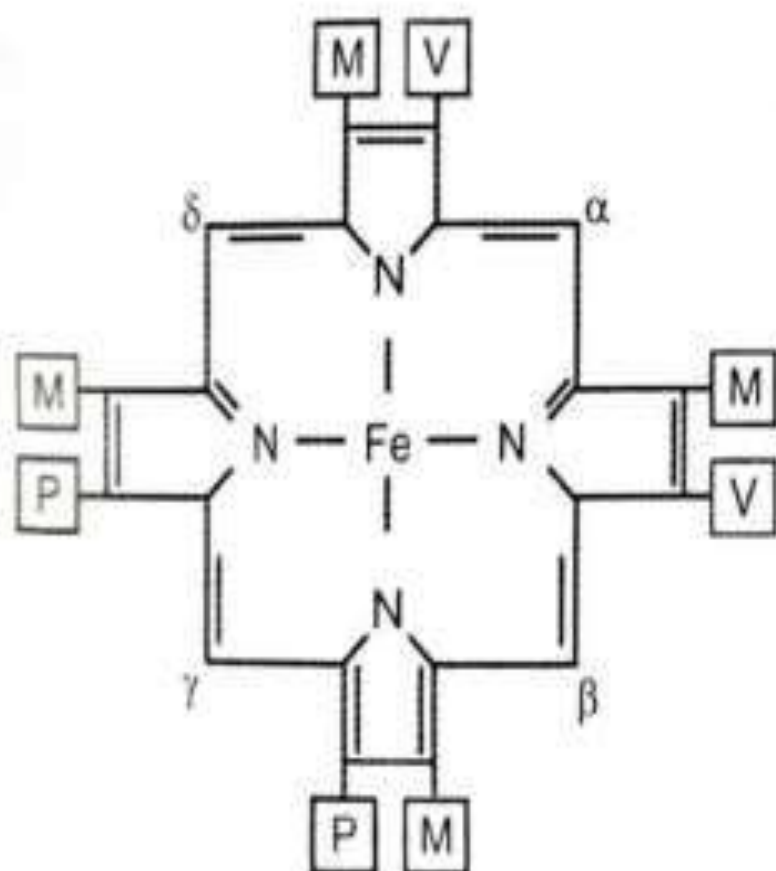
Examples of Some important Human Hemoproteins

1 – Hemoglobin (Hb) :

_ $\text{Hb} = \text{Heme} + \text{Globin}$

Heme is iron porphyrin called prosthetic group of globin .

M.Wt of hemoglobin is 65,000 D , Hb is responsible for transport the O_2 from lungs to all body tissues , and CO_2 from tissues to the lungs .



[M] = Methyl
 [V] = Vinyl
 [P] = Propionyl

2 – Myoglobin :

It is the respiratory pigments of muscles , it is O_2 storing molecules . During excess exercise , O_2 stored in myoglobin will be released and used by mitochondria to produce ATP to continue exercise . The molecule consist of single globin chain of 153 amino acids of M.Wt 1700 D .

3 – Cytochromes :

They are named as cyto b , cyto C_1 , cyto C , cyto aa_3 . They are a components of the respiratory chain , transfer the electrons (reducing equivalents) through the respiratory chain to the oxygen molecule .

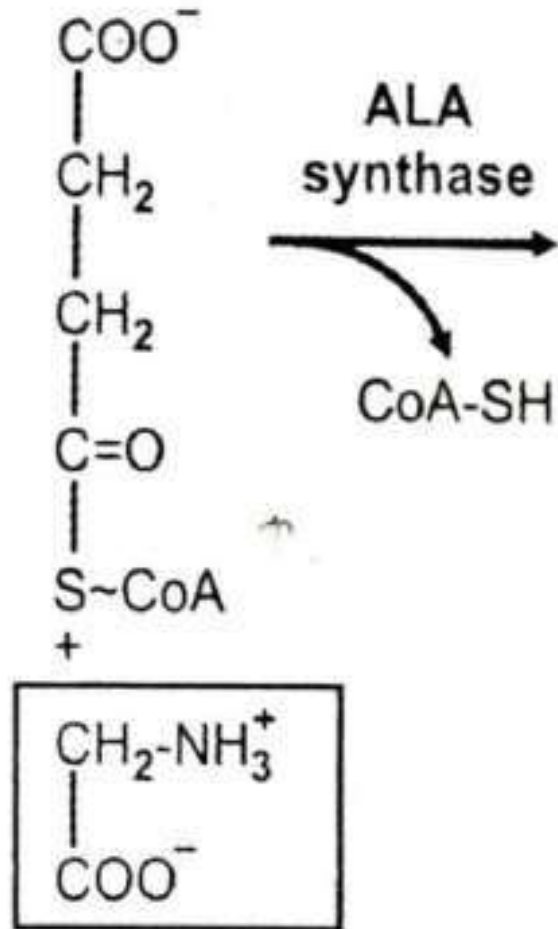
Biosynthesis of porphyrins and

Heme formation

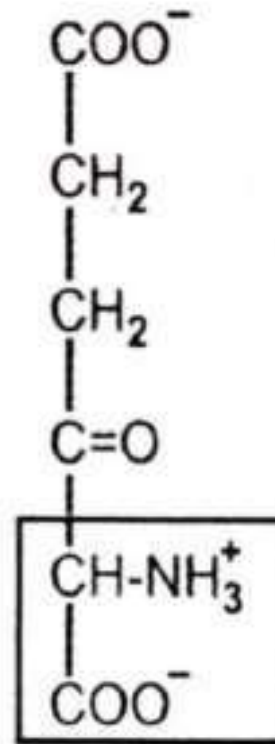
In living cells , porphyrin compounds are synthesized in all cells except cells which do not have mitochondria , for example erythrocytes .

- The starting substances are succinyl CoA , glycine , and pyridoxal phosphate (Vit B₆) . Succinyl CoA derived from krebs cycle , the enzyme catalyzes this reaction is amino levulinic acid (ALA) synthetase . The reaction occurs in the mitochondria , and the product is amino levulinic acid (ALA) .

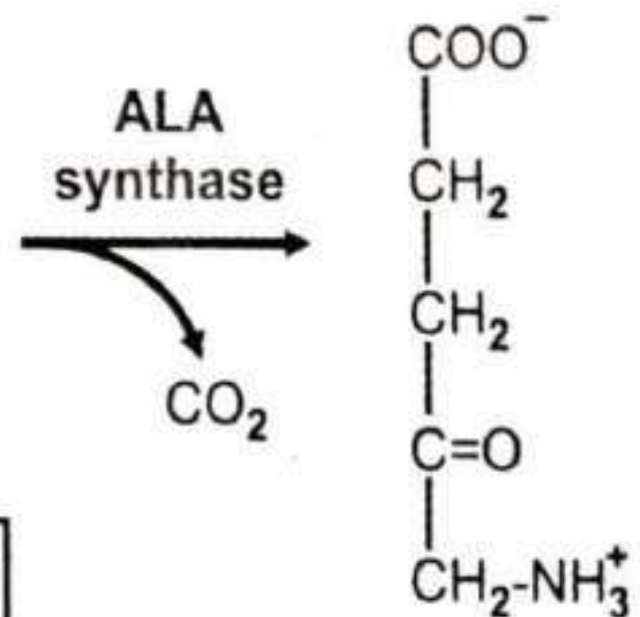
Succinyl CoA



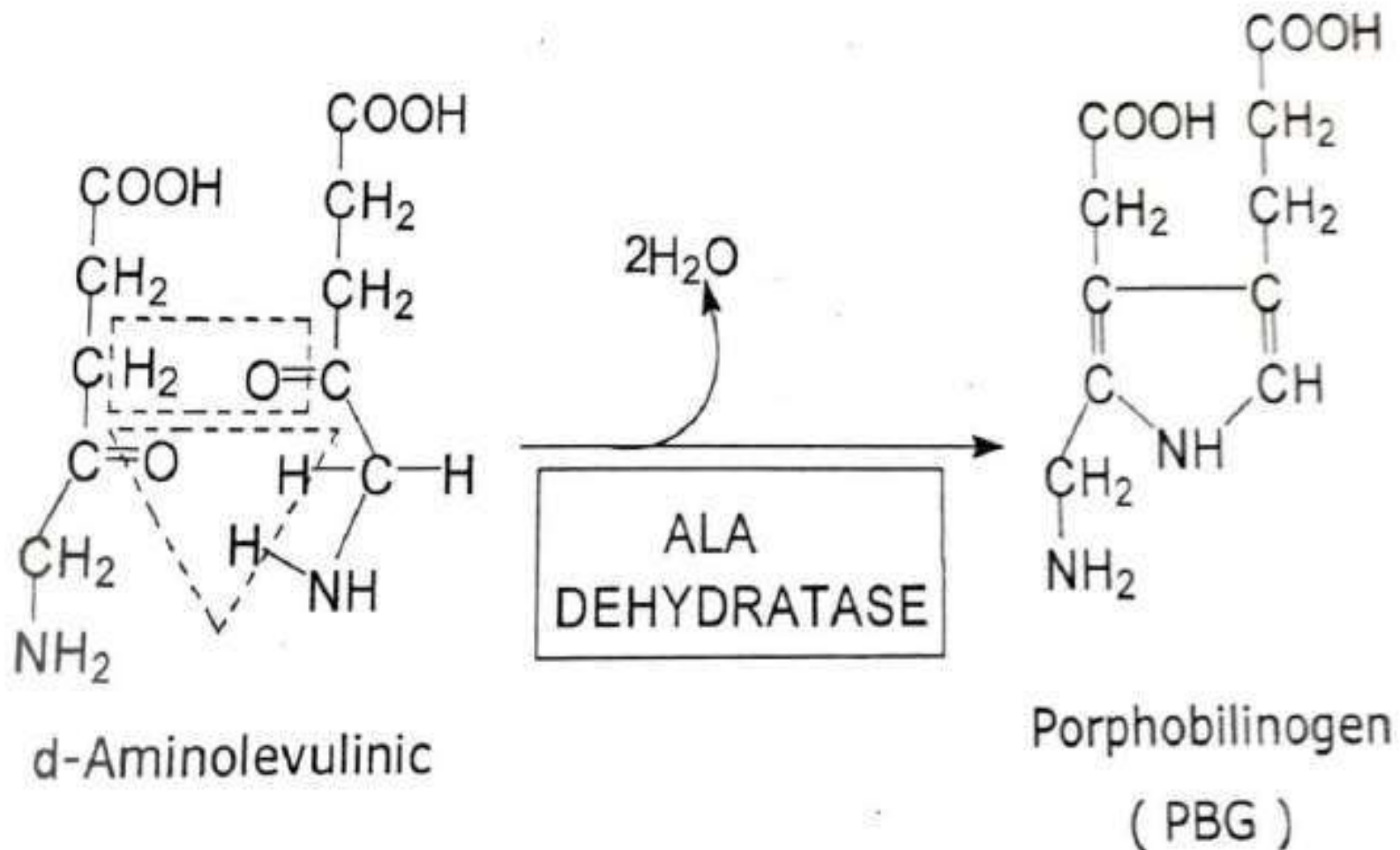
Glycine



Delta-amino
levulinic acid
(ALA)

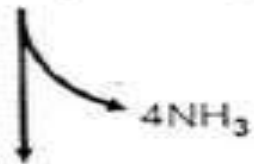


- Binding two molecules of ALA in the cytosol producing porphobilinogen as the first precursor of pyrrole compounds .



- condensation of 4 molecules of porphobilinogen results in the formation of the first porphyrin namely uroporphyrinogen which leads to other porphyrins synthesis : coproporphyrinogen and protoporphyrin .
- Protoporphyrin in the presence of ferrous ion (Fe^{+2}) , will be produced the heme which is the prosthetic group of hemoglobin.

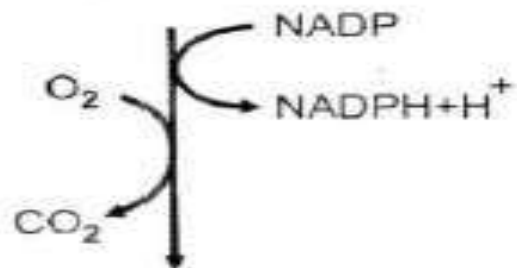
4 x Porphobilinogen



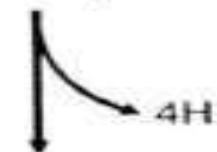
Uroporphyrinogen III
(UPG-III)



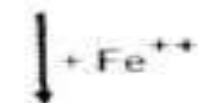
Coproporphyrinogen III
(CPG-III)



Protoporphyrinogen III
(PPG-III)

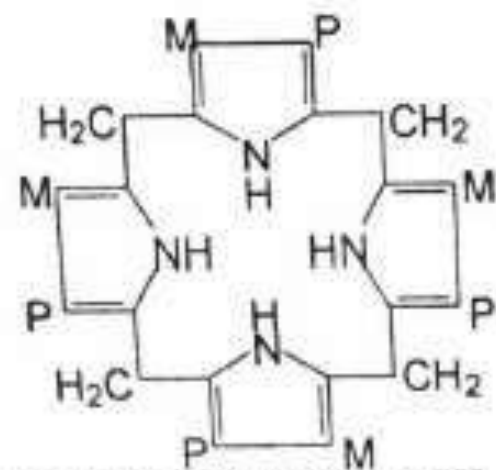


Protoporphyrin III

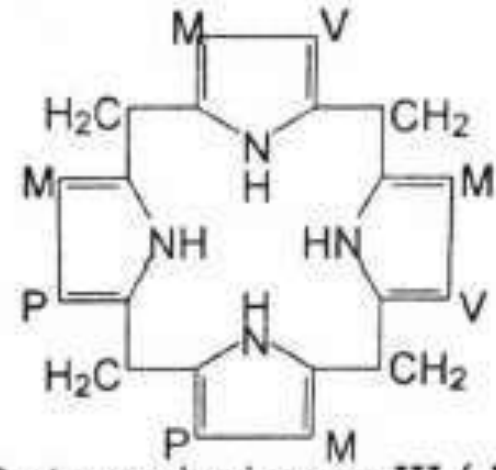
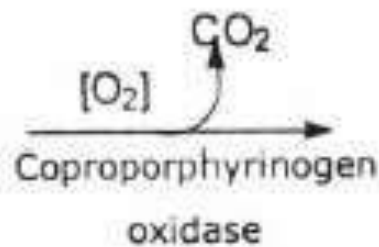


Heme

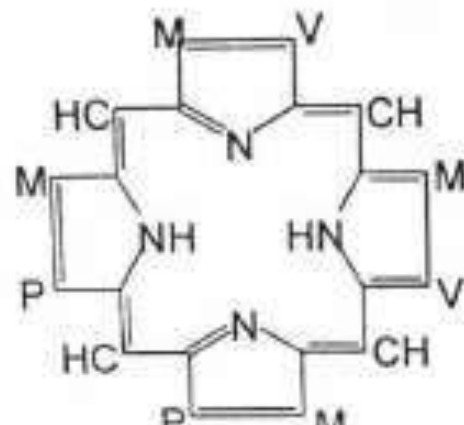
- About 85% of heme will be used for hemoglobin synthesis , 10% used for myoglobin synthesis , and 5% for the synthesis of cytochromes .



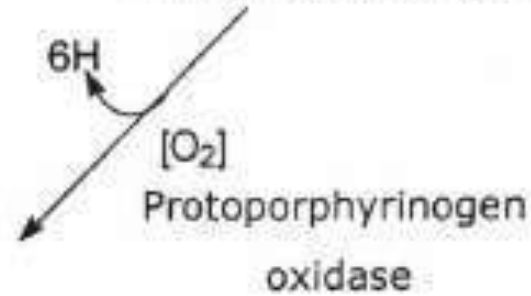
Coproporphyrinogen III



Protoporphyrinogen III (IX)



Protoporphyrin III (IX)



Structure of Hemoglobin Molecule :

Hemoglobin is synthesized in adults in the bone marrow , but during fetal life , it synthesized in the liver and spleen .

The most common types of normal hemoglobin are :

Hemoglobin A (HbA)

This is the most common type of hemoglobin found normally in adults , , which contains two α globin chains and two β globin chains . The α chain contain 141 amino acids and β chain contains 146 amino acids , each chain having heme group as prosthetic group .

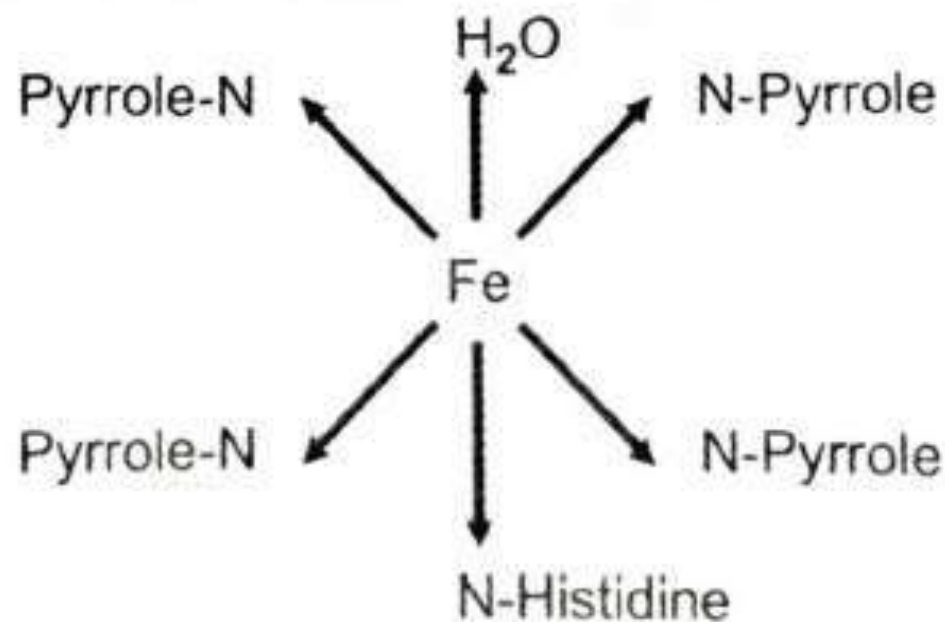
Hemoglobin F (Fetal hemoglobin) (HbF)

This type is normally found in fetuses and newborn babies . HbF is replaced by HbA shortly after birth , contains two α - and two γ - globin chains , the γ - chains contain about the same number of amino acids as β - chain .

Hemoglobin A₂ (HbA₂)

HbA₂ contains two α - and two δ - chains . It is a normal type of hemoglobin , and it present in normal adult blood in a ratio of 1 – 3% of total normal hemoglobin .

The capacity of Hb to bind O_2 depends on the heme, the central iron atom bounds to the four nitrogen atoms of pyrrole rings and the first nitrogen atom of a histidine residue of globin, the remaining valency of iron atom is linked with oxygen or water. So the iron is protected from oxidation (iron remains in the Fe^{+2}). When the Fe^{+2} in heme oxidized to ferric ion Fe^{+3} , **hematin** is formed, which loss the property of carrying the oxygen.



Affinity of Hb binding to O_2 is also affected by the presence of 2,3-Bisphosphoglycerate (2,3-BPG) , this compound causes a decrease affinity of Hb to oxygen in the tissues , so more oxygen comes to the tissues .

Without 2,3-BPG , Hb cannot transport O_2 efficiently and only 8% of its O_2 release in the tissues .

2,3- BPG binds more weakly to HbF than to adult HbA , therefore increasing the oxygen binding affinity of HbF and hence allows more O_2 to be transferred from mother to fetus .

Abnormal Hemoglobins

There are more than 300 types of abnormal hemoglobin . The most common are :

1- Hemoglobin S (HbS) Sickle Cell disease

Normally there is 2α and 2β globin chains , but glutamine is replaced by valine at position 6 of β chain. This causing sickling RBCs , tissue hypoxia , and leading to hemolytic anemia . The RBCs life span is 30 days instead of 120 days .

2- α -Thalassemia :

Or called mediteranian mild anemia , also called Thalassemia minor .

In this condition the Hb contains 4 β – and there is a defective in the synthesis of α – chains

In the normal condition , each person has 4 genes that responsible for the synthesis of α -globin chains .

In α - thalassemia , there is a mutation or absent of one or more of these genes . This leads to reduction in the hemoglobin synthesis which prevents enough oxygen from reaching the tissues . The red blood cells are destroyed at a faster rate leading to mild or severe anemia , which can cause pale skin , weakness , fatigue , and other complications .

3- β – Thalassemia :

Or Thalassemia major . It is common in our country “ Iraq “ . There are 2 specific genes responsible for the synthesis of beta - globin chains . Beta type characterized by the decrease or absence of synthesis of β - chains leading to synthesis of abnormal hemoglobin.

Severe anemia occur , liver and spleen enlargement , pale skin , and weak bones . These people will need repeated blood transfusions for treatment .

4- Methemoglobinemia :

In this case , histidine is replaced by tyrosine , so protection of iron in ferrous state will be lost , leading to oxidation of iron to ferric state and Hb will lose ability to transport oxygen .

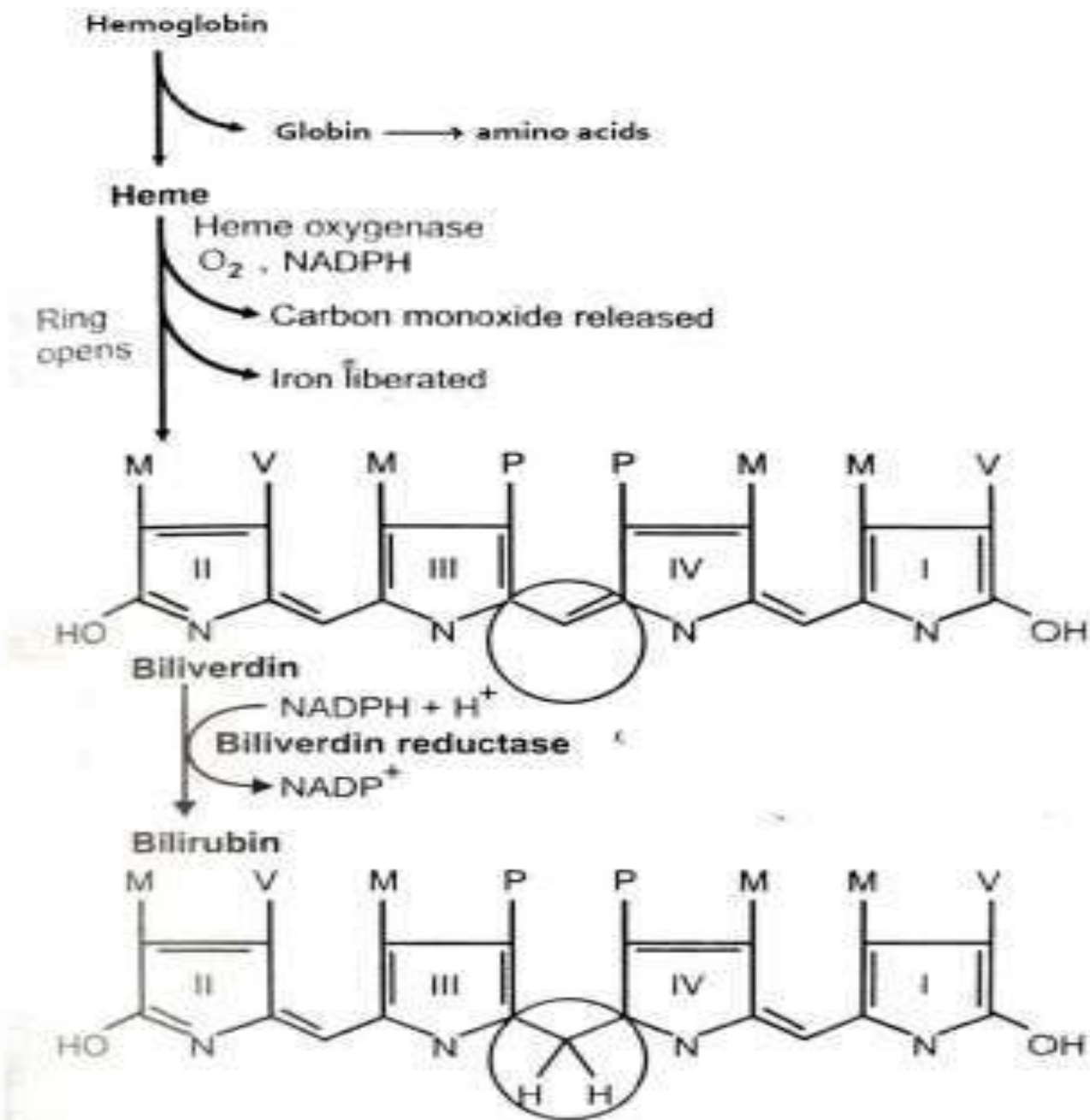
Various forms of hemoglobin :

- 1- Oxyhemoglobin (HbO_2) : Or fully oxygenated hemoglobin , iron in ferrous state (Fe^{+2}) .
- 2- Deoxy hemoglobin : Or reduced hemoglobin , it is Hb not combined with O_2 , also iron in reduced form (Fe^{+2}) .
- 3- Carboxy hemoglobin : It is Hb combined with CO_2 , the iron is in the ferrous state .
- 4- Methemoglobin : Iron is in the ferric state " Fe^{+3} ". It is either inborn abnormal Hb or due to presence of oxidizing agents like potassium ferricyanide .
- 5- Glycosylated hemoglobin (HbA_1C) : Glucose in RBC binds with hemoglobin forming glycosylated Hb . Normally , about 5% of glycosylated Hb found in blood . It reflects the concentration of blood glucose for 3 months ago and its measurement is important to follow up the diabetic patients .

Catabolism of Heme and Generation of Bilirubin

- The end product of Hb catabolism is a yellow pigments called bilirubin .
- Bilirubin has no function in the body, and excreted in the bile.
- When Hb is catabolized , globin chains are separated and hydrolyzed to its amino acids which are reused
- Iron liberated from heme and oxidized to ferric ion and taken up by transferrin .

- The porphyrin ring is broken down in the reticuloendothelial cells of liver , spleen , and bone marrow to a biliverdin which is green in color . Biliverdin is reduced to unconjugated bilirubin , a yellow pigments called indirect bilirubin .
- About 6 gm of Hb is catabolized daily , which form about 250 mg of bilirubin . Another 50mg of bilirubin is formed from myoglobin and other heme containing proteins .



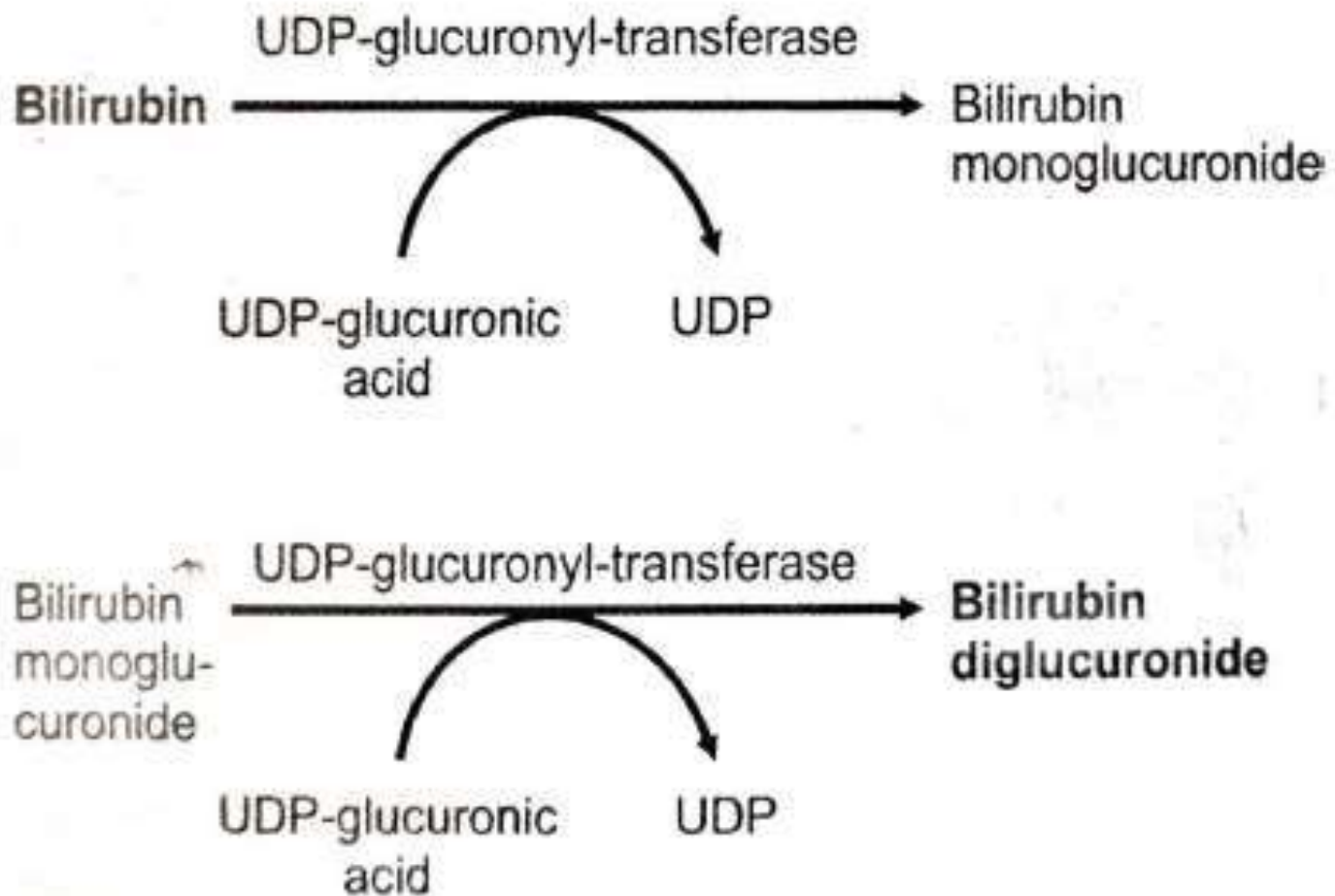
Liver takes up the bilirubin

Unconjugated Bilirubin formed in the reticuloendothelial cells is insoluble in water , so it transported in plasma bound with albumin . One molecule of albumin can bind 2 molecules of bilirubin .

When the albumin – bilirubin complex reach the surface of liver , bilirubin is taken up .

Conjugation in Liver

Inside the liver, bilirubin is conjugated with two molecules of glucuronic acid, to make it water soluble and it is called direct bilirubin.



Fate of conjugated Bilirubin is secreted into bile

The water soluble conjugated (direct) bilirubin is excreted into the bile

Conjugated Bilirubin Reduced by Intestinal Bacteria

The conjugated bilirubin reaches the large intestine . Intestinal bacteria deconjugate the conjugated bilirubin forming free bilirubin , which reduced by the bacteria to a colorless compounds called **urobilinogens** .

Enterohepatic Circulation

Small amount (about 20%) of urobilinogens is reabsorbed from intestine and returned to the liver by portal vein , and is again re-excreted through the liver (enterohepatic circulation) .

Final Excretion

Normally , most of the colorless Urobilinogens formed in the colon are oxidized to colored compounds called **urobilins** which are then excreted in the feces .

Darking of feces on standing in air , is due to oxidation of residual urobilinogens to urobilins .

Plasma Bilirubin

Normal plasma bilirubin level ranges from 0.2 – 0.8 mg / dL , while conjugated bilirubin is only 0 – 0.2 mg/ dL .

Jaundice

Jaundice means that there is excess amount of bilirubin in the blood , this leads to a yellow discoloration of the skin and eyes . Jaundice is not clinically visible until the bilirubin level reaches 2 – 3 mg/ dL .

Elevated Amounts of Unconjugated Bilirubin in Blood

Occur in number of Conditions : Some examples :

Neonatal “Physiologic Jaundice”

Jaundice is common in newborn babies because babies have a high number of red blood cells in their blood and have to broken down .

The liver of newborn baby is not completely developed , so it less effective at processing the bilirubin and removing it from the blood .

Normal indirect bilirubin would be under 5.2 mg/dL within the first 24 hours of birth .

Most jaundice goes away by 2 weeks . Phototherapy sometimes used to treat newborn jaundice through process called photo – oxidation . Photo – oxidation adds oxygen to the bilirubin , so it dissolves easily in water and easily removed from the blood .

Gilbert Disease

It is inherited caused by the defect in the uptake of bilirubin by the liver. Patient is asymptomatic (with no symptoms) except for the presence of mild jaundice , so the condition is harmless .

Toxic Hyperbilirubinemia

Unconjugated hyperbilirubinemia can result from liver cells dysfunction which caused by chloroform , carbon tetrachloride , hepatitis virus , cirrhosis , and mushroom poisoning . These disorders are due to hepatic cell damage , which impairs the conjugation process .

Obstructive Jaundice

Results from blockage of the hepatic or bile ducts , most often due to gallstone or to cancer of the pancreas . Because of the obstruction , conjugated bilirubin cannot be excreted , it thus go again into hepatic veins and appears in the blood and urine

Prof . Dr . Samera Alkatib

Regulation of blood glucose

Regulation of Blood Glucose

- The concentration of blood glucose during fast state is between 70 – 110 mg / dL.
- Fasting state means , glucose is estimated after overnight fast , i.e 8 hour after the food intake .

Auto-regulation of blood glucose :

- Following a good meal of carbohydrates , glucose is absorbed from intestine and enters the blood , the blood glucose rises to 120 – 140 mg /dL . The rise in blood glucose level will stimulate the secretion of insulin which lead to the taken up of glucose and utilizing it by most tissues .

Fasting State Regulation:

About 8-10 hours after a meal , blood glucose level normally falls to fasting levels . In this case , the secretion of insulin will decrease , and the secretion of glucagon and adrenaline will increase , and the liver will supply the blood with glucose by the breakdown of glycogen (**hepatic glycogenolysis**) , and by **gluconeogenesis** .

The plasma glucose level at these normal ranges depends on the balance between two processes :

Rate of glucose entering the blood

Rate of glucose removing from the blood

Factors leading to entry of glucose into the blood are :

- Absorption from intestine
- Glycogenolysis in liver
- Gluconeogenesis

Factors leading to removal of glucose from blood are :

- Glycolysis and krebs cycle in tissues
- Glycogenesis in liver and muscle
- Synthesis of T.G in adipose tissues
- Excretion in urine

Insulin hormone

- Insulin is the only hypoglycemic hormone , where the rest hormones are hyperglycemic in nature .
- Insulin is a protein consist of 51 amino acid , secreted by β -cells of the islets of Langerhans in the pancreas
- Insulin has anabolic action , activates the synthesis of glycogen , T.G , and proteins .

Metabolic Effects of Insulin on Carbohydrates :

- Increase the uptake of glucose by the cells .
- Stimulate the utilization of glucose through glycolysis and krebs cycle .
- Stimulate the glycogen synthesis (glycogenesis) in liver and muscle
- Inhibit the breakdown of glycogen (glycogenolysis) in liver by inhibiting C-AMP
- Inhibit gluconeogenesis .

Metabolic Effects of Insulin on Lipids :

- Inhibits the breakdown of T.G , by inhibiting the **hormone sensitive lipase (LPL)** enzyme in adipose tissues .
- Stimulate the synthesis of T.G in adipose tissues

Metabolic Effects of Insulin on Proteins :

- Insulin stimulate the synthesis of proteins in most tissues by stimulating the uptake of amino acids by the tissues . So insulin reduces the amino acids needed for the gluconeogenesis .

Diabetes Mellitus (DM)

It is a metabolic disease characterized by chronic hyperglycemia and abnormal metabolism of carbohydrates and lipids . This is due to absolute or relative insulin deficiency .

It is defined as the concentration of blood glucose in fasting state is greater than 140mg /dL .

Classification

Diabetic patients can be classified into 2 types according to the need for insulin :

***Type 1:* Insulin Dependent Diabetes Mellitus(IDDM)**

***Type2:* Non-Insulin Dependent Diabetes Mellitus (NIDDM)**

Type 1 : Insulin Dependent Diabetes Mellitus (IDDM)

- About 5% of total diabetic patients are of type 1 .
- It is due to decreased in insulin production , so the insulin level in blood is absent or very low .
- Type 1 appears in the early years of life , during the first or second decade of life .
- It is autoimmune disease usually stimulate by viral infection which has destroyed the β -cells of pancreas and therefore decreasing the production of insulin .
- The patients of this type are dependent on insulin injections which is necessary for treatment .

Type 2 : Non-Insulin Dependent Diabetes Mellitus (NIDDM)

- It is inherited hyperglycemia , caused by impaired the function of β -cells of pancreas , and hence fails to produce enough insulin .
- In some cases , it is due to insulin resistance , i.e. the patient have a normal level of insulin in blood , but it is not utilized due to decrease in the number of insulin receptors .
- This type is mainly occur in obese , and it seen in middle aged (above 45 year) .
- It is the common type , and about 95% of the diabetic patients are belonged it .
- Oral hypoglycemic drugs are mainly used in treatment type 2 . Insulin injections also used when oral drugs are not sufficient .

Chronic Complication of DM

- ***Vascular diseases*** : It is the common complication of diabetes which result in atherosclerosis or myocardial infraction .
- ***Complication in eye*** : Which include formation of cataract of lens and retinal diseases .
- ***Peripheral Neuropathy*** : Which result in risk of foot ulcers and gangrene .
- ***Nephropathy*** : Excretion of more than 300 mg /day of glucose in urine , indicates the presence of nephropathy , which can lead to renal damage .

Glycated Hemoglobin (HbA₁C)

- When blood glucose increase (hyperglycemia) , it will binding to proteins in a process called **glycation** .
- When glycation occur in tissues , it will lead to complication of diabetes mellitus , like Neuropathy , Nephropathy , and retinal diseases .
- When glycation occur in blood (hemoglobin) , it will form glycated hemoglobin which called **HbA₁C** .
- In HbA₁C , the glucose is attached to the valine of β -chain of hemoglobin.
- Measurement of HbA₁C is important for :

(1) *Diagnosis of diabetes mellitus*

(2) *Follow up of diabetic patients*

- The value of HbA₁C gives the actual mean of glucose level over 10-12 weeks ago.
- It is the best index to control the blood glucose level for long time.
- The binding of glucose with Hb is irreversible , so the glucose is not removed from Hb , and it remains inside the RBC through the life span of RBC (120 day).
- The measurement should be done every 3 months in all diabetic patients.

Values of HbA₁C :

- Normal range of HbA₁C is between (4% - 5.6%) or (68 – 110) mg / dL
- HbA₁C level between(5.7% - 6.4%) or (111 – 145)mg / dL mean there is a higher chance for getting diabetes .
- HbA₁C level of (6.5%) or higher , mean the person have diabetes .

Electron Transport Chain

Or

(Respiratory chain)

Definition:

A series of enzymes and coenzymes located in the inner membrane of mitochondria , arranged in the inner mitochondrial membrane according to increase its reducing potential.

Its function is to carry the electrons (reducing equivalents) from the substrate to the molecular oxygen . and release energy (ATP) .

Reducing equivalents (electrons) are produced by krebs cycle and beta-oxidation during oxidation reduction reactions occur in the matrix of mitochondria.

When the electrons pass from one coenzyme to another , the first is oxidized and the next is reduced , the final one is O_2 which is converted to H_2O .

The energy is released during this process and trapped as ATP molecules . This coupling of oxidation with phosphorylation is called **oxidative phosphorylation**.

Sequence of Respiratory Chain

The first member of ETC is **NAD⁺- dehydrogenase** .

NAD⁺ receive the electrons from substrate and become in reducing form NADH +H⁺ , and pass to CoQ .

NAD⁺ : Nicotinamide Adenine Dinucleotide



Mitochondrial **NAD⁺- dehydrogenase** are included :

- Pyruvate dehydrogenase
- Isocitrate dehydrogenase
- α keto glutarate dehydrogenase
- Malate dehydrogenase
- β hydroxy acyl CoA dehydrogenase
- Glutamate dehydrogenase

The second member of ETC is **FAD-dehydrogenase** .

This protein accepts electrons from substrate and convert to FADH_2 and pass to CoQ .

FAD : Flavin Adenine Dinucleotide



FAD-dehydrogenase includes :

- Succinate dehydrogenase
- Mitochondrial Glycerol-3 – P dehydrogenase
- Acyl CoA dehydrogenase

- The third member is **CoQ** , also known as **ubiquinone** made up of quinone

- its function is to transfer electrons from NADH and FADH_2 to the cytochromes

CoQ reduced to semiquinone (CoQH) and hydroquinone (CoQH_2) .

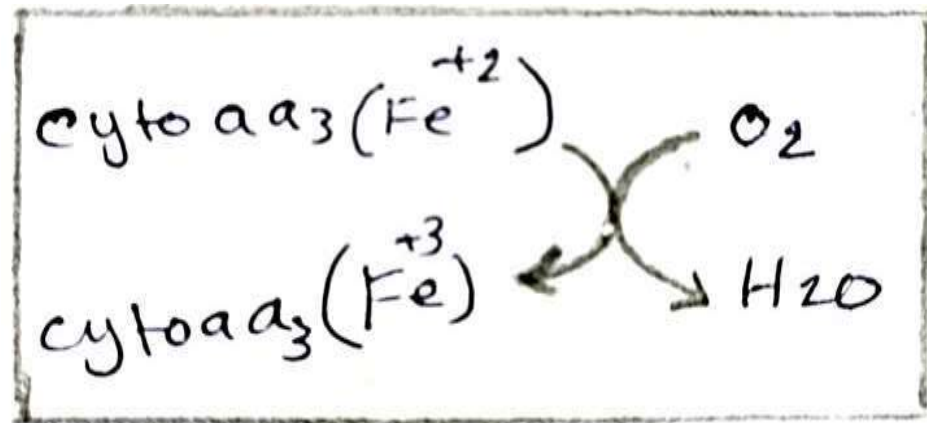
NAD-dehydrogenase -----> CoQ -----> CoQH -----|> CoQH_2

FAD-dehydrogenase

- The electrons then *transfer* to the **cytochromes** system (**b** , **c₁** , **c** , and **aa₃**).
- These enzymes are hemoproteins (contain 4 pyrrole rings and iron atom in the center) .
- During transfer of electrons , the iron in hem shuttles between Fe^{+2} and Fe^{+3} .

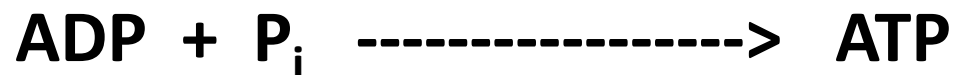
Cyto b -----> Cyto C₁-----> Cyto C -----> Cytoaa₃

- The electrons are then transfer from **cytochrome aa₃** to the last member (**O₂**) which immediately reduced to water . O₂ has a higher affinity for electrons .



Oxidative Phosphorylation

- When each pair of electrons transport from NADH or FADH_2 to the O_2 , free energy are formed , and this energy is enough to phosphorylate the ADP and produce ATP molecules .
- **This coupling of oxidation with phosphorylation is called oxidative phosphorylation .**

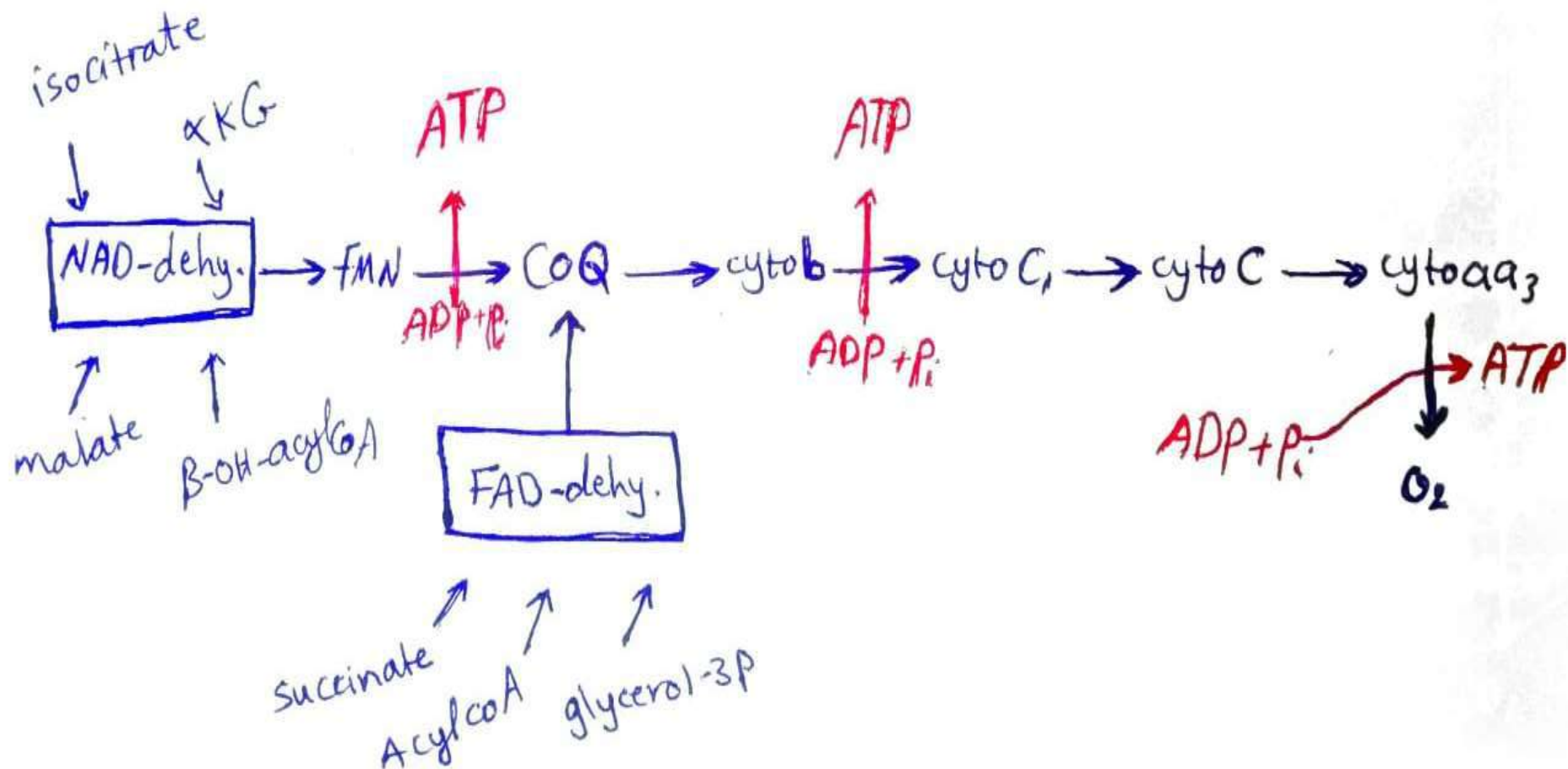


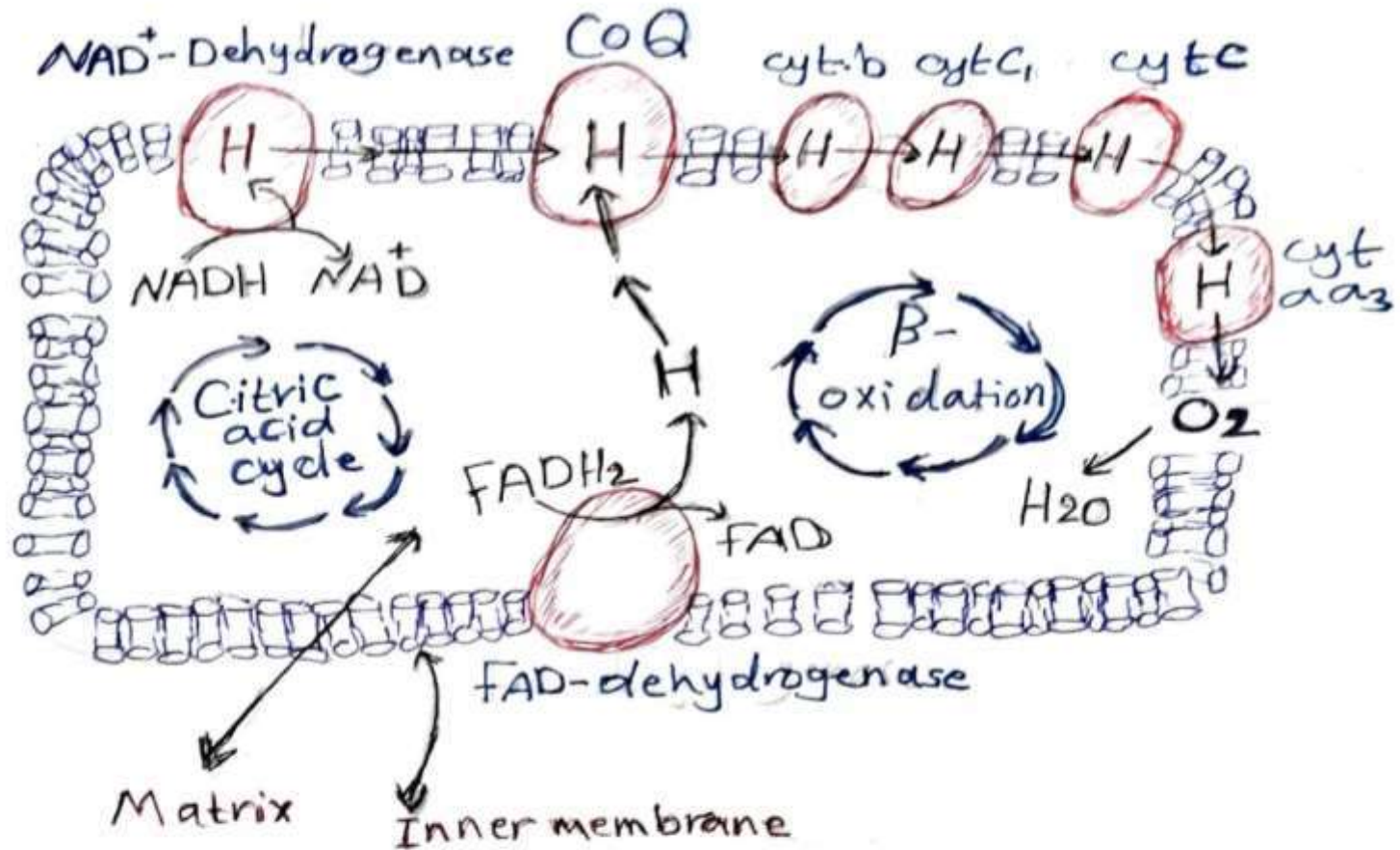
Sites Of ATP Production :

- Site (1) , NAD^+ -----> CoQ
- Site (2) , Cyto b -----> Cyto C_1
- Site (3) , Cyto aa_3 -----> O_2

- Each electron transport from NADH to O_2 ,will release 3ATP molecules.

- Each electron transport from FADH_2 to O_2 , will release 2ATP molecules.





Respiratory chain (Electron transport chain)

Enzymes

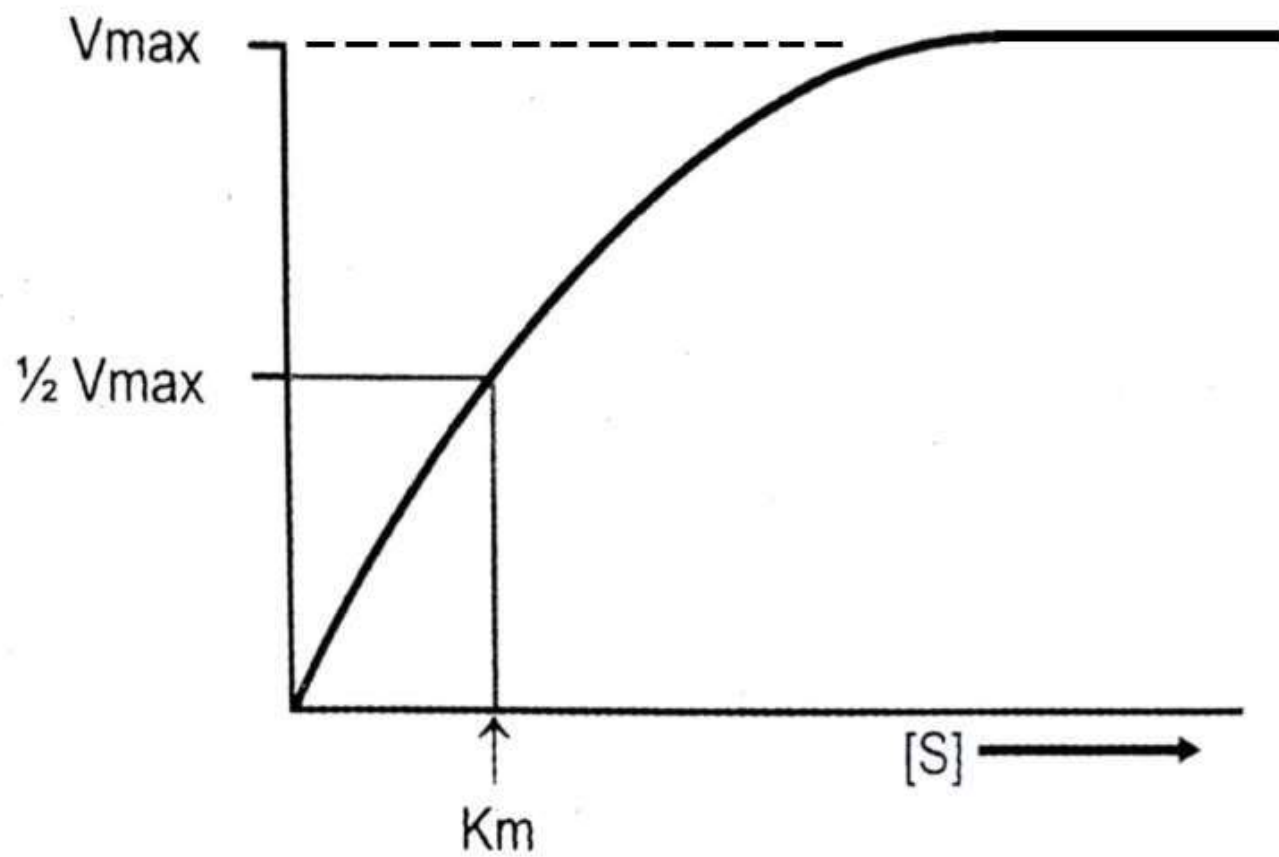
Part 2

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Factors affecting enzyme activity

Substrate concentration :

- The **Velocity of reaction (V)** or the **activity of enzyme** is define as the number of substrate molecules converted to product per unit time .
- The **activity of enzyme** increases with substrate concentration increase until a **maximum velocity (V_{\max})** is reached .
- Additional increase in substrate concentration , the velocity of reaction become constant , and this mean that all the active sites of enzyme molecules are saturated with substrate .



Michaelis-Menten Equation :

The Michaelis-Menten equation describes how the activity of enzyme varies with substrate concentration

$$v_0 = \frac{V_{\max} [S]}{K_m + [S]}$$

Where : V_0 = initial reaction velocity (enzyme activity)

V_{\max} = maximum velocity

K_m = Michaelis – Menten constant

$[S]$ = substrate concentration

Inhibition of Enzyme Activity :

Any substrate that can decrease the velocity of the reaction (decrease the enzyme activity) , is called inhibitor .

In general , there are 2 types of inhibition :

Competitive Inhibition

The inhibitor has a structure analog to the substrate , so the inhibitor binds to the same site that the substrate bind (active site) and competes with the substrate for the active site . Therefore the activity of enzyme is decreased .

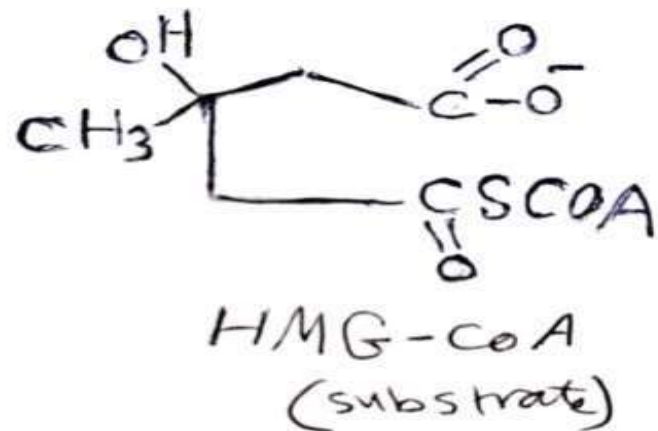
Competitive inhibition is reversible , so excess concentration of substrate will stopped the inhibition .

Clinical importance of competitive inhibition

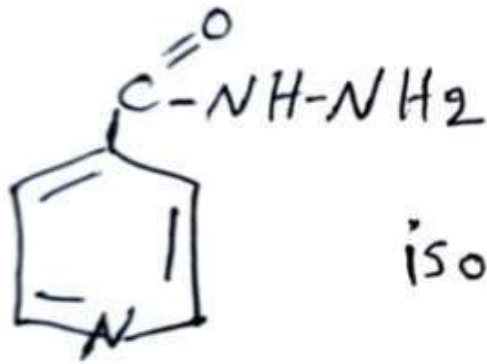
The action of many drugs explain the principle of competitive inhibition . Some examples are :-

(1)Statin drugs such as pravastatin and atorvastatin are anti-hypercholesterolemia , they are competitively inhibit the cholesterol synthesis .

Cholesterol synthesis is catalyzed by hydroxy methyl glutaryl CoA reductase (**HMG-CoA reductase**) . Statin drugs are structural analog of the HMG-CoA (the substrate of this enzyme) , and compete to inhibit HMG-CoA reductase , so these drugs inhibit cholesterol synthesis and decrease the cholesterol level in blood .



(2) Isoniazid , anti-tuberculosis drug : It has a structure similar to pyridoxal phosphate (a coenzyme derived from Vitamin B₆) . This drug inhibits pyridoxal kinase enzyme , and reduces the formation of pyridoxal phosphate causing Vitamin B₆ deficiency which lead to peripheral neuropathy .



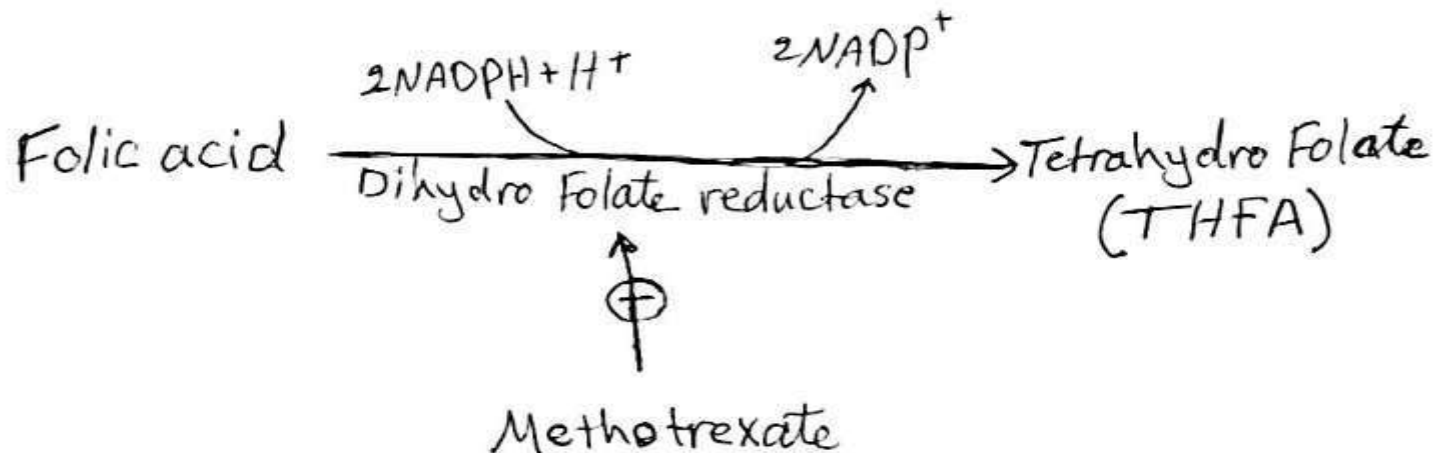
isonicotinic hydrazide
(isoniazid)

3)Methotrexate , a chemotherapy drug :

Its structure similar to **folic acid** . Folic acid is a substrate of **dihydrofolate reductase** ,
folic acid essential for nucleotides .

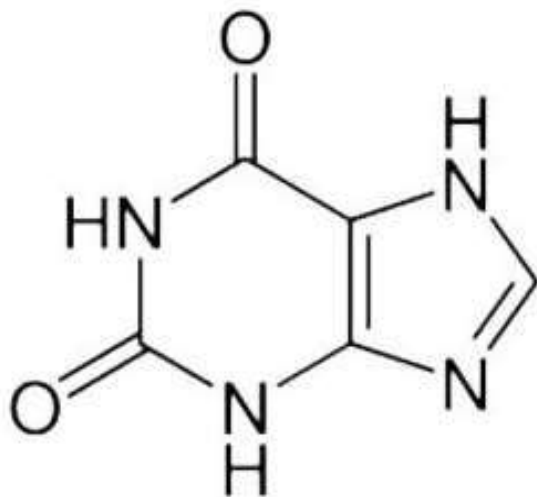
Methotrexate blocks **dihydrofolate reductase** and thereby inhibits the synthesis of nucleotides , this leads to inhibits the growing cells since these cells require DNA replication .

So methotrexate is often used in cancer chemotherapy

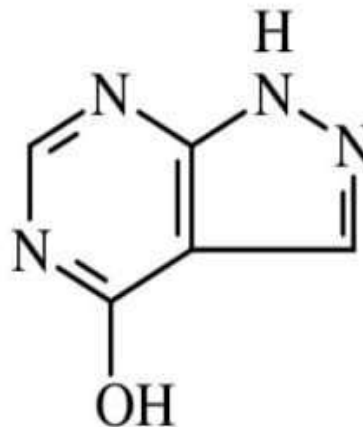


(4)Allopurinol drug for Gout treatment :

An inhibitor for uric acid synthesis , inhibits xanthine oxidase resulting in accumulation of hypoxanthine and xanthine which is more soluble than uric acid .



Xanthine



Allopurinol

Non-Competitive Inhibition

The inhibitor usually binds through a site other than the active site of substrate , it can binds either free enzyme or the ES complex and preventing the reaction.

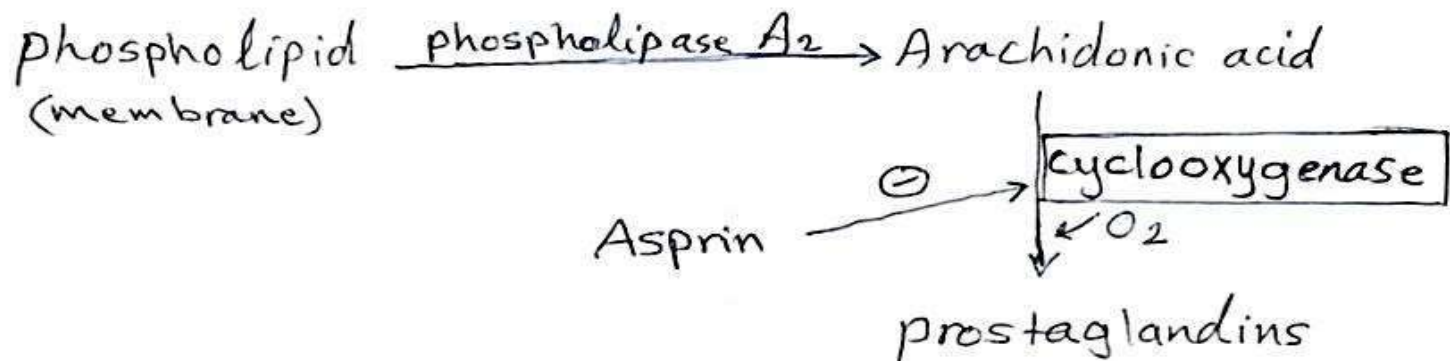
The inhibitor has a **structure differ to a substrate** , so there is no competition between substrate and inhibitor .

Noncompetitive inhibition is **irreversible** , so increase the substrate concentration does not return the activity of enzyme .

Examples :

(1)Cyanide (CN^-) inhibits cytochrome aa_3 and block the respiration.

(2)Asprin has anti-inflammatry action inhibits cyclooxygenase enzyme , which converts Arachidonic acid to Prostaglandins , so the synthesis of prostaglandins is inhibited and the inflammation keep aways.



(3)Angiotensin-Converting Enzyme (ACE) inhibitors such as Captopril and enalapril :

These drugs lower the blood pressure by blocking the enzyme converting angiotensin 1 to angiotensin 11 , which act a potent vasoconstrictor . so these drugs cause vasodilation and then reduce the blood pressure .

Allosteric enzymes :

Allosteric enzymes are enzymes have a site other than the active site called **allosteric site** (allo = other) .

Usually , the allosteric enzymes **catalyze the irreversible reactions** .

Allosteric enzymes are regulated by molecules called **effectors** or called **modulators** , which bind at the allosteric site of the enzyme.

The binding of the effector molecule can either increase the activity of enzyme , so it known **allosteric activator** . Or it can inhibit the activity of enzyme , so it called **allosteric inhibitor** .

- allosteric enzymes control the metabolic processes, therefore these enzymes are called **key enzymes** or **rate limiting enzymes** .

- Examples of allosteric enzymes :

Phospho fructo kinase (PFK) , is a rate limiting enzyme in glycolysis , activated by AMP and inhibited by ATP and citrate.

Isoenzymes

- Isoenzyme is enzyme synthesized in various tissues, so it found in various organs, but it catalyze the same reaction.
- has different molecular forms which differ in amino acids sequence and physical properties
- Isoenzyme in plasma may reflects the site of tissue damage , hence the study of isoenzymes is very useful to understand the diseases of different organs
- Isoenzymes consist of different subunits (polypeptide chains) in various combinations .

Examples:

Creatine kinase (CK)

synthesized in three organs called **CK₁** , **CK₂** , **CK₃** ,
each isoenzyme consist of 2 polypeptide chains ,**B** and **M**

BB found in brain (CK₁)

MB found in cardiac muscle (CK₂)

MM found in skeletal muscle (CK₃)

Creatine kinase catalyze the phosphorlyation of creatine

Lactate dehydrogenase (LDH)

it is a tetramer enzyme with 4 polypeptide chains (subunit) .

The subunits either **H** or **M** , so five isoenzymes are found in tissues :

H_4 seen in heart (LDH_1)

H_3M (LDH_2) , H_2M_2 (LDH_3) , HM_3 (LDH_4)

seen in different tissues

M_4 (LDH_5) seen in skeletal muscle

Lactate dehydrogenase catalyze the reduction of pyruvate to lactate.

Enzymes

Prof . Dr . Samera Alkatib

- Enzymes are **proteins** , catalyze the rate of chemical reactions . They enter the reaction and get out the reaction without any changes .
- Enzymes increase the chemical reactions at a short time and at the body temperature . Outside the body , chemical reactions take place for long time .
- All metabolic reactions in the cell need enzymes in order to occur in faster rates . Usually 1 molecule of enzyme can act on 1000 molecules per minute.
- The molecules at which enzymes may act , are called **substrates** . The enzyme converts the substrates into molecules called **products**

Substrates -----Enzyme-----> **Products**

- Lack of enzymes will block the metabolic pathway causing **inborn error disease** .
- Enzymes are synthesized inside the cell in very small quantities that could not be measure its concentration , so the **activity** of enzyme refers to the concentration of enzyme .
- International unit of enzyme (**IU**) or **enzyme activity** : define as the amount of enzyme that convert 1 micromole of substrate, or produce 1 micromole of product per minute per liter . It write **U/L**

Classification of enzymes

Old names , such as pepsin , trypsin , chymotrypsin , etc. . These **old names** do not refer to their functions or the kind of reaction , but are still used .

Later , the enzymes are named by adding the suffix (ase) to the substrate to describe the action of enzyme . For example lactase acts on lactose , maltase acts on maltose....

IUB SYSTEM

According to International Union of biochemistry (IUB) , the enzymes are classified into **six major classes** :

Class 1 : Oxidoreductases

These enzymes catalyze the oxidation of substrate together with the reduction of another substrate
(**oxidation – reduction reactions**).

Oxidation means the removal of hydrogen atom , reduction means the accept of hydrogen atom .



Oxidoreductase enzymes are classified into :

- **NAD⁺-dehydrogenases and NADP⁺ -dehydrogenases**
- **FAD –dehydrogenases**
- **Oxygenases**
- **Cytochromes b , C₁ , C , aa₃**
- **Ubiquinone (COQ)**

Class 2 : **Transferases**

This class of enzymes transfers one group (other than hydrogen) from the substrate to another substrate .

Example :



Class 3 : Hydrolyases

These enzymes catalyze the hydrolysis reactions , where the molecule is breaking down into 2 or more molecules by **addition of water**.



Class 4 : Lyases

These enzymes can hydrolyze the substrate without adding water . example :



Class 5 : Isomerase

These enzymes produce the **isomer** of substrate .

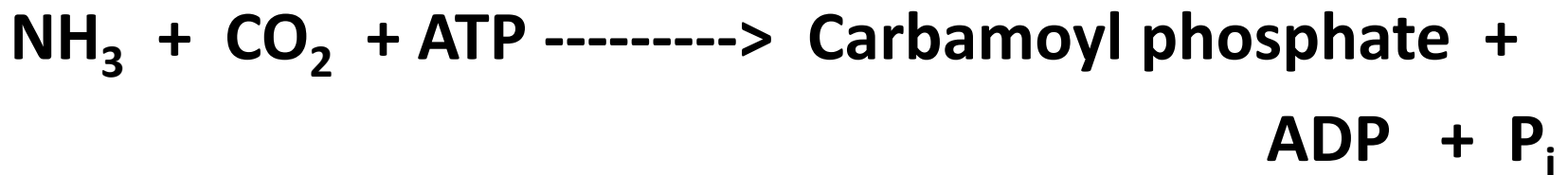
For example racemase , epimerase , cis-trans isomerase .

Glyceraldehyde-3-P Isomerase . **Dihydroxy**
acetone
phosphate

Class 6 : Ligases(Synthetases)

These enzymes catalyze the synthesis reactions , they link two substrates together with the addition of ATP .

Example , carbamoyl phosphate synthetase , glutamine synthetase .



Coenzyme and cofactor

Some enzymes are simple proteins (consist of polypeptide chain only) .

Other enzymes contain coenzyme or cofactor that help directly in substrate binding .

Coenzyme : Organic molecule with low molecular weight , binds loosely or tightly to the enzyme.

Coenzyme is essential for the activity of enzyme

Most coenzymes are derived form of vitamins B complex , such as NAD^+ , NADP^+ , FAD , FMN , CoA , pyridoxal phosphate ,...

Cofactor

metal ions require for the activity of enzymes , such as iron , copper , zinc , magnesium , molybdenum

Apoenzyme

enzyme lacks to its coenzyme or cofactor

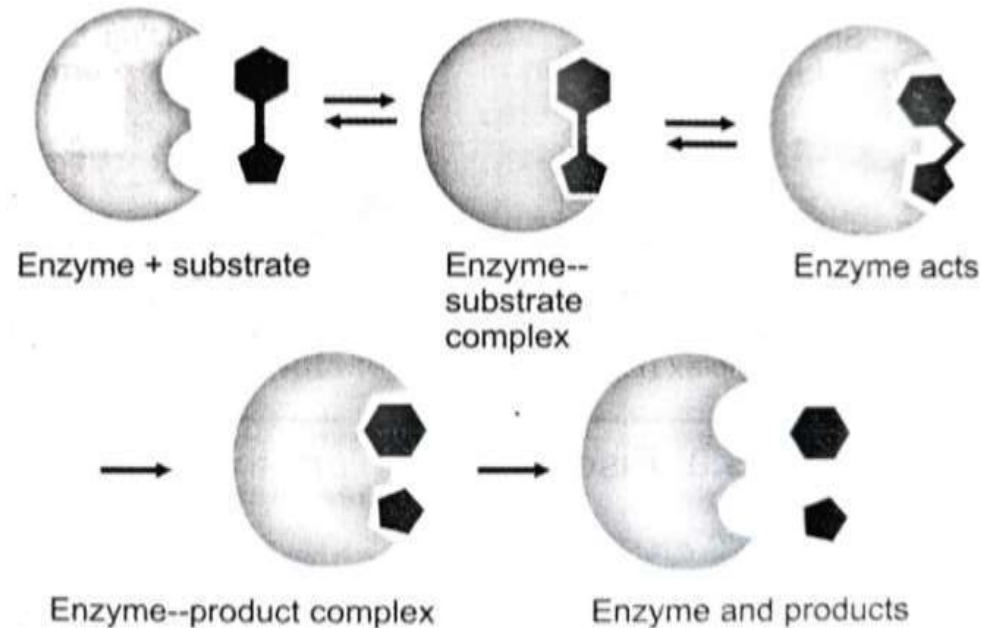
Holo-enzyme :

Enzyme with its coenzyme or cofactor .

Active Site

Small part of the enzyme acts as a site of substrate binding .

The substrate (S) binds with the enzyme (E) at the active site, and forming enzyme-substrate complex (ES) which is rapidly breaks down into product (P) and enzyme .



Sites of enzymes in the body

Inracellular enzymes :

Enzymes **synthesize in the cell and work in the same cell.**

Most enzymes are intracellular , but when there is a damage in the tissue, these enzymes are released in the blood and its levels become more higher .

Example :

enzymes of glycolysis , citric acid cycle , beta oxidation , amino acids metabolism and so on .

Extracellular enzymes :

Enzymes synthesize in the cell and work outside the cell .

These enzymes usually act on the breakdown the large molecules (polysaccharides , proteins , lipids ...) that would not be able to enter the cell

Example:

saliva amylase and pancreatic enzymes .

Functional plasma enzymes :

Enzymes present with their substrates in the blood at all time , **have a physiologic function in the blood .** These enzymes include **Lipoprotein Lipase(LPL) and blood clotting enzymes .**

Non-Functional plasma enzymes :

Enzymes that have no physiologic function in the blood . During diseases , these enzymes release from the tissues and increase in the blood, and reflect the site of tissue damage .