

Bio L1,2 : CARBOHYDRATES CHEMISTRY

CARBOHYDRATES:

organic material that contain **Carbon, Hydrogen & Oxygen**,
Some carbohydrates also contain **Nitrogen, Phosphorus or Sulphur**.

Definition: **Chemically**, Polyhydroxy (OH) aldehydes (CHO) or ketones(C=O) or compounds that produce them **on hydrolysis**.

Functions of CHO:

1. Main source of energy.
 2. Storage form (e.g. Starch in plants and glycogen in animals).
 3. Carbohydrates → fatty acids and glucogenic amino acids.
 4. Cellulose is structural unit in plants (Non digestable CHO in humans so serves as dietary fibers).
 5. Ribose and deoxyribose in the nucleotides of DNA and RNA and high energy phosphate compounds.
 6. Milk contains lactose
 7. Detoxification as glucuronic acid.
-
- ```

graph LR
 A[Energy] --- B[1. Main source of energy.
2. Storage form (e.g. Starch in plants and glycogen in animals).
3. Carbohydrates → fatty acids and glucogenic amino acids.
4. Cellulose is structural unit in plants (Non digestable CHO in humans so serves as dietary fibers).
5. Ribose and deoxyribose in the nucleotides of DNA and RNA and high energy phosphate compounds.]
 A --- C[6. Milk contains lactose
7. Detoxification as glucuronic acid.]
 A --- D[Others]
 B --- E[Structure]
 C --- F[Structure]
 D --- G[Others]

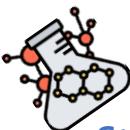
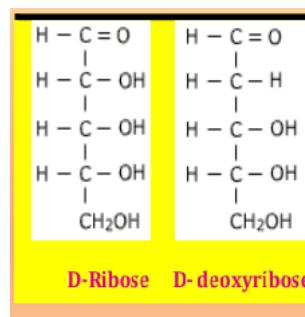
```

### Classification of CHO:

| <u>Mono</u> saccharides   | <u>Di</u> saccharides | <u>Poly</u> saccharides |
|---------------------------|-----------------------|-------------------------|
| 1 sugar unit              | 2 sugar units         | >10 sugar units         |
| Linked by glycosidic bond |                       |                         |

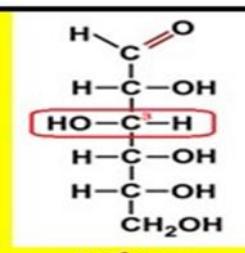
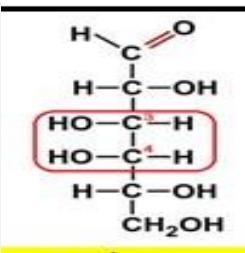
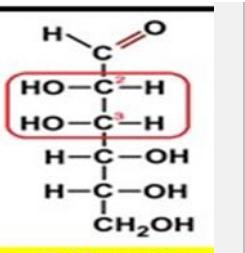
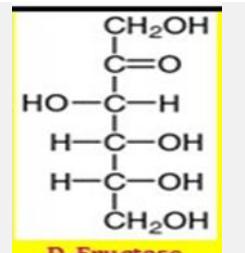
## MONOSACCHARIDES:

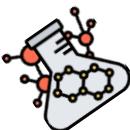
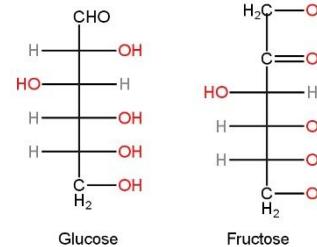
| Number of carbons | carbons<br>Monosaccharides | Active groups                   |                    |
|-------------------|----------------------------|---------------------------------|--------------------|
|                   |                            | Aldoses (CHO)                   | Ketoses (C=O)      |
| 3                 | Trioses                    | Glyceraldehyde                  | Dihydroxyacetone   |
| 4                 | Tetroses                   | Erythrose                       | Erythrulose        |
| 5                 | Pentoses                   | Ribose and deoxy ribose, Xylose | Ribulose, Xylulose |
| 6                 | Hexoses                    | Mannose, glucose, Galactose     | Fructose           |

Structure and importance of some monosaccharides:**Pentoses****Importance**

1. Enters in the structure of nucleotides of RNA and DNA
2. Enters in high energy phosphate compounds as ATP
3. Enters in coenzymes (NAD, NADP and flavoproteins)

Structure and importance of some monosaccharides:

|             | D -Glucose                                                                                                                                                        | D- Galactose                                                                                     | D- Mannose                                                                                       | D-Fructose                                                                                               |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Structures  | <br>D-Glucose                                                                    | <br>D-Galactose | <br>D-Mannose | <br>D-Fructose        |
| Nature      | aldohexoses                                                                                                                                                       |                                                                                                  |                                                                                                  | ketohexose                                                                                               |
| Other names | Grape sugar and dextrose                                                                                                                                          | Milk sugar                                                                                       |                                                                                                  | Fruit sugar & levulose                                                                                   |
| Sources     | 1. Free in honey and fruits especially grapes<br>2. Digestion of starch, glycogen, sucrose, maltose, lactose                                                      | Digestion of lactose in milk                                                                     | Found in many fruits                                                                             | 1. Free in honey and fruits<br>2. Digestion of sucrose                                                   |
| Importance  | 1. Major source of energy in the body<br>2. Main sugar in blood<br>3. Enter in the structure of disaccharides as maltose, sucrose and lactose and polysaccharides | 1. Enters in the structure of lactose in milk, glycolipids and glycoproteins                     | Acts as component of glycoproteins                                                               | 1. Source of energy for the sperms<br>2. Enters in the structure of sucrose And polysaccharides (inulin) |

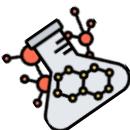
Differences between Glucose & Fructose:

|                                | Glucose                        | Fructose                        |
|--------------------------------|--------------------------------|---------------------------------|
| Nature                         | Aldo-hexose                    | Keto – hexose                   |
| Optical activity               | Dextrorotatory=(+) or (-)      | Levorotatory =(-) or (I)        |
| Another name                   | Dextrose                       | Levulose                        |
| Ketose test (heating with HCL) | No change                      | Red brown color                 |
| Distribution                   | Mainly in blood                | Mainly in semen                 |
| Function                       | Major source of Energy in body | Major source of energy in semen |

**NB:** All monosaccharides are reducing sugar.

MONOSACCHARIDES DERIVATIVES:I. Sugar Acids (oxidized derivatives)

|            | Aldonic acids (Gluconic acid)                                                                                                                                                   | Uronic acids (Glucuronic acid)                                                                                                                                                                   |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Importance | Estimation of blood glucose                                                                                                                                                     | <ol style="list-style-type: none"> <li>Synthesis of mucopolysaccharides</li> <li>Conjugates with toxic substances, drugs , hormones, bilirubin →nontoxic substances excreted in urine</li> </ol> |
|            | <br><b>Glucose</b><br><chem>O=C[C@H](O)[C@@H](O)[C@@H](O)[C@@H](O)[C@H](O)C</chem><br><br><b>Gluconic acid</b><br><chem>O=C[C@H](CO)[C@@H](O)[C@@H](O)[C@@H](O)[C@H](O)C</chem> | <br><b>Glucose</b><br><chem>O=C[C@H](O)[C@@H](O)[C@@H](O)[C@@H](O)[C@H](O)C</chem><br><br><b>Glucuronic acid</b><br><chem>O=C[C@H](CO)[C@@H](O)[C@@H](O)[C@@H](O)[C@H](CO)C</chem>               |

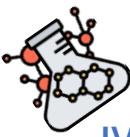


## II. Sugar Alcohols (reduced derivatives)

|            | Glycerol (glycerin)                                                                                                                | Sorbitol<br>(reduction of glucose and fructose)                                                                                                                                                                                  | Mannitol<br>(reduction of mannose)                                                                                                                                                                                               | Galactitol<br>(reduction of galactose)                                                                                                                                                                                           |
|------------|------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Importance | <ul style="list-style-type: none"> <li>1. Used in cosmetic preparations</li> <li>2. Used as vasodilator (nitroglycerin)</li> </ul> | <p>Accumulates in ocular lens in diabetes → cataract (lens opacity)</p>                                                                                                                                                          | <ul style="list-style-type: none"> <li>1. Used as diuretic</li> <li>2. Used to reduce intracranial tension (in brain tumors) and intraocular pressure (in acute glaucoma)</li> </ul>                                             | <p>Accumulates in ocular lens (in galactosemia) → cataract</p>                                                                                                                                                                   |
| Structure  | $\begin{array}{c} \text{CH}_2\text{OH} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{CH}_2\text{OH} \end{array}$               | $\begin{array}{c} \text{CH}_2\text{OH} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{HO}-\text{C}-\text{H} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{CH}_2\text{OH} \end{array}$ | $\begin{array}{c} \text{CH}_2\text{OH} \\   \\ \text{HO}-\text{C}-\text{H} \\   \\ \text{HO}-\text{C}-\text{H} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{CH}_2\text{OH} \end{array}$ | $\begin{array}{c} \text{CH}_2\text{OH} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{HO}-\text{C}-\text{H} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{H}-\text{C}-\text{OH} \\   \\ \text{CH}_2\text{OH} \end{array}$ |

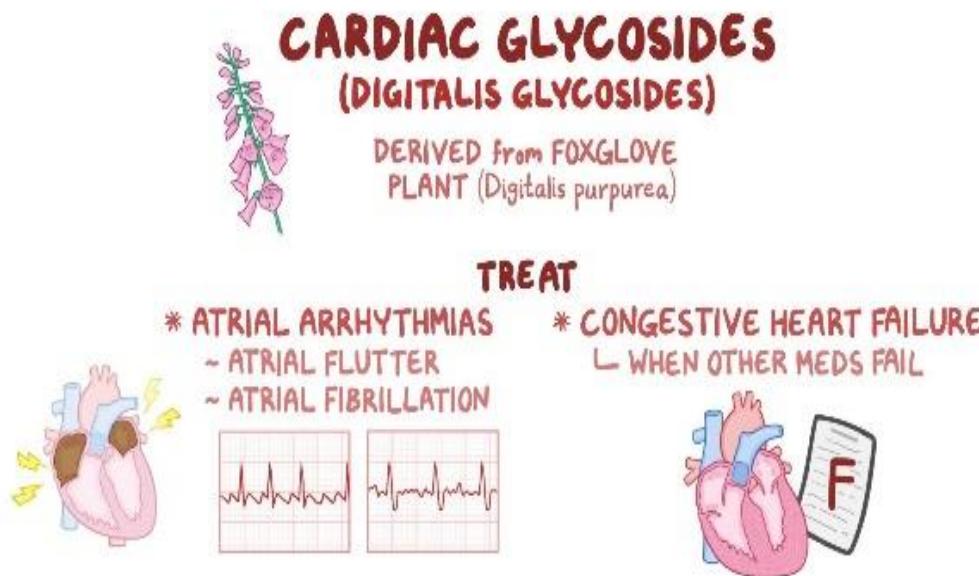
## III. Amino sugars:

|            | Glucosamine                                                                                                                                                                                               | Galactosamine                                                                                                                                                                                               | Mannosamine                                                                                                                                                                                                      |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            | $\begin{array}{c} \text{CHO} \\   \\ \text{H}-\text{NH}_2 \\   \\ \text{HO}-\text{H} \\   \\ \text{H}-\text{OH} \\   \\ \text{H}-\text{OH} \\   \\ \text{CH}_2\text{OH} \end{array}$ <p>D-glucosamine</p> | $\begin{array}{c} \text{CHO} \\   \\ \text{H}-\text{NH}_2 \\   \\ \text{HO}-\text{H} \\   \\ \text{H}-\text{OH} \\   \\ \text{H}-\text{OH} \\   \\ \text{CH}_2\text{OH} \end{array}$ <p>D-galactosamine</p> | $\begin{array}{c} \text{CHO} \\   \\ \text{H}_2\text{N}-\text{H} \\   \\ \text{HO}-\text{H} \\   \\ \text{H}-\text{OH} \\   \\ \text{H}-\text{OH} \\   \\ \text{CH}_2\text{OH} \end{array}$ <p>D-mannosamine</p> |
| Importance | Constituents of Glycoproteins & Glycosaminoglycans                                                                                                                                                        |                                                                                                                                                                                                             |                                                                                                                                                                                                                  |

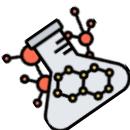


### IV. Glycosides:

- **Produced by:**
  1. bonding of one monosaccharide and another monosaccharide → Disaccharides
  2. bonding of one monosaccharide with non carbohydrate compound (aglycon) →:
    - a) Purine and pyrimidine nucleosides
    - b) Steroid glycosides
    - c) Digoxin
    - d) Glycolipids and glycoproteins
- **Importance:** Digoxin used in treatment of **congestive heart failure and cardiac arrhythmias**



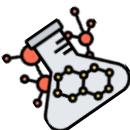
يَا طَالِبَ الْعِلْمِ لَا تَرْكِنْ إِلَى الْكُسْلِ  
واعجل فقد خلق الإنسان من عجل

**DISACCHARIDES**

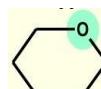
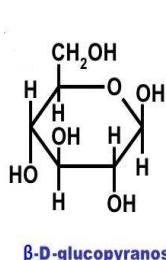
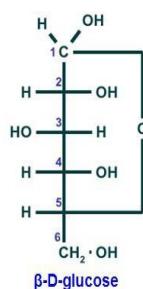
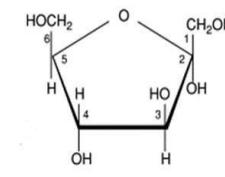
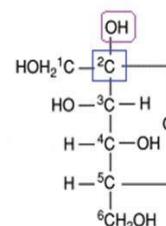
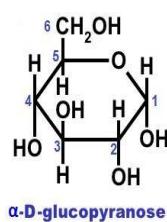
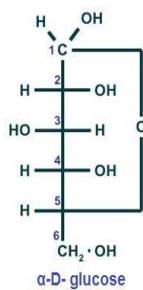
- Formed of: 2 monosaccharides connected together by glycosidic bond

|            | Sucrose                                                                                   | Lactose                           | Maltose                                                                       | Isomaltose                                                                        | Cellobiose                                        | Lactulose |
|------------|-------------------------------------------------------------------------------------------|-----------------------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|---------------------------------------------------|-----------|
| Other name | <ul style="list-style-type: none"> <li>Cane or beet sugar</li> <li>Table sugar</li> </ul> | Milk sugar                        | Malt sugar                                                                    |                                                                                   |                                                   |           |
| Source     | Can be obtained from sugar cane, sugar beet, also occurs in most fruits and vegetables    | Found in milk                     | present in malt<br>Produced during digestion of starch or glycogen by amylase | Produced during digestion of starch or glycogen by salivary or pancreatic amylase | partial hydrolysis of cellulose present in plants | Synthetic |
| Sweetness  | Very sweet sugar                                                                          | Not so sweet → not block appetite | Sweet sugar                                                                   |                                                                                   |                                                   |           |

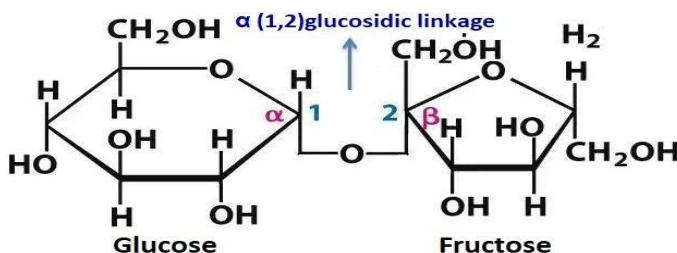
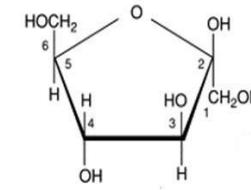
|                                                | Sucrose                   | Lactose                   | Maltose                  | Isomaltose            | Cellobiose                                                | Lactulose                 |
|------------------------------------------------|---------------------------|---------------------------|--------------------------|-----------------------|-----------------------------------------------------------|---------------------------|
| Monosaccharides units (products of hydrolysis) | D-glucose<br>D-fructose   | D-galactose<br>D-glucose  | α-D-glucose<br>D-glucose | As maltose            | D-glucose<br>D-glucose                                    | D-Galactose<br>D-fructose |
| Enzyme hydrolysis (Digestion by)               | Intestinal Sucrase enzyme | Intestinal lactase enzyme | Maltase enzyme           | Intestinal isomaltase | Not digested in humans (no enzyme that acts on p linkage) | No digested               |



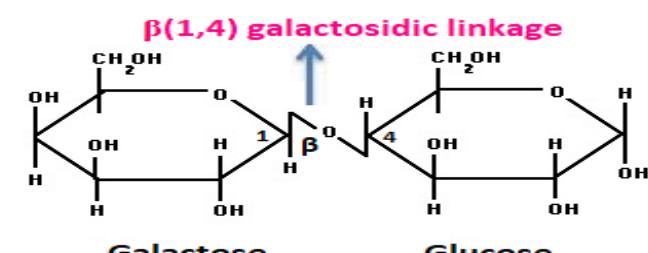
## Monosaccharide cyclic forms



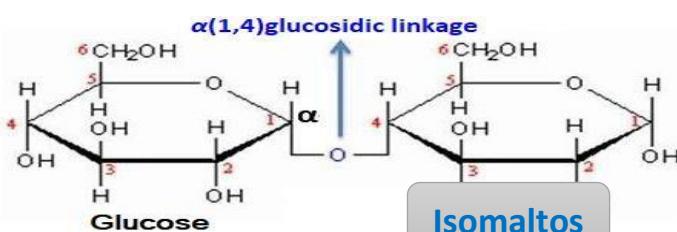
$\alpha$ -D-fructose  
(Fischer projection)



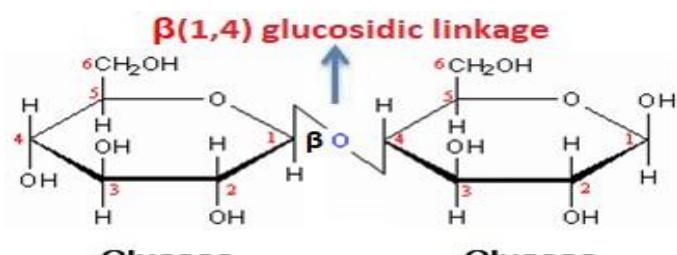
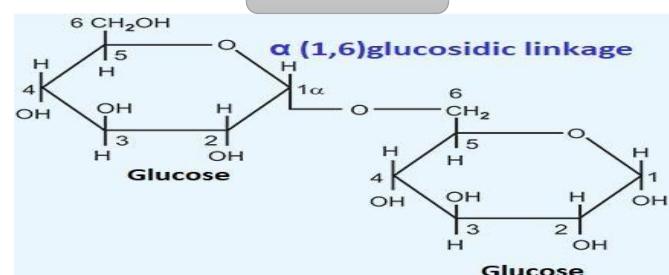
Sucrose



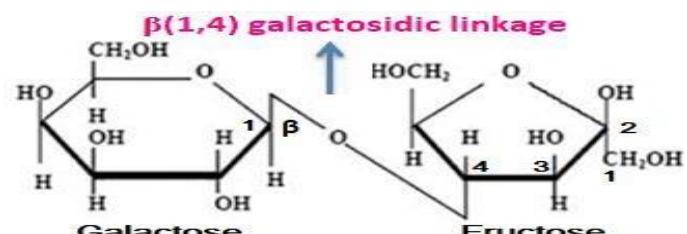
Lactose

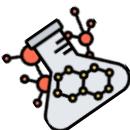


Isomaltos



Lactulose





❖ Explain: Sucrose is non reducing sugar.

As the reducing groups of both glucose and fructose are linked together by ( $\alpha,1\rightarrow 2$ glucosidic linkage).

❖ Short notes on Invert sugar:

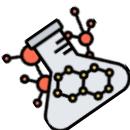
- ✓ Sucrose is Dextrorotatory (specific rotation is  $=66.5^\circ$ ) but on hydrolysis it is levorotatory as it is hydrolyzed by sucrase or invertase into a mixture of D-glucose ( $\alpha=52.5^\circ$ ) and D-fructose ( $\alpha=-92$ ) which is levorotatory.
- ✓ This change from dextro- to levo-rotatory is called inversion and the mixture of the resulting glucose and fructose is called invert sugar.
- ✓ The invert sugar is sweeter than sucrose. Honey is chiefly invert sugar.

❖ Explain: Sucrose is dextrorotatory but after hydrolysis levorotatory.

- ✓ Sucrose is Dextrorotatory (specific rotation is  $=66.5^\circ$ ) but on hydrolysis it is levorotatory as it is hydrolyzed by sucrase or invertase into a mixture of D-glucose ( $\alpha=52.5^\circ$ ) and D-fructose ( $\alpha=-92$ ) which is levorotatory.
- ✓ This change from dextro- to levo-rotatory is called inversion and the mixture of the resulting glucose and fructose is called invert sugar.

## ⊕ Importance of lactulose

- Treatment of constipation & ↓ plasma ammonia level in hepatic encephalopathy (taken by oral or rectal route).
- **Mechanism:** lactulose is not digested or absorbed in the small intestine, so it exerts its effect in the large intestine. It acts as osmotic substance causing diarrheal effect.. It also lowers the PH of the colonic lumen, thus converting ammonia ( $NH_3$ ) to ammonium ion ( $NH_4^+$ ).  $NH_4^+$  is not easily absorbed →



## Bio lecture 3 : polysaccharides

❖ Def of polysaccharides.: Carbohydrates of high molecular weight.

(>10 monosaccharide sugar units)

❖ Linkage: glycosidic; 1,2 - 1,3 - 1,4 or 1,6.

❖ Hydrolysis: acid or specific enz

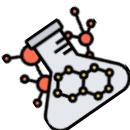
Hydrolysis

Monosaccharides or its derivatives

❖ Polysaccharides chemically & functionally

- Homogeneous Single sugar type (e.g. glucose units only)
- Heterogeneous Different sugar types associated with other subs.

| Homogeneous                                                     |            | Heterogeneous(mucopolysaccharides)                                                          |                                                                                                                                                                                 |               |
|-----------------------------------------------------------------|------------|---------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Glucosans                                                       | Fructosans | Neutral                                                                                     | Acidic                                                                                                                                                                          |               |
| Starch<br>Dextrin<br>Glycogen<br>Cellulose<br>Dextran<br>Chitin | Inulin     | NANA<br>Bl. gp subs<br>Gonadotrophin s, thyrotrophic H<br>$\alpha 1$ & $\alpha 2$ globulins | Non sulfated                                                                                                                                                                    | Hyaluronic a. |
|                                                                 |            | sulfated                                                                                    | <ul style="list-style-type: none"> <li>• Heparin</li> <li>• Heparan sulphate</li> <li>• Chondroitin sulfate</li> <li>• Keratan sulphate</li> <li>• Dermatan sulphate</li> </ul> |               |

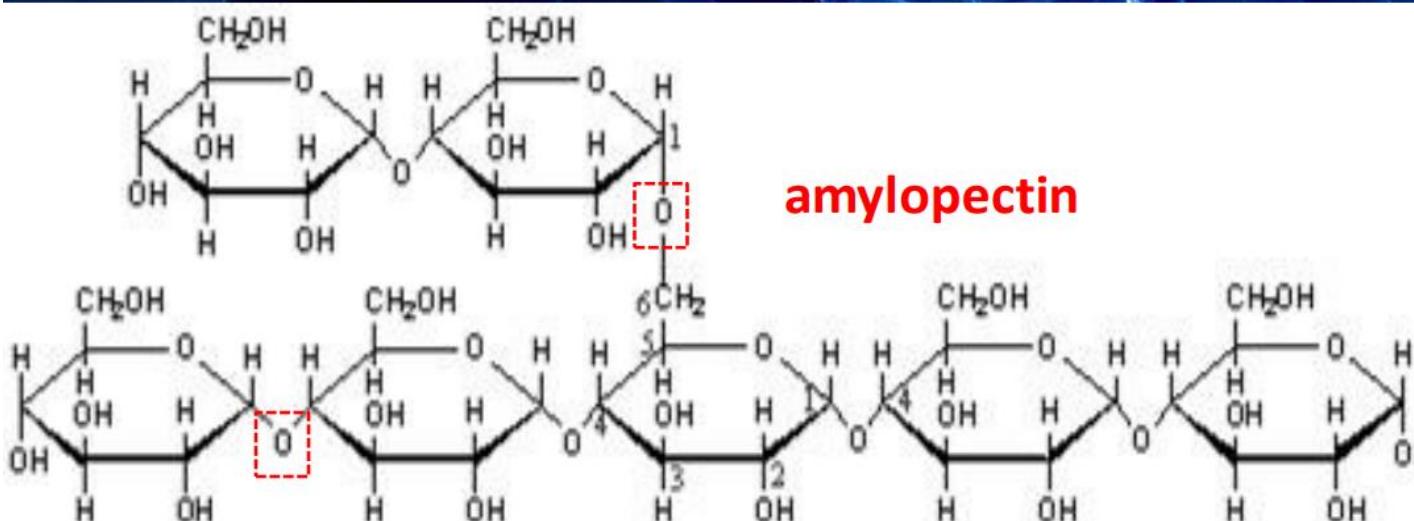
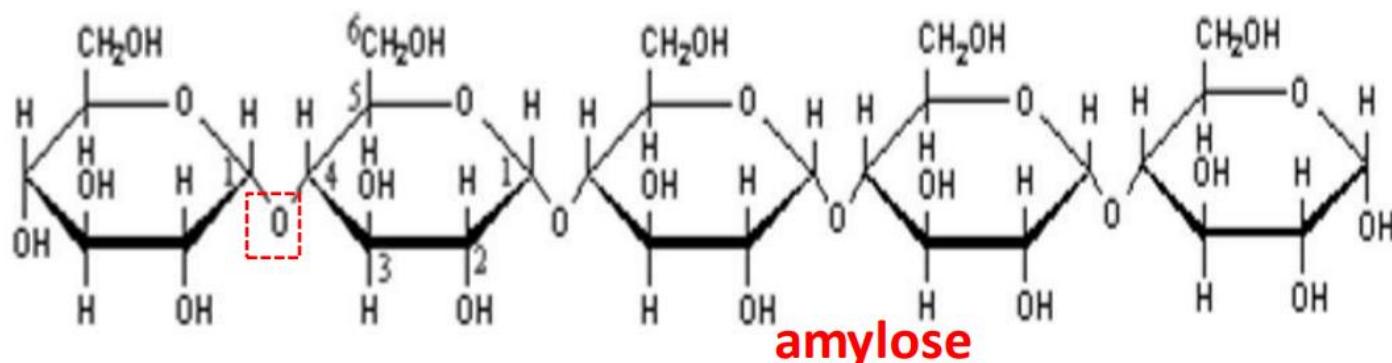


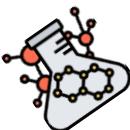
## ❖ Polysaccharides of biological importance

### 1) Starch: Glucosans, $\alpha$ -D-glucose units

- It is found in cereals, legumes & others.
- The most important food source of CHO (60%)
- - Insoluble in water → a suspension on heating, this suspension → colloidal solution
- It consists of amylose & amylopectin

| Differences  | Amylose            | Amylopectin       |
|--------------|--------------------|-------------------|
| Percentage   | 15-20%             | 80-85%            |
| Chain        | Long, non-branched | Highly branched   |
| Site of bond | $\alpha$ -1,4      | $\alpha$ -1,4&1,6 |
| Iodine test  | Blue color         | Purple to red     |





❖ **Hydrolysis of starch:-**

1. Acid hydrolysis:(Dilute mineral acids)
2. Enzymes hydrolysis:  $\alpha$ -amylase (salivary & pancreatic).

| Differences        | Salivary amylase              | Pancreatic amylase            |
|--------------------|-------------------------------|-------------------------------|
| Source             | Saliva                        | Pancreatic juice              |
| Optimum pH         | 6.8 (slight acidic)           | 7.5 (alkaline)                |
| Activator ions     | Cl <sup>-</sup>               | HCO <sub>3</sub> <sup>-</sup> |
| Site of action     | Mouth & short time in stomach | Intestine                     |
| Digestion Products | Incomplete (Dextrin mainly)   | Complete digestion (Maltose)  |

❖ **Products of Starch Hydrolysis:**

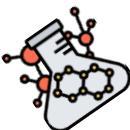
- Starch (**blue color iodin test**)
- Then, Amylodextrin (**purple color iodin test**)
- Then, Erythrodextrin (**red color iodin test**)
- Then, Achrodextrin (no color iodin test)
- Then, Maltose ( $\alpha$ -1,4) & Isomaltose(  $\alpha$ -1,6 )
- Then, Glucose (complete hydrolysis of starch)

**2) Dextrin:**

- It is produced by partial hydrolysis of starch.
- It gives different colors with iodine as:
- Amylodextrin → **Purple color**
- Erythrodextrin → **Red color**
- Achrodextrin → **Colorless**

**3) Dextran (Glucosan):**

- Storage polysaccharide of yeast & bacteria.
- Glucose units only linked Mainly by  $\alpha$  1,6 linkage.
- Occasional branches ( $\alpha$ -1,2,  $\alpha$ -1,3 or  $\alpha$ -1,4) depending on the species.
- Dextran solutions are given intravenously after blood loss due to **high viscosity, low osmotic pressure and they remain in blood for few hours.**



### ❖ Disadvantage:

- Interfere with blood grouping cross matching. So cross-matching must be done before dextran administration in case of hemorrhage, as blood transfusion may be required.
- Some patients show hypersensitivity to dextran.
- slight prolongation of bleeding time may occur with administration of large doses.
- 

### 4) Glycogen (animal starch): (Glucosan)

- It is a non-reducing sugar.
- It gives **Pink** color with iodine.
- It is similar in its structure to amylopectin
- Highly branched formed of  $\alpha$ 1,4 link &  $\alpha$ 1,6 at the branching point
- It is stored in liver & muscle

### 5) Inulin: (Fructosan)

- It is found in many plants e.g. artichokes dahlia, onion & garlic.
- On hydrolysis: it gives D-fructose units ( $\beta$ -1,2).
- It is used as a test for measuring of glomerular filtration rate (GFR).

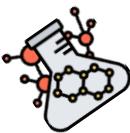
## N.B.

Inulin has all the properties of an ideal marker for GFR:

- It is freely filtered by the glomerulus,
- It is not secreted or reabsorbed in the tubules,
- It is not synthesized or metabolized by the kidney.

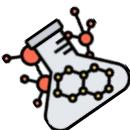
### 6) Cellulose: (Glucosans)

- Insoluble in water
- Unbranched polysaccharides; it is long straight chains of  **$\beta$ -glucose** units linked by  $\beta$ -1, 4 glucosidic bond
- The most abundant structural unit in plants.
- Complete hydrolysis: [acid or enzyme (cellulase)]  $\beta$ -D-glucose units.
- Partial hydrolysis: cellobiose (disaccharide)



- Many mammals (e.g. human) cannot digest cellulose due to absence of enzymes that attack the  $\beta$  link (absence of cellulase enzyme).
- ❖ **The importance of Cellulose**
- It acts as a laxative (prevents constipation): It increases the bulk of stool & has the ability to absorb water. It stimulates intestinal peristalses.
- Being a constituent of dietary fibers: ↓ absorb toxic compounds ↓ the incidence of cancer colon
- A source of Energy in herbivores [their gut contains bacterial enzymes for the  $\beta$  link (cellulase)]

| Difference           | Starch                                                               | Cellulose                                                                                                 | Glycogen                                                                     |
|----------------------|----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Units                | $\alpha$ -glucose                                                    | $\beta$ -glucose                                                                                          | $\alpha$ - glucose                                                           |
| Chains               | Straight,<br>branched.                                               | Straight only                                                                                             | Straight,<br>branched                                                        |
| Glycosidic Link      | $\alpha$ -1,4 & ( $\alpha$ -1,6<br>branch p)                         | B- 1,4                                                                                                    | $\alpha$ -1,4 & ( $\alpha$ -1,6<br>branch p)                                 |
| Digest. in human     | Saliv., pancr.<br>amylases.                                          | Not digested<br>(nocellulase)                                                                             | Saliv., pancr.<br>amylases                                                   |
| I <sub>2</sub> React | Blue color                                                           | No color                                                                                                  | Pink color                                                                   |
| Functions            | 1. Storage CHO in<br>plants 2. Major<br>source of CHO<br>for animals | 1. Supportive in<br>plants.<br>2. Laxative<br>3. ↓ absorb. of<br>toxic subs. 4.<br>Energy<br>(herbivores) | 1. Storage CHO in<br>animals 2. Source<br>of Energy for ms<br>(ms contract.) |



## Bio L4 : CHO chemistry Heteropolysaccharides

### MUCOPOLYSACCHARIDES (Glycosaminoglycans GAGs)

#### I. Neutral mucopolysaccharides:

- Present in **mucous** secretion.
- Formed of **protein & polysaccharides**.
- Contain acetyl hexosamines but **no uronic acid**.

#### II. Acidic mucopolysaccharides:

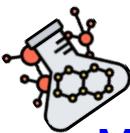
a- Non-sulfated

N.B. L-fucose is deoxyhexose at C6 ( $C_6H_{12}O_5$ )

b- Sulfated

NANA: Pyruvate + mannosamine

| Heterogeneous (mucopolysaccharides GAGs)                 |                 |                     |
|----------------------------------------------------------|-----------------|---------------------|
| I. Neutral                                               | II. Acidic      |                     |
| NANA in tissue of vertebrate & bacteria                  | A. Non-sulfated | Hyaluronic a.       |
| Bl. gp subs. ( <u>L-fucose</u> is important constituent) | B. sulfated     | Heparin             |
| Gonadotrophins & thyrotrophic H                          |                 | Heparan sulphate    |
| $\alpha 1$ & $\alpha 2$ globulins                        |                 | Chondroitin sulfate |
| Ovalbumin                                                |                 | Keratan sulphate    |
| Fibrinogen                                               |                 | Dermatan sulphate   |



## Mucopolysaccharides (negatively-charged)

- Most GAG are present extracellular except heparin
- They act as lubricant and cushion for other tissues as they absorb large amount of water
- On compression of GAG; water is squeezed out and they occupy smaller volume. When the compression is released they return to their original volume. This property is called **resilience** of synovial fluid and vitrous humor of the eye.

## II. Acidic mucopolysaccharides

Formed of (repeated disaccharide units):

- 1- amino-sugar acids OR amino- sugars
- 2- Uronic acid (glucuronic or iduronic)  
OR monosaccharide Linked by glycosidic bond

### A. Non-sulfated mucopolysaccharides :

#### 1. Hyaluronic acid

Repeating units of **N-acetyl glucosamine** & **B-glucuronic a.**

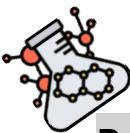
**Functions :**

- Forms the **cement substance** between tissues.
- Present in **synovial fluid** (lubricant facilitates joint movement)
- Makes cartilage **compressible**.
- Makes **ECM loose** (by the ability to attract H<sub>2</sub>O).
- Permits **cell migration** during wound repair & morphogenesis.

**N.B.**

- It facilitates cell migration; being produced in increased amount by **tumor cells**; so facilitates migration through ECM & spread of tumor.
- Hyaluronidase secreted by certain bacteria causes destruction of this cement subs. (hyaluronic a) so help spread of infection (**spreading factor**).
- Hyaluronidase is present in **acrosomal cap** of sperm & invades the tissues of the ova causing destruction of hyaluronic a. & its fertilization.
- **Morphogenesis:** cell differentiation into tissues & organs in the embryo.



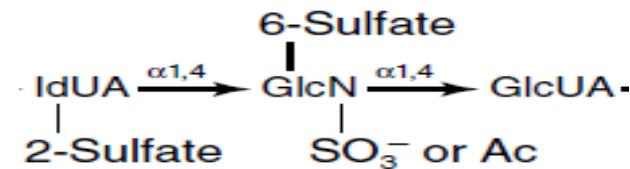


## B. Sulfated mucopolysaccharides :

### 1- Heparin :

repeating units of:

- Sulfated glucosamine &
- Sulfated glucuronic a. (or L-iduronic a.)
- linked by  $\alpha$ -1,4 glycosidic bond.
- Formed by mast cells (intracellular), located along the blood vessel wall in many tissues like heart, lung, liver, kidney, skin & spleen.
- Its concentration in blood is very low.



**N.B.** Iduronic acid is C5 epimer of glucuronic acid  
(differ in the position of -OH at C5)

### Functions of heparin :

#### 1. Anticoagulant :

It activates antithrombin III &  
It Inhibits blood clotting factors II, VII, IX & X.

#### 2. Plasma clearance from lipids :

It activates lipoprotein lipase that digests plasma lipids.  
(heparin & lipoprotein lipase are clearing factors)

### 2- Heparan sulphate :

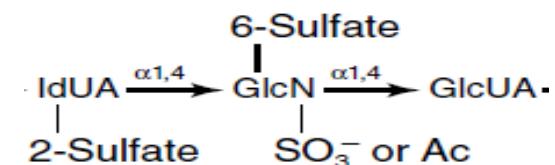
It differs from heparin in the amount of uronic a.  
& the sulphate attached to glucosamine

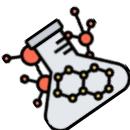
(more glucuronic a. but less sulphated glucosamine).

- It is a component of ECM in the form of proteoglycans.

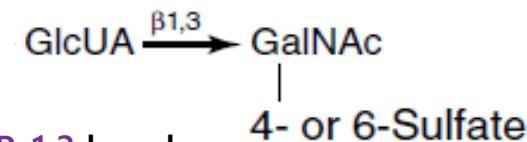
It has a role in:

1. Cell-cell interaction &
2. Cell membrane receptors.



**3- Chondroitin sulfate :**

repeating units of :

N- acetyl galactosamine &  $\beta$ -glucuronic a. linked by  $\beta$ -1,3 bond.

Types: 3 (A, B &amp; C)

**1- Chondroitin sulfate A :** sulfate ester gp of N- acetyl galactosamineat C4  $\rightarrow$  Chondroitin-4-sulfate Aat C6  $\rightarrow$  Chondroitin-6-sulfate C

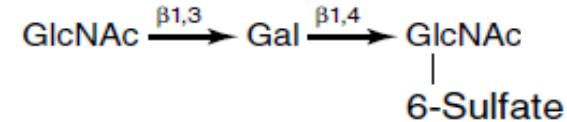
Chondroitin itself is a minor component of ECM but its sulfate ester (A & C) are major component of **cartilage, bone, cornea** & other connective tissues.

**2- Chondroitin sulfate B :** It yields upon hydrolysis

L-iduronic a. instead of D-glucuronic a.

**4- Keratan sulphate :**

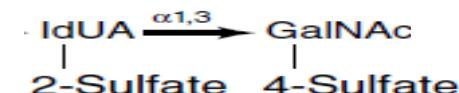
repeating units of:



- Galactose and N-acetyl glucosamine linked together by  $\beta$ - bond.
- No uronic acid
- Present in cornea (to make it transparent), cartilage and tendons.

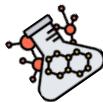
**5- Dermatan sulphate :**

repeating units of:

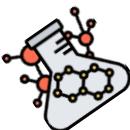


- L-iduronic acid and N-acetyl galactosamine linked together by  $\alpha$ -1,3 bond.
- Present in blood vessels, heart, cornea, sclera & skin.  
It maintains the shape of sclera

N.B. When proteins are connected to acidic mucopolysaccharides  $\rightarrow$  proteoglycans



| Points   | Hyaluronic a                                     | Heparin                                                                                 | Heparan S                                           | Chondroitin S                                               | Keratan S                                   | Dermatan S                                                           |
|----------|--------------------------------------------------|-----------------------------------------------------------------------------------------|-----------------------------------------------------|-------------------------------------------------------------|---------------------------------------------|----------------------------------------------------------------------|
| Amino S. | Glucosamine                                      | Glucosamine                                                                             | Glucosamine                                         | Galactosamine                                               | Glucosamine                                 | Galactosamine                                                        |
| Uronic a | Glucuronic a                                     | Glucuronic +<br>Iduronic                                                                | Glucuronic +<br>Iduronic                            | Glucuronic +<br>Iduronic                                    | Galactose<br>(No uronic a)                  | Iduronic a                                                           |
| Sulfate  | Absent                                           | Present                                                                                 | Present                                             | Present                                                     | Present                                     | Present                                                              |
| Bonds    | $\beta$ -1,3 & $\beta$ -1,4                      | $\alpha$ -1,4                                                                           | $\alpha$ -1,4                                       | $\beta$ -1,3                                                | $\beta$ -1,3 & $\beta$ -1,4                 | $\alpha$ -1,3                                                        |
| Sites    | - S.C. tissues<br>- Ovum Wall<br>-Synovial fluid | -CT <u>Mast cells</u><br>(liver, spleen, kidney, bone marrow) but least conc. in blood. | EXTRA-CELLULAR Matrix (ECM)                         | - Matrix of cartilage<br>- Tendons<br>- Ligaments<br>- Bone | - <u>Cornea</u> .<br>-Cartilage<br>-Tendons | -Blood vessels<br>-Heart.<br>- Cornea,<br>- <u>Sclera</u><br>- Skin. |
| Function | Protective for tissues                           | Anticoagulant<br>Lipid clearance from plasma                                            | - Cell-cell interaction<br>-Cell membrane receptors | Supportive                                                  | Supportive<br>Transparency of cornea        | Supportive<br>Maintains the shape of sclera                          |



## CBL1 : Mucopolysaccharidosis

### Def :

A group of inherited inborn errors of metabolism, progressive lysosomal storage disorders (1:25000 live births).

### Age of onset :

initially normal development with the abnormality appear in infancy or later in childhood.

### Incidence of subtypes :

The most common subtype is MPS III, followed by MPS I and MPS II

### Inheritance :

All MPS are autosomal recessive (AR) except MPS II (Hunter syndrome) which is X-linked (occurs in males only).

### Cause and pathogenesis

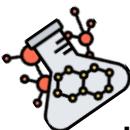
- Mutation in the genes coding for the lysosomal enzymes that degrade GAG →
- Variable expression of mutated enzymes →
- Variable residual enzyme activity (complete absence, deficiency or malfunction) →
- Accumulation of GAG in lysosomes interfering with cell function causing permanent progressive damage in cells, tissues and organs →
- Variable severity of disease with characteristic pattern of clinical, radiologic and biochemical abnormalities

### N.B.

Within this pattern, specific diseases can be recognized resulting from intracellular accumulation of different degradation products.

### As a general role,

- Impaired degradation of heparan sulfate is more closely associated with mental deficiency.



- Impaired degradation of dermatan sulphate, chondroitin sulfate, & keratan sulfate is more closely associated with **mesenchymal abnormalities**.
- Variable severity of disease is manifest e.g. in MPS I ;
  - homozygous or double heterozygous **non-sense** mutation (stop codon) → severe form of Hurler disease.
  - **mis-sense** mutation → preserve some residual enzyme activity (mild form of Hurler disease).

### Clinical abnormality of MPS :

#### 1. Physical features :

- **Coarse facial features** (due to storage of GAGs in soft tissues of the face)
- **Skeletal abnormalities** : early, prominent feature
- Most patients show progressive change in size and shape of bone, involving almost all bones as :-
  - ✓ **short stature** with disproportionate short trunk in all MPS except MPS IS
  - ✓ **Spine deformity** (kyphosis, scoliosis & lordosis)
- Joint stiffness is common in MPS except MPS IV (hyperlax)

#### 2. Neurologic : intellectual changes & behaviour disability

#### 3. Hepato-splenomegaly

#### 4. Respiratory : Obstructive airway disease with frequent infections

#### 5. Cardiovascular : valve disease, angina, heart failure

#### 6. Eye : corneal clouding, glaucoma, retinal degeneration

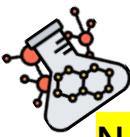
#### 7. Ear : deafness

Generally the patients present in one of three ways :

1.Dysmorphic syndrome (MPSI, MPSII, MPSVI)

2.Learning difficulties, behavioral disturbance ,dementia and mild somatic abnormalities (MPSIII)

3.Severe bone dysplasia (MPSIV)

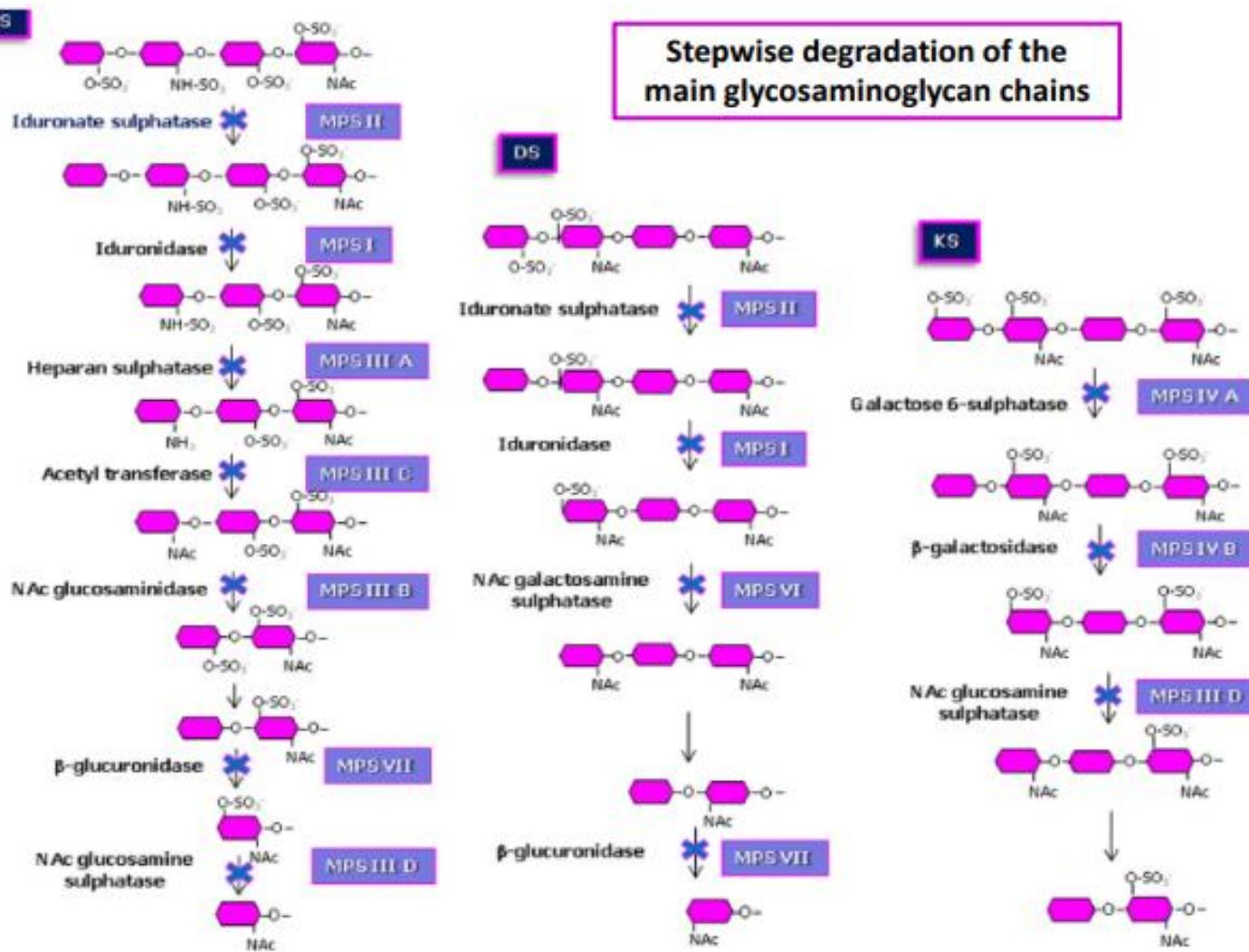
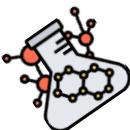


N.B.

1. Heparansulfate degradation is impaired in MPS types I-H, I H/S, II, III & VII → Show mental retardation, however mild MPS II may show no mental deficiency.
2. Absence of **corneal opacity** in MPS II & IX & not common in MPS III & Fine in MPS IV
3. MPSVII presents commonly at birth with **hydrops fetalis** (severe fatal disease usually die at birth )

| Subtypes & Disease        | Enzyme defect                          | Affected GAGs & Urinary MPS   |
|---------------------------|----------------------------------------|-------------------------------|
| MPS I                     | α-L-iduronidase                        | DS & HS                       |
| MPS I-H (Hurler)          | Severe                                 |                               |
| MPS I-H/S (Hurler/Scheie) | Moderate                               |                               |
| MPS I-S (V) (Scheie)      | Mild (dermatan sulfate only in urine)  | DS                            |
| MPS II (Hunter)           | Iduronate-2-sulfatase                  | DS & HS                       |
| MPS III (Sanfillipo)      | A ) Heparan-Sulfate sulfatase          | HS                            |
|                           | B ) α-N-acetyl Glucosaminidase         |                               |
|                           | C ) Acetyl transferase                 |                               |
|                           | D ) N- acetyl glucosamine 6-sulfatase  |                               |
| MPS IV (Morquio)          | A ) N-acetyl galactosamine 6-sulfatase | KS & CS                       |
|                           | B ) β-galactosidase.                   | KS                            |
| MPS VI (Maroteaux-Lamy)   | N- acetyl galactosamine 4-sulfatase    | DS                            |
| MPS VII (Sly)             | β-Glucuronidase                        | DS, HS & CS                   |
| MPS IX                    | Hyaluronidase 1                        | Hyaluronic acid (< in plasma) |

DS: dermatan sulphate, HS: heparin sulfate, KS: keratan sulfate & CS: chondroitin sulfate



### Laboratory diagnosis :

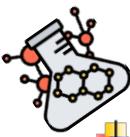
#### 1. Urinary GAG analysis (24 hour urine) :

- ✓ Quantitative assay for the total amount of GAG; elevated level in age-matched normal subjects
- ✓ Qualitative assay (determine type of elevated GAG)

#### 2. Enzyme activity assay in WBCs, fibroblast or serum

#### 3. Molecular genetic testing for mutation in the genes coding for the defective enzyme

#### 4. Increased plasma hyaluronan level in type IX



### Other tests that may be done :

5. Prenatal diagnosis using amniotic fluid cells (Amniocentesis) or chorionic villus biopsy.

### 6. Genetic counselling

N.B.

- Urinary GAG can be used for disease monitoring
- When a sibling of MPS patient is identified, the other undiagnosed siblings should undergo the same clinical history & laboratory testing.

### Treatment :

1. Enzyme replacement therapy: used in MPS I & II but can not prevent neurological damage
2. Bone marrow & cord blood transplantation : limited success (macrophage enzymes degrade GAG)
3. Treatment of various clinical manifestations

### Case I

A seven year old boy is brought to the physician with severe mental retardation, on asking the mother; several members of the mother's and father's family had mental retardation. The mother also noticed that this child is very active, restless and frequently get into troubles in his school.

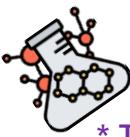
On examination; the boy appeared restless with delayed incomprehensive speech, coarse facial features and normal other examination. The lab tests showed elevated heparan sulphate.

### Questions

- What is the most likely diagnosis?
- What is the inheritance pattern of this disorder?
- What are the other causes of MPS?

### Answers

1. MPS III (Sanfillipo syndrome)
2. Autosomal recessive (AR)
3. Write all other types of MPS; Hurler (MPS I), Hunter (MPS II), Morquio (IV), Maroteaux-Lamy (VI), Sly (VII), hyaluronidase deficiency (IX)



\* **The first explanation for diagnosis:** MPS present in one of three ways :

1. As a dysmorphic syndrome ( e.g. MPS IH, MPS II, MPS VI).
2. With learning difficulties, behavioral disturbance and dementia and mild somatic abnormalities (MPS III)
3. As a severe bone dysplasia (MPS IV)

\* **The second explanation** presence of heparan sulfate in urine (the only metabolite present)

### Case 2 :

An infant presented with corneal clouding. His urine examination showed dermatan sulphate and heparan sulphate. He was diagnosed as Hurler syndrome.

- One of the following enzymes has decreased activity to confirm the suspected diagnosis :
- a.  $\alpha$ -L-iduronidase      b.  $\alpha$ -glucuronidase      c. Glycosyl transferas      d. Iduronte sulfatase

**Answer :** a.  $\alpha$ -L-iduronidase

### Case 3

- A fifteen month old white female was brought to the paediatrician because of upper respiratory tract infection. On examination; the girl was noticed to have short stature, some corneal clouding, coarse facial features, some hearing loss, developmental delay. It was suspected to has MPS.
  - Which of the following is the least likely to affect her :
- a. Hurler syndrome (MPS I)      b. Hunter syndrome (MPS II)  
 C. Morquio syndrome (MPS IV)      d. Sly syndrome (MPS VII)  
 e. Sanfillipo syndrome (MPS III)

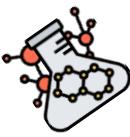
**Answers** b. Hunter syndrome (MPS II)

**Explanation :** All are autosomal recessive except hunter syndrome. Hunter syndrome is X linked recessive and thus is almost exclusively seen in males. This case is female and thus not expected to have an X linked disorder.

### Case 4

A 3-year-old male with coarse facial features, progressive loss of motor skills, hepatosplenomegaly & chronic diarrhea is suspected of having Hunter syndrome (MPS II).

- Which of the following monosaccharide residues would be expected to be found at the non reducing end of GAGs in this patient urine :
- a) N-acetyl glucosamine.      b) N-acetyl galactosamine.



- c) Glucuronate.      d) Iduronate.      e) Iduronate 2- sulfate.

**Answers:** e) Iduronate 2- sulfate

**Explanation :** Since the patient is suspected of having Hunter syndrome (MPS II) with deficiency of iduronate 2- sulfatase, iduronate 2- sulfate would be expected to be found at the non reducing end of GAGs in this patient urine.

### Case 5

An 11-year-old boy was referred to the outpatient department for routine examination. Medical history reported that he had frequent respiratory infections and generalized weakness with easy fatigability. On examination, the patient had retarded growth with a short stature with mild mental retardation and hepatomegaly. Bony deformities, including kyphosis and rotated legs. The case was diagnosed as MPS II after the suitable lab investigations were done.

- What do you think the laboratory investigations were done to diagnose MPS II and their results?
- What is the treatment of this case?

**Answers :**

a) Lab. investigations for MPS II and their results:

#### 1. Urinary GAG analyses:

- Quantitative GAG assay: revealed elevation of GAG compared to GAG levels in age-matched normal subjects.
- Qualitative GAG assays: revealed the type of GAG excreted (dermatan sulfate and heparan sulfate).

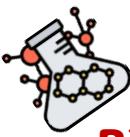
2. Enzyme activity assays: Iduronate-2-sulfatase enzyme activity in WBCs, fibroblasts or serum was decreased.

3. Molecular genetic testing: of mutation in the gene coding Iduronate-2-sulfatase enzyme was found

b) Treatment of this MPS II case :

- Enzyme replacement therapy.
- Treatment of different clinical manifestations

”يا طالب العلم لا تركن إلى الكسل  
واعجل فقد خلق الإنسان من عجل“



## Bio L 11 : CHO Metabolism\_ Introduction and Glycolysis 1

### Carbohydrate metabolism

- Metabolism, the sum of the chemical reactions that take place within each cell of a living organism and that provide energy for vital processes and for synthesizing new organic material.

#### Fate of absorbed sugars:

Monosaccharides pass through portal blood to the liver where fructose and galactose are converted to glucose. The only sugar in blood is glucose. Glucose is then passed to all tissues

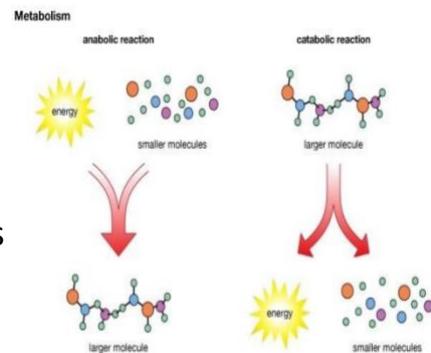
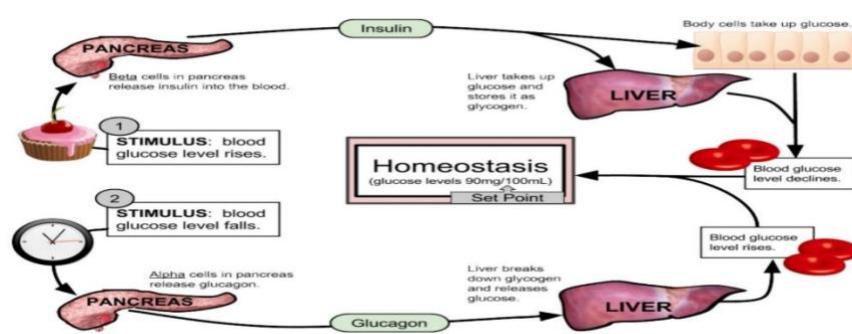
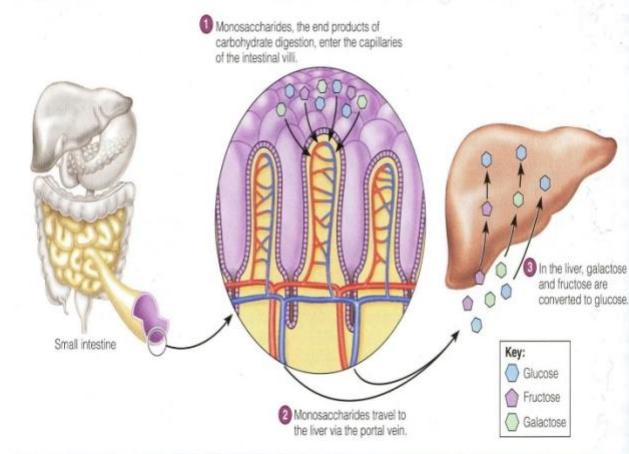
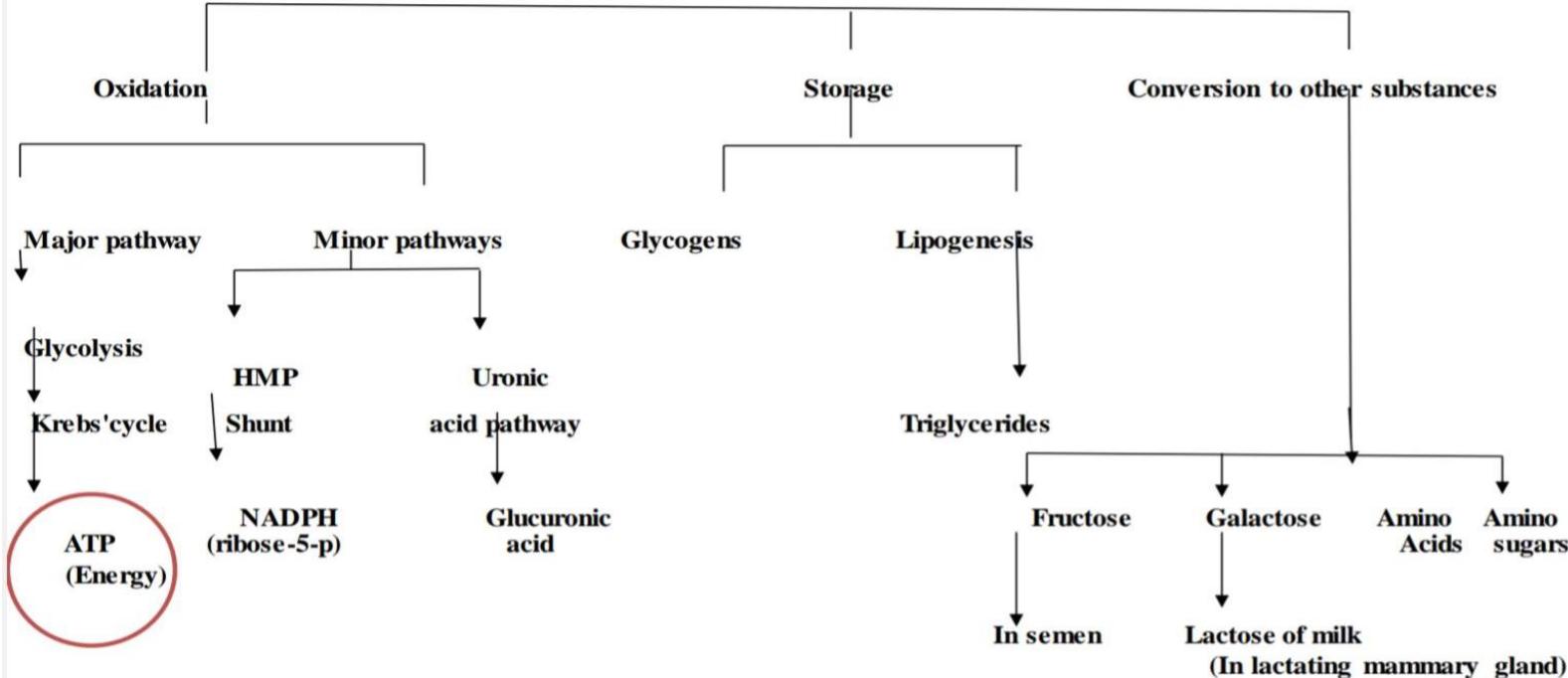
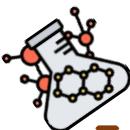


FIGURE 4-11 Absorption of Monosaccharides



#### Fate of Absorbed Glucose



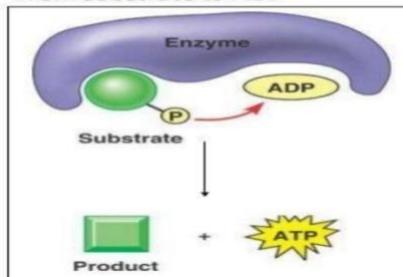


## Two methods of ATP synthesis(phosphorylation)

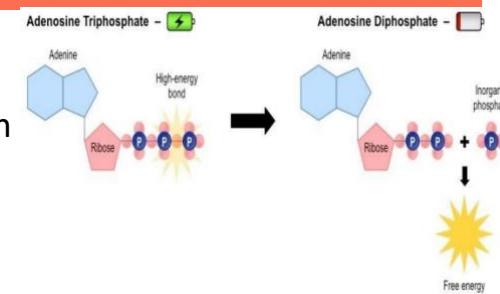
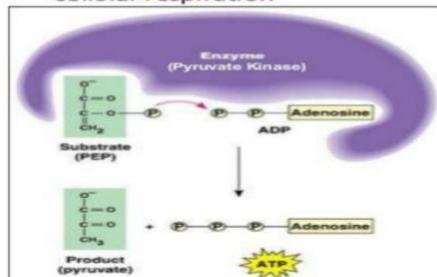
1-Substrate level phosphorylation    2-Oxidative phosphorylation

### Substrate-level Phosphorylation

An enzyme transfers phosphate from substrate to ADP



Example from glycolysis in cellular respiration



### I. The major pathway of glucose oxidation

Divided into 2 phases:

A. Anaerobic phase (anaerobic glycolysis).

B. Aerobic phase (aerobic glycolysis, oxidative decarboxylation of pyruvate, Krebs' cycle).

#### A. Glycolysis (Embden-meyerhof pathway)

**Definition:** It is oxidation of glucose or glycogen to pyruvic acid (in presence of O<sub>2</sub>) or lactic acid (in absence of O<sub>2</sub>).

**Site:** It occurs in the cytosol of every cell. Glucose can only give lactic acid in:

1-RBCs (no mitochondria).    2-Exercising muscles (O<sub>2</sub> lack).

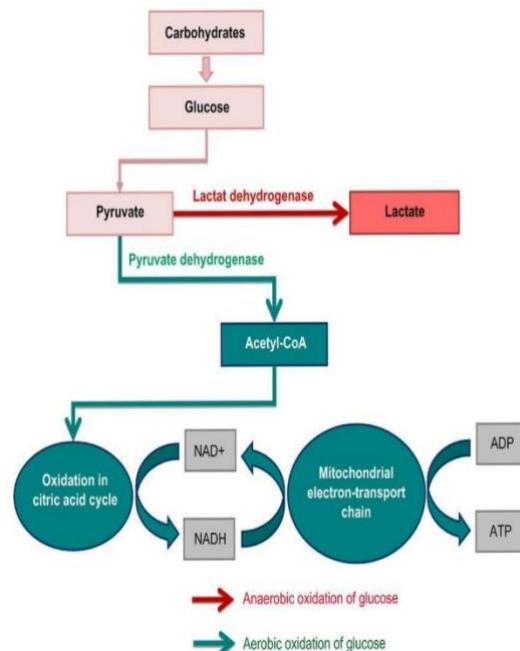
**Steps:** The steps of glycolysis can be classified into two phases:

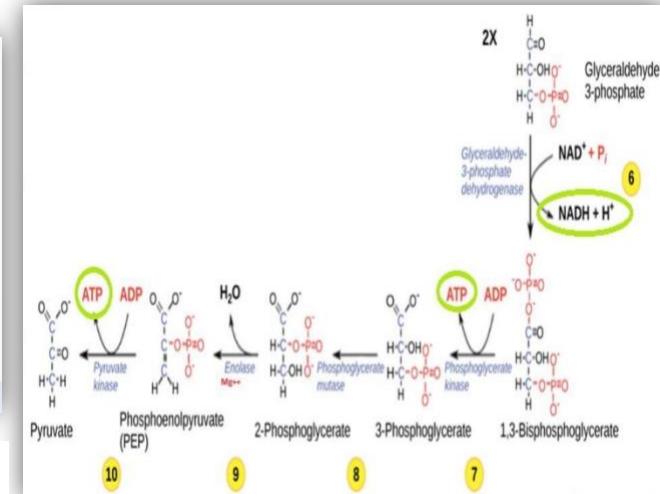
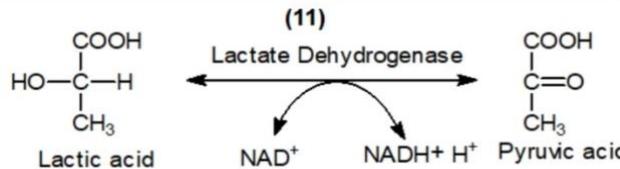
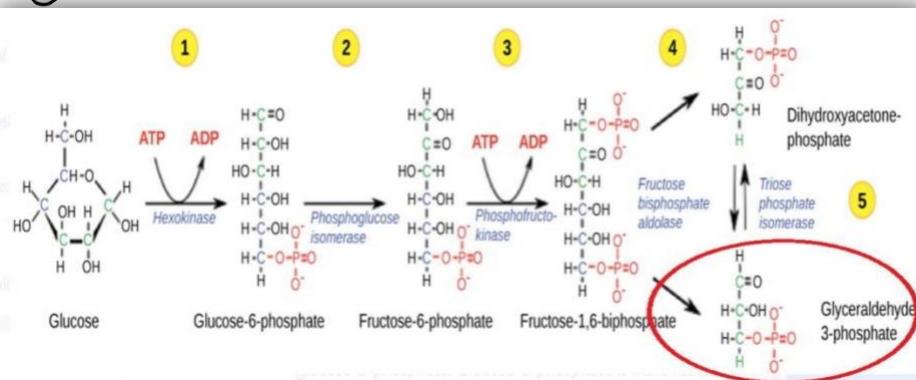
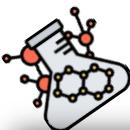
**A-Phase one:** investment phase

- In this phase glucose is converted into two molecules of glyceraldehydes-3-phosphate.

**B- Phase two:** energy production

- In this phase the 2 molecules of glyceraldehydes-3-phosphate are converted into two molecules of pyruvate (aerobic) or lactate (anaerobic).





### Energy gain of glycolysis:

#### Energy consumed:

**Step (1) by glucokinase:** One ATP is lost (spared if we start with glycogen).

**Step (3) by phosphofructokinase:** One ATP is lost.

♡ So, the total lost 2 ATPs

#### Energy gained:

**Step (6) by glyceraldehyde -3 P dehydrogenase:** 2 NADH+H+

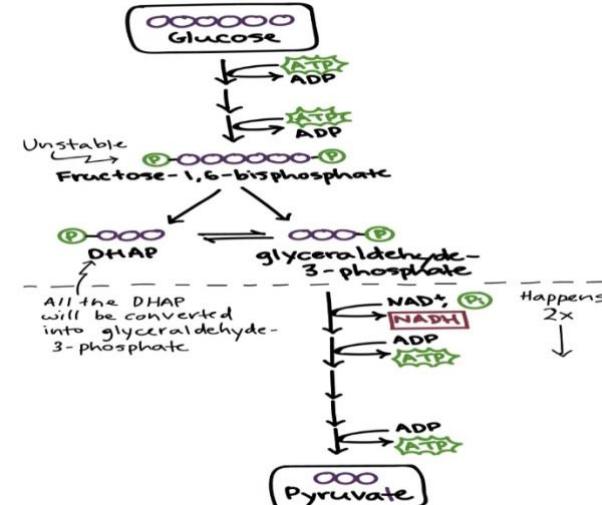
- (6 ATPs) gained only in the presence of O<sub>2</sub>

**Step (7) by phosphoglycerokinase:** 2 ATPs gained.

**Step (10) by pyruvate kinase:** 2 ATPs gained. So, the total gains 10 ATPs.

• So, Energy gained under anaerobic condition: (.e.) Glucose to 2 molecules of lactic acid is 2 ATPs and 3 ATPs if we start with glycogen.

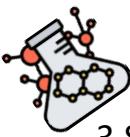
• Energy gained under aerobic condition: (i.e.) Glucose to 2 molecules of pyruvic acid and 2 NADH +H+ equal to 2 ATPs + 6 ATPs (from 2 NADH+H+) = 8 ATPs.



### Importance and functions of glycolysis

1. It is the only source of energy for RBCs (2 ATPs)

2. The main source of energy for exercising muscles.

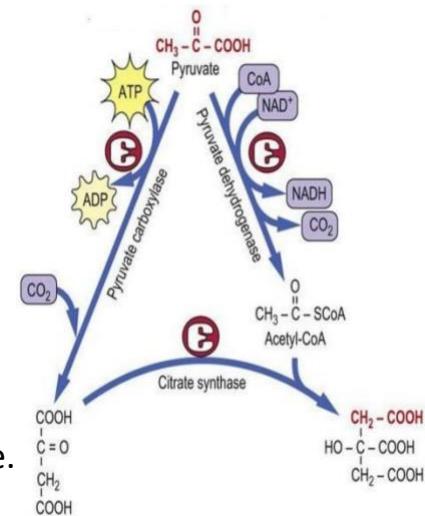


3. Supply small amount of energy to all cells : 2 ATPs in absence of O<sub>2</sub> & 8 ATPs in presence of O<sub>2</sub>
4. Supply pyruvic acid which gives acetyl-CoA & Oxaloacetic acid for Krebs' cycle.
5. Supply dihydroxy acetone phosphate (DHAP) which gives glycerol-3-P which is important in synthesis of triacylglycerol and phospholipids
6. Supply pyruvic acid which gives by transamination alanine A A.
7. Supply 3 -Phosphoglycerate which gives serine AA.

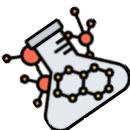
### Comment on Glycolysis:

1. All reactions of glycolysis are reversible except steps 1,3,10
2. Glucose is phosphorylated to G-6-P either by glucokinase or hexokinase.

### Differences between hexokinase and glucokinase



| Points of differences          | Hexokinase                                                                                 | Glucokinase                                                                                  |
|--------------------------------|--------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| <b>1. Specificity</b>          | <b>Non specific:</b> Acts on hexoses (glucose, fructose, galactose)                        | <b>Specific:</b> acts only on glucose                                                        |
| <b>2. Site</b>                 | Present in all cells except Liver & pancreas.                                              | Present only in liver and pancreas                                                           |
| <b>3. Km</b>                   | <b>Low Km:</b> It has high affinity for glucose so can act at low concentration of glucose | <b>High Km:</b> It has low affinity to glucose so acts only at high concentration of glucose |
| <b>4. Induction</b>            | <b>Not inducible:</b> Insulin no effect                                                    | <b>Inducible:</b> insulin induce its synthesis                                               |
| <b>5. Feed-back inhibition</b> | Affected by feedback inhibition<br><br>G hexokinase → G-6-P<br>                            | Not affected by G-6-P                                                                        |



# Regulation of Glycolysis

The rate glycolysis is regulated to meet two major cellular needs:

- (1) the production of ATP, and
- (2) the provision of building blocks for synthetic reactions.

There are three control sites in glycolysis - the reactions catalyzed by

- **hexokinase**,
- **phosphofructokinase 1**, and
- **pyruvate kinase**

These reactions are **irreversible**.

Their activities are regulated

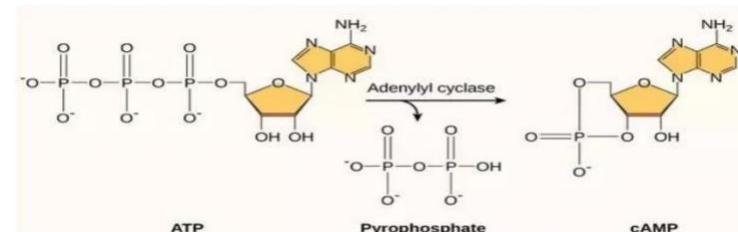
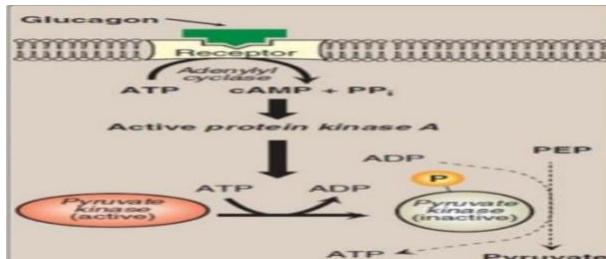
- by the reversible binding of **allosteric effectors**
- by **covalent modification**
- by the regulation of **transcription** (change of the enzymes amounts).

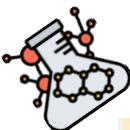
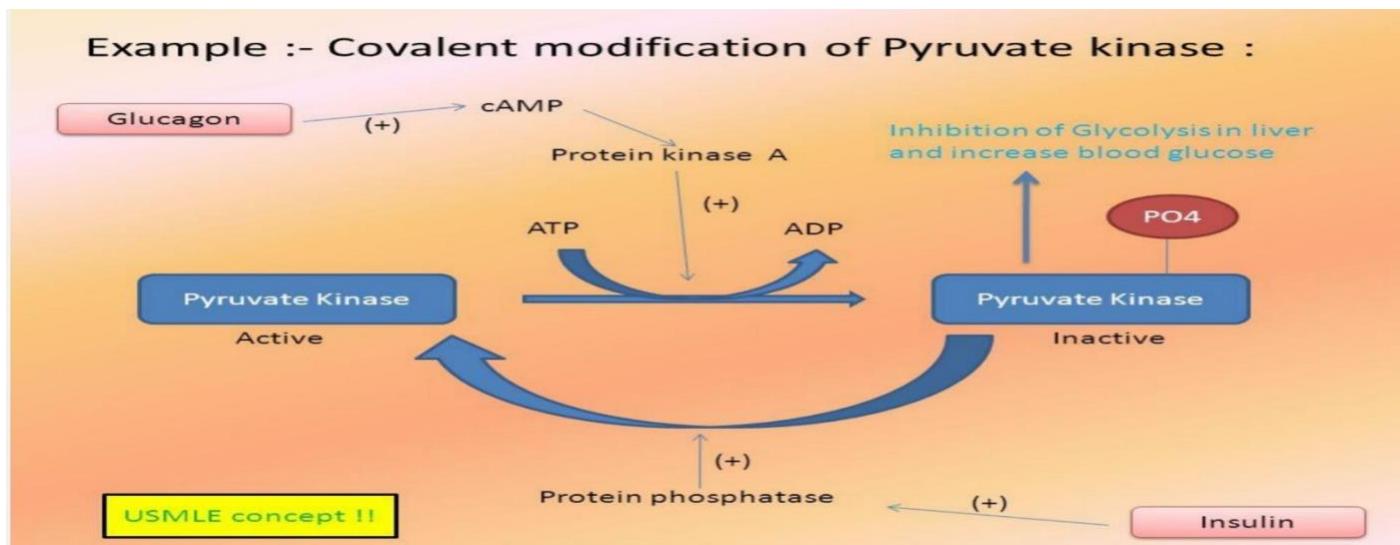
The time required for allosteric control, regulation by phosphorylation, and transcriptional control is typically in milliseconds, seconds, and hours, respectively.

## A-Allosteric regulation

- **GK (Glucokinase)**: No regulation.
- **Hexokinase** is allosterically inhibited by G-6-P.
- **PFK (Phosphofructokinase)**:
  - Allosterically **activated** by fructose-2,6-bis-phosphate, AMP& ADP
  - Allosterically **inhibited** by ATP & Citrates.
- **PK (Pyruvate kinase)**:
  - Allosterically **activated** by Fructose-1,6- bis-phosphate
  - Allosterically **inhibited** by ATP

## B-Covalent modification:




**Example :- Covalent modification of Pyruvate kinase :**


### C- Induction and Repression of the

#### key enzymes:

- Insulin induces (increases) the synthesis of these enzymes, while glucagon and adrenaline inhibit their synthesis.

#### Feeding status & hormonal regulation:

##### Carbohydrates feeding:

- Intake of carbohydrates **stimulates** insulin secretion which leads to:
- **Increase** glucose uptake by tissues.
- **Increase** synthesis of GK, PFK & PK.
- **Increase** activity of PK by dephosphorylation. So,
- carbohydrates feeding **stimulate** Glycolysis.

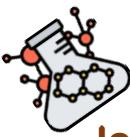
##### Fasting (starvation): It leads to:

- **Decrease** insulin and decrease glucose uptake by tissues.
- **Increase** glucagon and adrenaline leads to:
  - A. Decrease synthesis of GK, PFK & PK.
  - B. Decrease activity of PK by phosphorylation.

So, fasting inhibits glycolysis.

#### Inhibition of Glycolysis in vitro:

**Fluorides:** They inhibits enolase enzyme (binds to Mg++) so we add fluoride as anticoagulant for blood sample to estimate its blood glucose.



**Iodoacetate:** It blocks the SH group at the active site of glyceraldehydes-3-phosphate dehydrogenase enzyme.

**Arsenite:** It inhibits ATP formation.

### Pasteur effect:

**Definition:** It is the inhibition of glycolysis by Oxygen.

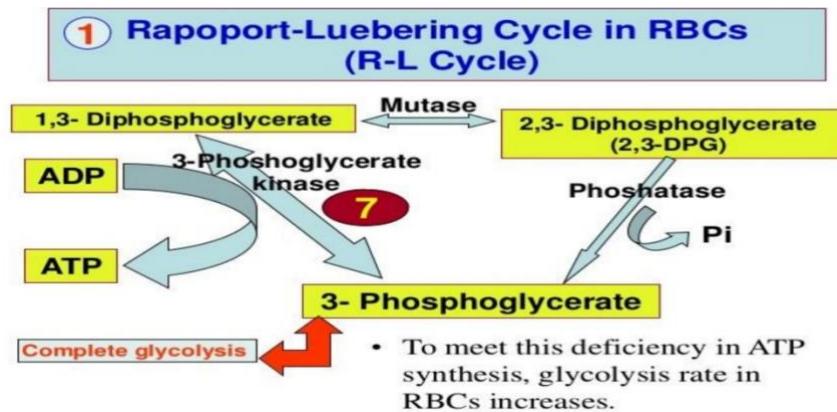
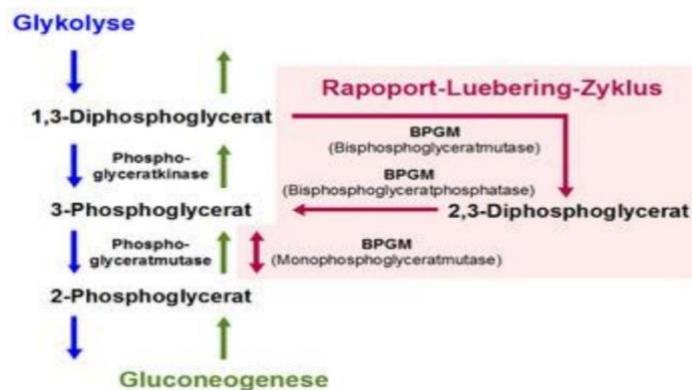
**1. In presence of oxygen:** Stimulation of Krebs' so citric acid is increased leading to inhibition of glycolysis. Increase ATP leading to inhibition of glycolysis.

**2. In absence of oxygen:** Glycolysis is increased which is an important part of the defense against cerebral anoxia.

### Glycolysis in red blood cells:

- RBCs have no mitochondria so, glucose oxidation by Glycolysis give 2 lactic acids and only 2 ATPs. Sometimes Glycolysis in RBCs gives no ATP. RBCs need 2, 3 bisphosphoglycerate as its increase will decrease the oxygen affinity for hemoglobin to oxygen and helping oxygen delivery to tissues.

- RBCs 1, 3 diphosphoglycerate is changed by mutase to 2, 3 diphosphoglycerate which by phosphatase is changed to 3-phosphoglycerate to continue glycolysis till pyruvic acid.

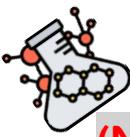


### Importance of 2, 3 Bisphosphoglycerate (2, 3 DPG):

- 2, 3 DPG attaches to HB causing a decrease in its affinity for oxygen. So, 2, 3 DPG helps oxygen delivery to tissues.
- 2, 3 DPG causes shift of the oxygen dissociation curve of HB to the right.

**In Hypoxia (decrease oxygen), 2, 3 DPG is increased in it level.**

- 2, 3 DPG is increased in high altitude and in chronic lung diseases, and in chronic anemia.



(N.B.)

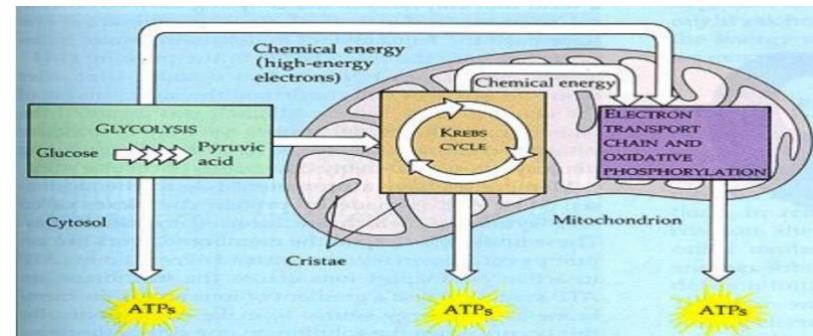
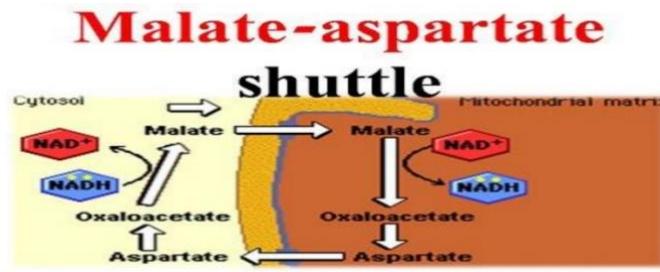
❖ Congenital deficiency in hexokinase enzyme in RBCs leading to hemolytic anemia as there is no ATP will be formed as no glycolysis.

❖ Congenital deficiency in pyruvate kinase enzyme in RBCs leading to hemolytic anemia as ATP will be decreased.

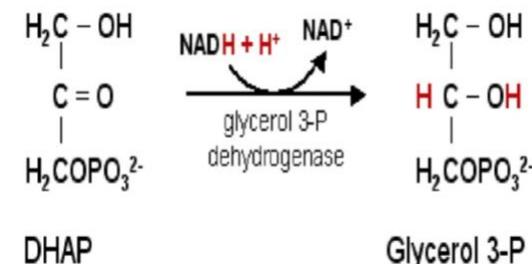
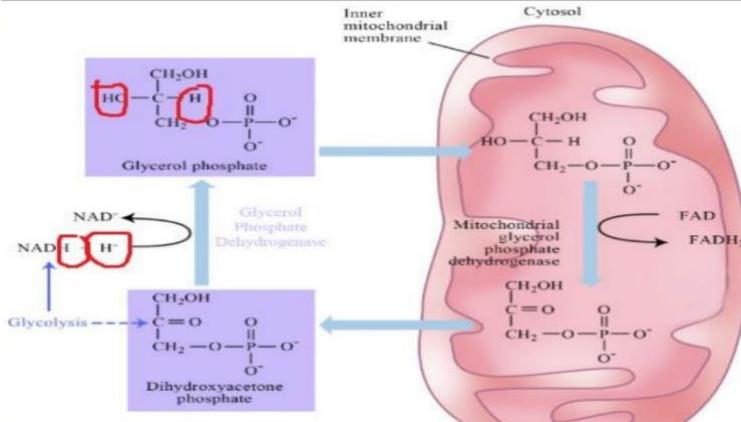
### The transfer of NADH+H+ through mitochondrial membrane

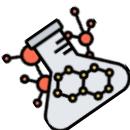
- The mitochondrial membrane is impermeable to NADH+H+
- There is certain shuttles to transfer H atoms across mitochondrial membrane.

#### 1-Malate shuttle



#### 2-Glycerophosphate shuttle

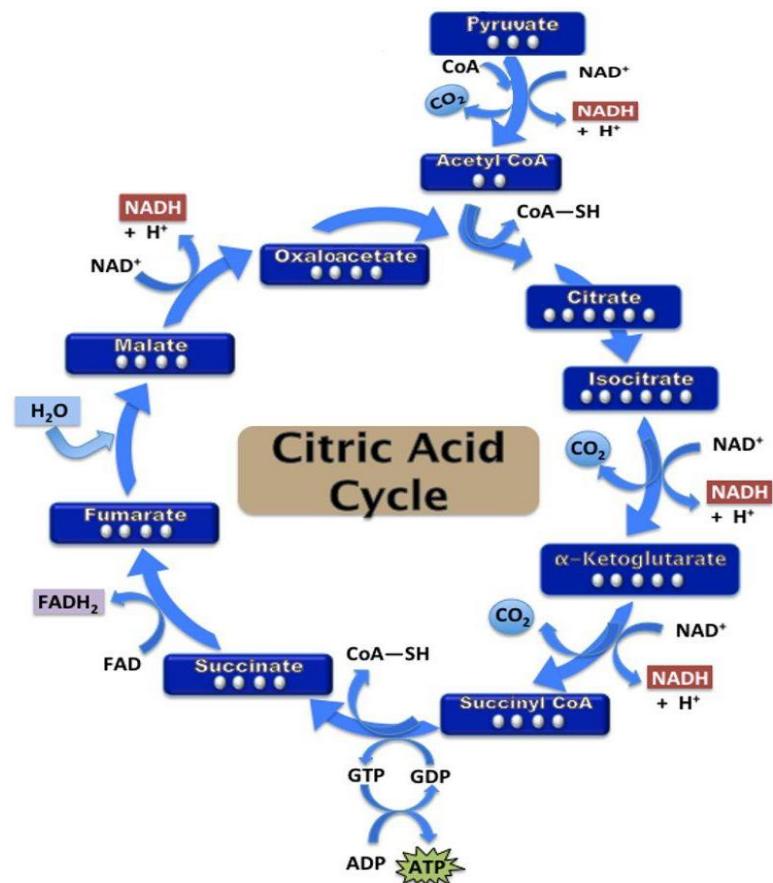




# L16: Integration of metabolism & TCA Cycle

## Citric Acid Cycle

- ❖ Takes place inside mitochondria
- ❖ The rate of the TCA cycle matches the need for ATP.
- ❖ High ATP levels decrease the activities of 2 enzymes:
  - 1) Citrate synthase
  - 2) Isocitrate dehydrogenase
- ❖ High NADH inhibits:
  - 1) Citrate synthase
  - 2) Isocitrate dehydrogenase
  - 3)  $\alpha$ -ketoglutarate dehydrogenase.
- ❖ Ensures that the rate of the citric acid cycle matches the need for ATP

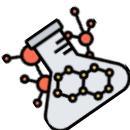


## Net energy gain in TCA

| Enzyme                                              | Number of reducing equivalent   | ATP Gain |
|-----------------------------------------------------|---------------------------------|----------|
| Isocitrate dehydrogenase                            | NADH                            | 3 ATP    |
| $\alpha$ -Ketoglutarate dehydrogenase               | NADH                            | 3 ATP    |
| Succinate dehydrogenase                             | $\text{FADH}_2$                 | 2 ATP    |
| Malate dehydrogenase                                | NADH                            | 3 ATP    |
| Succinate thiokinase                                | Substrate level phosphorylation | 1 ATP    |
| Net gain or ATP per molecule of acetyl CoA = 12 ATP |                                 |          |

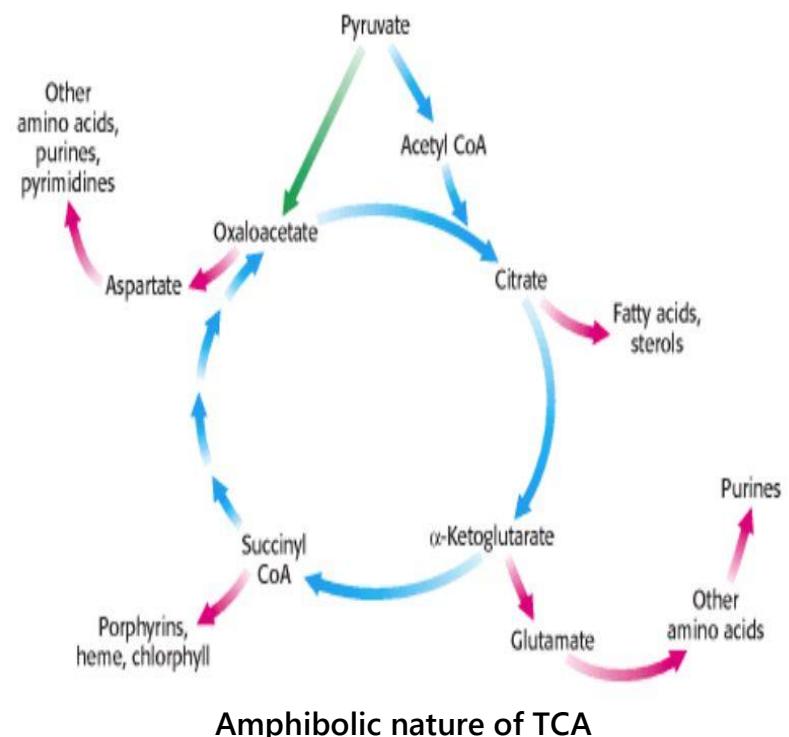
## Summary of total energy yield of complete oxidation of 1 glucose molecule

| Step                     | Coenzyme          | ATP Yield | Source of ATP                                      |
|--------------------------|-------------------|-----------|----------------------------------------------------|
| Glycolysis - Stage 1     | -                 | -2        | Phosphorylation of glucose and fructose uses 2 ATP |
| Glycolysis - Stage 2     | -                 | 4         | Substrate level phosphorylation                    |
|                          | 2 NADH            | 6         | Oxidative phosphorylation                          |
| Pyruvate metabolism      | 2 NADH            | 6         | Oxidative phosphorylation                          |
| TCA cycle                | -                 | 2         | Substrate level phosphorylation                    |
|                          | 6 NADH            | 18        | Oxidative phosphorylation                          |
|                          | 2 $\text{FADH}_2$ | 4         | Oxidative phosphorylation                          |
| Total ATP Yield = 38 ATP |                   |           |                                                    |



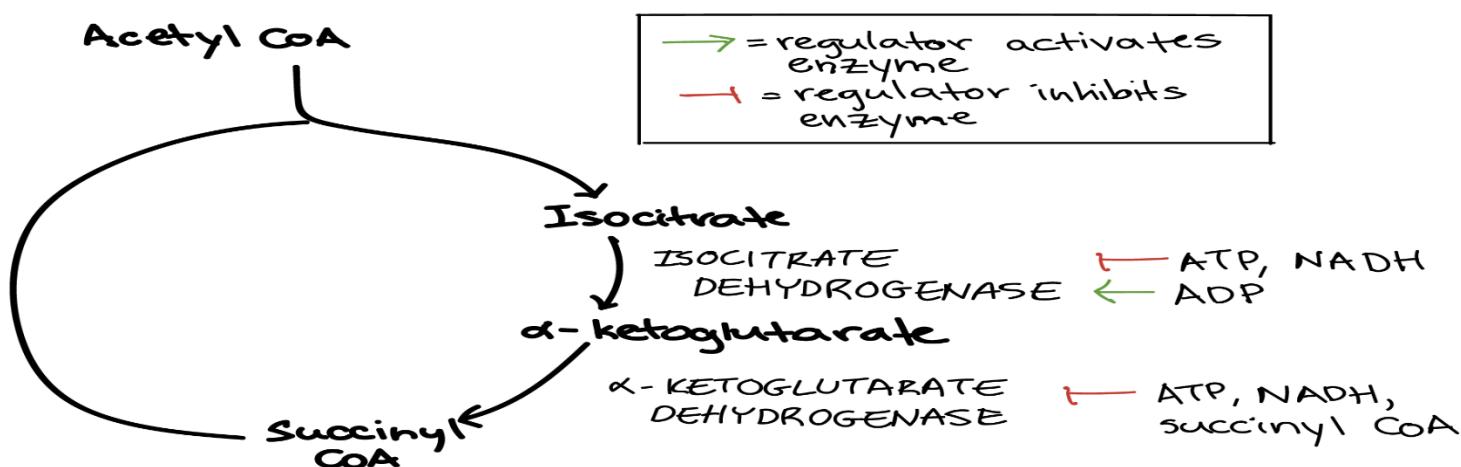
## Functions of the citric acid cycle:

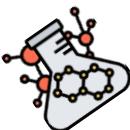
- ❖ It is the final common oxidative pathway that oxidizes acetyl CoA to CO<sub>2</sub>.
- ❖ It is the source of reduced co-enzymes that provide the substrate for the respiratory chain.
- ❖ It acts as a link between catabolic and anabolic pathways (amphibolic role).
- ❖ It provides precursors for synthesis of amino acids and nucleotides.
- ❖ Components of the cycle have a direct or indirect controlling effect on key enzymes of other pathways



## Significance of TCA cycle

- ❖ Complete oxidation of acetyl CoA.
- ❖ ATP generation.
- ❖ Final common oxidative pathway.
- ❖ Integration of major metabolic pathways.
- ❖ Fat is burned on the wick of carbohydrates.
- ❖ Excess carbohydrates are converted as neutral fat.
- ❖ No net synthesis of carbohydrates from fat





### In vitro inhibition of TCA cycle

#### 1) Fluoroacetate (F<sub>l</sub>-CH<sub>2</sub>-COSCoA)

❖ Inhibits aconitase enzyme.

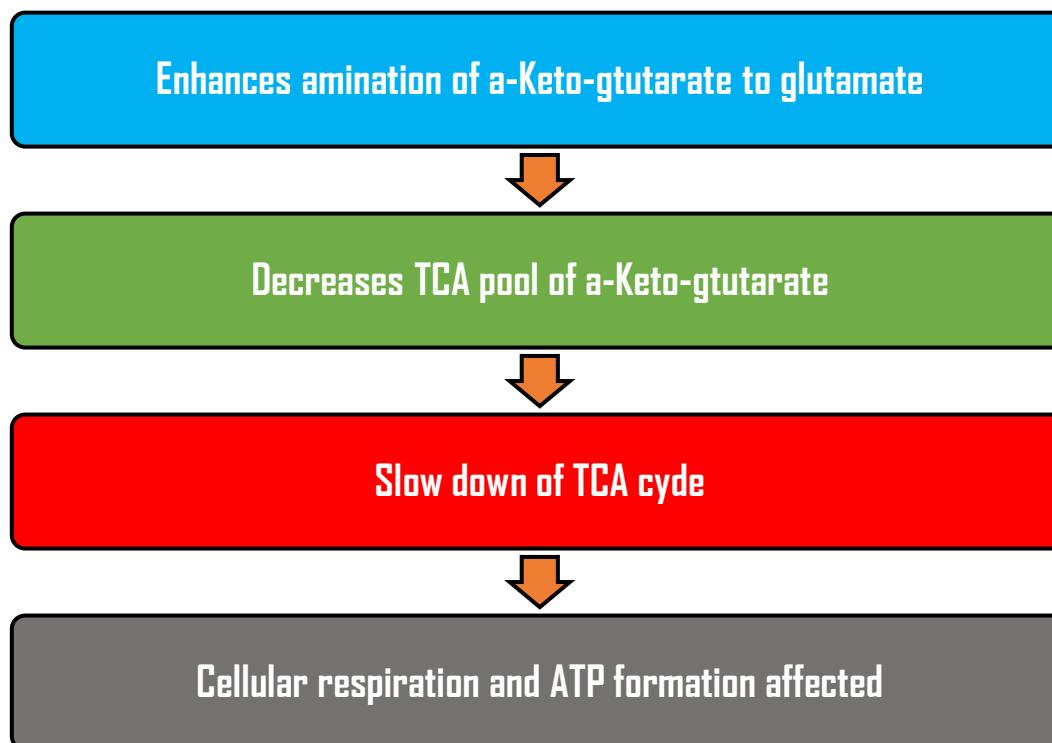
#### 2) Arsenate

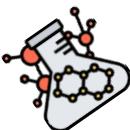
❖ Inhibits a-ketoglutarate dehydrogenase enzyme.

#### 3) Malonic acid:

❖ Inhibits succinate dehydrogenase enzyme (competitive inhibition).

### Toxicity of Ammonia Biochemical changes in brain





## Bio L17 : CHO metabolism\_minor pathways of glucose oxidation

### II. Minor Pathways for Glucose Oxidation

#### A. Hexose Monophosphate Shunt (HMP-shunt, Pentose shunt pathway or direct oxidative Pathway)

**Definition:** It is an alternative minor pathway for glucose Oxidation involving the formation of pentoses as Intermediates .

#### Importance of HMP shunt

1.Production of NADPH+H+ , NOT NAD, (Reduced Coenzyme II). 2.Production of pentoses 5 –P (ribulose, xylulose & ribose 5-P)

#### Site:

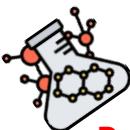
- The enzymes are located in cytosol of special tissues.
- The tissues such as liver, adipose tissue, adrenal gland, Ovaries, testes and lactating mammary gland are highly Active in HMP shunt. Most of these tissues are involved in Biosynthesis of fatty acids and steroids which are dependent On supply of NADPH.
- RBCs Keeps cell membrane resistant to hemolysis.
- Retina, cornea, lens (source of energy & reduction of retinal To retinol).

#### Steps:

Divided into 2 phases: oxidative and non Oxidative phase

#### A.Oxidative phase:

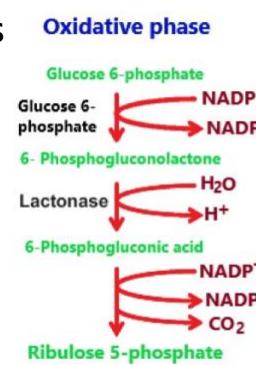
- It is irreversible. It catalyzes conversion of 6 Molecules of glucose to 6 molecules of ribulose-5- Phosphate
- Glucose-6-p is oxidized by glucose 6-phosphate Dehydrogenase(G6PD), 6- up Dehydrogenase
- NADPH is formed in these reactions.



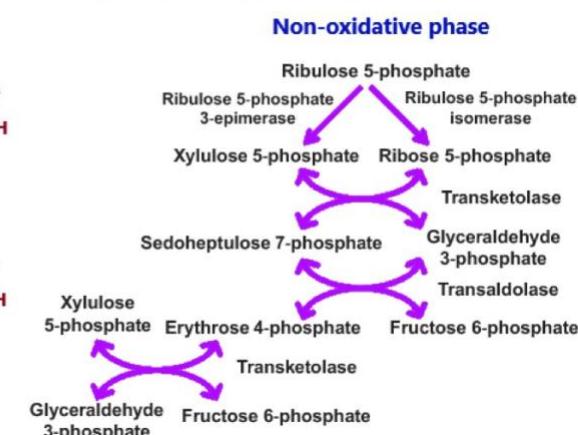
**B. Non oxidative phase:** It is reversible. It catalyzes conversion of 6Molecules of pentoses produced by phase one into 5 molecules of glucose 6 phosphate.

N.B

- Transketolase: transfers 2 carbon units from a ketose to an Aldose. It is TPP(vitamin B1) dependent.
- Transaldolase: transfer 3 carbon unit from ketose to an Aldose.



### Pentose phosphate pathway

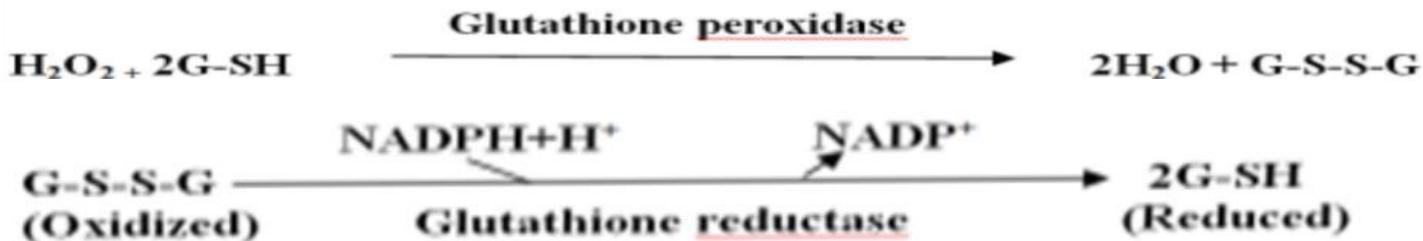


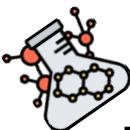
### Functions of HMP shunt:

- HMP can account for complete oxidation of glucose where it is converted to 6 molecules of  $\text{CO}_2$  and 12 molecules of  $\text{NADPH} + \text{H}^+$ .
- It provides the body with ribose -5-p which forms Phosphoribosyl pyrophosphate (PRPP) for synthesis of Nucleotides and nucleic acids. In tissues (muscles) where nonOxidative HMP is not active due to deficiency of the Dehydrogenases of the oxidative phase and in cases of Favism (deficiency of G6PD), pentoses are formed by Reversal of the non oxidative phase.
- It is the main source of  $\text{NADPH} + \text{H}^+$  required for the Reaction of many reductases and hydroxylases.
- Rarely in the eye HMP shunt is used for ATP production

### A-Reductases use of $\text{NADPH} + \text{H}^+$

- Glutathione reductase and glutathione peroxidase which are Important for removal of  $\text{H}_2\text{O}_2$ .  $\text{H}_2\text{O}_2$  Is powerful oxidant That produce damage of cellular DNA, proteins and Phospholipids.





- Reductase for fatty acids synthesis.
- Retinal reductase.
- Folate and dihydrofolate reductase.
- HMG –CoA reductase for cholesterol synthesis.

### B- Hydroxylases use of NADPH+H<sup>+</sup>

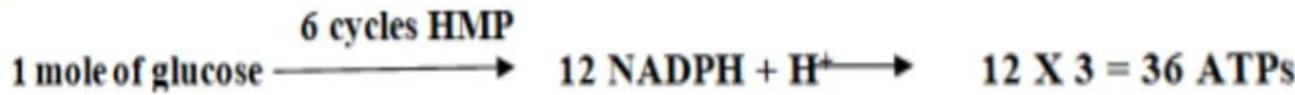
- Hydroxylases of steroid synthesis.
- Phenyl alanine hydroxylase.
- Tryptophan hydroxylase.
- Synthesis of clacitriol.

### C- NADPH+H<sup>+</sup> oxidase

## Favism

**Definition:** A genetically inherited disease characterized by increased fragility of RBCs & hemolysis occurs after the intake of some drugs (aspirin, sulfa & some antimalarial drugs) or after intake of fava beans.

**Cause:** Deficiency of G-6-PD enzyme, so RBCs capacity to protect itself from oxidative damage is markedly decreased due to decrease concentration of NADPH+H<sup>+</sup>



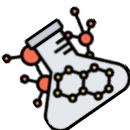
### Regulation of HMP shunt:

#### 1-Allosteric regulation:

NADPH+H<sup>+</sup> allosterically inhibit G-6-P dehydrogenase and 6-phospho-gluconate dehydrogenase. So, high NADH+H<sup>+</sup> Leads to inhibition of HMP shunt.

#### 2-Induction:

CHO feeding increases insulin which leads to induction of Synthesis of both dehydrogenases leading to activation of HMP Shunt. Fasting decreases insulin leading to repression Of synthesis of both Dehydrogenases, so HMP is inhibited.



## Differences between glycolysis and HMP-shunt

| Points of differences         | Glycolysis          | HMP –Shunt                     |
|-------------------------------|---------------------|--------------------------------|
| 1- H-Carrier                  | NAD                 | NADP                           |
| 2- CO <sub>2</sub> production | No CO <sub>2</sub>  | CO <sub>2</sub> is produced    |
| 3- ATP-formation              | ATP is produced     | No ATP.                        |
| 4- Site                       | Cytosol of all cell | Cytosol of certain tissues     |
| 5- Function:                  | Energy              | NADPH+H <sup>+</sup> and R-5-P |

### B. Uronic acid pathway ( Glucuronic acid pathway )

**Definition:** It is an alternative minor oxidative pathway for Glucose involving the formation of active UDP-glucuronic Acid as intermediate.

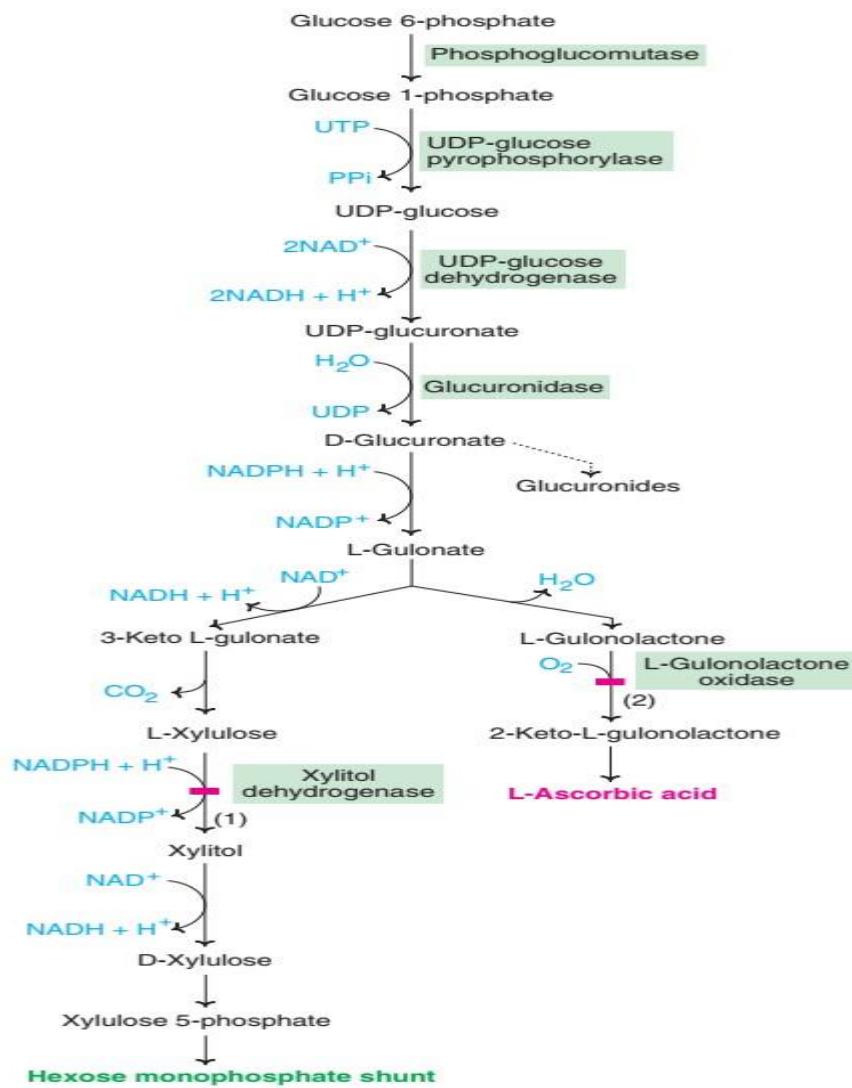
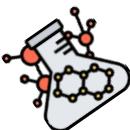
**Site:** Cytosol of liver mainly

**Importance:**

1-Formation of vitamin C (L-ascorbic acid): This occurs in Some lower animals (not in human or guinea pigs).

2-Formation of UDP– glucuronic acid the active donor of glucuronic acid for:

- Detoxification of alcohols, phenols & aromatic acids and steroid hormones.
- Conjugation to bilirubin to form bilirubin diglucuronide; the conjugated bilirubin (direct bilirubin) which is soluble and is excreted in the bile.
- Synthesis of glycosaminoglycans (GAGs) e.g. heparin and chondroitin sulfate.



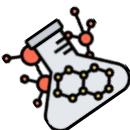
### Essential pentosuria:

- It is an inborn error of metabolism caused By deficiency of L-xylulose reductase which Converts L-xylulose to xylitol.

L-xylulose is Not metabolized and is excreted in large Amounts in urine.

L-xylulose is a reducing Sugar.

- It is a harmless condition needs no Treatment



## L20: Glycogen metabolism

### Glycogenesis:

**Definition:** It is the formation of glycogen from glucose. <<Glucose → Glycogen>>

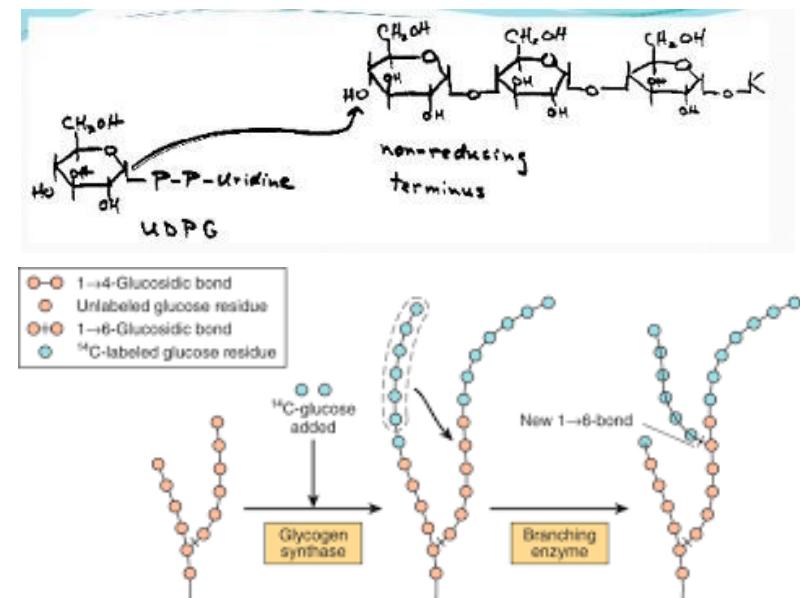
**Site:** It occurs in the cytosol of cells (except RBCs) especially in **liver & muscles**.

### Importance:

- Storage of excess glucose, or other hexoses taken in food.

### Steps:

- UDPG** acts as a substrate for glycogen synthesis.
- Glucose is transferred from UDPG to a glycogen primer forming  **$\alpha$ -1, 4-glucosidic bonds**.
- The reaction is catalyzed by the enzyme **glycogen synthase** (the key enzyme) whose function is to elongate short branches in the glycogen tree up to 12 glucose units.
- The **branching enzyme** transfers some of glucose units from the end of a long branch to one of glucose units in the middle of an adjacent long branch forming  **$\alpha$ -1,6-glucosidic bond**.
- A new branch appears on which glycogen synthase can act again.

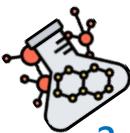


### Regulation of glycogenesis:

#### 1. Covalent modification:

Glycogen synthase is the key enzyme. It is present in two forms:

- Glycogen synthase (active form) which is dephosphorylated.
- Glycogen synthase (inactive form) which is phosphorylated.



### 2. Induction and repression of the key enzyme:

- Carbohydrates feeding induce insulin synthesis for the key enzyme (induction) so, glycogenesis is stimulated.
- Fasting decrease insulin and increase ant-insulin leading to decrease synthesis of the key enzyme (repression) and hence glycogenesis is inhibited.

### 3. Allosteric Regulation:

- Glycogen synthase is allosterically activated by Glucose -6-P.
- allosterically inhibited by glycogen molecule.

## Glycogenolysis:

**Definition:** It is the breakdown of glycogen into glucose or glucose 6-phosphate.

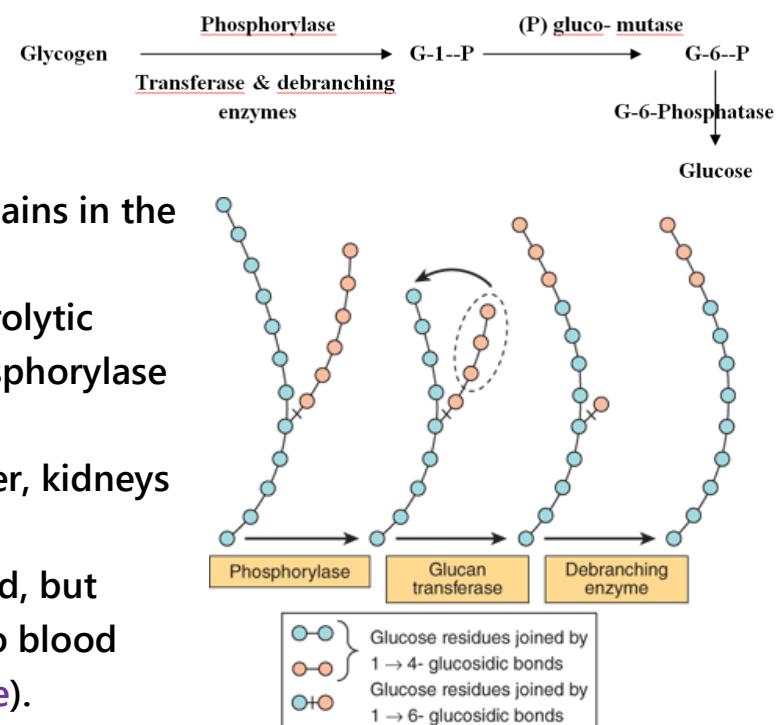
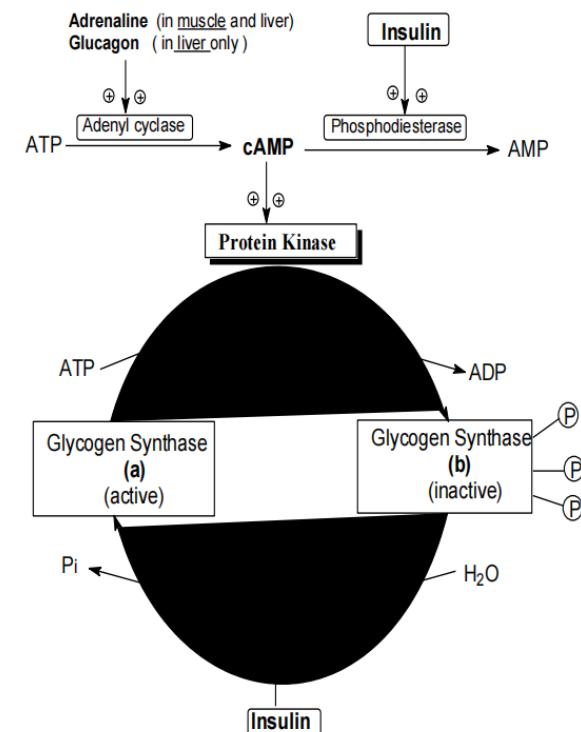
**Site:** It occurs in cytosol of cells (except RBCs) especially in liver & muscles.

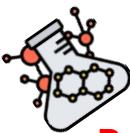
## Importance:

- In liver: It supplies blood glucose **during fasting** less than 18 hours.
- In muscles: It is source of energy during exercise.

## Steps:

- The **phosphorylase** enzyme (the key enzyme) catalyzes the removal of glucose residues from the outermost chains in the form of **G-1-P**.
- Debranching enzyme catalyzes the hydrolytic splitting of the  **$\alpha$ -1, 6- bond** so, the phosphorylase can act again.
- (N.B.) G-6-Phosphatase is present in liver, kidneys &intestines but NOT in muscles.
- Liver glycogen can give glucose to blood, but Muscle glycogen cannot give glucose to blood directly (may give it through **Cori's cycle**).





## Regulation of glycogenolysis:

### 1. Covalent modification:

Phosphorylase is the key enzyme. It is present in two forms:

- Phosphorylase (active form) which is phosphorylated.
- Phosphorylase (inactive form) which is dephosphorylated.

### 2. Induction and repression of the key enzyme:

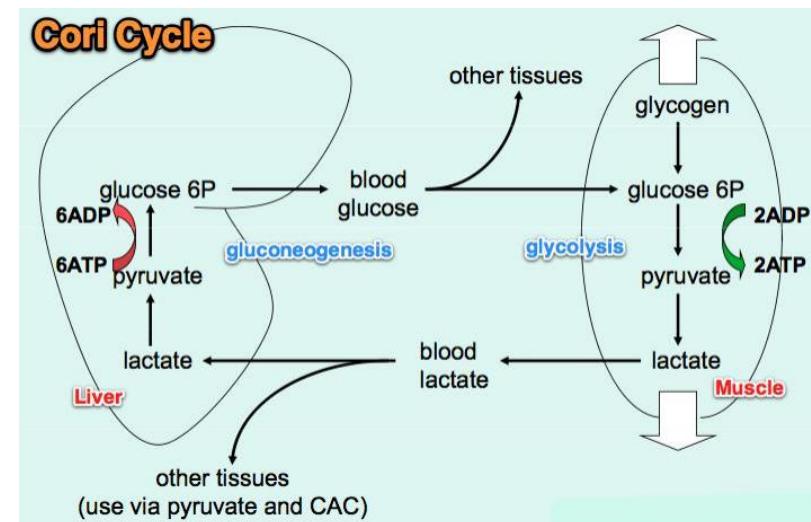
- Carbohydrates feeding induce insulin which leads to decrease synthesis of key enzyme (repression) so glycogenolysis is inhibited.
- Fasting decrease insulin and increase anti-insulin which increase synthesis of key enzyme (induction) so glycogenolysis is stimulated.

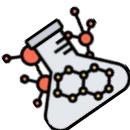
### 3. Allosteric Regulation:

- Muscle phosphorylase is allosterically activated by AMP which is increased during muscular exercise.

## Cori's (glucose -lactate ) cycle :

- In contracting muscle glycogen gives G-6-P which by glycolysis gives lactate as there is no O<sub>2</sub>
- Lactate diffuses to blood and goes to the liver where lactate is converted to glucose by gluconeogenesis.
- Glucose can return back to muscles





# Bio L21&22 : Gluconeogenesis

## Definition

- ❖ It is the formation of glucose or glycogen from non-carbohydrate sources.

## Sources (substrates)

### 1) Proteins

- ❖ Are most important (58% of proteins are convertible to glucose)
- ❖ 100 g of protein give about 58g of glucose

### 2) Fats

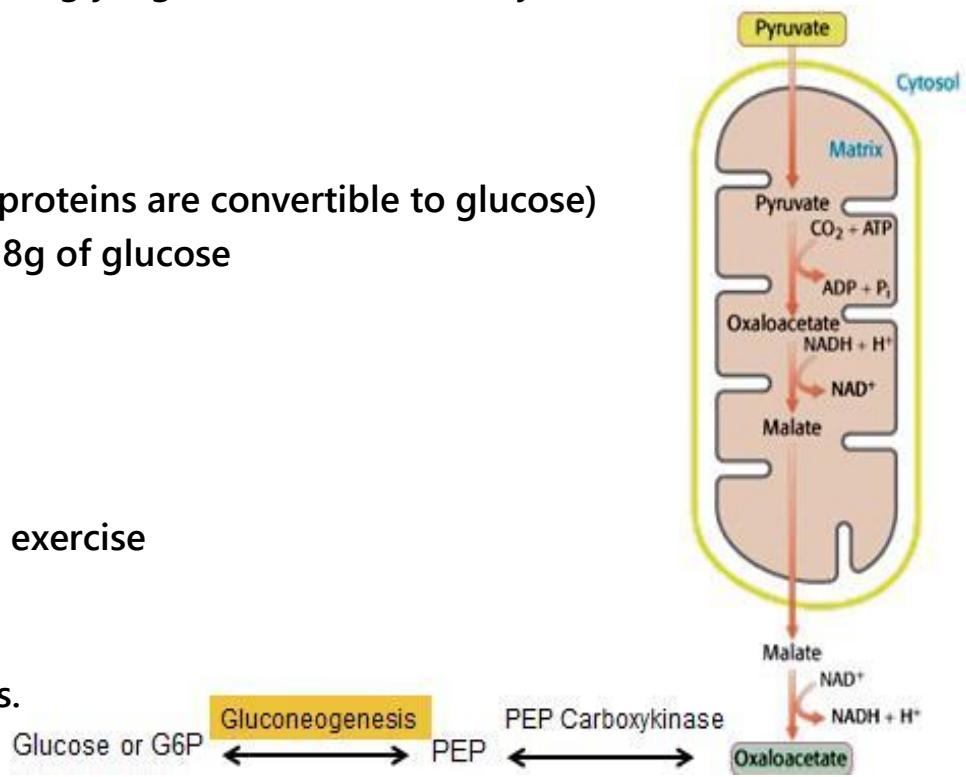
- ❖ Glycerol.
- ❖ Odd chain fatty acids (rare).

### 3) Lactate

- ❖ From RBCs & muscles during exercise

## Site of Gluconeogenesis

- ❖ Chiefly in liver also in kidneys.
- ❖ Little in skeletal muscles.
- ❖ Not in heart, smooth muscles & fat cells because fructose-1,6-bisphosphatase deficient
- ❖ Starts in mitochondria and completed in Cytosol

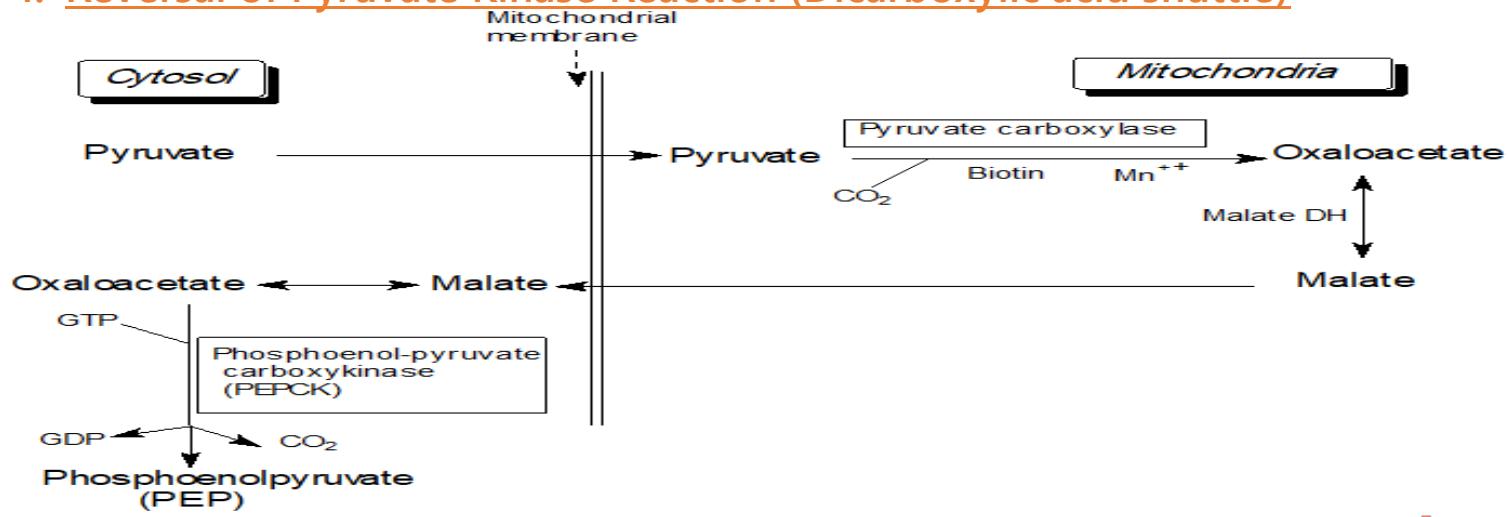


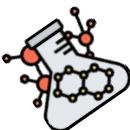
## Steps

- ❖ It is essentially the reversal of glycolysis.

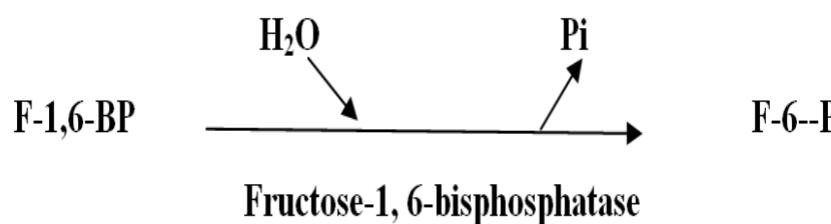
### 1) From lactate & pyruvate.

#### 1. Reversal of Pyruvate Kinase Reaction (Dicarboxylic acid shuttle)

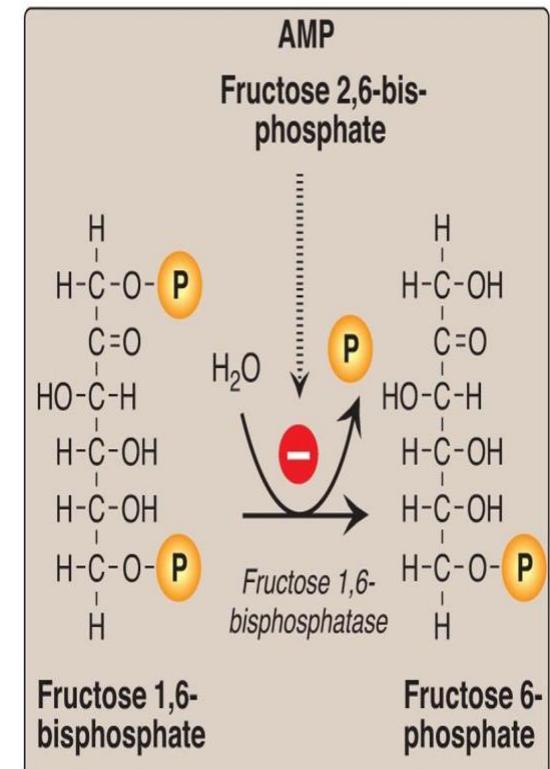
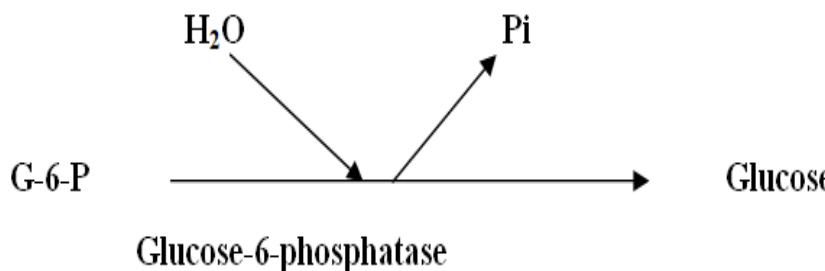




## 2. Reversal of the phosphofructokinase reaction:



## 3. Reversal of the Hexokinase Reaction



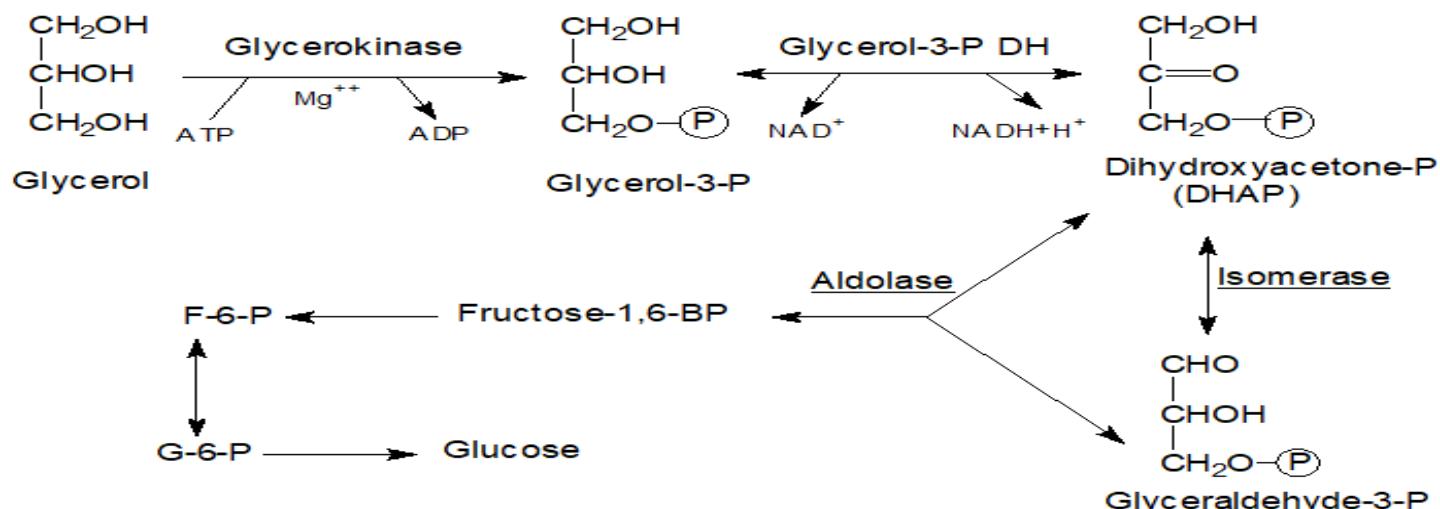
Dephosphorylation of fructose 1,6-bisphosphate

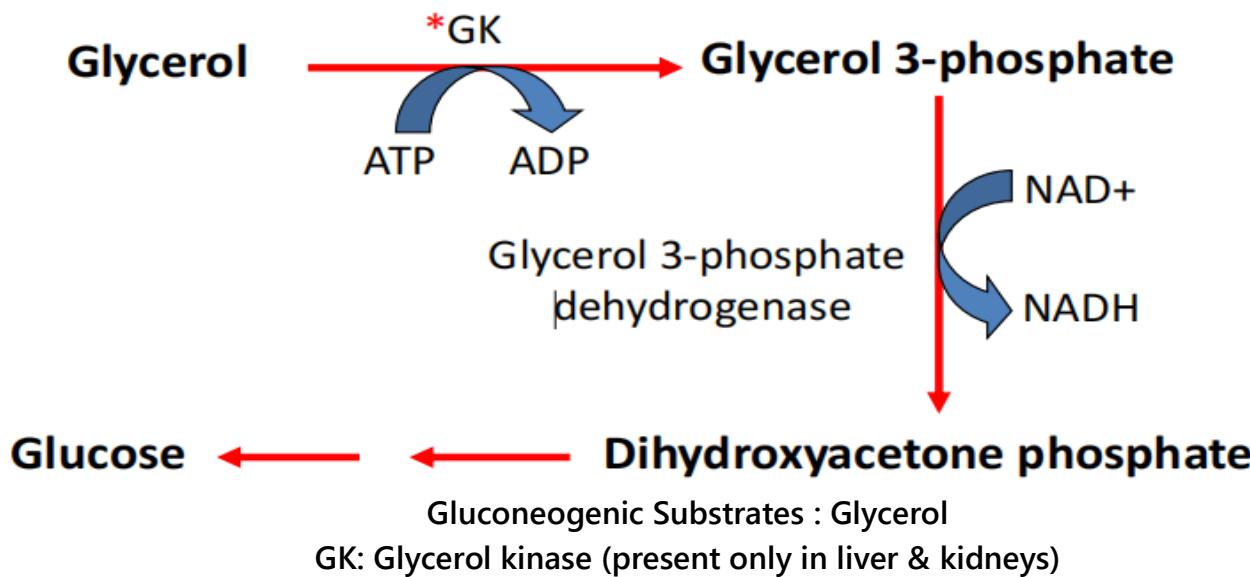
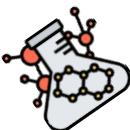
Fructose 1,6-bisphosphatase  $\neq$  PFK-1

### A. The opposing enzymatic differences between Glycolysis and Gluconeogenesis

| Enzyme | Glycolysis                | Gluconeogenesis                                                   |
|--------|---------------------------|-------------------------------------------------------------------|
| 1      | Glucokinase or Hexokinase | Glucose -6- phosphatase.                                          |
| 2      | Phosphofructokinase       | Fructose-1, 6- Bisphosphatase                                     |
| 3      | Pyruvate kinase           | Pyruvate carboxylase & phosphoenolpyruvate carboxykinase (PEPCK). |

## 2) From glycerol



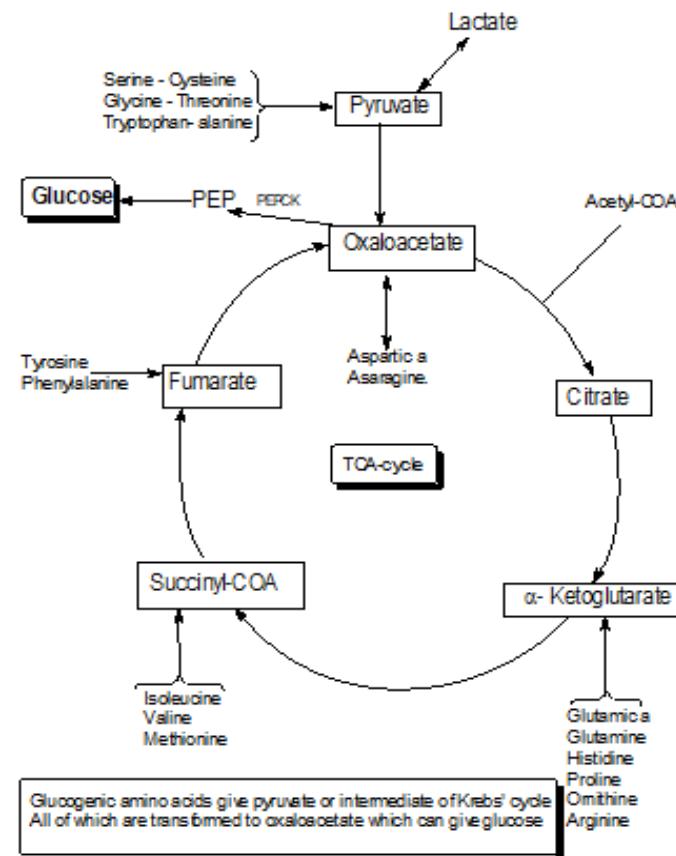


### 3) From odd chain fatty acids

- ❖ It is rare conversion

### 4) From proteins

- ❖ All glucogenic and mixed amino acids can give glucose (i.e. all amino acid except leucine).
- ❖ Amino acids give pyruvic acid or intermediate of Krebs' cycle, both can be converted to oxaloacetate which by PEPCK can give phosphoenolpyruvate.
- ❖ PEP by reversal of glycolysis can form glucose or glycogen.



### Importance of gluconeogenesis

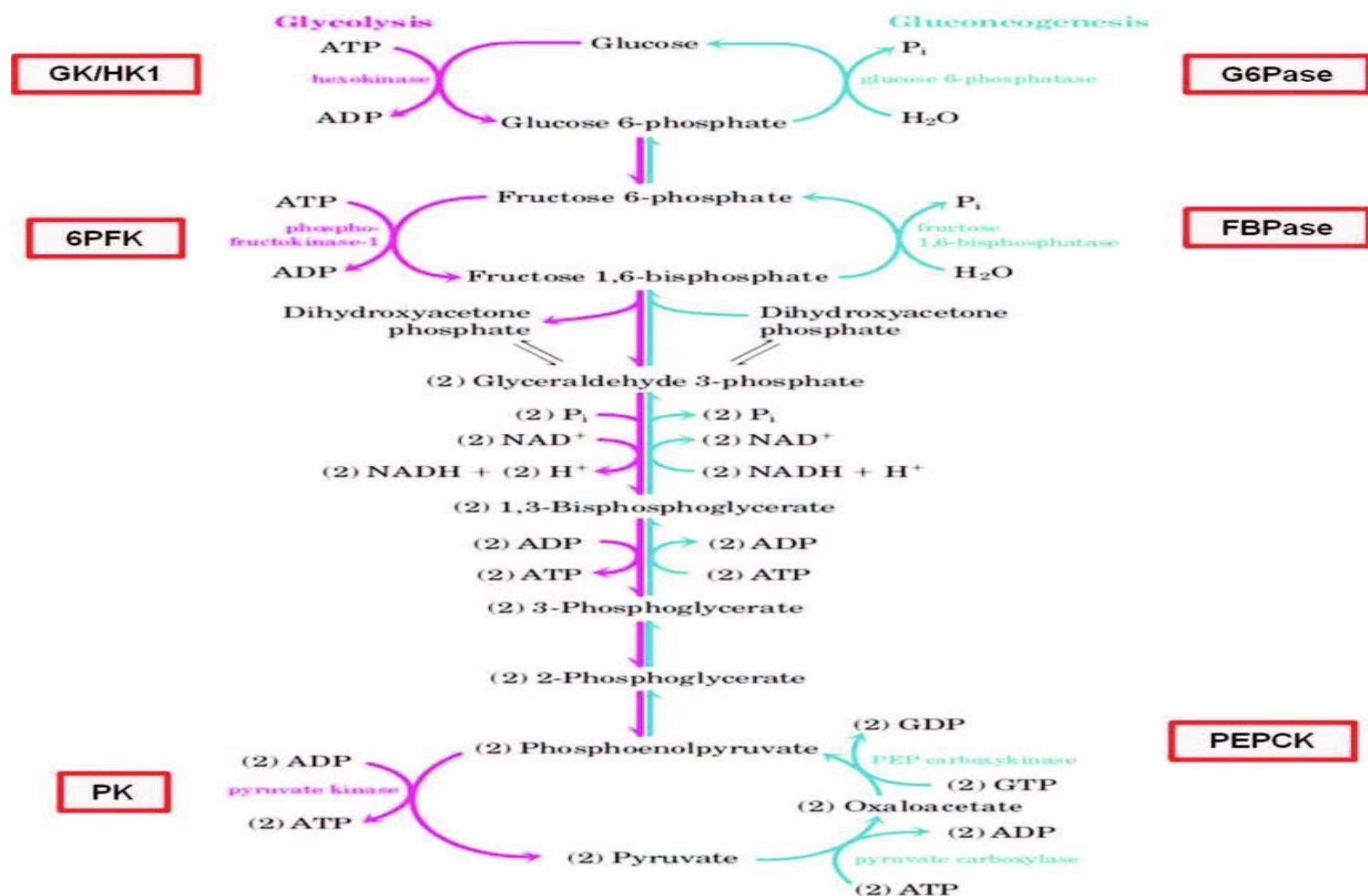
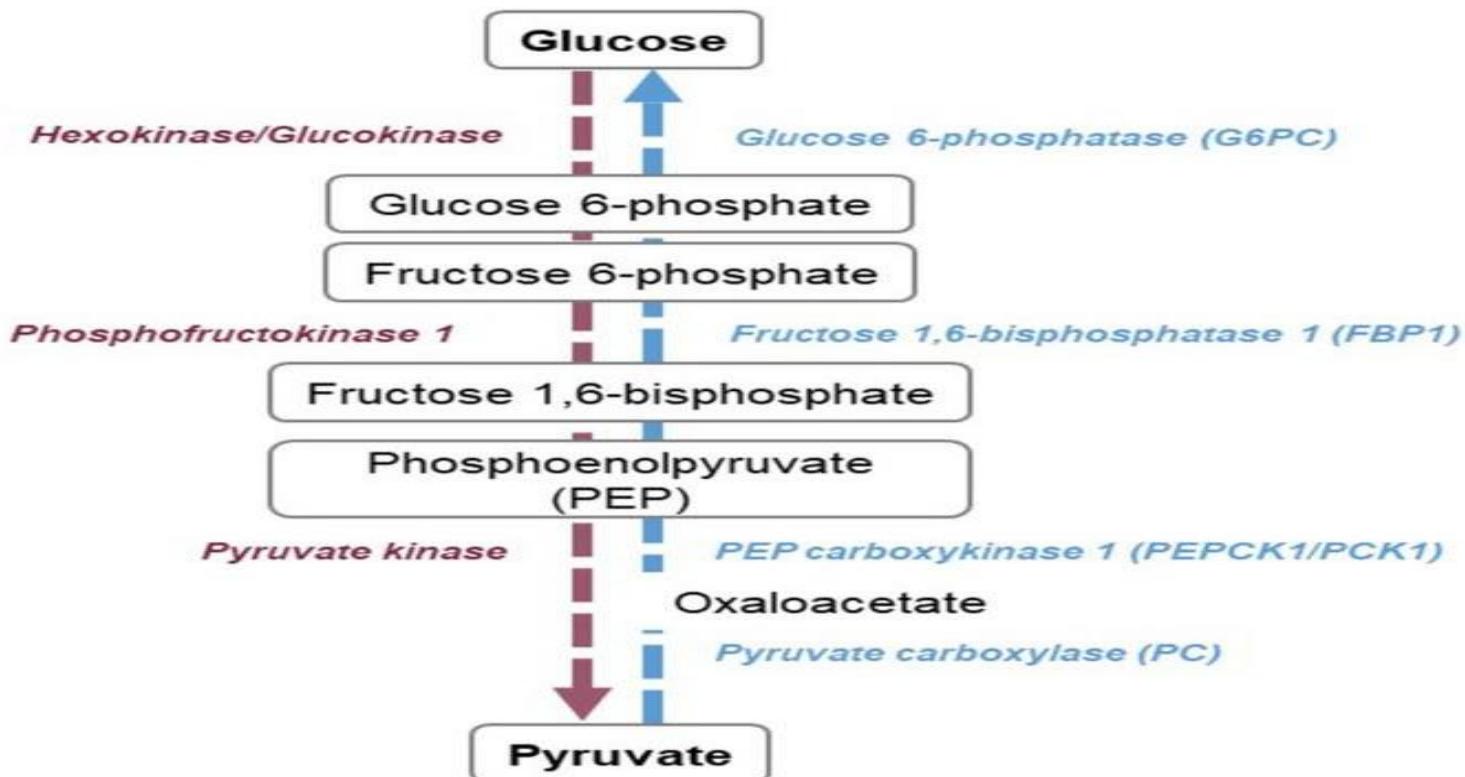
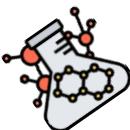
#### 1) Supplies blood glucose during

fasting more than 18 hours, Blood glucose is important as a source of:

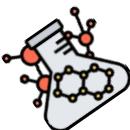
- ❖ Energy in brain, RBCs & muscle exercise.
- ❖ Oxaloacetate in all tissues for Krebs' cycle.

#### 2) Removes

- ❖ Lactate & Pyruvate from RBCs & exercising muscle.
- ❖ Glycerol absorbed from the intestines & produced by adipose tissues.



### Gluconeogenesis Vs. Glycolysis



## Enzymes of Gluconeogenesis:

1. Pyruvate carboxylase (PC).
2. Phosphoenolpyruvate carboxykinase (PEPCK).
3. Fructose 1, 6 –Bisphosphatase (F-1, 6- BPtase; the key enzyme).
4. Glucose-6-phosphatase (G-6-Ptase).

## REGULATION OF GLUCONEOGENESIS:

1. Availability of the substrate
2. Allosteric regulation
3. Induction repression
4. Covalent modifications (PFK-2)

### Gluconeogenesis: Regulation

- Availability of the substrate

high CHO feeding  $\downarrow$  Gluconeogenesis &  $\uparrow$  Glycolysis  
 starvation  $\uparrow$  Gluconeogenesis &  $\downarrow$  Glycolysis

- Allosteric:

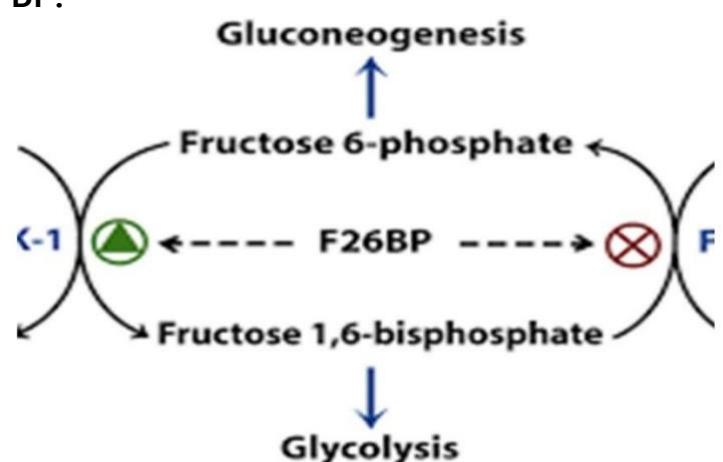
Acetyl CoA + (Pyruvate carboxylase)

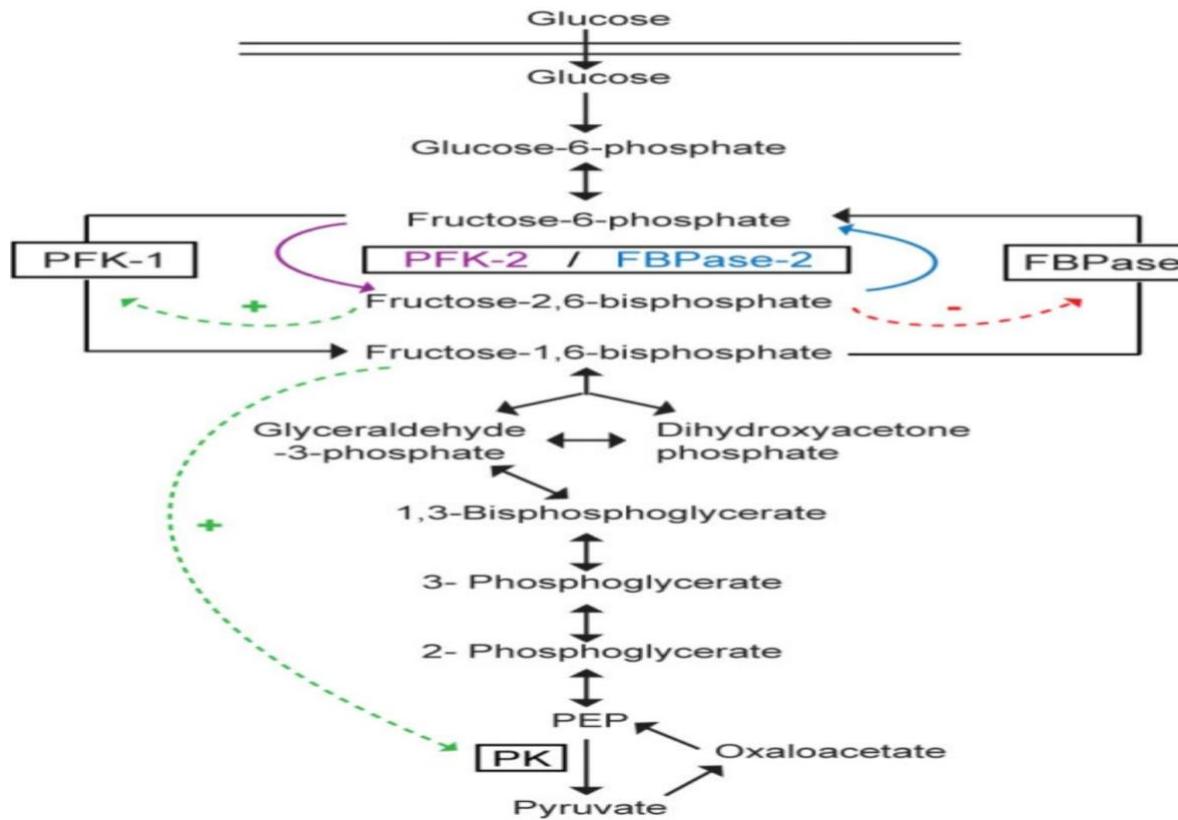
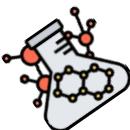
F 2,6-Bisphosphate  $\xrightarrow{(-)}$  F 1,6-bisphosphatase

### 2. Allosteric regulation

- The key enzyme of gluconeogenesis: F-1, 6-Bisphosphatase

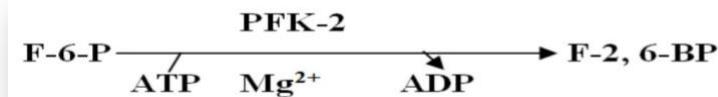
which is allosterically inhibited by F-2, 6– BP.





### Fructose-2, 6-Bisphosphate: [F-2, 6-BP]

- Fructose-2, 6-Bisphosphate: [F-2,6-BP] is formed by phosphorylation of F-6-P by the enzyme phosphofructokinase-2 (PFK-2).

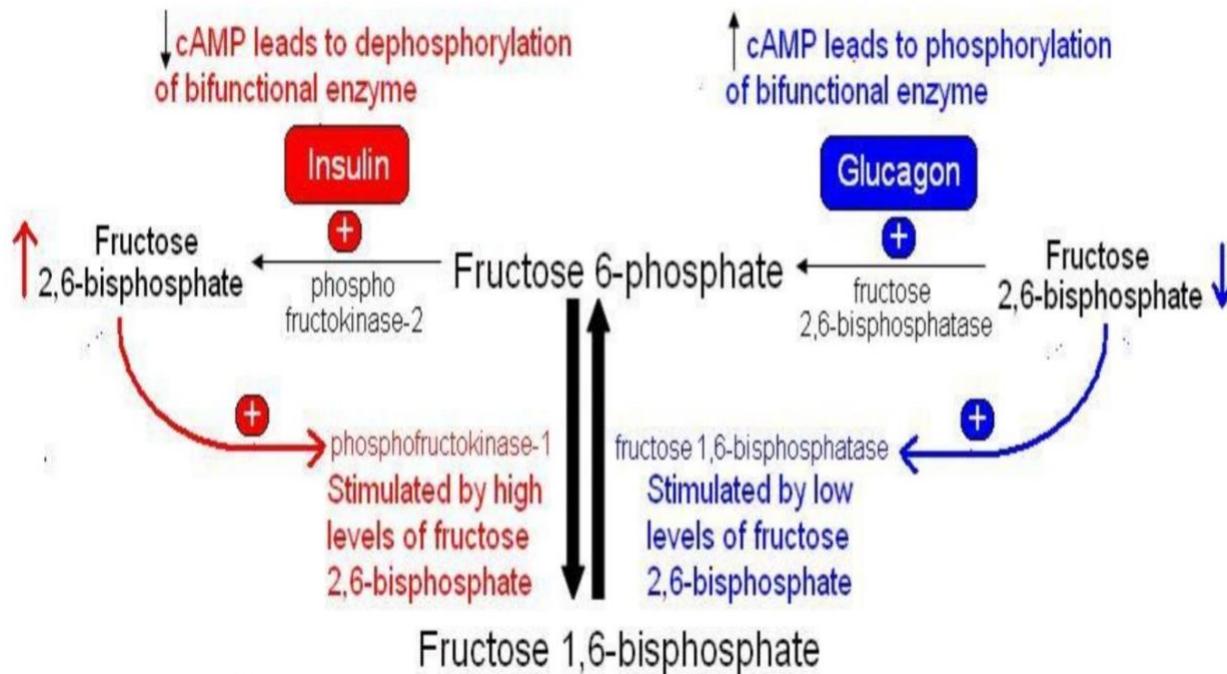
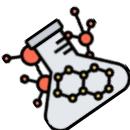


- CHO Feeding increase insulin & increase F-2,6 BP. It allosterically stimulates PFK-1 and inhibits F-1, 6-BPtase → stimulates Glycolysis and inhibits Gluconeogenesis. So, glycolysis and Gluconeogenesis can't occur at the same time.

- Fasting → ↑↑ adrenaline & glucagon → ↑↑ cAMP → ↓↓ F-2, 6-BP → Stimulation of F-1, 6-BPtase → Stimulation of gluconeogenesis.

### 3. Induction and Repression of the key enzymes:

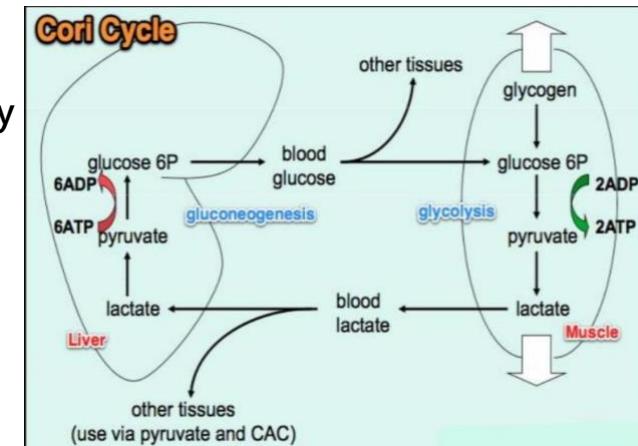
- After carbohydrate feeding, insulin/glucagon (I/G ratio) increases and it decreases the synthesis (repression) of enzymes of gluconeogenesis.
- During fasting, I/G ratio decreases. This increase the synthesis (induction) of enzymes of gluconeogenesis.



### Cori's (glucose -lactate ) cycle :

In contracting muscle glycogen gives G-6-P which by glycolysis gives lactate as there is no O<sub>2</sub>. Lactate diffuses to blood and goes to the liver where lactate is converted to glucose by gluconeogenesis.

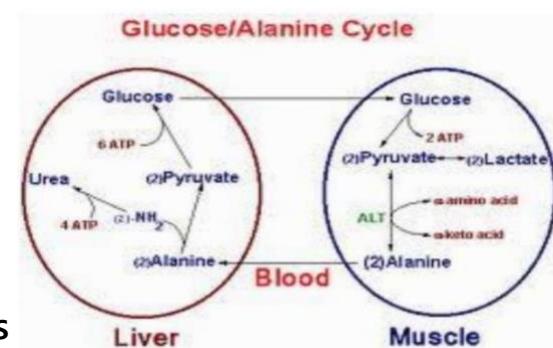
• Glucose can return back to muscles



### Glucose Alanine cycle:

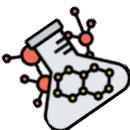
If pyruvic acid in muscle is changed to alanine by transamination, it goes to the blood then to liver where alanine is changed again to pyruvic by transamination.

By gluconeogenesis, alanine gives glucose which diffuses from liver to blood to contracting muscle again to supply energy.

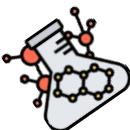


### Take Home Messages

- Gluconeogenesis is an important pathway for glucose production from non-carbohydrate sources during prolonged fasting.



- Lactate, glycerol and glucogenic amino acids are the major gluconeogenic substrates.
- Gluconeogenesis is not a simple reversal of glycolysis. In fact, gluconeogenesis requires 4 unique reactions to circumvent the 3 irreversible reactions of glycolysis.
- Gluconeogenesis and glycolysis are reciprocally controlled, allowing efficient glucose metabolism.
- It is mainly anabolic pathway that consumes ATP for the synthesis of glucose.



## Bio TUT8: Bioenergetics and Metabolism

### What is Metabolism?

Metabolism is:

All intracellular biochemical reactions that use the simplest units of nutrients (Glucose-Fatty acids-glycerol and amino acids) for either:  
 building up (**anabolism**)  
 or  
 breakdown (**catabolism**).

- ▶ CHO CHO formed from glucose.
- ▶ Fat Fats (TG) formed from glycerol and FA.
- ▶ Proteins Proteins formed from AA.

| Metabolism                                                                                                        |                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| Catabolism                                                                                                        | Anabolism                                                                                                        |
| Breakdown (Degradation) of complex molecules.                                                                     | Building up (Synthesis) of complex molecules.                                                                    |
| <b>Exergonic</b> = releases energy.                                                                               | <b>Endergonic</b> = consumes energy.                                                                             |
| e.g. <ul style="list-style-type: none"> <li>• Oxidation of glucose</li> <li>• Oxidation of fatty acids</li> </ul> | e.g. <ul style="list-style-type: none"> <li>• Biosynthesis of glycogen, triacylglycerols and proteins</li> </ul> |

**Catabolism of the main metabolites occurs in three stages:**

#### Stage I:

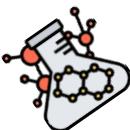
- Hydrolysis of polysaccharides into monosaccharides,
  - triglycerides into glycerol and fatty acids,
  - proteins into amino acids.
- This stage usually produces minimum amount of energy.

#### Stage II:

- Conversion of monosaccharides, glycerol, fatty acids and amino acids into active acetate.
- This stage produces some energy,

#### Stage III:

- Oxidation of active acetate in Krebs cycle into CO<sub>2</sub>and H<sub>2</sub>O and reduced coenzymes (hydrogen carriers).
- release maximum amount of energy via respiratory chain.



## Energy

- ❖ It is the capacity to do work or the ability to make a change
- ❖ The energy in our body have two types
  - 1-heat energy → body temp.
  - 2-Free energy → trapped or collected as high  $\textcircled{P}$  bond

### ATP:

It is the useful part of energy available to do work. it is needed for building macromolecules, transmission of nerve impulse, contraction of muscles, etc.

#### Formation of ATP

- 1-Substrate Level Phosphorylation
- 2-Oxidative Phosphorylation (Respiratory chain level)

Mechanisms of collection of released energy: Released energy is collected in the form of ATP at two levels:

| Oxidative Phosphorylation                                                                                                                       | Substrate Level Phosphorylation                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>• <math>\text{SH}_2 + \text{NAD} \longrightarrow \text{S} + \text{NADH}^+ + \text{H}^+</math></li> </ul> | $\text{S}-\text{P} + \text{ADP} \xrightarrow{\text{Kinase}} \text{S} + \text{ATP}$                                                                                                               |
| The main source of energy.                                                                                                                      | Very small amount.                                                                                                                                                                               |
| It occurs in mitochondria by the Respiratory Chain enzymes [=Electron Transport Chain (ETC) enzymes].                                           | Few reactions can form ATP at substrate level: e.g. <ol style="list-style-type: none"> <li>1. Phosphoglycerate Kinase.</li> <li>2. Pyruvate Kinase.</li> <li>3. Succinate thiokinase.</li> </ol> |

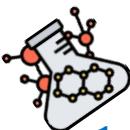
## Respiratory Chain

**Oxidation process** accompanied with liberation of energy  $\Delta G$ , that stored as high energy phosphate bond  $\sim \text{P}$  (ATP) for further uses

The process includes:

1-Oxidation

2- Phosphorylation

**1- Oxidation:**

-Oxidation involves the loss of electrons and reduction involves the gain of electrons.

-gradual transfer of hydrogen and electron from Low Redox potential to oxygen (high Redox potential).

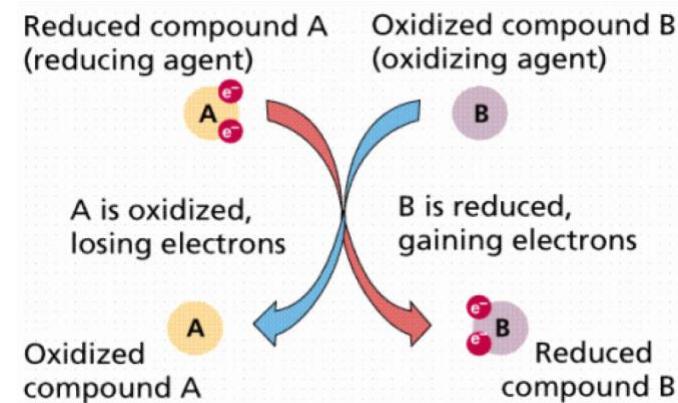
**Redox:** a chemical reaction which involves both a reduction process and a complementary oxidation process, two key concepts involved with electron transfer processes.

**Redox potential:** the ability to accept an electron. The electron transfers from components with low Redox potential to components with high Redox potential.

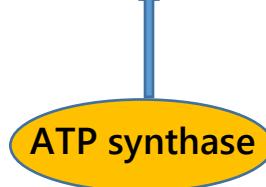
**Electronegativity:**

A measure of the tendency of an atom to attract electrons in a covalent bond.

Each enzyme contains metal ions, either **iron(Fe)** or **copper(Cu)** in their heme-group that allow passage of electrons through them from less electronegative to more electronegative.

**2-Phosphorylation:**

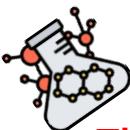
The energy liberated from oxidation stored in the form of high energy phosphate bonds ~ P (ATP).

**Respiratory chain**

It is a group of oxidation reduction reaction through them the **hydrogen** is gradually transferred to **oxygen** to form water and liberate **energy ΔG** which stored as high ~ P (ATP)

**Oxidative –phosphorylation process**

Site: Inner mitochondrial membrane enzymes



## The Electron Transport Chain

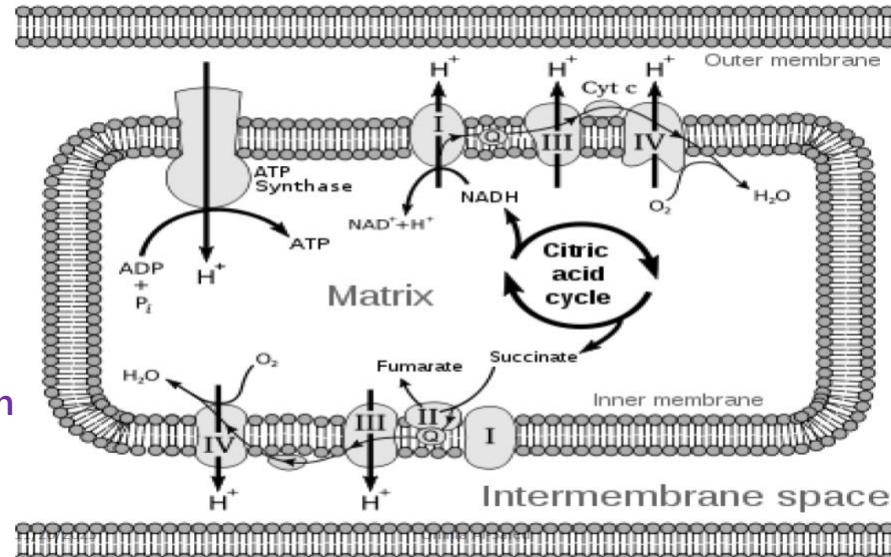


### The Electron Transport Chain [ETC]:

- Electron transport chain or respiratory chain consists of series of hydrogen and electron carriers located in the inner mitochondrial membrane.
- Its components are arranged from less electronegative to more electronegative to transfer hydrogen derived from different oxidation reactions between series of hydrogen and electron carrier's coenzymes (less electronegative) to give it finally to oxygen (more electronegative) to form water and release energy.
- Part of the energy is used to form high energy phosphate bond (ATP).

### The components of electron transport chain:

They are formed in the form of 4 complexes, 2 mobile elements (CoQ and cytochrome c) and ATP synthase (complex V).



#### Complex I:

It consists of FMN, NADH+H dehydrogenase, many polypeptides, and **7 iron sulfur (7FeS)** groups. FMN transfers hydrogen from NADH+H to CoQ forming (CoQH<sub>2</sub>) **with the production of one ATP**.

#### Complex II:

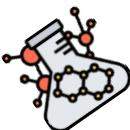
It consists of FAD, succinate dehydrogenase, and **(2 FeS)** groups. FAD transfers hydrogen from succinate to CoQ to form CoQH<sub>2</sub> **without production of ATP**.

#### Complex III:

It is formed of cytochromes b and **one** iron sulfur (**1FeS**) group. It transfers electrons from CoQH<sub>2</sub> to cytochrome c **releasing two protons with the production of one ATP**.

#### Complex IV:

It consists of: cytochromes a-a3 also called cytochrome oxidase and **(2 copper)** atoms. It **transfers electrons from cytochrome c to oxygen which combines with the two protons to form water with the production of 1/2 ATP**.



## Cytochromes

- ❖ Cytochromes are enzymes that contain heme as a prosthetic group.
- ❖ The cytochrome iron atom is reversibly converted from ferric ( $\text{Fe}^{3+}$ ) to its ferrous ( $\text{Fe}^{2+}$ ) state as a normal part of its function as reversible carrier of electrons.
- ❖ Electrons are passed down the chain from coenzyme Q to cytochromes:  $b$ ,  $c$ , and  $a+a_3$  and finally to  $\text{O}_2$ .

## Comments on respiratory chain:

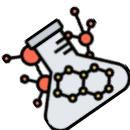
- The enzymes of respiratory chain are oxido-reductases as there is simultaneous oxidation and reduction in each step.
- Oxidation-reduction by NAD, FAD and Co Q occurs by transfer of hydrogen.
- Oxidation by cyt. b, C and a-a<sub>3</sub> occurs by transfer of electrons.
- Oxidation of NADH + H<sup>+</sup> in respiratory chain produces 2.5 ATP i.e. starting by  $\text{NADH} + \text{H}^+ \rightarrow 2.5 \text{ ATP}$ .
- Oxidation of FADH<sub>2</sub> in respiratory chain → only give 1.5 ATP.

## Chemiosmotic theory of ATP synthesis:

- ❖ Protons are pumped through complexes I, III&IV from mitochondrial matrix to space between the inner and outer membranes (Intermembrane space), creating potential difference across the inner membrane which is impermeable to protons.
- ❖ ATP synthase enzyme (complex V) consists of two subunits, F<sub>0</sub> and F<sub>1</sub>. Through the F<sub>0</sub> subunit, protons return to the mitochondrial matrix according to their concentration gradients, The F<sub>1</sub> subunit couples ADP with Pi to form ATP.

## Oxidative Phosphorylation

- ❖ The liberated free energy is used by ATP Synthase (Complex V) for the phosphorylation of ADP to form ATP:  
$$\text{ADP} + \text{Pi} \rightarrow \text{ATP}$$
- ❖ This is called coupled “Oxidative Phosphorylation”; because the oxidation of the substrate and the phosphorylation of ADP are coupled together and must occur at the same time.



### ATP transporter (Translocator):

Its function is the translocation of ATP formed in respiratory chain **from the mitochondria to cytoplasm in exchange of ADP** to avoid inhibition of ETC by the accumulated ATP.

### Regulation of respiratory chain:

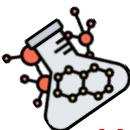
- ❖ Due to coupling of oxidation with phosphorylation, when one of the two processes is inhibited the other will be also inhibited.
- ❖ It is inhibited under anaerobic condition (in absence of oxygen).
- ❖ It is inhibited by ATP (excess energy in the cell) and is stimulated by ADP.
- ❖ **CO** and cyanide inhibit cytochrome aa<sub>3</sub> at complex IV.
- ❖ It is also inhibited by some Insecticides and some antibiotics.

### P/O ratio:

- ❖ It is the ratio between inorganic phosphates consumed to form ATP in relation to oxygen atom reduced forming water in the respiratory chain.
- ❖ In case of oxidation of **NADH+H<sup>+</sup>** it is 2.5/1 (2.5 ATPs formed at 3 coupling sites),
- ❖ In case of oxidation of **FADH<sub>2</sub>** it is 1.5/1(1.5 ATPs formed at 2 coupling sites).

### NADH and FADH<sub>2</sub>

- Oxidation of one mole of NADH in ETC results in a free energy sufficient to synthesize **2.5 ATP**
- In the mitochondrial ETC **2.5 moles of ATP** can be formed per pair of electrons transferred from reduced nicotinamide adenine dinucleotide (NADH) to oxygen.
- Oxidation of one mole of FADH<sub>2</sub> in ETC results in a free energy sufficient to synthesize **1.5 ATP**
- In the mitochondrial ETC **1.5 moles of ATP** can be formed per pair of electrons transferred from reduced Flavin adenine dinucleotide (FADH<sub>2</sub>) to oxygen.



## Uncouplers of oxidative phosphorylation

- Uncouplers are substances that inhibit the oxidative phosphorylation in ETC.  
They dissociate oxidation from phosphorylation.
- Uncouplers **allows** electron transport to proceed **without ATP synthesis**.
- The oxidation of hydrogen with oxygen to form H<sub>2</sub>O proceeds **while no phosphorylation of ADP to ATP**.
- Uncouplers allow leakage or transport of H<sup>+</sup> across the membrane, thus collapsing the proton gradient for ATP synthesis.
- The free energy liberated during oxidation is lost as **heat**, so uncouplers cause the body temperature to rise (cause hotness).

### Examples of uncouplers of Oxidative phosphorylation

- **Bilirubin:** in abnormal high levels as in jaundice
- **Calcium:** in large dose
- **Thyroid hormones(T3&T4):** in abnormal high levels as in hyperthyroidism
- **Toxins:** of some bacteria and fungus
- **Thermogenin** (called uncoupling protein) in brown adipose tissue

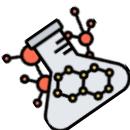
## Inhibitors of electron transport chain

Selective inhibition of various components of the ETC can be achieved by using a variety of substances, some of which are used as **poisons** (e.g. insecticides), others as drugs

### Examples:

- **Hydrogen cyanide (HCN),**
- **Carbon monoxide (CO),**
- **sodium azide** inhibit cytochrome oxidase.

They are fatal



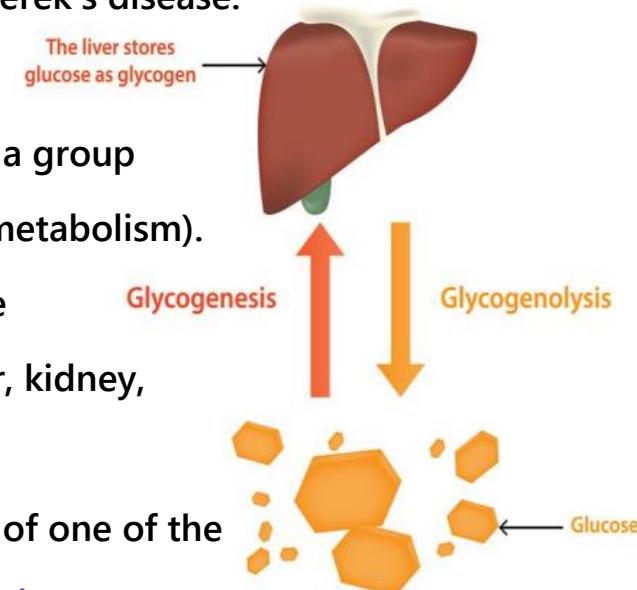
## Bio CBL4 : Glycogen storage diseases

### Learning outcomes

- Define glycogen storage diseases.
- Describe the pathophysiology of von Gierek's disease.
- Describe the presentation of a patient with von Gierek's disease.

### What is glycogen storage diseases ?

- Glycogen storage diseases or **glycogenesis** form a group of inherited congenital disorders (inborn errors of metabolism).
- They are characterized by the deposition of large amounts of **abnormal forms of glycogen** in the liver, kidney, heart, or skeletal muscles.
- They result from mutation, leading to deficiency of one of the enzymes of **glycogenolysis**, **glycogenesis**, or **glycolysis**.



### What is the inheritance pattern of glycogen storage diseases?

|            |                                                                                                                                 |  |
|------------|---------------------------------------------------------------------------------------------------------------------------------|--|
| CASE STUDY | <b>GLYCOGEN STORAGE DISEASE</b><br>* 15 SUBTYPES<br>↳ ALL RESULT in INABILITY to:<br><br>BREAK DOWN ← X GLYCOGEN → X SYNTHESIZE |  |
| PHYSIOLOGY |                                                                                                                                 |  |

**CASE STUDY**

**PHYSIOLOGY**

**PATHOLOGY**

**TYPE I**

**TYPE II**

**TYPE III**

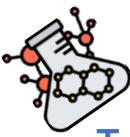
**TYPE V**

**REVIEW**

**SUMMARY**

\* MOST HIGH YIELD: TYPES I, II, III, & V  
↳ AUTOSOMAL RECESSIVE DISEASES  
↳ INDIVIDUAL NEEDS to INHERIT TWO COPIES  
of MUTATED GENE (ONE from EACH PARENT) to DEVELOP

**HIGH YIELD**

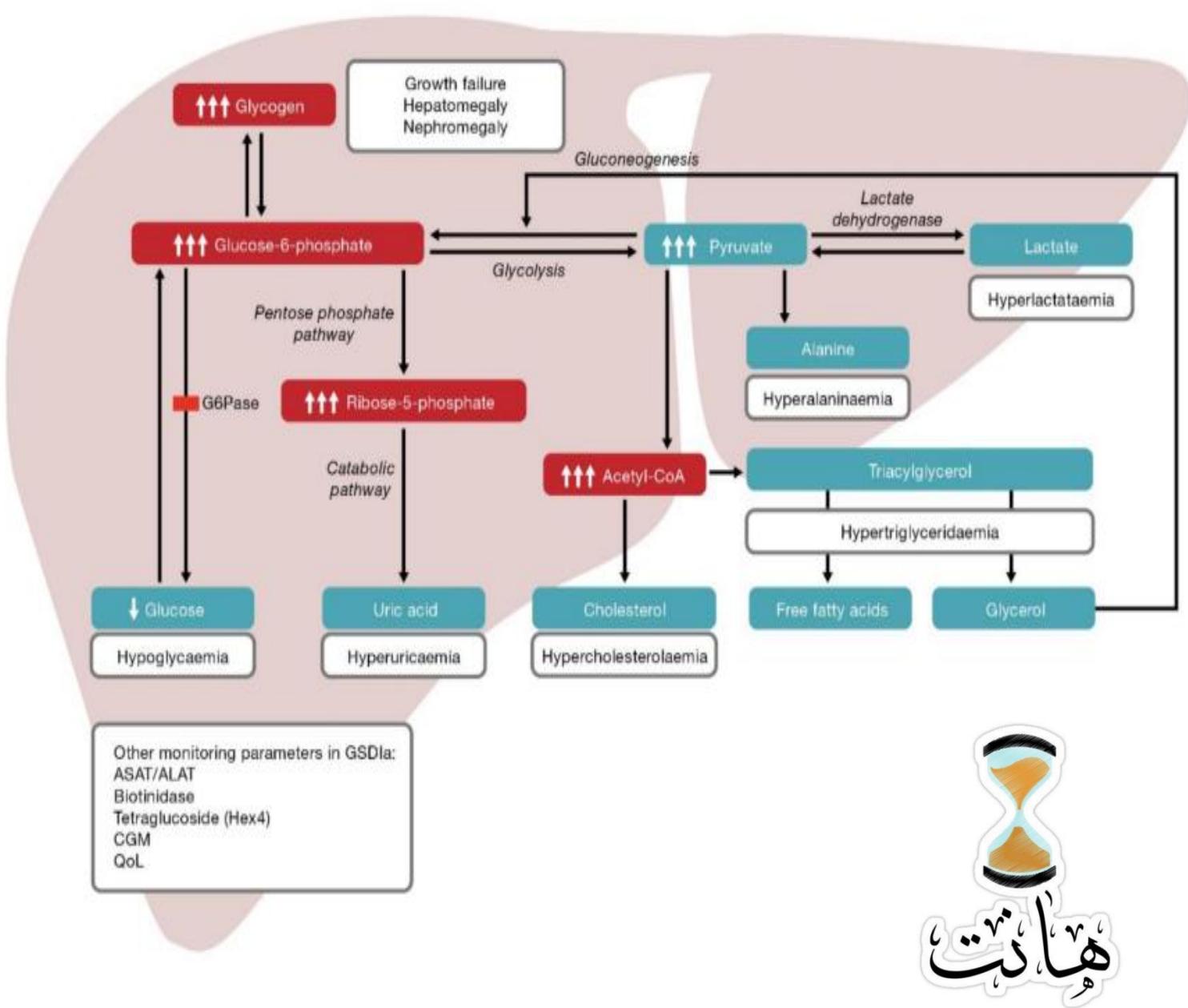


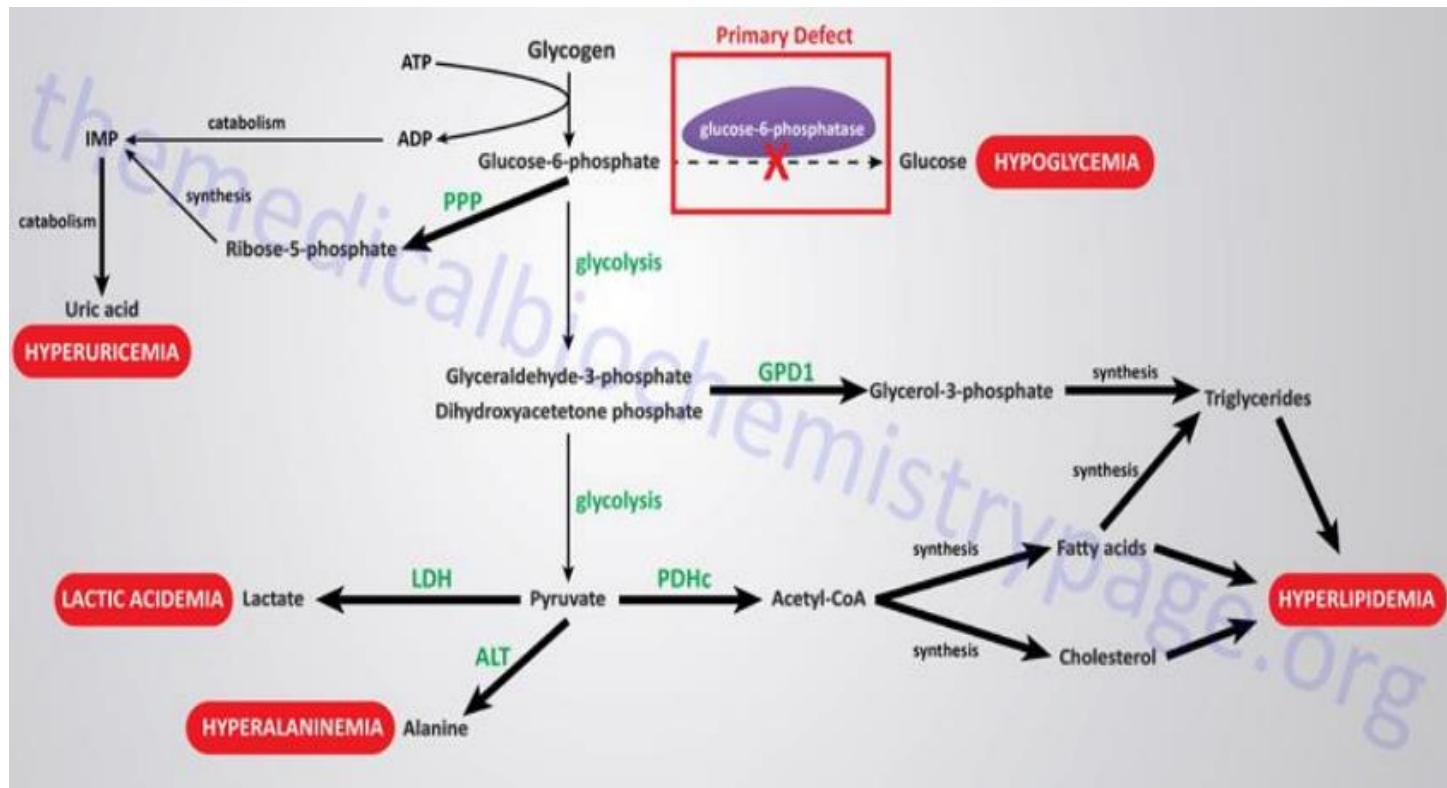
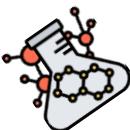
## Type I: Von Gierke's disease

- ✓ This is the commonest glycogen storage disease and is usually manifested during the early year of life.
- ✓ It is caused by deficiency of glucose 6- phosphatase (type Ia) in the **liver**, **kidney**, and **intestine**.

## What are the symptoms and signs of von Gierke's disease?

- It is characterized by accumulation of large amounts of glycogen and **enlargement of the liver and kidney**.
- **Fasting hypoglycemia** develops rapidly because glycogenolysis and gluconeogenesis cannot supply blood glucose.





## Lactic acidemia

Failure of gluconeogenesis.

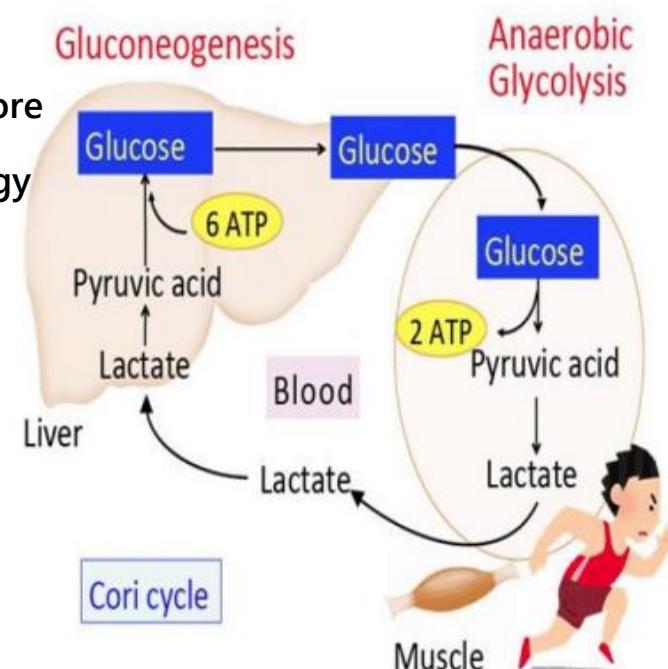
Glucose is not synthesized from lactate produced in muscle.

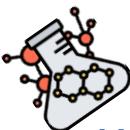
Lactate level in blood increases and the PH is lowered (lactic acidosis).

## Hyperlipidemia

\* There is blockade in gluconeogenesis. Hence more fat is mobilized from adipose tissue to meet energy requirements of the body.

\* This results in increased plasma free fatty acids leads to fatty liver, hypertriacylglycerolemia , hypercholesterolemia.





## Hyperuricemia (gout)

Glucose 6-phosphate that accumulates is diverted to pentose phosphate pathway.  
leading to increased synthesis of ribose phosphates.

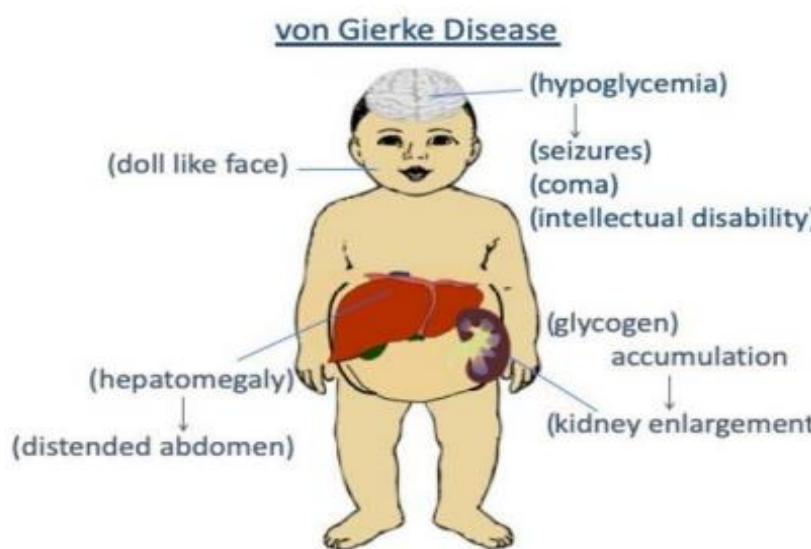


which increases the synthesis of phosphoribosyl pyrophosphate and enhance the metabolism of purine nucleotides to uric acid.



Elevated plasma levels of uric acid (hyperuricemia) are often associated with gouty arthritis (painful joints).

CP



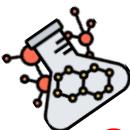
# GSD1 von Gierke Disease

Pathophysiology

Dr.Chat Perche MD, PhD

ممکن ماقننسی  
كل شوي؟





## Case study

A 12-year-old girl who had a grossly enlarged abdomen reported to OPD. Her development had been slow; she sat at the age of 1 year, walked unassisted at the age of 2 years, and was doing poorly in the school. The liver was enlarged, firm and was descended into pelvis. Laboratory investigations report revealed, low blood glucose, low PH, high lactate, triglycerides and high free fatty acids. The liver biopsy revealed high glycogen content .

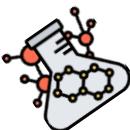
What is the probable diagnosis in this case? What is the biochemical defect in this disease? Explain the biochemical reason for above clinical signs and symptoms.

- a. Hepatomegaly:
- b. Fasting hypoglycemia:
- c. Lactic acidosis:
- d. Hypertriacylglycerolemia :

## Formative questions

Von Gierke's disease is caused by defective :

- a. Branching enzyme
- b.  $\alpha$  1,4 glucosidase
- c. Phosphorylase
- d. Glucose-6-phosphatase

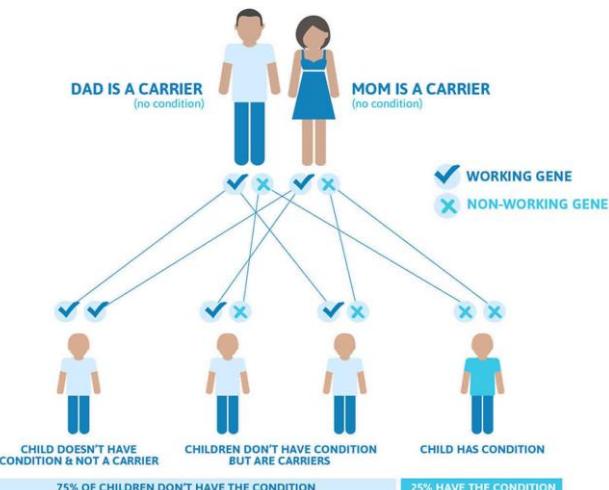


## CBL3 Inborn errors of galactose and fructose metabolism

### what is galactosemia?

- Galactose forms a small part of carbohydrate intake, being present in diet only in the form of disaccharide **lactose** found in milk.
- Galactosemia is a rare, autosomal recessive hereditary disorder of carbohydrate metabolism that affects the body's ability to convert galactose into glucose.
- Galactosemia and galactosuria occur after the intake of galactose or lactose.

**Autosomal Recessive Inheritance Pattern**



### Which enzyme deficiency causes galactosemia?

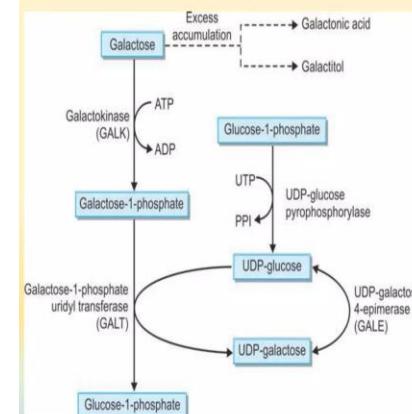
Galactosemia usually caused by deficiency of **galactose-1-phosphate uridyl transferase**, and rarely **galactokinase** or **UDP-galactose 4-epimerase**.

### What are the signs and symptoms of galactosemia?

#### 1-Cataract in galactosemia

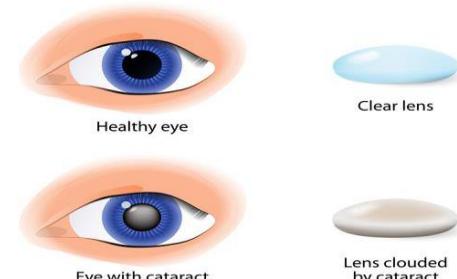
- It is principally due to conversion of galactose to its conversion of galactose to its alcohol galactitol (alcohol galactitol (dulcitol) by the enzyme galactose reductase).
- Galactitol cannot leave the eye lens, leading to accumulation of excess water and osmotic damage.

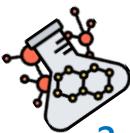
### Galactosemia



Galactose-1-phosphate uridyl transferase (GALT) deficiency is the most common enzyme deficiency that causes galactosemia.

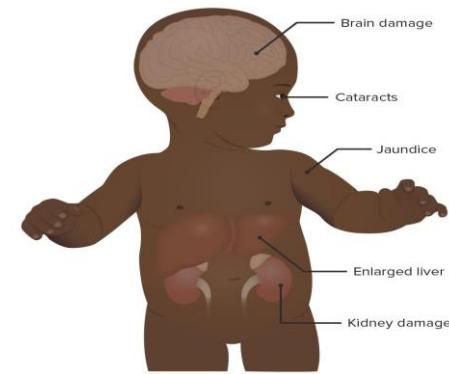
### Cataract





## 2-Can galactosemia cause hypoglycemia?

- Deficiency of the enzyme galactose 1-phosphate uridyl transferase leads to attacks of **hypoglycemia** after galactose or lactose feeding.
- This is probably due to accumulation of galactose 1-phosphate, leading to sequestration of phosphate needed for glycogenolysis.



## What is the treatment of galactosemia?

- a) The only way to treat galactosemia is immediate and total removal of galactose from the diet.
- b) Newborns can be treated by artificial milk that does not contain lactose.

## Case study

A 44-month-old male child presents with the history of repeated loss of consciousness and refusal to feed especially milk and milk containing diet. On examination, baby was found to be mild icteric and bilateral cataract was detected. Liver was palpable below costal margin.

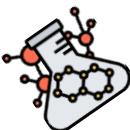
- Blood sugar: 72mg/dl (normal random blood glucose = 80-140mg/dl).
- Plasma free galactose: 129mg/dl (normal = < 20mg/dl).
- RBC galactose-1-phosphate level: phosphate level: 54mg/dl (normal = <11mg/dl).

1. What is the possible diagnosis in this case?
2. What is the biochemical defect in this disease?

Deficiency of enzyme galactose-1-phosphate uridyl transferase

3. Explain the biochemical reason for above clinical signs and symptoms.
  - a) Hepatomegaly: lack of this enzyme impairs galactose metabolism results in accumulation of galactose-1-phosphate in liver which results in hepatomegaly.
  - b) Recurrent hypoglycemic attack.
  - c) Cataract: cataract in the baby is due to reduction of metabolized galactose
4. What is the treatment regime suggested for this child?

Treatment of this child is lactose and galactose-free diet.



### **Formative questions**

1) Which of the following enzymes are not involved in galactose metabolism?

- a. Galactokinase
- b. Glucokinase
- c. Galactose-1-phosphate uridyl transferase
- d. UDP-galactose 4-epimerase.

2) Which of the following enzymes is defective in galactosemia —a fatal genetic disorder in infants?

- a. Galactose 1 phosphatase
- b. Phosphoglucomutase
- c. Galactose reductase
- d. Galactose-1-phosphate uridyl transferase

3) Galactose-1-phosphate uridyl transferase

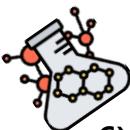
- a. Essential for glucose metabolism
- b. Its deficiency is usually associated with fructosemia
- c. Its substrate is galactose
- d. Its deficiency leads to mental retardation, liver cell failure and

4) Galactose is converted to dulcitol by the enzyme called

- a. Galactose reductase
- b. Galactokinase
- c. UDP--galactose galactose 4-epimerase
- d. Galactose-1-phosphate uridyl transferase

5) Increased galactose level in the blood circulation is named as

- a. Galactosemia
- b. Galactosuria
- c. Hyperglycemia
- d. Glucosuria



6) Increased galactose level in urine is referred to as

- a. Galactosemia
- b. Galactosuria
- c. Hyperglycemia
- d. Glucosuria

7) The tissues, functions are impaired by the accumulation of galactose, captivated in

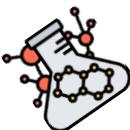
- a. Liver
- b. Stomach
- c. Placenta
- d. Seminal vesicles

8) A breast-fed infant began to vomit frequently and lose weight. Several days later she developed jaundice, hepatomegaly and bilateral cataract. What is the possible diagnosis for these symptoms?

- a. Galactosemia
- b. Von-Gierke's disease
- c. Hereditary fructose intolerance
- d. Gaucher disease

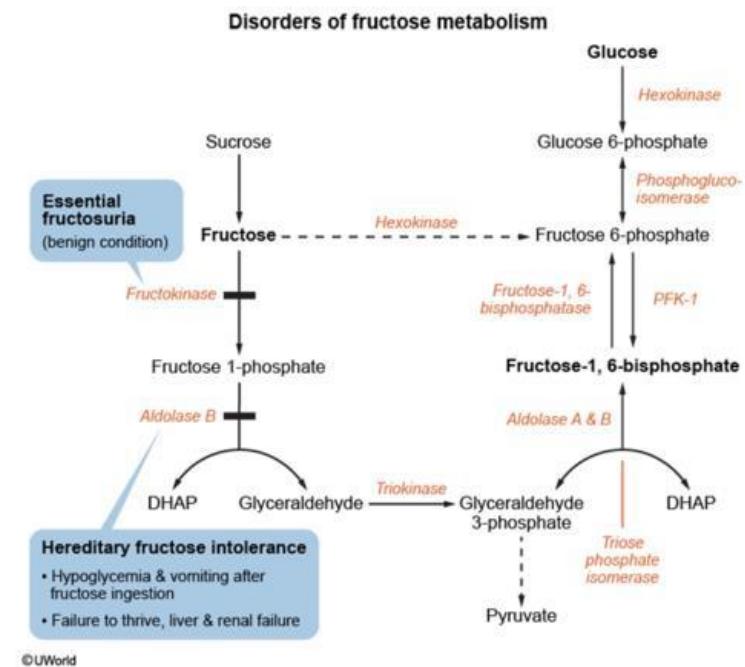
### Answers:

- |     |     |
|-----|-----|
| 1-b | 6-b |
| 2-d | 7-a |
| 3-d | 8-a |
| 4-a |     |
| 5-a |     |



### What is essential fructosuria in biochemistry?

- Fructose forms 20% of our carbohydrate diet. It is found in sucrose (table sugar and molasses, honey, and some fruits).
- Essential fructosuria or benign fructosuria is an autosomal recessive disorder caused by deficiency of the enzyme fructokinase.
- It is characterized by slow utilization of fructose, leading to **fructosemia** and **fructosuria** after the ingestion of fructose or sucrose



### What causes hereditary fructose intolerance?

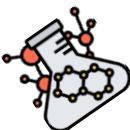
- Hereditary fructose intolerance is another inborn error in metabolism of fructose.
- It is an autosomal recessive disease caused by deficiency of the enzyme aldolase B

### What are the symptoms of hereditary fructose intolerance?

- It is characterized by fructosemia and fructosuria due to slow utilization of fructose.
- It attacks of **hypoglycemia** follow the intake of fructose or sucrose.
- This is probably due to the accumulation of fructose 1-phosphate, leading to sequestration of phosphate needed for glycogenolysis.

### What is the treatment of hereditary fructose intolerance?

The standard therapy is a fructose-free diet. This involves exclusion of anything containing fructose or sucrose.



## Case study

A 3-year-old boy was brought to the emergency department after several episodes of vomiting and lethargy. After a careful history, it was observed, that these episodes occur after ingestion of certain types of food, especially high in fructose. His blood sugar was checked in the emergency department and was extremely low (42mg/dl). The test for reducing sugar in urine was positive.

### 1. What is the most likely diagnosis?

The child was diagnosed with hereditary fructose intolerance (HFI).

### 2. What is HFI? Is it genetic or environmental?

### 3. What is the biochemical defect in this disease?

### 4. Explain the biochemical reason for low blood sugar.

## Formative questions

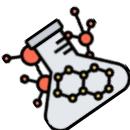
### 1) Hereditary fructose intolerance is due to

- a. Deficiency of Aldolase A
- b. Deficiency of Aldolase B
- c. Deficiency of fructokinase
- d. Deficiency of fructose reductase

**Answer: b**

❖ Name the disease resulting from deficiency of fructokinase enzyme?

❖ List one manifestation of hereditary fructose intolerance?



## TUT:9 Integration of metabolism

- Metabolism is a continuous process, with thousands of Reactions, simultaneously occurring in the Jiving cell.

- Though metabolism of each of major food nutrients, viz.

Carbohydrates,

Lipids and

Proteins

- Have been considered separately for the sake of Convenience, it actually takes place simultaneously in The intact animal and are closely interrelated to one Another.
- The metabolic processes involving these three major food Nutrients and their interrelationship can be broadly Divided into three stages.



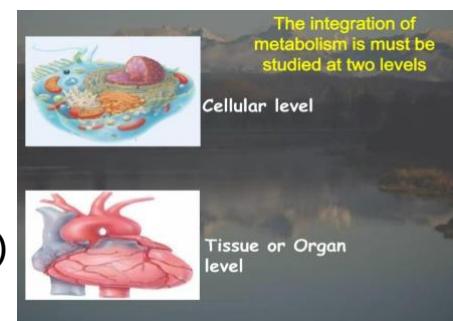
## INTEGRATION OF METABOLISM

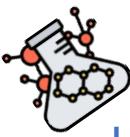
**Definition-** The co-ordination between three metabolites (carbohydrates, lipids and proteins) called Integration Of Metabolism.

### Significance of Integration of metabolism

- It ensures a supply of suitable fuel for all Tissues, at all the time (from the fully fed state To the totally starved state)
- Under positive caloric balance,

A significant proportion of the food energy intake is Stored as either glycogen or fat





- Under negative caloric balance,

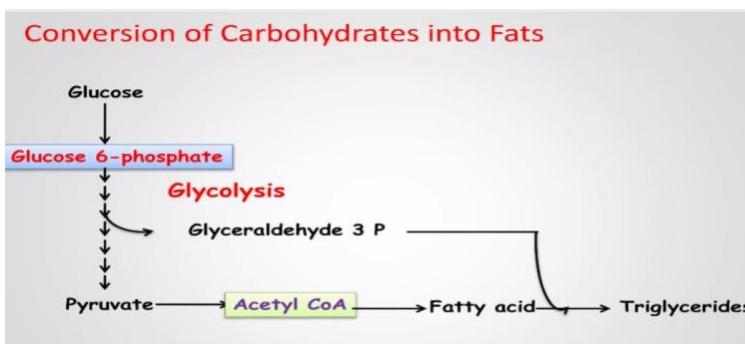
Fatty acids are oxidised in preference to glucose, to Spare glucose for those tissues (Brain & RBCs) that Require it under all conditions.

### INTER CONVERSION BETWEEN 3 PRINCIPAL COMPONENTS

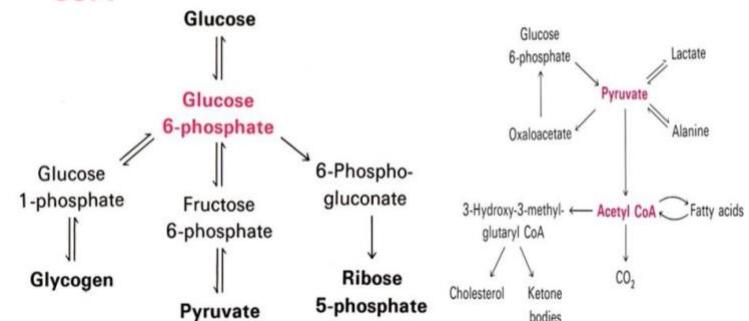
- Conversion of carbohydrates into fats and fats into Carbohydrates.
- Conversion of carbohydrates into proteins and proteins into Carbohydrates.
- Conversion of proteins into fats and fats can't be converted into proteins.

### Major Metabolic Pathways and their Control Sites

- Glycolysis
- Gluconeogenesis
- Citric Acid Cycle
- Pentose Phosphate Pathway
- Glycogen Synthesis and Degradation
- Fatty Acid Synthesis and Degradation.

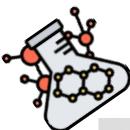


### Key Junctions: G-6-P, Pyruvate, and Acetyl CoA

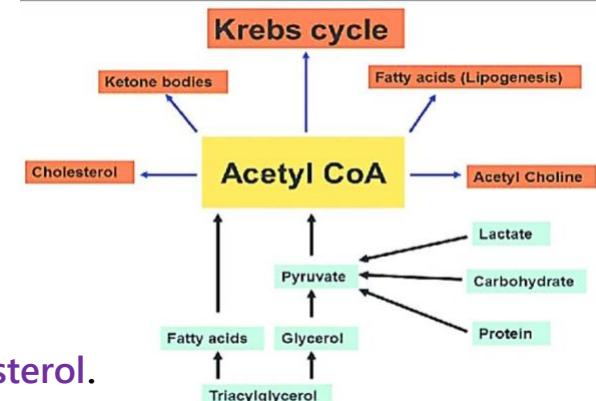


### Conversion of Fatty Acids to Carbohydrate

- There is no net conversion of fatty acids to glucose or Glycogen take place.
- Only odd chain fatty acids are glucogenic as it forms a Molecule of propionyl-CoA upon D-oxidation.
- Propionyl-CoA can be converted to succinyl-CoA, an Intermediate of citric acid cycle, which can be converted to Glucose by gluconeogenesis.



- Pyruvate end product of Glycolysis is **Oxidatively decarboxylated to Acetyl- COA**
- Acetyl-coA is then utilized via TCA Cycle
- Acetyl-coA of Glucose when excess is Diverted and used for **biosynthesis of Fatty acids and Cholesterol**.
- Glyceraldehyde-3-phosphate an Intermediate of **Glycolysis of Glucose IS a source for Glycerol production.**
- **Glycerol** obtained from Glucose is Utilized during Lipogenesis ,for **Biosynthesis of Triacylglycerol and Phospholipid biosynthesis.**



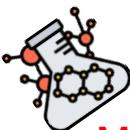
### Integration of Metabolism at Tissue or Organ Level .

Integration of metabolism at tissue or organ level Includes the inter-relationship of different tissues and Organs to meet metabolic demands for the whole body.

- Role of Liver
- Role of Skeletal Muscle
- Role of Adipose Tissue
- Role of Heart Muscle
- Role of Brain

**Liver is a Glucostatic organ regulates blood Glucose In all conditions.**

- In a well fed condition Liver stores excess Free Glucose as Glycogen.
- emergency condition In Liver Glycogen is Degraded via Glycogenolysis and Biosynthesizes Glucose via Gluconeogenesis.



### Major roles of the liver 🍹

1. Maintenance of blood glucose levels.

2. During the fed state,

- Converts and stores excess glucose as glycogen.
- Converts it to fatty acids.

3. During the fasting state,

Provides glucose for the body by : Glycogenolysis and Gluconeogenesis.

4. During starvation,

It synthesizes ketone bodies and supplies to the peripheral Tissues as a source of energy.

5. It serves as the major site of fatty acid synthesis.

### Muscles 💪

- In a normal metabolic state muscle uses

Glucose and Fatty acids as main sources of Energy.

- In a well fed state muscles has large stores

of Glycogen (3/4<sup>th</sup>)

- In contracting muscles during severe Exercise

in anaerobic condition Glycolysis ends as Lactate.

- Later Lactate is metabolized by Converting it

into Glucose after carried Through blood in Liver via Gluconeogenesis(Cori cycle).

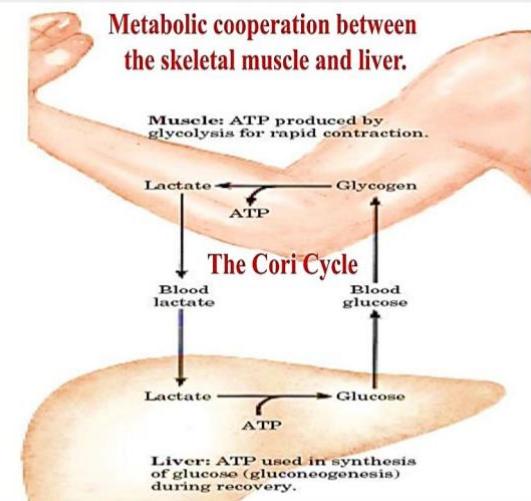
- In resting muscles, fatty acids are the major source of energy.

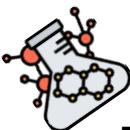
- This use spare glucose to be used by brain and erythrocytes.

### Role of Skeletal Muscle 💪

- Skeletal muscle maintains large stores of glycogen, Which provide energy during exercise.

- During starvation, free fatty acids and ketone bodies Supplied by liver are oxidized in preference to glucose in Muscle.





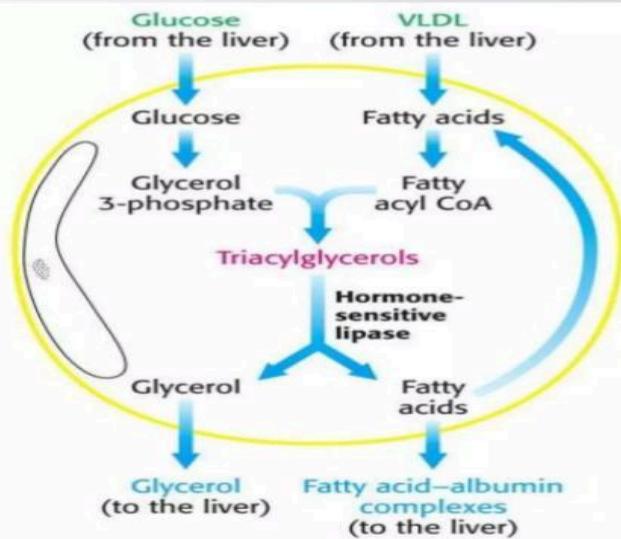
- The protein present in muscle may be used as a fuel source, if no other fuel is available.
- Pyruvate, the product of glycolysis in the skeletal muscle, may be converted to either lactate or alanine

### Adipose tissue

- TAG can be abundantly and unlimitedly stored in adipocytes.
- TAG serve as a reserve store of energy during well fed condition.
- TAG is the majority fuel for this tissue.

## Role of Adipose Tissue

- Amorphous tissue widely distributed about the body
- Triacylglycerols are stored in tissue (enormous reservoir of metabolic fuel)
- continuous synthesis and breakdown of triacylglycerols, controlled by **hormone-sensitive lipase**
- Needs glucose to synthesize TAG;
- Glucose level determines if fatty acids are released into blood

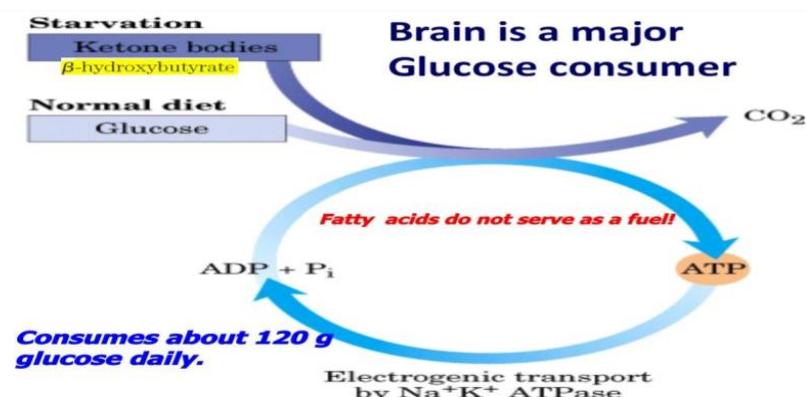


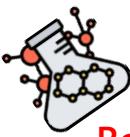
### Metabolic Profile of Brain

60-70 % of bodies utilization

of Glucosels by Brain.

In starvation -> Ketone bodies can Replace Glucose.

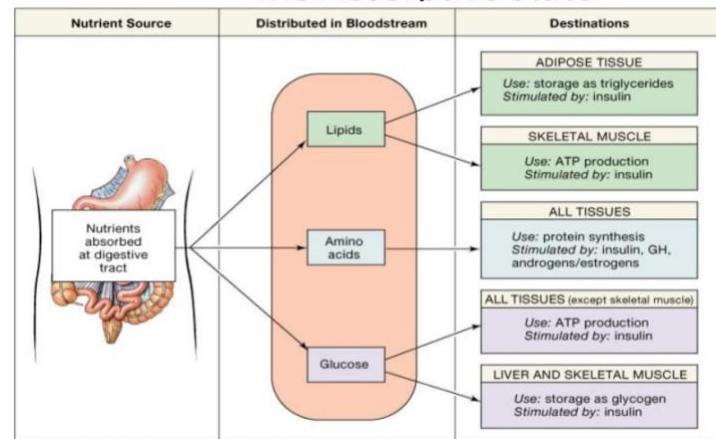




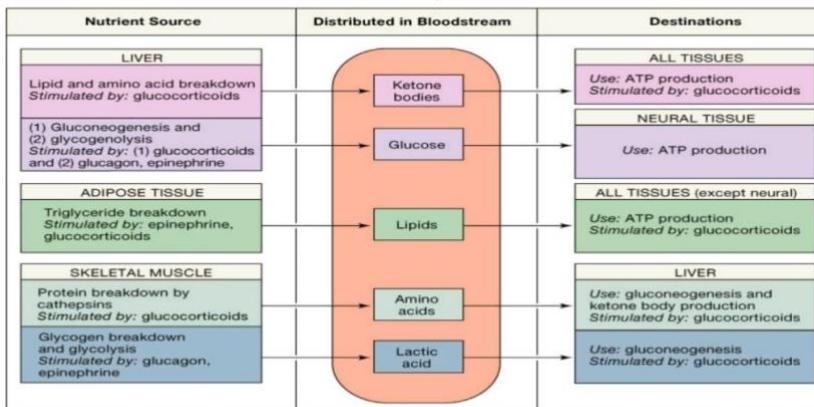
## Role of heart □ muscle

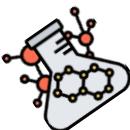
- ♥ The activity of heart muscle is constant and rhythmic
- ♥ The heart functions as a completely aerobic organ and is very rich in mitochondria prefers fatty acid as fuel.
- ♥ Continually nourished with oxygen and free fatty acid ,glucose ,or ketone bodies as fuel.

The Absorptive State



The Postabsorptive State





## Bio L5,6 : Lipids chemistry

### Definition

❖ Lipids are organic compounds, containing C ,H , O, formed mainly from alcohol and fatty acids(FA) combined together by ester linkage . They are relatively insoluble in water ,but soluble in fat solvents as ether ,benzene and chloroform.

### Functions

- 1) Lipids enter in the **structure of cell membrane**.
- 2) They supply the body with **energy** as one gm of fats gives 9.3 K. calories. (Note: a calorie is defined as the heat needed to raise the temperature of one liter of water one degree centigrade).
- 3) Lipids keep the **body temperature** as they act as insulator.
- 4) Lipids help in **fixation** of internal organs. So, in extensive diet regimen or in prolonged starvation, organ ptosis occurs.
- 5) They act as **laxative** in constipating patients.
- 6) Lipids in addition to muscles give the **body contour**.
- 7) Lipids make the **food palatable**.                            8) Help in **absorption** fat soluble vitamins
- 9) **Supply** the body with **essential fatty acids** & eicosanoid acids.

### Classification of lipids

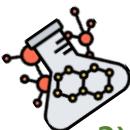
Based on the chemical nature, Lipids are classified into the followings

#### 1) Simple lipids

- ❖ They are **esters of fatty acids with glycerol or other higher alcohols**.
- ❖ They are subclassified into:
  1. Neutral fats
    - ❖ They are formed of **alcohol & fatty acids (F.A)**.
    - ❖ The alcohol in neutral fats is **restricted to glycerol only**.
  2. Waxes
    - ❖ Also, They are formed of **alcohol & F.A.**                            ❖ The alcohol must be **higher than glycerol**

#### 2) Compound, complex or structural lipids

- ❖ They are formed of **simple lipids** in addition to other substances or groups which may be **phosphoric acid, carbohydrates, and proteins** to give **phospholipids, glycolipids, and lipoprotein**, respectively.



### 3) Derived Lipids

❖ These types of lipids are derived from simple & compound lipids by hydrolysis.

### 4) Substances associated with lipids

❖ These are substances present **associated with lipids** as **fat soluble vitamins** (A, D, E & K) and **sterols** as cholesterol and provitamins as carotenes.

## Simple lipids

❖ Triacylglycerol (Neutral fats)

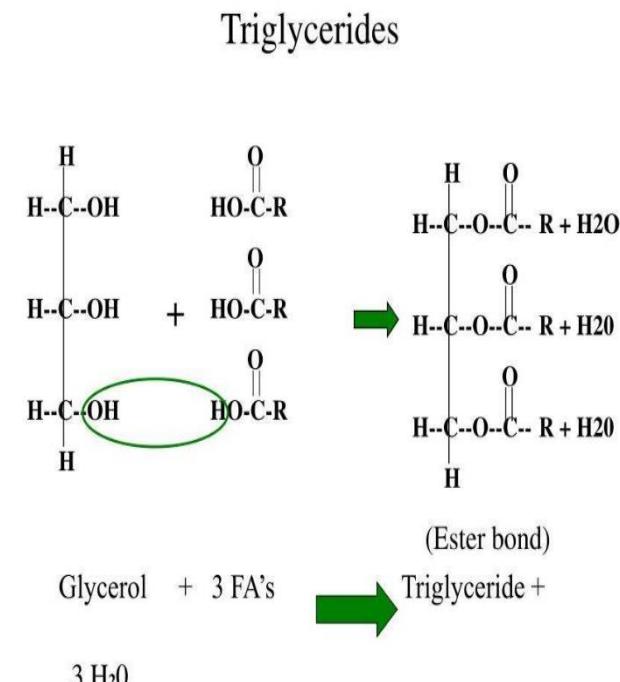
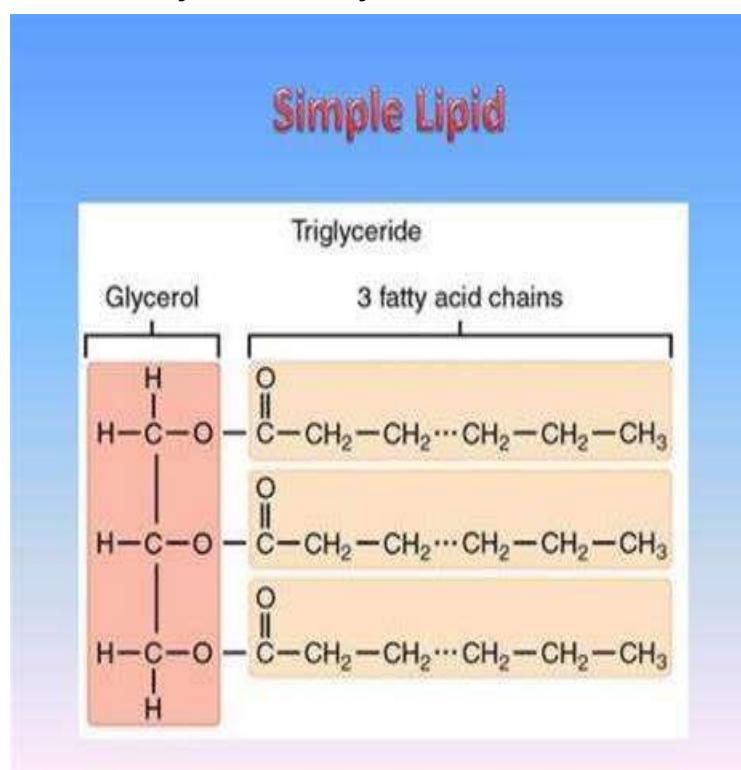
❖ Waxes

### Triacylglycerol(Neutral fats-TAG)

❖ They are esters of 3 fatty acids with glycerol.

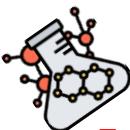
❖ The simplest naturally occurring lipids TAG.

❖ N.B. Glycerol :trihydric alcohol ,which has 3 carbon atoms and 3(OH)groups



### Importance of glycerol

- 1) It has a **nutritive function** as it is a product of digestion.
- 2) It enters in many **pharmaceuticals and cosmetic preparation**.
- 3) It enters in the preparation of **nitroglycerin** which is a coronary vasodilator as well as **an explosive** in the manufacture of dynamite



## Fatty acids

- ❖ Fatty acids are aliphatic carboxylic acids and building blocks of most lipids, made of long chain organic acids having **one polar carboxyl group(head)** and a **non-polar hydrocarbon chain (tail)**.
- ❖ The latter makes them water insoluble.
- ❖ They are not found free in nature but found as esterified forms .
- ❖ Their general structural formula is **R-COOH**.
- ❖ **The COOH (carboxylic group)** represents the functional group which makes F.A. **hydrophilic**.
- ❖ **The R group represents** the hydrocarbon chain which gives them **hydrophobic properties**
- ❖ OR the general structural formula of FA represented as **CH<sub>3</sub>- (CH<sub>2</sub>)<sub>n</sub>-COOH**, where (n) equals the number of methylene -(CH<sub>2</sub>) groups between methyl(CH<sub>3</sub>) and carboxyl groups (COOH)

Depending on the R group the fatty acid may vary there are 2 types

- ❖ Saturated fatty acids: No double bonds
- ❖ Unsaturated fatty acids: Contain double bonds

### 1) Saturated fatty acids(-CH-CH-)

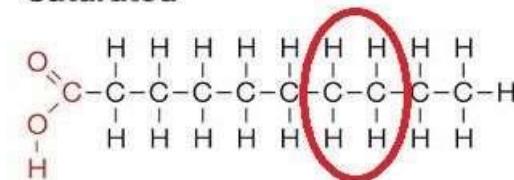
- ❖ Butyric acid(4 C) = CH<sub>3</sub>-CH<sub>2</sub>-CH<sub>2</sub>-COOH
- ❖ Caproic acid(6 C) =CH<sub>3</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-COOH
- ❖ Palmitic acid (16 C)=CH<sub>3</sub>-(CH<sub>2</sub>)<sub>14</sub>-COOH
- ❖ Stearic acid (18 C)=CH<sub>3</sub>-(CH<sub>2</sub>)<sub>16</sub>-COOH

### 2) Unsaturated fatty acids(-CH=CH-)

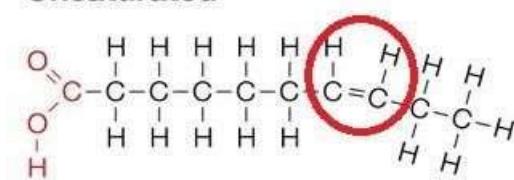
#### 1. Mono-enoic or monounsaturated FA

- ❖ Contain one double bond e.g. oleic acid (18: 1,9) (**ω9 family**)
- ❖ Monounsaturated FA are non-essential and can be synthesized by the body

**Saturated**



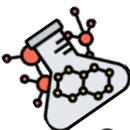
**Unsaturated**



**\*Palmitoleic (unsaturated palmitic acid): 16:1:w7**  
 $\text{CH}_3 - (\text{CH}_2)_5 - \underset{10}{\text{CH}} = \underset{9}{\text{CH}} - (\text{CH}_2)_7 - \text{COOH}$

**\*Oleic (unsaturated stearic): 18:1:w9**  
 $\text{CH}_3 - (\text{CH}_2)_7 - \underset{10}{\text{CH}} = \underset{9}{\text{CH}} - (\text{CH}_2)_7 - \text{COOH}$

**\*Nervonic (unsaturated lignoceric): 24:1:w9**  
 $\text{CH}_3 - (\text{CH}_2)_7 - \underset{16}{\text{CH}} = \underset{15}{\text{CH}} - (\text{CH}_2)_{13} - \text{COOH}$



## 2. Poly-enoic or Polyunsaturated FA

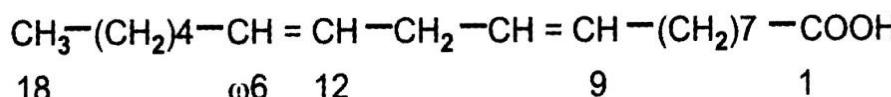
- Contain more than one double bond

A. Dienoic FA :Linoleic acid(18:2,9,12) ,2 double bonds ( $\omega 6$  family).

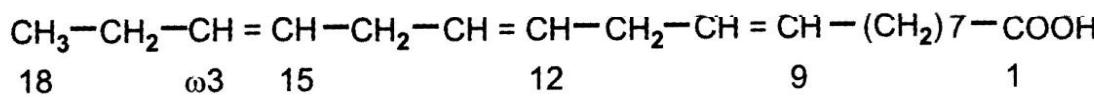
B. Tri-enoic FA: Linolenic acid(18:3,9,12,15) ,3 double bonds. ( $\omega 3$ family)

C. Tetra-enoic FA: Arachidonic acid (20:4,5,8,11,14) ,4 double bonds .( $\omega 6$  family)

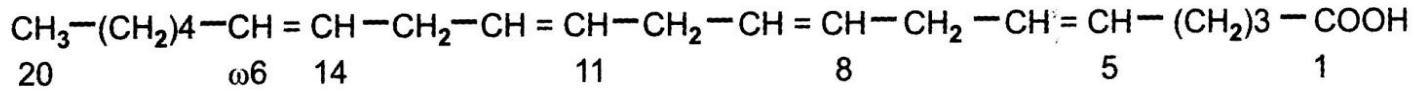
**Linoleic (C<sub>18</sub>) Δ9, 12 (two double bonds) ( $\omega 6$  family)**



**Linolenic (C<sub>18</sub>) Δ9, 12, 15 (three double bonds) ( $\omega 3$  family)**



**Arachidonic (C<sub>20</sub>) Δ5, 8, 11, 14 (four double bonds) ( $\omega 6$  family)**



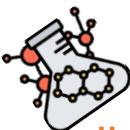
## Differences between monoethenoid & polyethenoid F.A.

| Points of differences  | Monoethenoid F.A                                                          | Polyethenoid F.A                                                              |
|------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Number of double bonds | ❖ One double bond                                                         | ❖ More than one double                                                        |
| Synthesis by the body  | ❖ Can be synthesized                                                      | ❖ Cannot be synthesized                                                       |
| Examples               | ❖ Palmitoleic acid(C16).<br>❖ Oleic acid (C18).<br>❖ Nervonic acid (C 24) | ❖ Linoleic acid (C18).<br>❖ Linolenic acid (C18).<br>❖ Arachidonic acid (C20) |

## Clinical significance of polyunsaturated FA (PUFA)

I. **Linoleic, ( $\omega 6$ ), linolenic ( $\omega 3$ ) and arachidonic acids ( $\omega 6$ ) are called essential fatty acids**

- They cannot be synthesized by the body, must be taken in diet and essential for normal growth.
- They enter in the structure of phospholipids and cholesterol esters.
- They enter in the structure of cell membrane and required for fluidity of membrane structure.
- They occur in high concentration in the reproductive organs.
- They protect against atherosclerosis and coronary heart disease by lowering of free cholesterol and LDL .



### II. Eicosanoids (Eicosa = twenty) are derived from 20C arachidonic acid.

- ❖ They are poly-enoic fatty acids.
- ❖ They are **prostanoid** (prostaglandins, prostacyclins & thromboxanes) and **leukotrienes**.
- ❖ Arachidonic acid is the **precursor of prostaglandins**.
- ❖ It can be synthesized from linoleic acid if the latter supplied in the diet

### Rancidity

#### Definition

- ❖ It is the change of color, odor & taste of fats & oils.
- ❖ If fat is exposed to moisture, oxygen, heat, light and certain metals such as copper, it develops a bad odor and taste and is said to be rancid.

#### Types

##### There are three types of rancidity

###### 1) Oxidative rancidity

- ❖ Occurs in fats containing unsaturated fatty acids.
- ❖ The polyunsaturated F.A. in fats add oxygen, forming **peroxides** that break into short chain aldehydes and acids, giving fat a bad flavor.
- ❖ In addition, peroxides are destructive to the fat-soluble vitamins.
- ❖ Oxidative rancidity is **inhibited by phenols and by vitamin E**

###### 2) Hydrolytic rancidity

- ❖ This type of rancidity is due to partial hydrolysis of triacylglycerols by traces of hydrolytic enzymes.
- ❖ It occurs for example in butter due to its content of water.
- ❖ Also, some bacteria secrete lipase enzyme which causes partial hydrolysis of fats & oils.
- ❖ **Hydrolysis liberates free F.A. which give bad odor & taste.**

###### 3) Ketonic rancidity

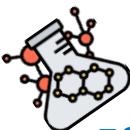
- ❖ This type of rancidity is due to effect of some fungi as *Aspergillus Niger* on fats.
- ❖ The products of rancid fats are ketones of bad taste & odor.

#### Prevention of rancidity

- ❖ Keep fats or oils far from humidity.
- ❖ Use of antioxidants like vitamin E

#### How to detect Rancidity

- ❖ Bad odor and taste.
- ❖ High acid number



## Effect of Rancid fat

- ❖ Rancid fat is toxic
- ❖ Rancidity destroys fat soluble vitamins and essential fatty acids.

## Acid number

- ❖ It is the number of mg of KOH required to neutralize free FA (FFA) present in one gm of fats or oils.
- ❖ Rancidity produces FFA so, it gives high acid number than the non-rancid fats or oils.
- ❖ Pure triacylglycerols have acid number equal to zero. Why?

## Compound lipids

- ❖ Esters of fatty acids with alcohols together with some other head groups .

### 1) Phospholipids

- ❖ Containing phosphoric acid residue.
- 1. Glycero-phospholipids: The alcohol is **glycerol**.
- 2. Sphingo-phospholipids: The alcohol is sphingosine ,e.g. **sphingomyelin**

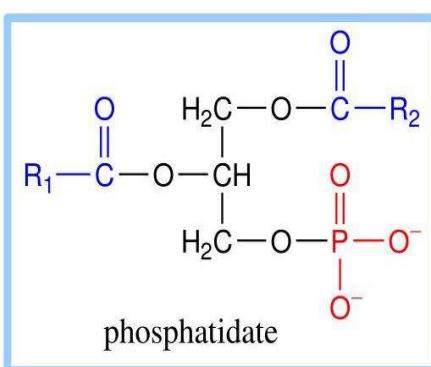
### 2) Lipoproteins

- ❖ Lipids containing lipid part and protein part(apoproteins).

### 3) Others (Glycolipids)

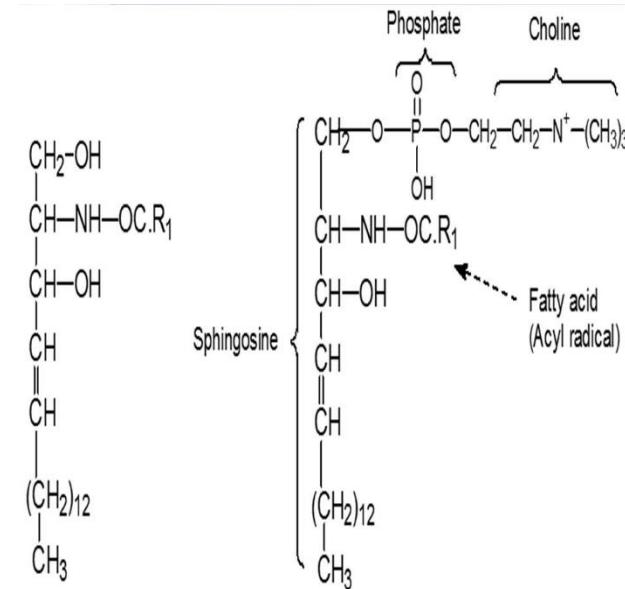
- ❖ Lipids containing fatty acid , sphingosine.
- ❖ And carbohydrate residue.

## Phosphatidate

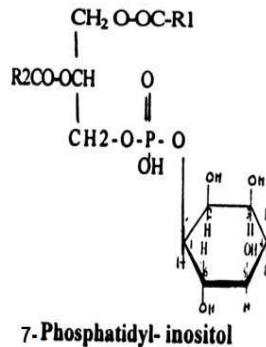
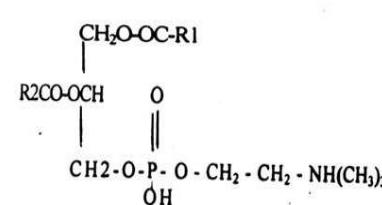
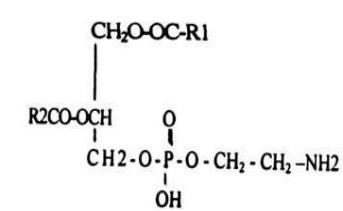
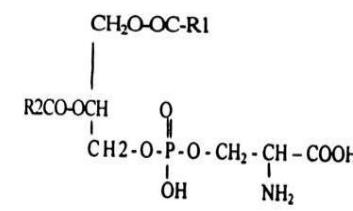


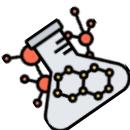
In **phosphatidate**:

- ♦ **fatty acids** are esterified to hydroxyls on **C1 & C2**
- ♦ the **C3** hydroxyl is esterified to **P<sub>i</sub>**.



Sphingomyelin





## Lipoproteins and Proteolipids

- ❖ They are present in cell membrane, mitochondria and plasma.
- ❖ Plasma lipoproteins convert water insoluble lipids into water soluble complexes.
- ❖ This facilitates transport of lipids between blood and different tissues.
- ❖ The protein fractions called apolipoproteins or apoproteins (apo A, B48, B100,C,D.&E)

### 1) Lipoproteins

- ❖ Are arranged as lipid part to **the interior** of the molecule and the protein part to **the exterior** of the molecule.
- ❖ This gives the structure a property of its **solubility in water** (NB: lipoproteins are water-soluble).

### 2) Proteolipids

- ❖ Are mainly distributed in nervous tissues.
- ❖ Proteolipids are arranged as lipid part surrounding the protein part.
- ❖ This structure makes proteolipids **insoluble in water** but soluble in fat solvents

### Classification Plasma lipoproteins (by ultracentrifugation)

- ❖ Five major groups (fractions) of lipoproteins have been identified as important physiologically and in clinical diagnosis.

### These are

#### 1) Chylomicrons (CM):

- ❖ They are derived from **intestinal absorption** of triacylglycerols and other lipids.

#### 2) Very low-density lipoproteins (VLDL, or pre- $\beta$ -lipoproteins):

- ❖ They are derived from **the liver** for the export of triacylglycerols.

#### 3) Low density lipoproteins (LDL or $\beta$ -lipoprotein)

#### 4) High-density lipoproteins (HDL or $\alpha$ -lipoprotein)

#### 5) Albumin + FFA (NEFA): (FFA was carried by albumin)

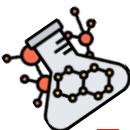
### Derived lipids

- ❖ These lipids are derived from both simple & compound lipids by hydrolysis.

### Substances associated with lipids

### Definition

- ❖ These substances are present in association with lipids.



### Types

- 1) Vitamins:
  - ❖ As fat-soluble vitamins (A, D, E & K).
- 2) Carotenoids:
  - ❖ As carotenes.
- 3) Steroids:
  - ❖ As sterols, bile acids and steroids.

### Steroids

#### Definition

- ❖ These are a group of compounds that contain ring
- ❖ The ring called cyclopentano-perhydrophenanthrene ring (**steroid nucleus**).

#### Subtypes

- ❖ Steroids include sterols, bile acids and steroid hormones.

#### General criteria of the steroids

- 1) All steroids are derived from C17 cyclopentano-perhydro-phenanthrene nucleus
- 2) Natural steroids contain:
  - A. **At C3** : Oxygen (O = or OH -) .
  - B. **At C10 & C13**: Methyl groups.
  - C. **At C17**: Side chain .
  - D. **Ring A & B** may contain double bond but **ring C & D** are always saturated

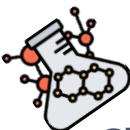
### Sterols

- ❖ These are **steroid alcohols** containing OH at C3 and methyl group at C10 and C13 and side chain at C17.

### Cholesterol

#### Source

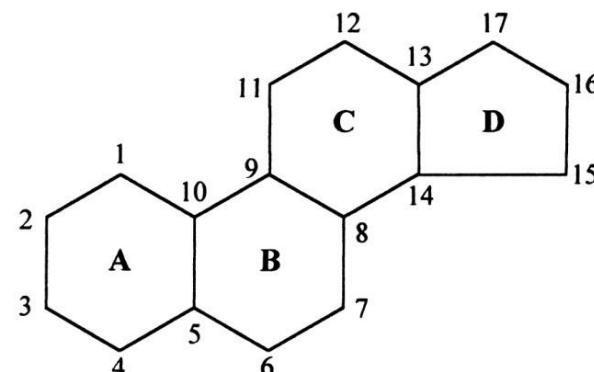
- ❖ It is of animal sources only not of plant sources.
- ❖ Present in cell membrane, brain, adrenal cortex, ovaries, testis, blood and egg yolk are rich sources of cholesterol.



## Chemical formula

The structure formula is characterized by:

- ❖ A hydroxyl group at C3.
- ❖ A double bond between C5 and C6.
- ❖ It contains 8 asymmetric carbon atoms.
- ❖ 2 methyl groups at C 10&13.
- ❖ Long side chain at C17.



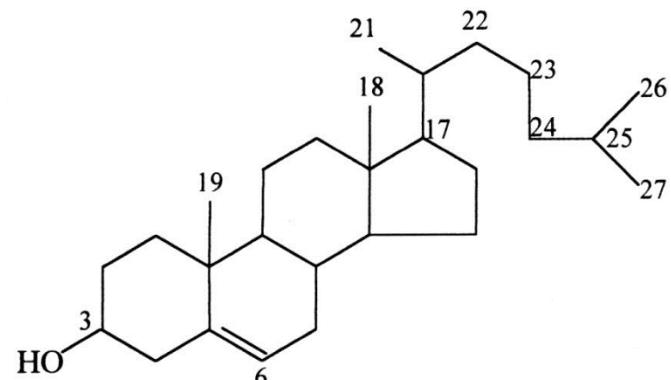
## Blood cholesterol

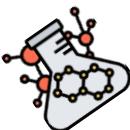
It occurs in the blood in 2 forms:

- ❖ free form and esterified form.
- ❖ The level of blood cholesterol is 150-200 mg/dl

## Importance of cholesterol

- ❖ It enters in the structure of cell membranes
- ❖ It is oxidized in liver, intestine & skin to give 7-dehydrocholesterol which is the precursor of vitamin D3.
- ❖ Also, cholesterol is the precursor of steroid hormones & bile acids.
- ❖ Abnormal accumulation of cholesterol in the wall of the blood vessels gives rise to atherosclerosis.



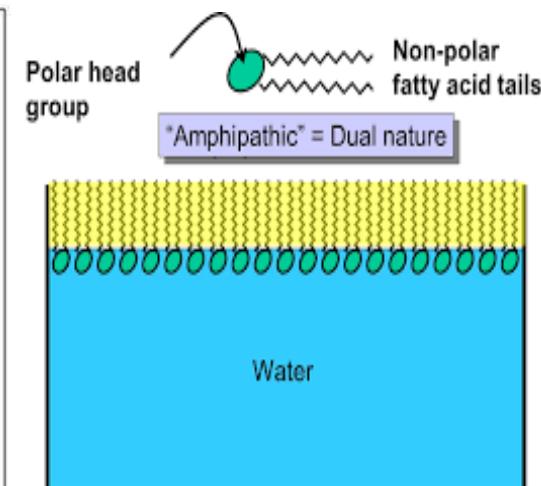
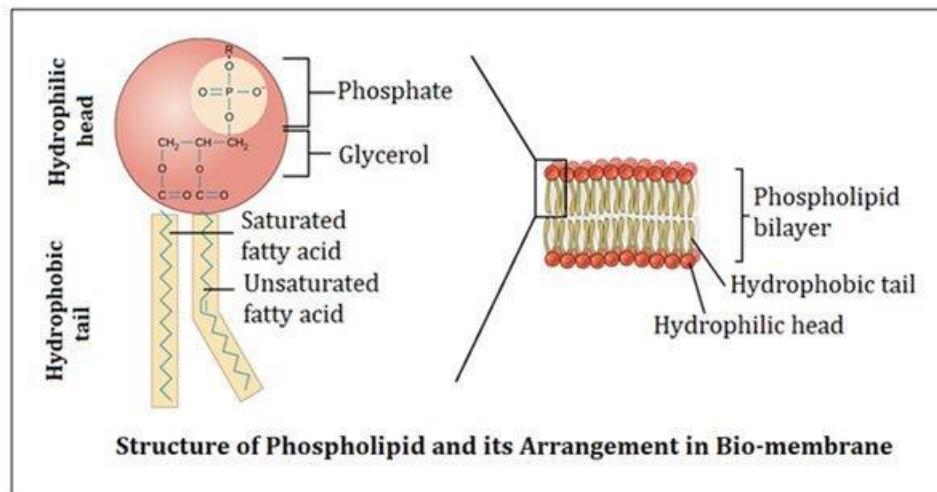


# Bio TUT4 : Phospholipid Chemistry And Metabolism

## Importance in diet

- ❖ Phospholipids are present in high concentrations in the brain, egg yolk, liver & kidney.
- ❖ They are not essential dietary components.
- ❖ Phospholipids are complex (compound) lipids.

## Phospholipids are amphipathic



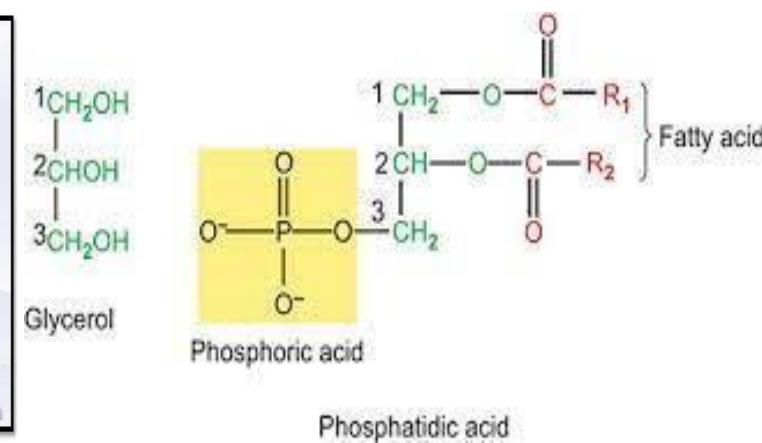
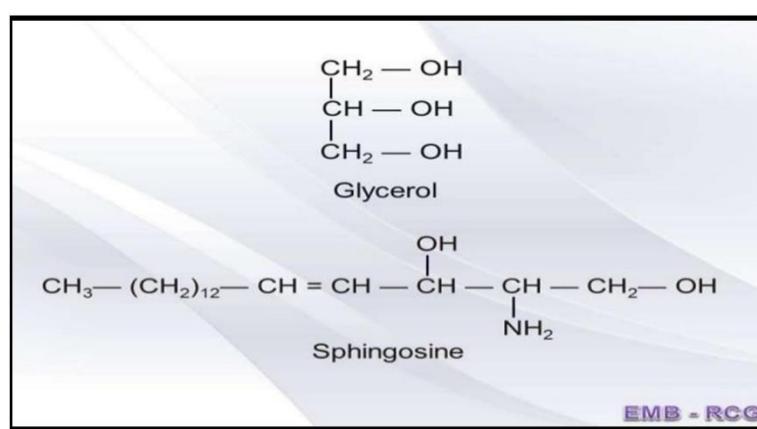
## Classification

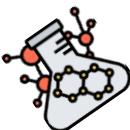
### 1) Glycerophospholipid or phosphoglyceride

- ❖ Phosphatidylcholine (lecitin)
- ❖ Phosphatidylethanolamine(Cephalin)
- ❖ Phosphatidylserine
- ❖ Phosphatidylinositol
- ❖ Plasmalogens
- ❖ Lysophospholipids
- ❖ Cardiolipin

### 2) Sphingophospholipid

- ❖ Sphingomyelins





## Functions of Lecithin

1) Lecithin enters in the structure of cell membrane. It is the most abundant phospholipid in cell membrane.

2) Lecithin acts as lipotropic factor i.e. prevent accumulation of fat in liver (fatty liver)

3) Lecithin prevents gall stones: Lecithin in bile solubilizes cholesterol and prevent cholesterol stones in gall bladder.

4) Lecithin acts as body store of choline. Choline is important for nerve transmission and transmethylation.

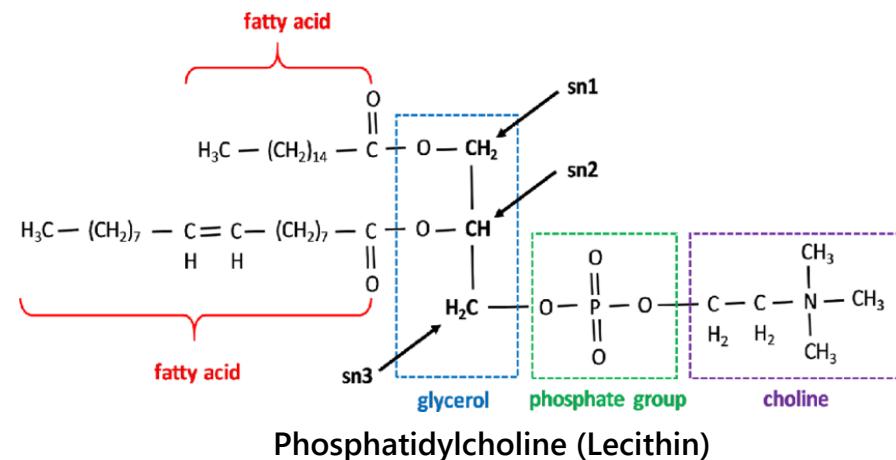
5) Lecithin forms cholesterol esters (in the presence of LCAT enzyme): Lecithin + Cholesterol → Cholesterol ester + Lysolecithin

❖ Cholesterol esters is transported to the liver and excreted with bile. This prevents atherosclerosis.

6) Dipalmitoyl lecithin (i.e. lecithin containing 2 palmitic acid residues) acts as a surfactant in the lung

❖ It is continuously secreted by the lung cells in the alveolar wall, forming monolayer over the watery surface of the alveolus.

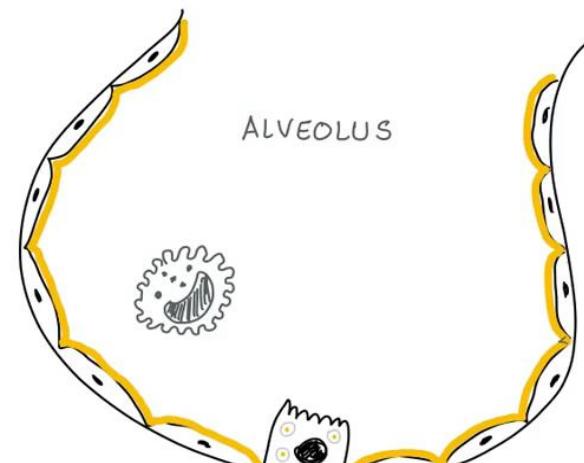
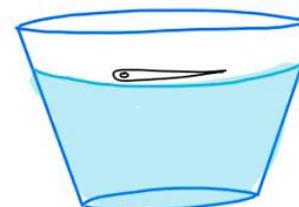
❖ It lowers the surface tension and prevents the alveoli from collapse.

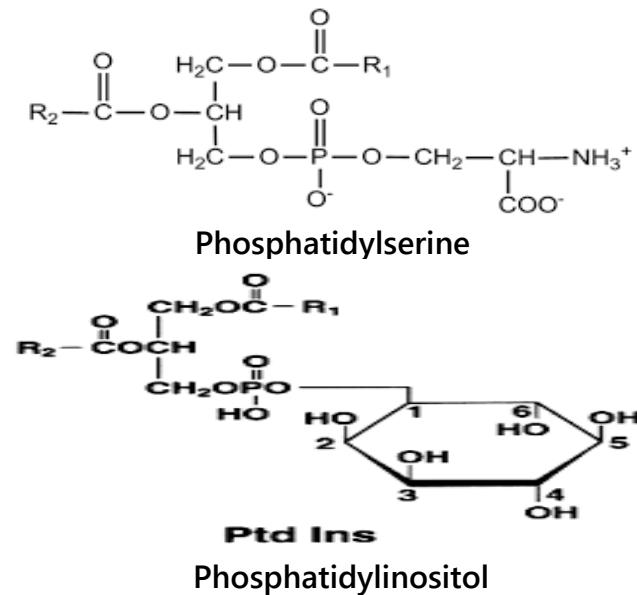
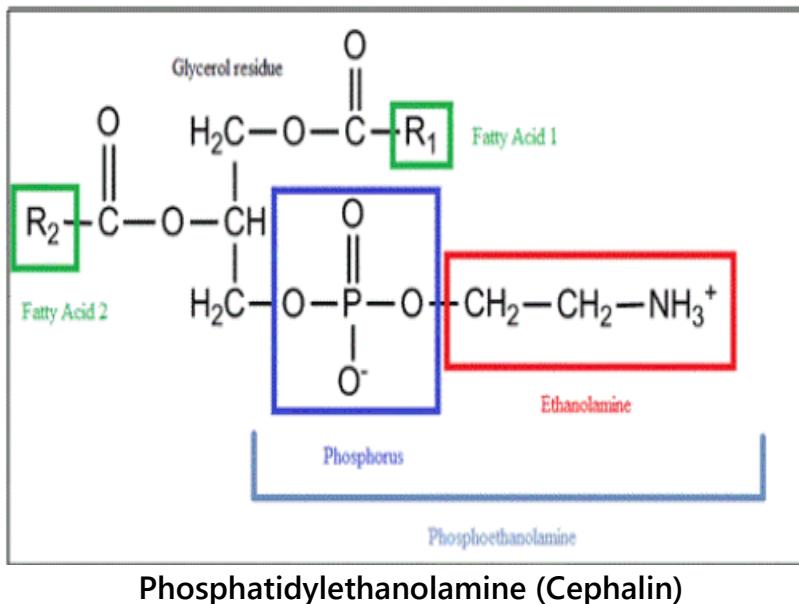
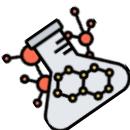


Lecithin is a natural surfactant

# Surfactant

anti Surface tension





### Plasmalogens

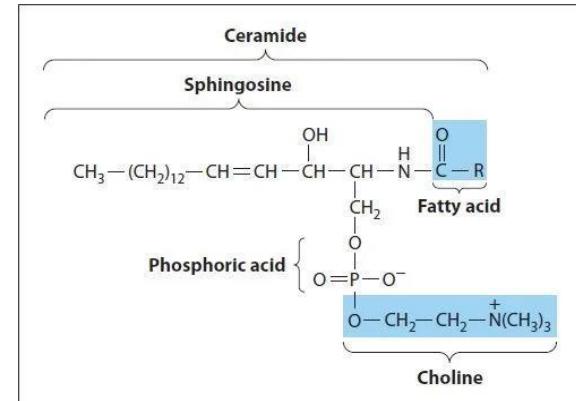
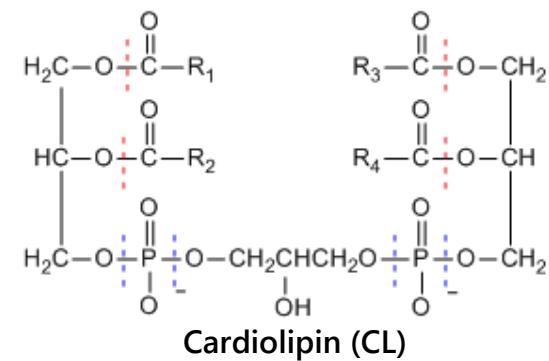
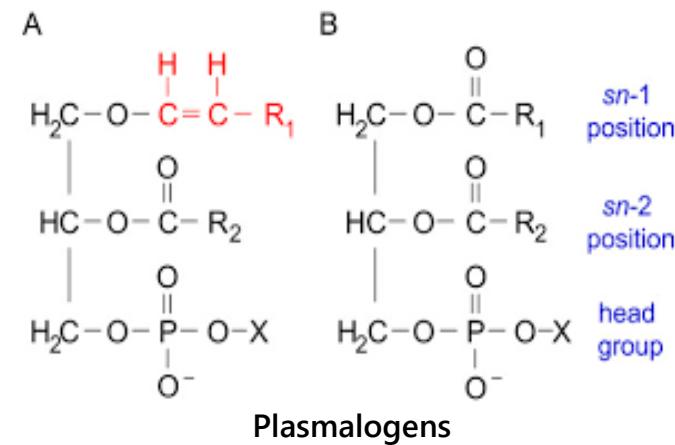
- The pathway for synthesis of plasmalogens occurs in the peroxisomes.
- It contains a vinyl-ether and an ester bond at the sn-1 and sn-2 positions, respectively, in the glycerol backbone.
- They constitutes about 10% of the phospholipids present in brain and muscles.

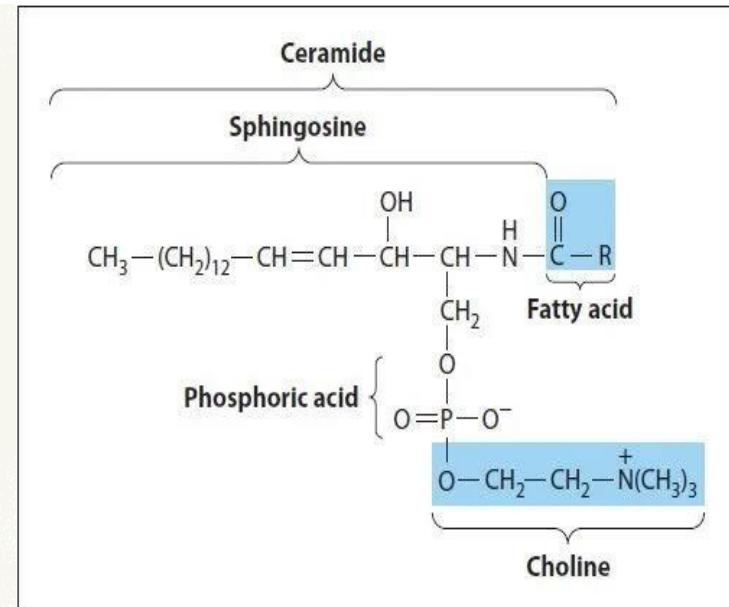
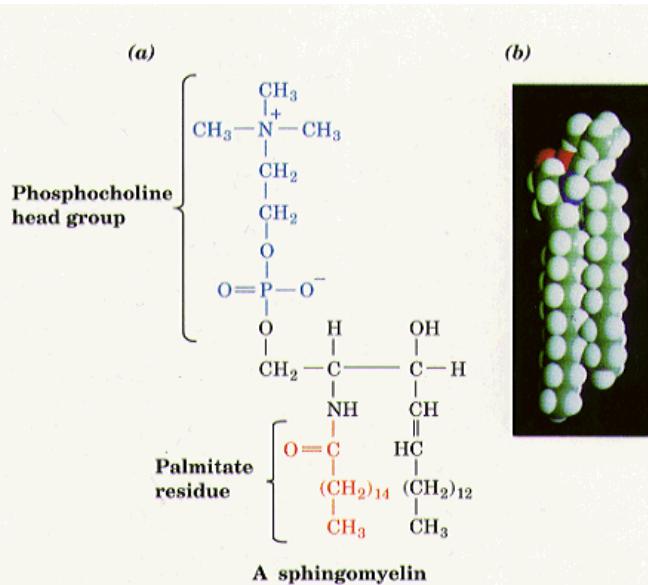
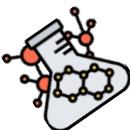
### Cardiolipin (CL)

- Each CL possesses a glycerol backbone connected to two phosphatidyl lipids.
- Cardiolipin is the major lipid in mitochondrial membrane
- It stimulates antibody formation i.e. antigenic

### Sphingomyelin

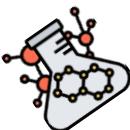
- Sphingomyelin consists of phosphocholine head group, a sphingosine, and a fatty acid.
- It is one of the few membrane phospholipids not synthesized from glycerol.
- The sphingosine & fatty acid (usually unsaturated) can collectively be categorized as a ceramide.





## Summary of functions of phospholipids

- 1) Phospholipids form an essential component of plasma lipoproteins.
- 2) Phospholipids enter into the structure of cell membranes, myelin sheath, mitochondria and lung alveoli.
- 3) Source of arachidonic acid. This is the substrate for the formation of prostanoids, leukotrienes and lipoxins in the cell.
- 4) Hypersensitivity and inflammatory reactions
- 5) Phosphatidic acid is produced as an intermediate in the synthesis of triacylglycerols and phospholipids.
- 6) Cephalin is one of activating factors of coagulation mechanism.
- 7) Phosphatidylserine plays a role in cell cycle signaling, specifically in relation to apoptosis
- 8) Phosphatidylinositol acts as precursor of second messenger (inositol triphosphate), mediating hormonal action inside cells.
- 9) The lecithin-sphingomyelin ratio (L/S ratio or L-S ratio) is the traditional standard for fetal lung maturity testing



## Formative questions

1) Lecithin and cephalin are:

- A. Neutral fats
- B. Glycolipids
- C. Waxes
- D. Phospholipids

2) Lecithin is phospholipid its main component (s) is/are?

- A. Glycerol + 2 fatty acids + H<sub>3</sub>PO<sub>4</sub> + Choline
- B. Glycerol + H<sub>2</sub>PO<sub>4</sub> + Choline
- C. Glycerol + 3 fatty acids
- D. Only glycerol

3) What is the other name of lecithin?

- A. Phosphatidylcholine
- B. Phosphatidylserine
- C. Cardiolipin
- D. Sphingomyelin

4) Plasmalogen synthesis takes place in

- A. Rough endoplasmic reticulum
- B. Smooth endoplasmic reticulum
- C. Mitochondria
- D. Peroxisome

5) Which of the following is a cephalin

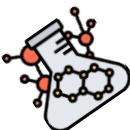
- A. Phosphatidylserine
- B. Phosphatidylethanolamine
- C. Phosphatidylcholine
- D. Phosphatidylinositol

6) Which of the following lipids act as lungs surfactants?

- A. Phosphatidylcholine
- B. Phosphatidylethanolamine
- C. Ceramide
- D. Phosphatidylinositol

7) A phospholipid consists of a

- A. Polar phosphate hydrophilic tail group and hydrophobic fatty acid head
- B. Non-polar phosphate containing head group and polar fatty acid tail
- C. Polar phosphate containing hydrophilic head group and hydrophobic fatty acid tail
- D. Non-polar phosphate containing head group and non-polar fatty acid tail



**8) Which of the following is mostly present in mitochondrial membranes?**

- A. Lecithin
- B. Cephalin
- C. Cardiolipin
- D. Ceramide

**9) Which of the following phospholipids plays an important role in apoptosis?**

- A. Dipalmitoyl lecithin
- B. Cardiolipin
- C. Phosphatidylinositol
- D. Phosphatidylserine

**10) Which of the following phospholipids play an important role in mitochondrial function**

- A. Dipalmitoyl lecithin
- B. Cardiolipin
- C. Phosphatidylinositol
- D. Phosphatidylserine

**11) Lecithin/cholesterol acyltransferase (LCAT converts cholesterol to cholesteryl esters)**

- A. True
- B. False

**12) Classify phospholipids**

---

---

---

---

**13) Enumerate two examples for glycerophospholipids**

---

---

---

---

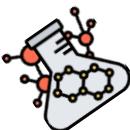
**14) Name one example for sphingophospholipids**

---

---

---

---



# L23&24 Fatty acid synthesis & Lipogenesis

## Lipogenesis

### Definition:

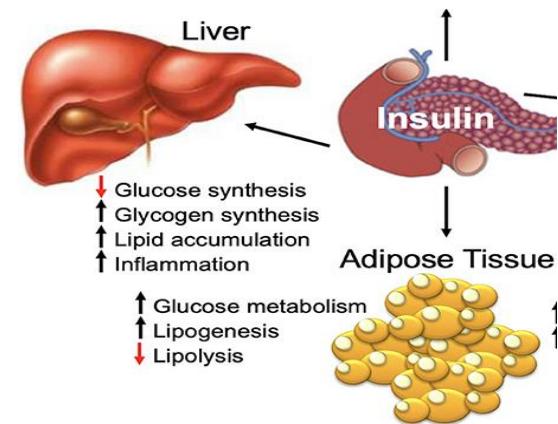
- It is the **biosynthesis of Triacylglycerols (TAGs)** in human body.
- Synthesis of fatty acids and esterification with glycerol.

### Conditions Favoring Lipogenesis:

- Excess of Free Glucose after heavy Carbohydrate meals.
- Insulin promotes Lipogenesis

Thus

- Lipogenesis occur in a well fed condition To transform free excess Glucose to Acetyl-CoA further into Fatty acids.
- Fatty acids are stored as TAG
- Storageable form of Lipid is (TAG).
- TAG in Adiposecytes can be stored in unlimited amounts.



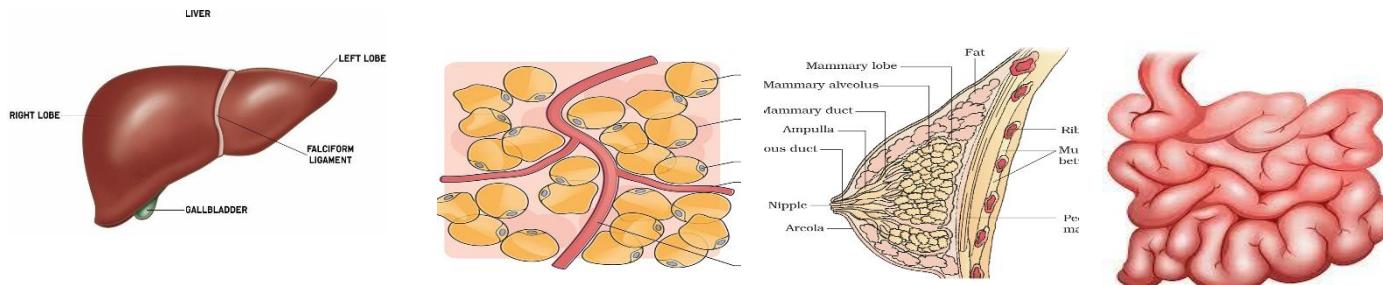
### Site of Lipogenesis:

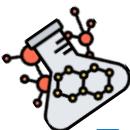
Predominant site for Lipogenesis is **cytoplasm** of

1-Liver.      2- Adipose tissue

### Other tissues for Lipogenesis:

1-Small intestine      2- Mammary glands





## Types of fatty acid synthesis

|                                | Extra-mitochondrial  | Microsomal                                                 | Mitochondrial                                                           |
|--------------------------------|----------------------|------------------------------------------------------------|-------------------------------------------------------------------------|
| 1-Site                         | Cytoplasm            | Endoplasmic reticulum.                                     | Mitochondria                                                            |
| 2-Synthesis                    | de novo              | Elongation of palmitate to longer FA (i.e. C16 to C22,C24) | Elongation of shorter or medium to long chain F.A (i.e. C10,C12 to C16) |
| 3-Source of added carbon atoms | Malonyl CoA          | Malonyl CoA                                                | Acetyl CoA                                                              |
| 4-Carriers                     | Acyl carrier protein | CoASH                                                      | CoASH                                                                   |
| 5-FA. synthase                 | Multi enzyme Complex | Separate enzymes                                           | Separate enzymes                                                        |
| 6-Coenzyme                     | NADPH+H              | NADPH+H                                                    | NADH+H+<br>NADPH+H+                                                     |

### Fatty Acid Synthesis needs

1-Acetyl CoA

2-Malonyl CoA

3-NADPH+H

4-Fatty acid synthase complex

#### Problem:

Fatty acid biosynthesis takes place in the cytosol.

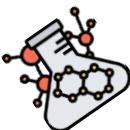
Acetyl-CoA is mainly in the Mitochondria



How is acetyl-CoA made available to the cytosolic fatty acyl synthase?

#### Solution:

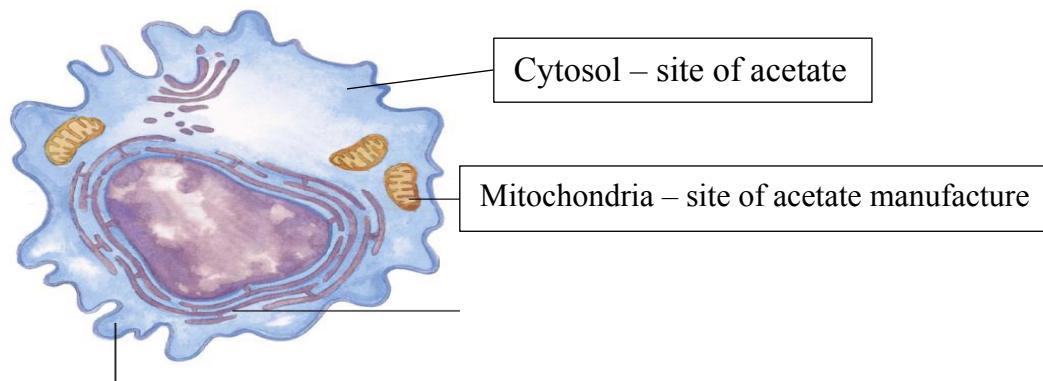
Acetyl-CoA is delivered to cytosol from the mitochondria as Citrate



## 1-Fatty acid synthesis requires considerable amounts of acetyl-CoA

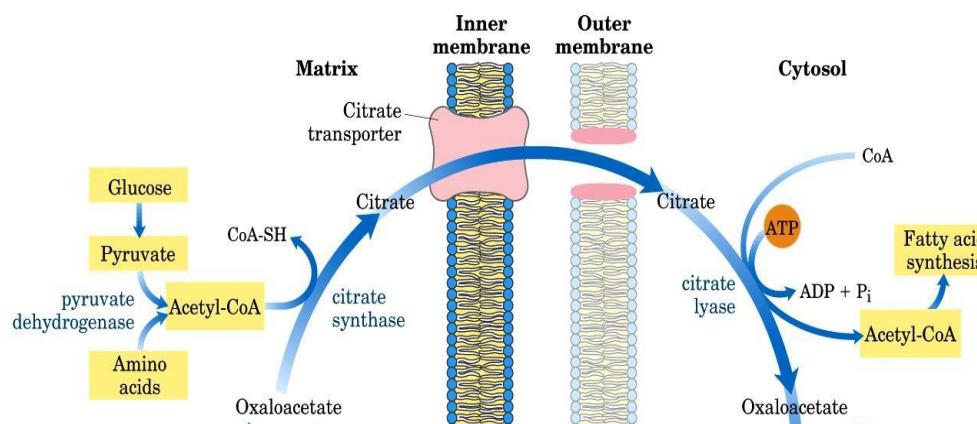
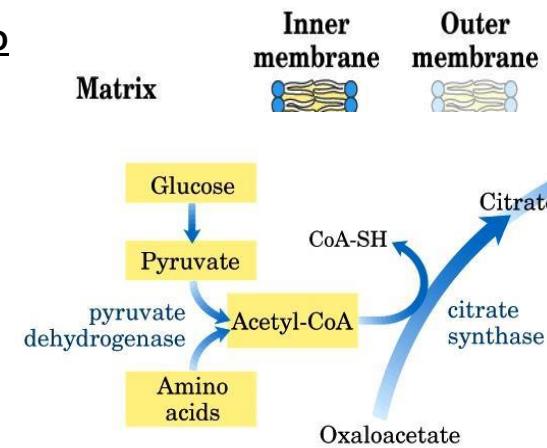


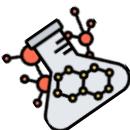
- Nearly all acetyl-CoA used in fatty acid synthesis is formed in mitochondria from **pyruvate oxidation**.
- So acetate must go from the mitochondria to the cytosol



### Acetate is shuttled out of mitochondria as citrate

- The mitochondrial inner membrane is impermeable to acetyl-CoA
- Intra-mitochondrial acetyl-CoA first reacts with oxaloacetate to form citrate, in the TCA cycle catalyzed by citrate synthase.
- Citrate then passes into the cytosol through the mitochondrial inner membrane on the **citrate transporter**.
- In the cytosol, citrate is cleaved by citrate lyase regenerating acetyl-CoA.





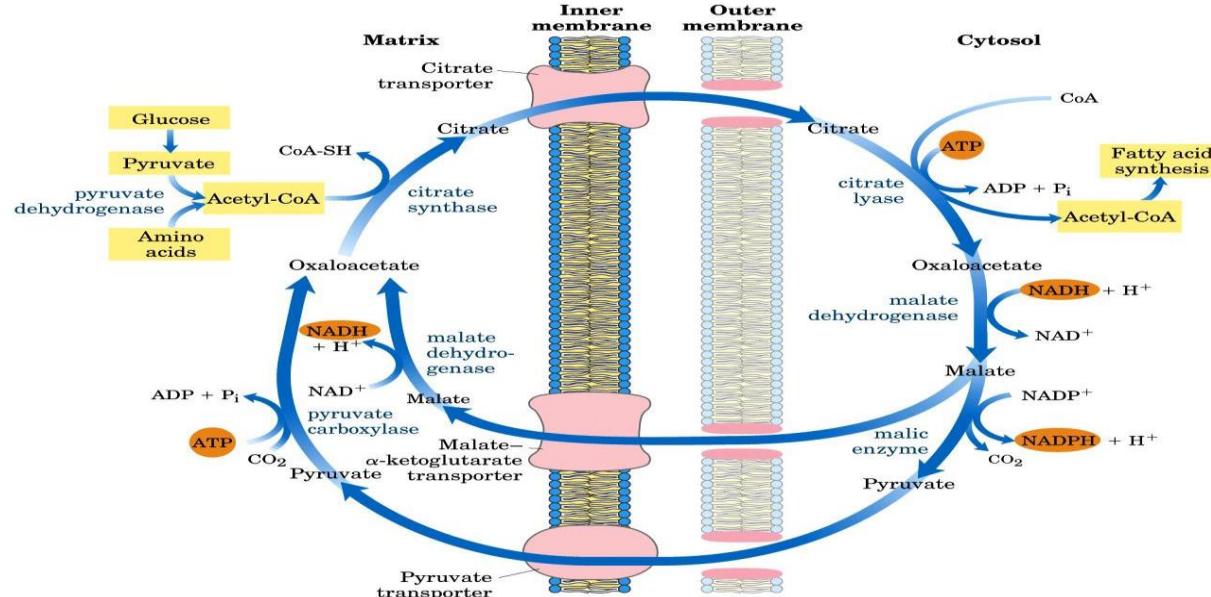
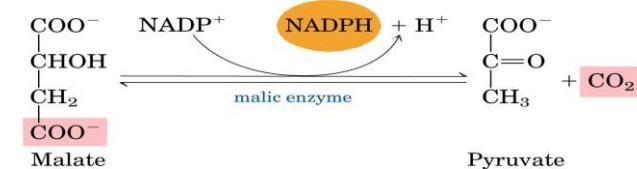
## 2-Fatty acid synthesis requires considerable amounts of NADPH + H+



- In hepatocytes and adipocytes, cytosolic NADPH is largely generated by the malic enzyme and by the pentose phosphate pathway.

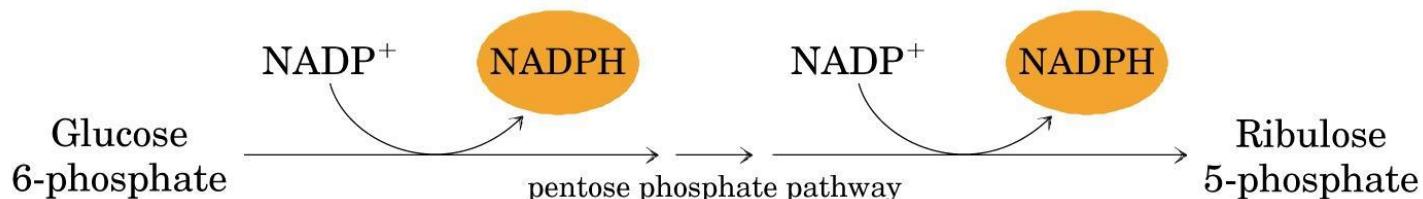
### 1-The malic enzyme

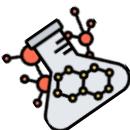
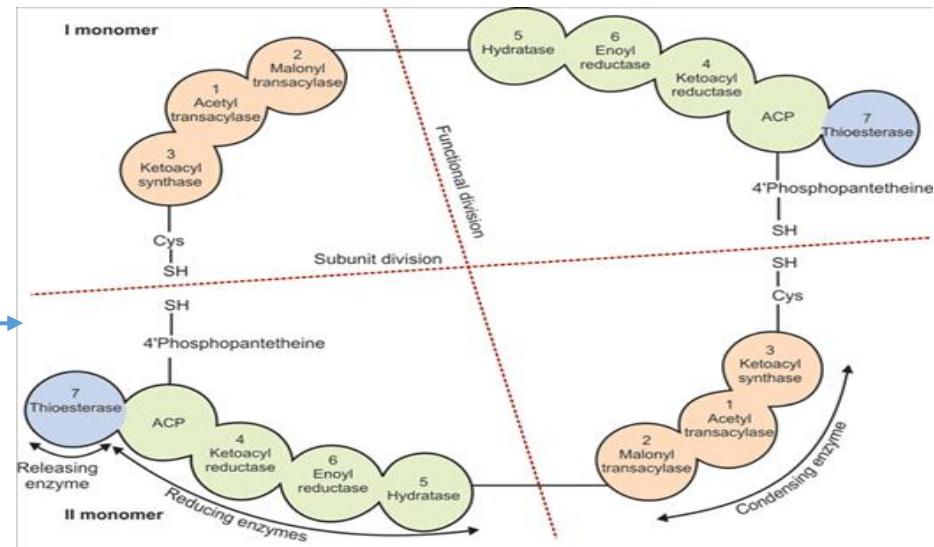
- The pyruvate produced in the reaction reenters the mitochondrion
- The other product --oxaloacetate cannot return to the mitochondrial matrix directly.
- Instead, oxaloacetate is reduced to malate



### 2-The pentose phosphate pathway

In hepatocytes and the mammary gland of lactating animals, the NADPH is supplied primarily by the pentose phosphate pathway.

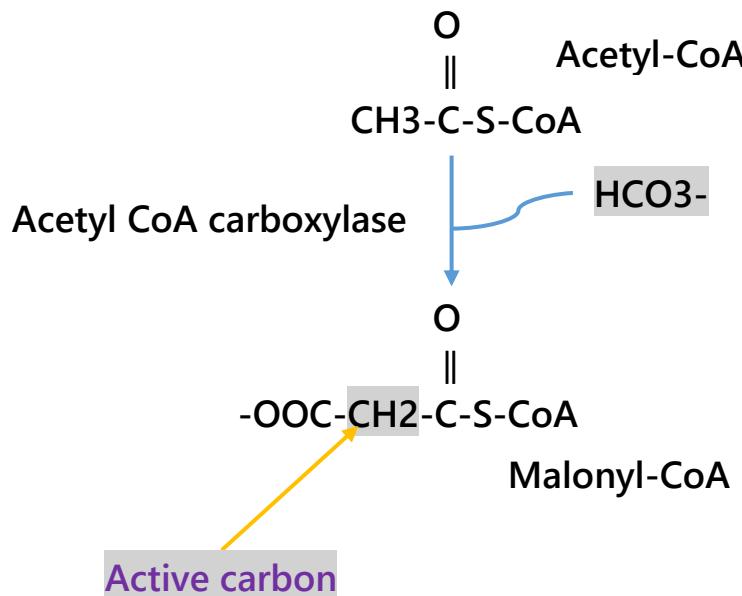


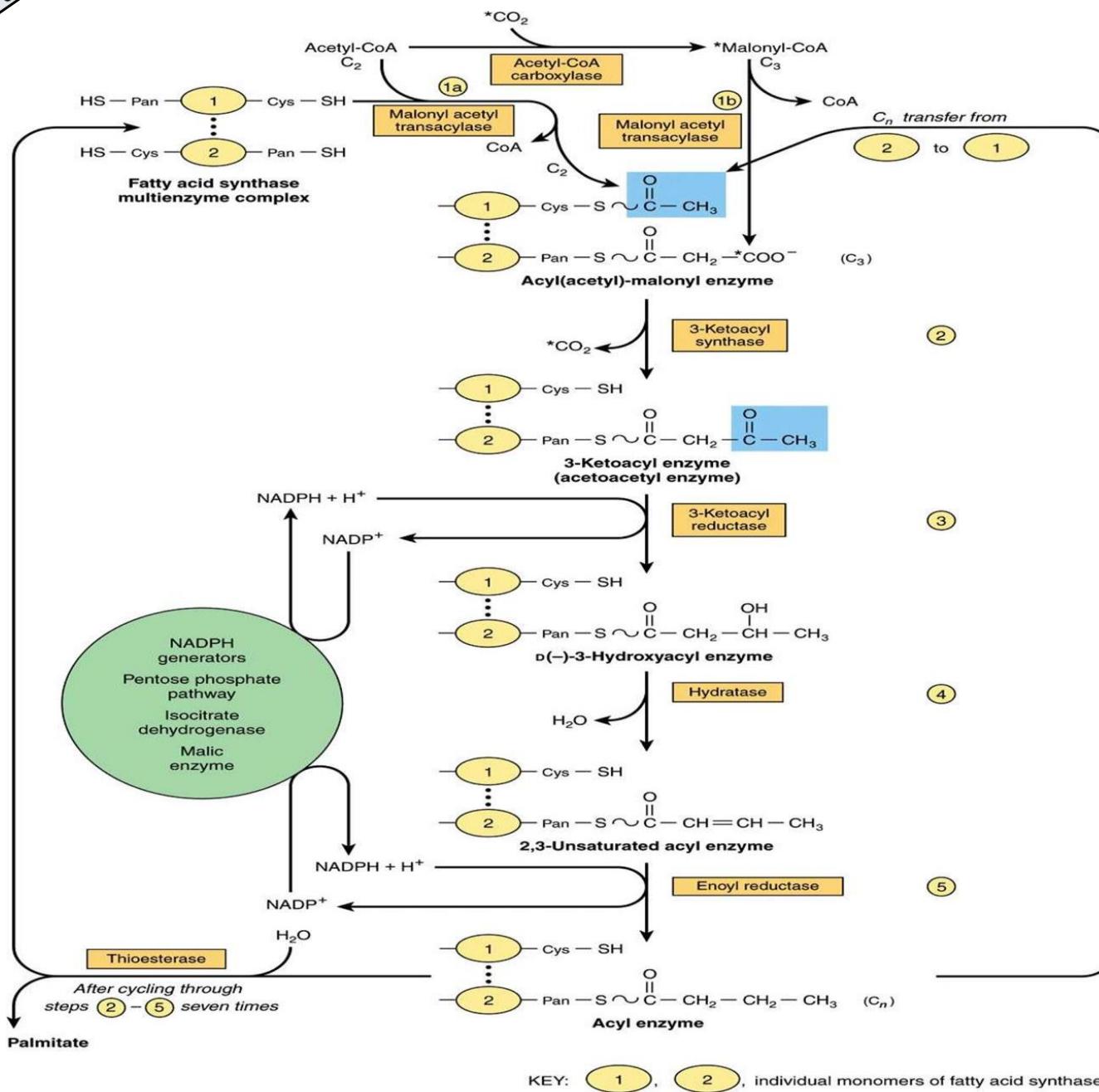
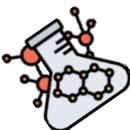
3-Cytosolic Isocitrate dehydrogenase**Fatty Acid Synthesis Steps:**

- |              |               |
|--------------|---------------|
| 1-Activation | 2-Initiation  |
| 3-Elongation | 4-Termination |

**1-Activation**

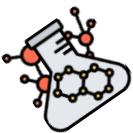
-The carboxylation of acetyl-CoA yields malonyl-CoA





### The result of fatty acyl synthase activity

- Seven cycles of condensation and reduction produce **the 16-carbon saturated palmitoyl group**, still bound to ACP.
- Chain elongation usually stops at this point, and free palmitate is released from the ACP molecule by hydrolytic activity in the synthase complex.



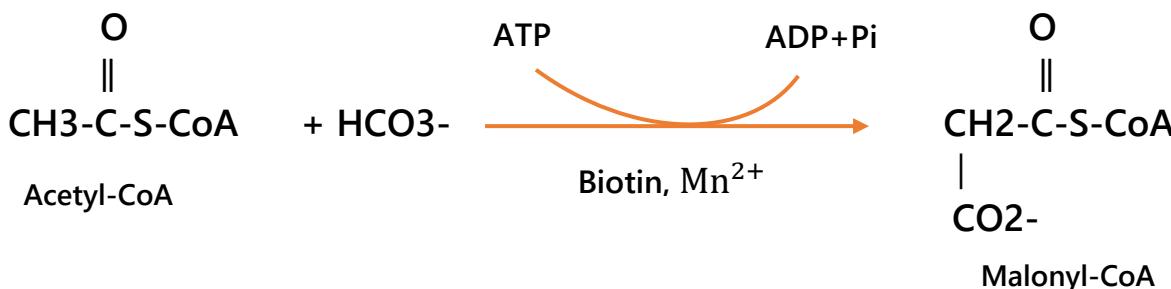
## Synthesis of TAGs

## Regulation Of Fatty Acid Biosynthesis

## 1-Availability of the substrate

- High Carbohydrate
  - High Lipid Diet
  - Acyl-CoA Inhibits Pyruvate Dehydrogenase

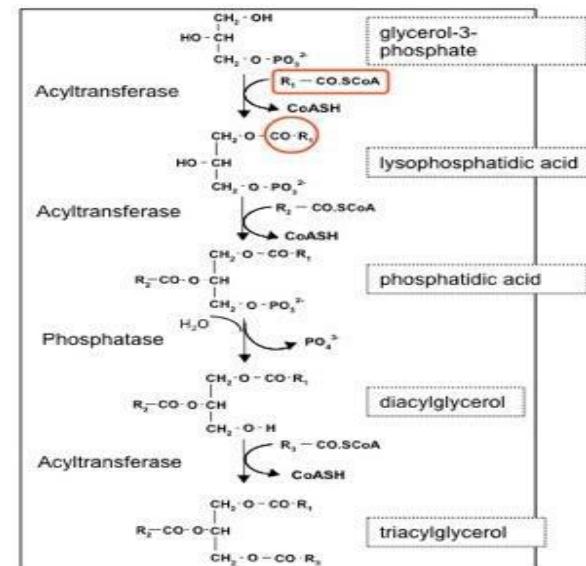
- Enzyme **Acetyl-CoA Carboxylase** Is Key Enzyme Of De Novo Fatty acid Synthesis.
  - Carboxylation of Acetyl-CoA to form Malonyl-CoA is an **Irreversible, committed step** in Fatty acid biosynthesis
  - Committed Step of Fatty Acid Synthesis
    - Carboxylation of Acetyl CoA to Malonyl CoA
    - By Acetyl CoA Carboxylase – Biotin

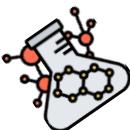


## Modes of Regulation of Acetyl CoA Carboxylase of FA Biosynthesis

## Acetyl-CoA Carboxylase is regulated by 3 modes:

- 1) Induction /repression by Hormones
  - 2) Allosteric Control
  - 3) Covalent Modification





## 1) Induction /repression of ACC

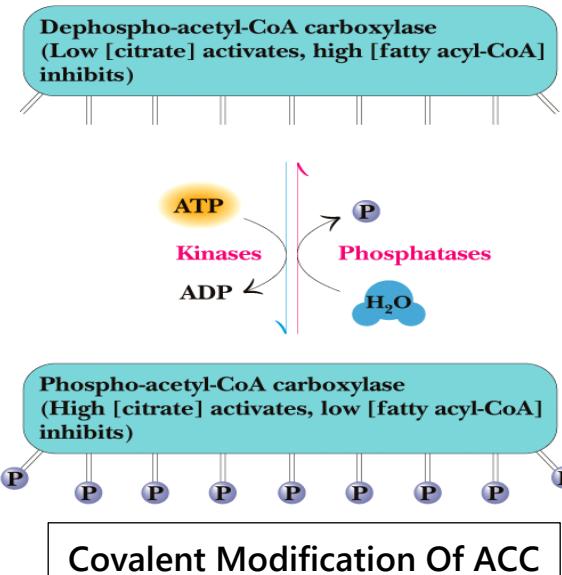
- Induced by Insulin → Insulin activates ACC
- Repressed by Glucagon → Glucagon inhibits ACC

## 2) Allosteric regulation of ACC

- Activated by: Citrate
- Inhibited by: Long Chain Fatty Acid

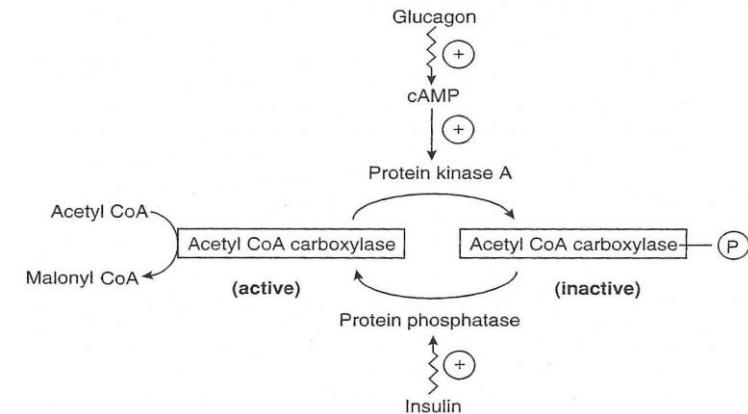
## 3) Covalent Modification of Acetyl-CoA Carboxylase (ACC)

- ACC is Activated by: Dephosphorylation
- ACC is Inhibited by: Phosphorylation



## Covalent Regulation of Acetyl CoA Carboxylase

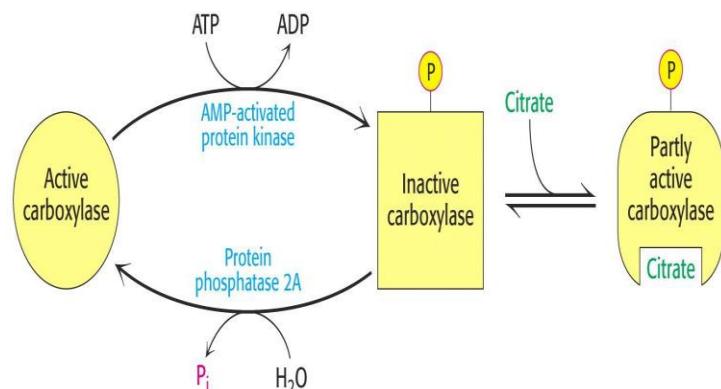
- Activation of ACC
- In a well Fed state:
  - Insulin induces Protein Phosphatase
  - Activates ACC by Dephosphorylation
- Inactivation of ACC
- In a Starved state:
  - Glucagon increases cAMP
  - Activates Protein kinase A
  - Inactivates ACC by Phosphorylation

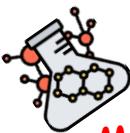


N.B:

Biosynthesis and Degradation of Fatty Acid are Reciprocally Regulated

Very Well Coordination and Regulation of Lipolysis and Lipogenesis is A Healthy Lipid Metabolism





**Match each with the description below.**

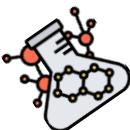
- |                 |               |                |
|-----------------|---------------|----------------|
| 1) Mitochondria | 2) cytosol    | 3) glucagon    |
| 4) Insulin      | 5) acetyl ACP | 6) malonyl ACP |

### Answers

- A. site of de novo fatty acid synthesis  
B. site of  $\beta$  oxidation  
C. starting material for lipogenesis  
D. compound added to elongate acyl-ACP  
E. activates  $\beta$  oxidation  
F. activates lipogenesis

- |                 |
|-----------------|
| 2) cytosol      |
| 1) mitochondria |
| 5) acetyl ACP,  |
| 6) malonyl ACP  |
| 3) glucagon     |
| 4) insulin      |





## L25 & 26 : Lipid metabolism \_Lipolysis and fatty acid oxidation

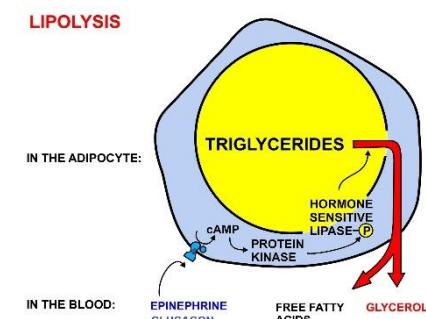
### LIPOLYSIS (MOBILIZATION OF TRIACYLGLYCEROLS)

#### ➤ Mobilization of Triacylglycerols:

In the Adipose Tissue, Triacylglycerols are broken down to Free Fatty Acids and Glycerol (fatty acids are hydrolyzed initially from C1 or C3 of the fat)

#### ➤ Hormone Sensitive Lipase:

- Cleaves a Fatty Acid from Triacylglycerol, then other Lipase complete the process.
- Fatty Acids are released into the blood to be carried by Serum Albumin.



The hydrolysis of TG is done by 3 tissue lipases:

1. Hormone sensitive lipase initiates the process of lipolysis in the adipose tissue
2. Diacylglycerol lipase.
3. Monoacylglycerol lipase.

**Diacylglycerol and monoacylglycerol lipases** rapidly complete the hydrolysis of di and monoacylglycerols releasing free fatty acids and glycerol



### REGULATION OF LIPOLYSIS

#### a) Short Term

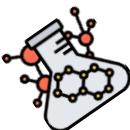
- Substrate Availability
- Covalent Modification (Phosphorylation)
- Allosteric Modification

#### b) Long Term:

- Change in Rate of Protein (Enzyme) Synthesis or Degradation

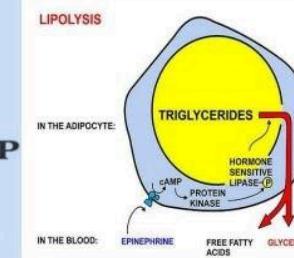
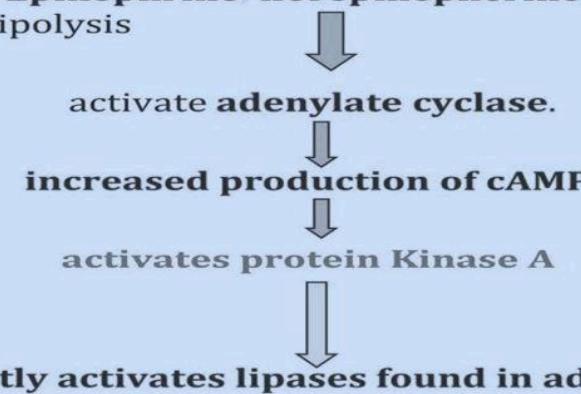
### HORMONAL CONTROL OF LIPOLYSIS \ LIPOLYSIS IS UNDER HORMONAL CONTROL

| Lipolytic hormones                                                                                                                                                                                       | Antilipolytic Hormone |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| <ul style="list-style-type: none"> <li>• Epinephrine</li> <li>• Norepinephrine</li> <li>• Glucagon</li> <li>• Adrenocorticotropic hormone (ACTH)</li> <li>• Thyroid stimulating hormone (TSH)</li> </ul> | Insulin               |



The activity of ***hormone sensitive lipase*** in the adipose cells is regulated by different hormones.

- Glucagon, Epinephrine, norepinephrine stimulate lipolysis

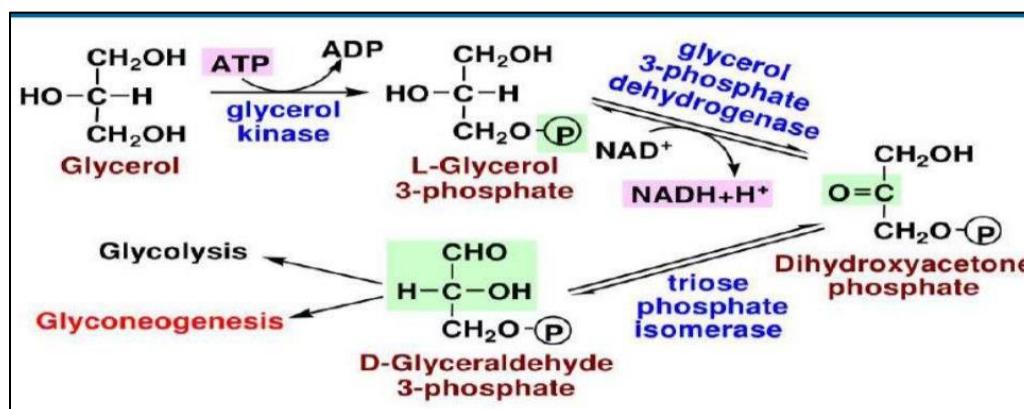


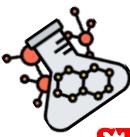
## FAT MOBILIZATION

- The triacylglycerol stored in the adipocytes are hydrolyzed by lipases, to produce free fatty acids (FFA) and glycerol, which are released to the blood, this process is called fat mobilization.
- The fatty acids are thus released diffusively from the adipocyte into the blood, where they bind to the **SERUM ALBUMIN**

## GLYCEROL METABOLISM:

Place : Liver, Kidney, Intestine.





## STAGES AND REACTION STEPS OF BETA OXIDATION OF FATTY ACIDS

### STAGE I: ACTIVATION OF LONG CHAIN FATTY ACID (ACYL CHAIN) TO ACYL-COA IN CYTOSOL

- IS A PREPARATIVE PHASE
- PALMITATE TO PALMITOYL-COA IN CYTOSOL

### STAGE II:

TRANSLOCATION OF ACTIVATED FATTY ACID FROM CYTOSOL INTO MITOCHONDRIAL MATRIX THROUGH ROLE OF CARNITINE (CARNITINE SHUTTLE).

### STAGE III: STEPS OF BETA OXIDATION PROPER IN MITOCHONDRIAL MATRIX:

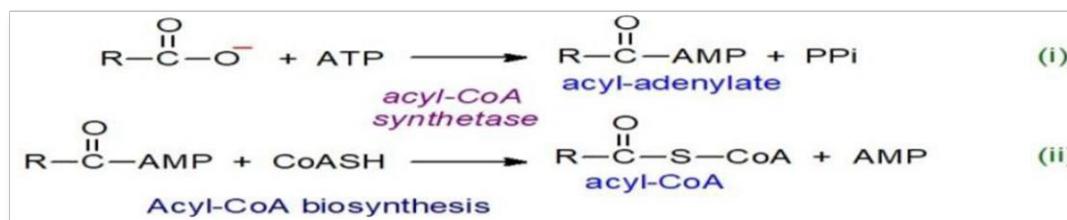
- OXIDATION REACTION
- HYDRATION REACTION
- OXIDATION REACTION
- CLEAVAGE REACTION

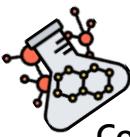
### STAGE I : SITE OF FATTY ACID :

- Activation of FATTY ACID(ACYL CHAIN) IS ACTIVATED IN CYTOSOL TO ACYL-COA
- Requirements of FA Activation:
  - **Enzyme:** Thiokinase /Acyl CoA Synthetase
  - **Coenzymes/Cofactors:**
    - ✓ CoA-SH derived from Pantothenic acid
    - ✓ ATP
    - ✓ Magnesium ions ( $Mg^{++}$ )

### Fatty acid Activation:

- Activation of Fatty Acids is Esterification of Fatty Acid with Coenzyme A
- In presence of Acyl-CoA Synthetase (Thiokinase) forming an activated Fatty Acid as Acyl-CoA
- This process is **ATP-dependent**
- There are different Acyl-CoA Synthetase for fatty acids of different chain lengths.

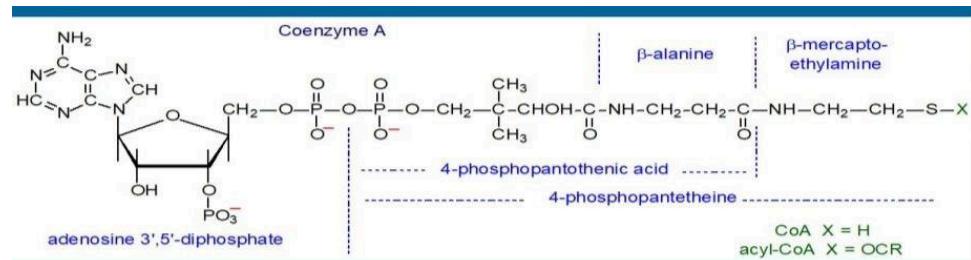




## Data Zone 43

## Biochemistry

CoA Helps in Activation of Fatty Acid :

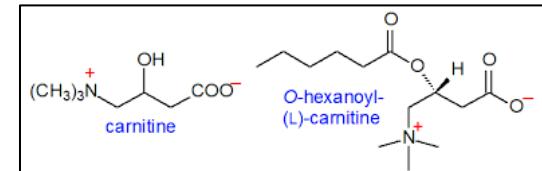


### STAGE II

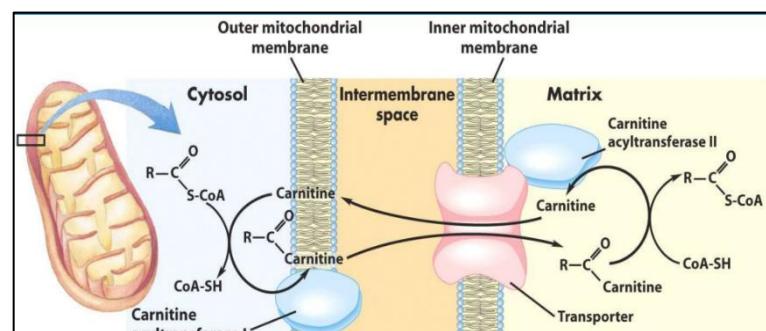
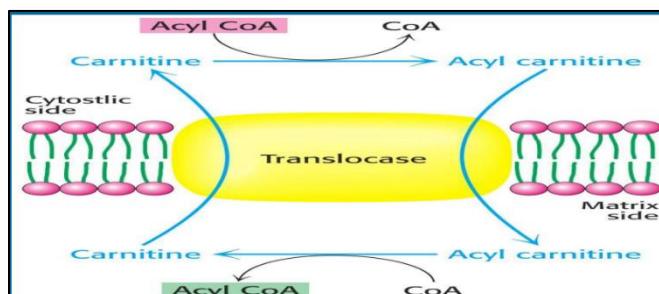
- Translocation of Acyl-CoA from Cytosol into Mitochondrial Matrix with the Help of **CARNITINE**
- $\beta$ -oxidation Proper Occurs in Mitochondrial Matrix
- CoA is a Complex Structure
- Inner Mitochondrial Membrane is Impermeable to CoA
- Long-chain Fatty Acids more than 12 Carbon Atoms Cannot be Directly Translocated into the Mitochondrial Matrix.
- However Short Chain Fatty Acids are Directly Translocated into the Mitochondrial Matrix
- Long chain Acyl CoA Traverses an Inner Mitochondrial Membrane with a Special Transport Mechanism called **CARNITINE SHUTTLE**.

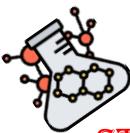
### WHAT IS Carnitine ?

- Carnitine is a Functional, Non Protein Nitrogenous (NPN) substance
- Carnitine is Synthesized in the Body by Amino Acids Lysine and Methionine (3-Hydroxy 4-Tri Methyl Ammonium Butyrate)



### Mechanism of Carnitine in Transport of Fatty Acyl CoA from Cytosol to Mitochondrial Matrix

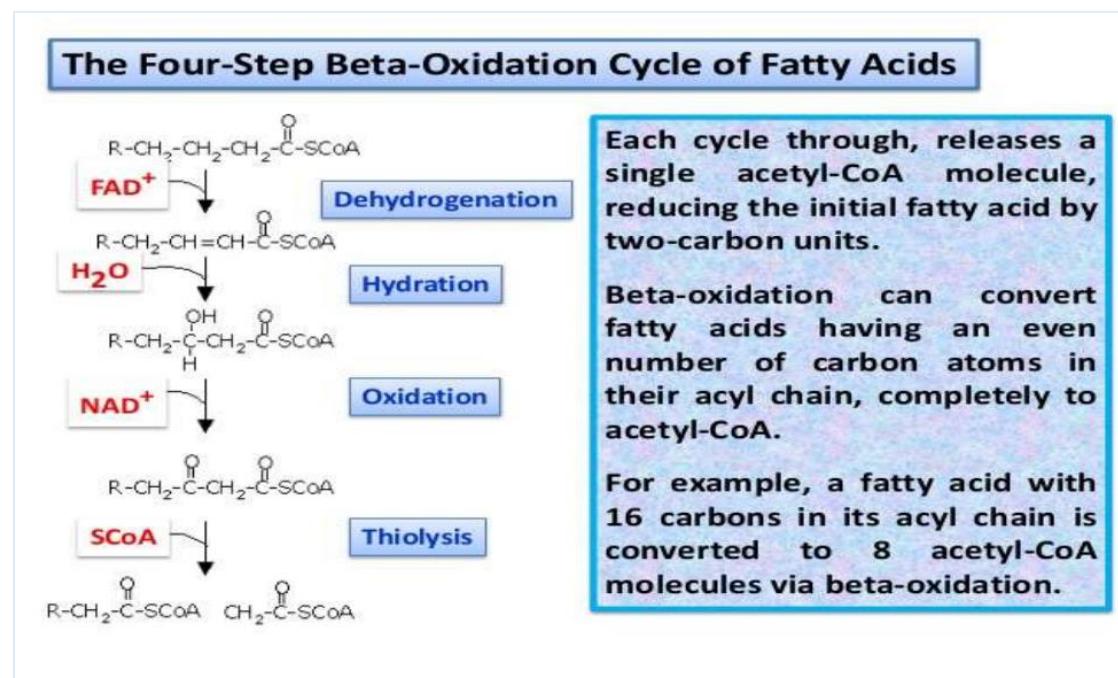


STAGE III: STEPS OF BETA OXIDATION PROPER/CYCLE IN MITOCHONDRIAL MATRIX

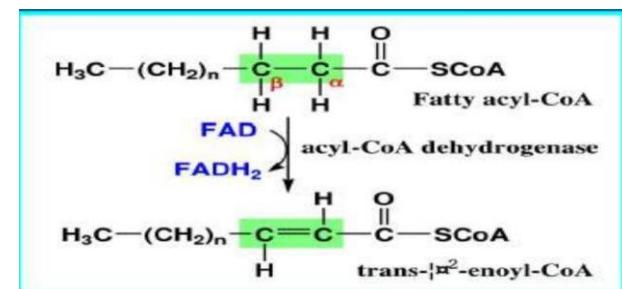
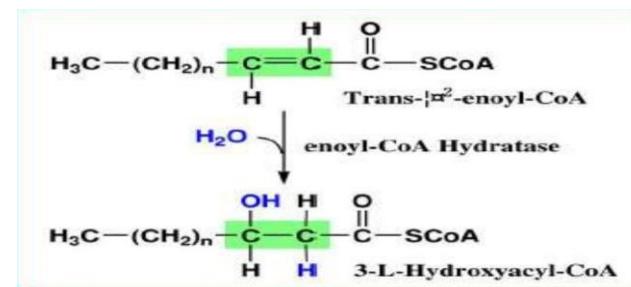
a) B-oxidation means B-C Reaction

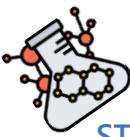
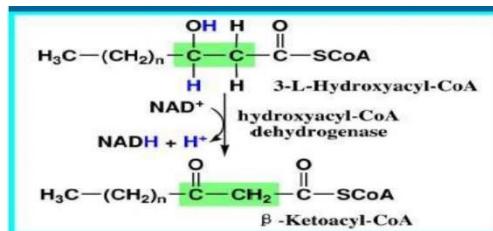
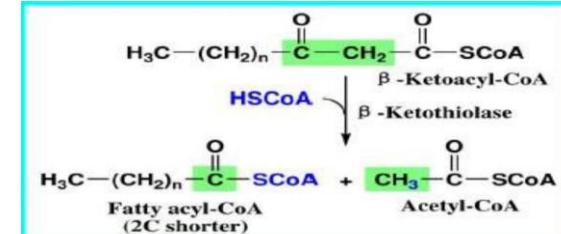
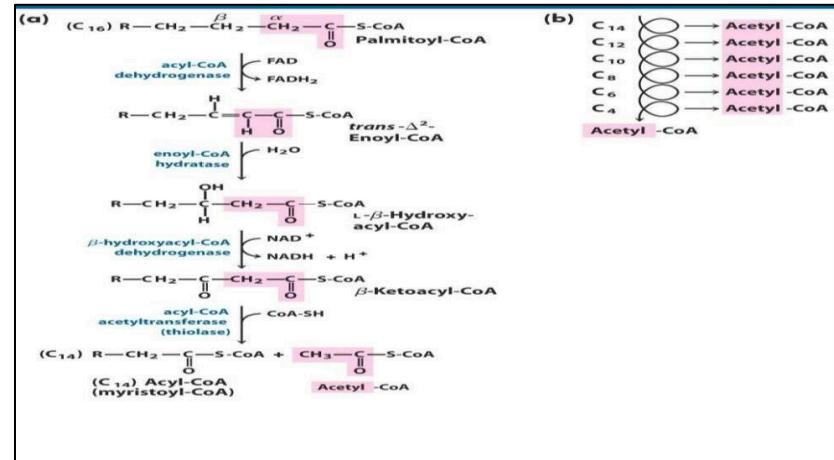
4 Steps in one round:

- ✓ Step 1: Dehydrogenation (FAD linked Acyl CoA Dehydrogenase)
- ✓ Step 2: Hydration (Enoyl CoA Hydratase)
- ✓ Step 3: Dehydrogenation (NAD linked  $\beta$  hydroxy Acyl CoA Dehydrogenase)
- ✓ Step 4: Thiolytic cleavage (Keto Thiolase)

STEP 1. DEHYDROGENATION:

- There are different Acyl-CoA Dehydrogenases:
  1. Short Chain Fatty acids (4-6 C)
  2. Medium Chain Fatty Acids (6-10 C)
  3. Long (12-18 C)
  4. Very Long Chain Fatty Acids (22 and more)
- Acyl CoA Dehydrogenase is inhibited by Hypoglycin A (from Ackee Fruit).

STEP 2. HYDRATION:

STEP 3. DEHYDROGENATIONStep 4. Thiolytic Cleavage**B-OXIDATION OF FATTY ACIDS:****B-OXIDATION SUMMARY ONE CYCLE:**

Fatty acyl CoA + FAD + NAD<sup>+</sup> + HS CoA  $\rightarrow$  Fatty acyl CoA (2C less) + FADH<sub>2</sub> + NADH + H<sup>+</sup> + acetyl CoA

Products Of Each Turn of Beta Oxidation Proper

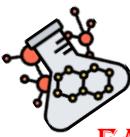
Each turn/cycle of  $\beta$  oxidation proper generates one molecule each of:

- ✓ FADH<sub>2</sub>
- ✓ NADH+H<sup>+</sup>
- ✓ Acetyl CoA
- ✓ Fatty Acyl CoA (with 2 carbons shorter each round)

CYCLES OF B-OXIDATION:

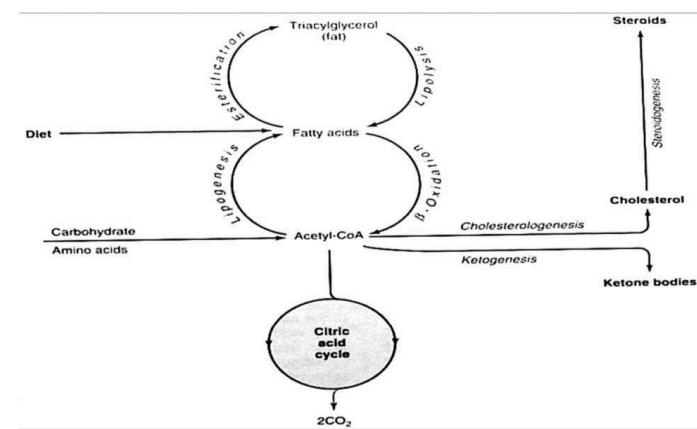
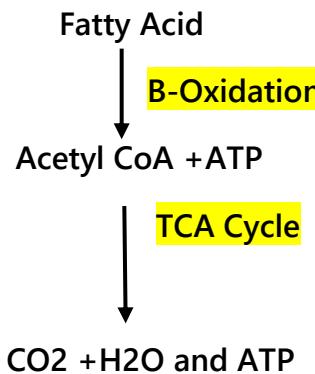
The length of fatty acid determines the No of oxidations & Total No of Acetyl CoA groups :

| Carbons in Fatty Acid | Acetyl CoA (C/2) | $\beta$ -Oxidation Cycles (C/2 - 1) |
|-----------------------|------------------|-------------------------------------|
| 12                    | 6                | 5                                   |
| 14                    | 7                | 6                                   |
| 16                    | 8                | 7                                   |
| 18                    | 9                | 8                                   |



## FATE OF THE PRODUCTS OF $\beta$ -OXIDATION OF FATTY ACID

- NADH+H<sup>+</sup> and FADH<sub>2</sub> - are Reoxidized in ETC to Generate ATP
- Acetyl CoA - Enters the Citric Acid Cycle (TCA cycle) for its Complete Oxidation
- Acyl CoA – Undergoes the Next Turn/Cycle of  $\beta$ -oxidation Proper

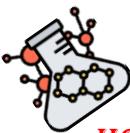


## ENERGETICS OF FATTY ACID BETA OXIDATION e.g. Palmitic (16C)

- $\beta$ -oxidation of Palmitic acid will be repeated in 7 cycles producing 8 molecules of Acetyl COA
- In each cycle 1 FADH<sub>2</sub> and 1 NADH+H<sup>+</sup> is produced and will be transported to the respiratory chain/ETC.
- FADH<sub>2</sub> 2 ATP  $\ominus$  NADH + H<sup>+</sup> 3 ATP
- Thus, Each cycle of  $\beta$ -oxidation 05 ATP So 7 cycles of  $\beta$ -oxidation  $5 \times 7 = 35$  ATP
- 1 Acetyl CoA Yields 12 ATPs via TCA Cycle (8 Acetyl CoA)  $\rightarrow 8 \times 12 = 96$  ATP
- 1 ATP converted to AMP during activation of Palmitic acid to Palmitoyl-CoA is equivalent to 2ATPs utilized, hence
  - 2 ATP are utilized in the activation of Fatty acid
  - Energy gain = Energy produced - Energy utilized
  - $35 \text{ ATP} + 96 \text{ ATP} - 2 \text{ ATP} = 129 \text{ ATP}$



انا جاي اقولك  
 كل سعي يا  
 صاحبى مفيش  
 موجود بيبقىع.



## HORMONAL REGULATION OF $\beta$ - OXIDATION OF FATTY ACIDS

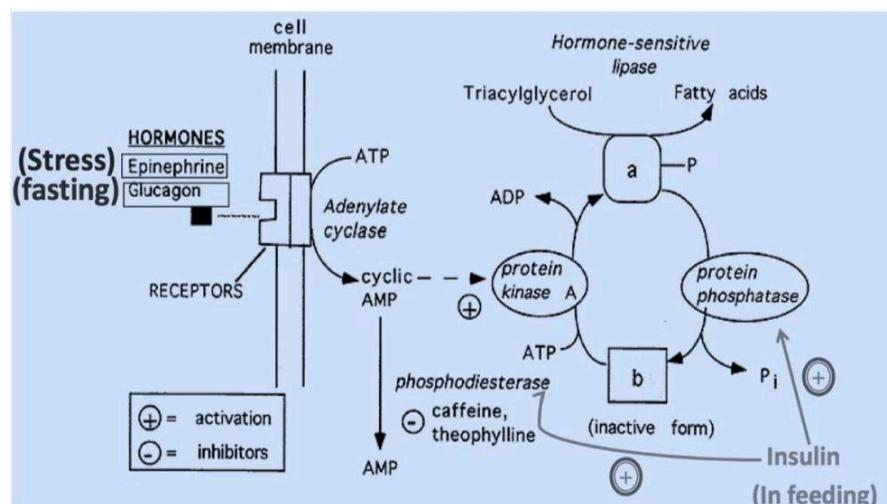
- Lipolysis and  $\beta$  Oxidation of Fatty Acids are well Regulated under Hormonal Influence

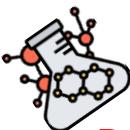
### INSULIN

- Insulin is Secreted in Well Fed Condition
- Insulin Inhibits Lipolysis of Adipose Fat (TAG) and Mobilization of Free Fatty Acids by Stimulating Phosphodiesterase Enzyme which inhibits Hormone Sensitive Lipase by Dephosphorylation
- Decreases  $\beta$  Oxidation of Fatty Acids

### GLUCAGON AND EPINEPHRINE:

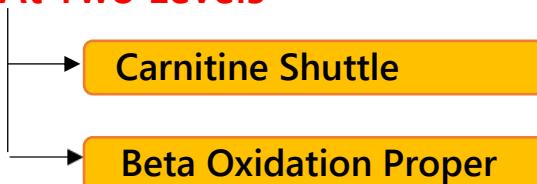
- Glucagon and Epinephrine Stimulate Lipolysis in Emergency Conditions when Cellular or Blood Glucose Lowers Down
- Stimulate Hormone sensitive Lipase and hydrolyzes depot Fat (TAG)
- Free Fatty Acids are Mobilized out into Blood
- Increases  $\beta$  Oxidation of Fatty Acids





## Regulation of Beta Oxidation of Fatty Acid

### At Two Levels



### Regulation at Carnitine Shuttle

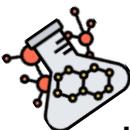
- Transport of Fatty Acyl CoA from Cytosol into Mitochondrial Matrix Via Carnitine Shuttle Is a Rate-limiting step.
- Malonyl-CoA (Intermediate of Lipogenesis) is an Inhibitor of Carnitine Acyl Transferase I

### Regulation at Beta Oxidation Proper

- Acyl-CoA Dehydrogenase is Regulatory or Key Enzyme of Beta Oxidation of Fatty Acids.

### Significance of Beta Oxidation of a Fatty acid

- Beta Oxidation Cycles Helps in Cleaving and Shortening of a Long Chain Fatty Acids.
- Oxidation of Beta Carbon Atom of a Fatty Acid Transforms Stronger Bond Between Alpha and Beta Carbon Atom to a Weaker Bond.
- Transformation to a Weaker Bond Helps in Easy Cleavage between Alpha and Beta Carbon.
- During  $\beta$  Oxidation there is Dehydrogenation of Beta Carbon atom ( $\text{CH}_2$  to  $\text{C}=\text{O}$ ).
- Hydrogen Atoms Removed During  $\beta$ - Oxidation Are Temporarily Accepted by the Oxidized Coenzymes (FAD and NAD) to form Reduced Coenzymes.
- Reduced Coenzymes Then Finally Enter ETC and Get Reoxidized.
- The byproduct of ETC is ATP.
- Thus  $\beta$ -Oxidation of FA Metabolizes a Long Chain Fatty Acid with Liberation of Chemical Form of Energy ATP for Cellular Activities.
- Complete  $\beta$ -oxidation of one Palmitic FA. Yields 129 Mol. of ATP.



- Large Energy Yield is Consequence of the Highly Reduced State of the Carbon in Fatty Acids.
- This Makes FA the Fuel of Choice.

### Disorders of Beta Oxidation:

- A- Carnitine deficiency.
- B- Sudden infant death syndrome (SIDS).
- C- Jamaican vomiting sickness.

#### Carnitine deficiency

Either due to:

- 1- Primary or Secondary Carnitine Deficiency.
- 2- Carnitine Transferase Deficiency
- 3- Translocase Activity Deficiency

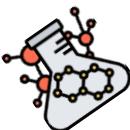
All are Related to Disease State

#### Biochemical Consequences of Carnitine Shuttle Defect

- ❖ Defect in Carnitine Shuttle System.
- ❖ No Beta Oxidation of Fatty Acids.
- ❖ No ATP Generation.
- ❖ All ATP Dependent Processes will be Ceased.
- ❖ Cell Deaths.
- ❖ Organ Failures.

#### Carnitine Shuttle Defects

- Affects normal function of Muscles, Kidney and Heart.
- Symptoms include Muscle cramping during exercise, severe weakness and death.
- Muscle weakness occurs since they are related with Fatty acid oxidation for long term energy source.
- Individuals with Carnitine Shuttle Defects Should be supplemented with a Diet with Medium Chain fatty acids, Since MCFAs do not Require Carnitine Shuttle to Enter Mitochondria.

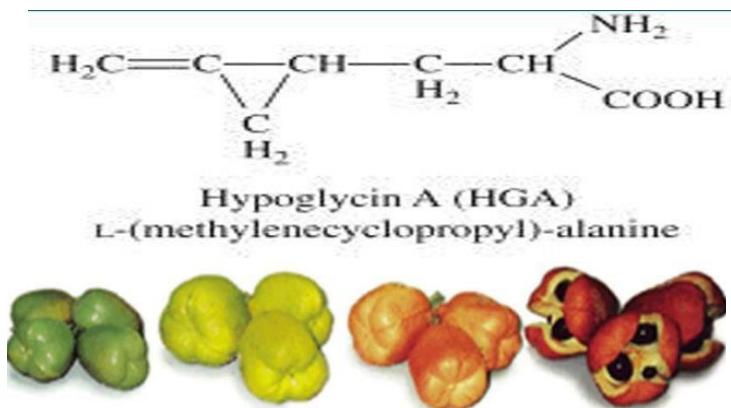


## Sudden Infant Death Syndrome (SIDS)

- SIDS is a Congenital Rare Disorder with an Incidence of 1 in 10,000 births.
- Due to congenital defect of Enzyme Acyl-CoA Dehydrogenase Result in Deficiency of Acyl-CoA Dehydrogenase.
- Blocks  $\beta$  Oxidation of Fatty Acids.
- Stops Supply of Energy in Form of ATPs in Fasting Condition.
- Leads to Unexpected Infant Death.

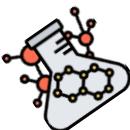
## Jamaican Vomiting Sickness

- Is due to ingestion of unripe Ackee fruit by people in Jamaica.
- Ackee Fruit is Rich in Hypoglycin -A.
- Hypoglycin is an inhibitor of regulatory Enzyme  $\beta$  Oxidation Proper Acyl-CoA Dehydrogenase.
- Jamaican Vomiting Disease Leads to:
  - Severe Vomiting (throwing out).
  - Hypoglycemia.
  - Water Electrolyte Imbalance.
  - Convulsions.
  - Coma.
  - Death.



## Beta Oxidation of Odd Chain Saturated Fatty Acids

- Ingestion of Odd Chain Fatty Acids Are **Less Common in Human Body**.
- Odd Chain Fatty Acids Are Formed by Some Bacteria in the Stomachs of Ruminants and the **Human Colon**.
- $\beta$ -oxidation of odd chain Saturated Fatty Acid occurs **Same as Even Chain Fatty Acid Oxidation**.
- Releasing Acetyl CoA (2C) in every turn.
- **Until the final Thiolase cleavage**.
- Which results in a **3 Carbon Acyl-CoA /Propionyl-CoA** in Last Cycle and Last Step of Beta Oxidation.
- **Propionyl CoA (3C) Is Converted into Succinyl CoA (4C) A TCA intermediate.**



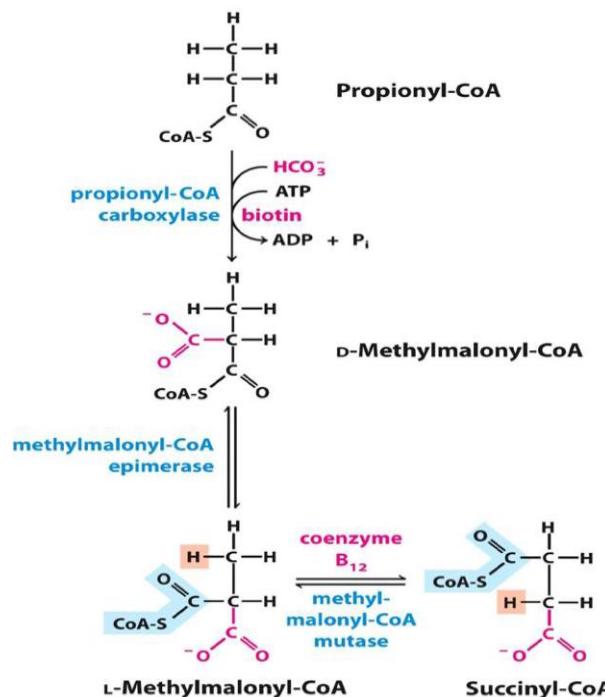
## Propionyl CoA Metabolism is Dependent on

## Three Enzymes

- Carboxylase
- Epimerase
- Mutase

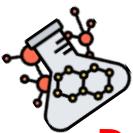
## Two Vitamin B Complex Members

- Biotin
- Vitamin B12



## Fates of Succinyl CoA

- ❖ Enters TCA Cycle and Get Metabolized.
- ❖ Serve as **Glucogenic Precursor** for Glucose Biosynthesis in Emergency Condition.
- ❖ Used as a **Precursor for Heme Biosynthesis**.
- ❖ Involves in **Thiophorase Reaction of Ketolysis**.



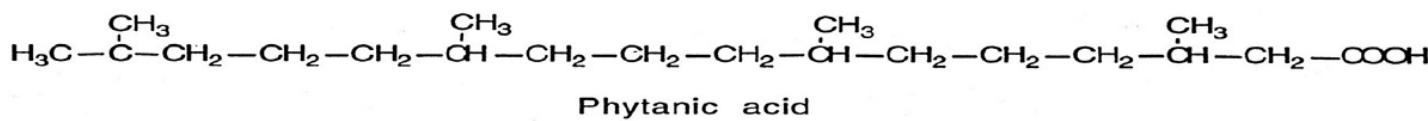
## Defects in Propionyl CoA Metabolism

- ❖ Deficiency of Enzyme Propionyl-CoA Carboxylase will block the metabolism of Propionyl-CoA.
  - ❖ Accumulates Propionyl-CoA in blood leading to **Propionicacidemia**.
  - ❖ **Deficiency of Vitamin B Complex Members** affects Propionyl CoA metabolism to Succinyl -CoA.
  - ❖ **Vitamin B12** deficiency blocks the **Mutase Reaction** Accumulates L-Methyl Malonyl-CoA leading to **Methyl Malonylaciduria**.

## Alpha Oxidation of Branched-Chain FA

## Phytanic Acid Oxidation

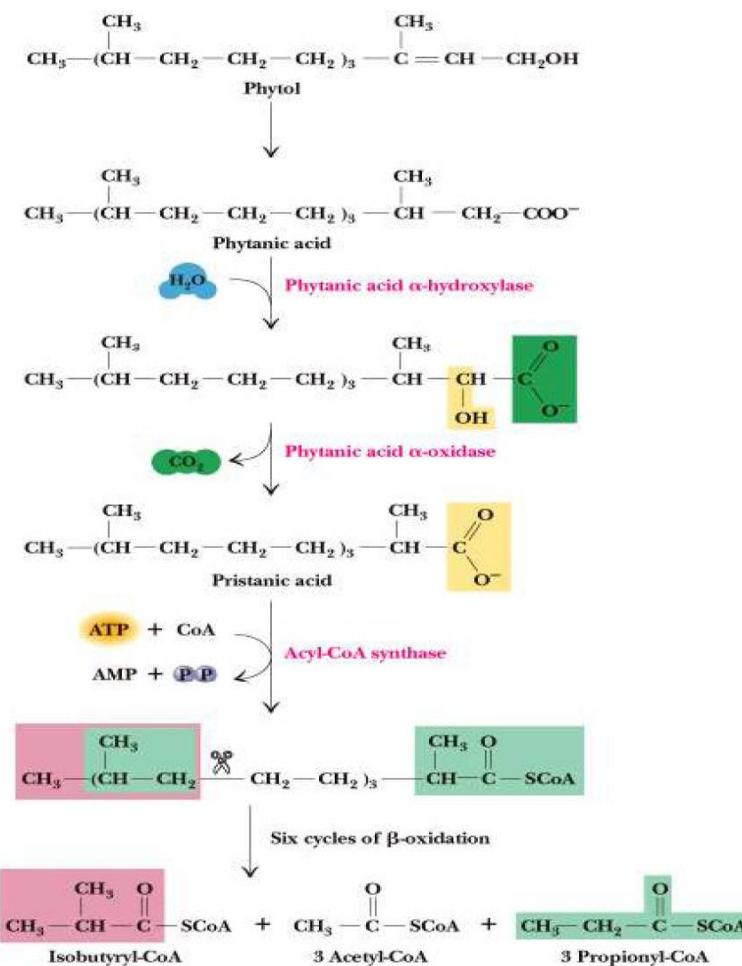
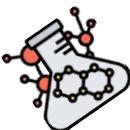
- ❖ Source of Phytanic Acid in Human Body is through Ingestion of Animal Foods.
  - ❖ Phytanic acid is a Breakdown Product of Phytol Component of Plant Chlorophyll.
  - ❖ Phytanic acid (or 3,7,11,15-tetramethyl hexadecanoic acid) is a 16 Carbon Branched Chain Fatty Acid.
  - ❖ Has Four Methyl branches at odd-number carbons 3,7,11 and 15 which is Not Good Substrates for  $\beta$ -oxidation.



- ❖ Alpha Oxidation of Phytanic Acid Takes Place in Peroxisomes.
  - ❖ Initially Phytanic acid is converted to Pristanic acid and Further Present it for Beta Oxidation Process.

## ❖ During $\alpha$ Oxidation:

- Hydroxylation at  $\alpha$  Carbon to  $\alpha$  Hydroxy Acyl-CoA in presence of Enzyme **Hydroxylase** This reaction is **Vitamin C Dependent**.
  - $\alpha$  Hydroxy Acyl-CoA is then Oxidized to  $\alpha$  Keto Acyl-CoA.
  - **Ketonic Group at  $\alpha$  Carbon** Atom is Decarboxylated Yielding CO<sub>2</sub> mol. and a Fatty Acid with One Carbon Atom Less **Pristanic Acid**.
  - Which is Further Metabolized Via Beta Oxidation Process to Generate Propionyl-CoA.



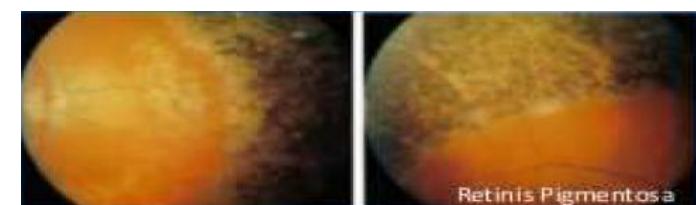
## Disorders Associated with Defective $\alpha$ Oxidation of Phytanic Acid Refsums Disease

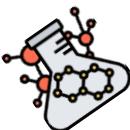
- ❖ Rare Autosomal Recessive Disease.
- ❖ Deficiency of Enzyme **Phytanic acid  $\alpha$  Oxidase**.
- ❖ No Oxidation of Phytanic acid.
- ❖ Accumulation of Phytanic acid in Brain cells and other Tissues.
- ❖ Brain Dysfunction and Neurological Disorders.
- ❖ Management by Avoid eating diet contain Phytol /Phytanic acid.

## Symptoms

### How do I know if I have Refsum Disease?

- ❖ Impaired eyesight (Retinitis Pigmentosa)
  - Loss of night vision in childhood → disrupted peripheral vision → blindness.
  - Most apparent and serious symptom.
- ❖ Deafness
  - Apparent later in life.
- ❖ Loss of smell (Anosmia)
  - Always apparent in patient.





- ❖ Balance or coordination problems (Ataxia).
  - Apparent later in life.
- ❖ Dry, scaly skin (Ichthyosis).
  - Apparent later in life
- ❖ Heartbeat abnormalities
  - (Cardiac arrhythmias).
- ❖ Shortened fingers or toes
  - Disease usually apparent in childhood, although sometimes symptoms may not develop until 40s or 50s.

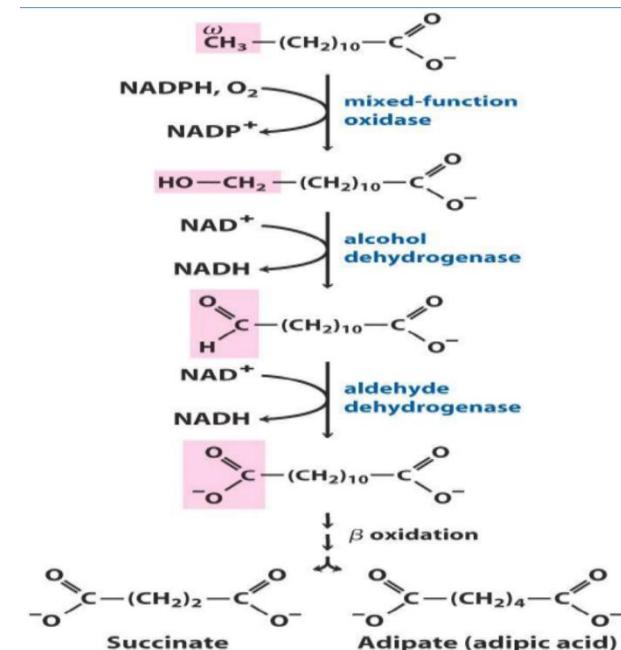


### Omega Oxidation of Fatty Acids

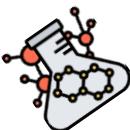
- ❖ Minor Alternative Oxidative Pathway.
- ❖ Occurs in Endoplasmic Reticulum of Many Tissues Spec. Liver.
- ❖ Takes Place When There is Defect in  $\beta$  Oxidation of Fatty Acid.
- ❖ Oxidation of Omega Carbon atom ( $\text{CH}_3$ ) of a Fatty Acid.
- ❖  $\omega$  Carbon ( $\text{CH}_3$ ) of a Fatty Acid is Transformed to  $-\text{COOH}$ .
- ❖ Formed Dicarboxylic Acid Further Undergoes  $\beta$  Oxidation to more short Dicarboxylic Acids **Adipic acid and Succinic acid**.
- ❖ Which are more Polar and can be Excreted in Urine.
- ❖ So  $\omega$  Oxidation facilitates excretion of accumulated Fatty Acids due to Defective Normal  $\beta$  Oxidation in Cells.

#### Omega Oxidation of a Fatty acid takes place with

- ❖ **Hydroxylation Reaction** (catalyzed by NADPH+H<sup>+</sup> dependent Cytochrome P450) sy
- ❖ **Oxidation Reaction**



$\omega$  = Omega, last letter in Greek alphabet

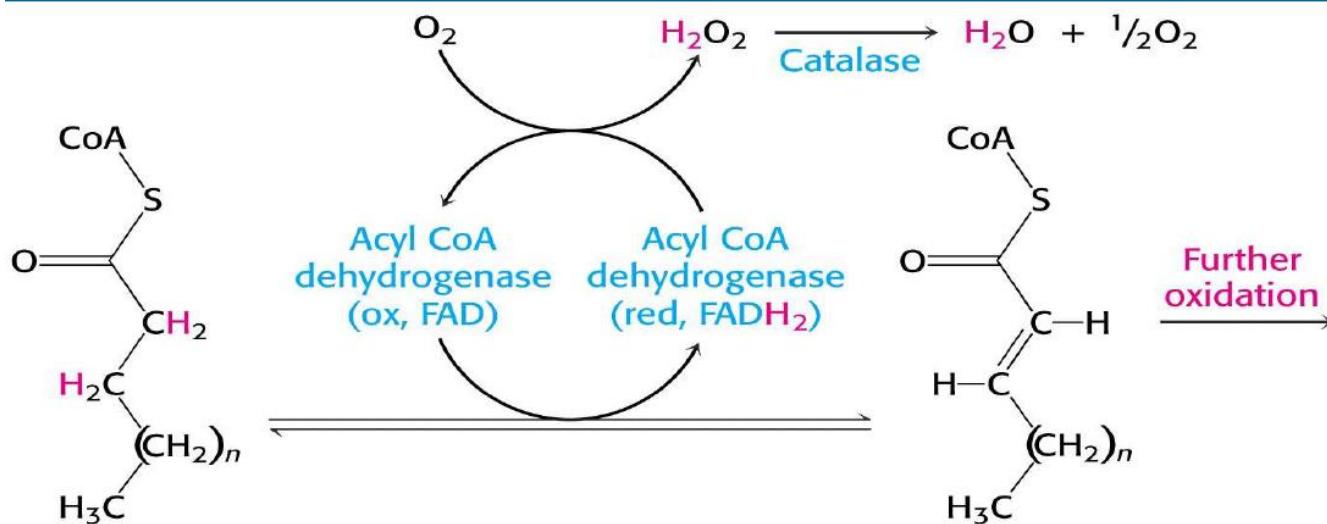
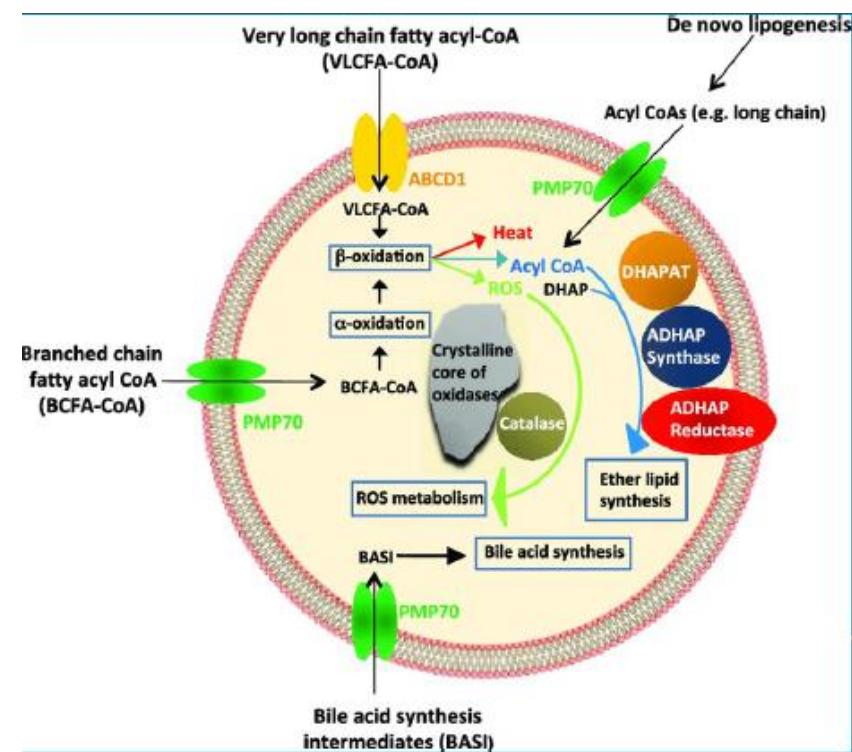


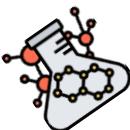
## Oxidation of Very Long Chain Fatty Acids (VLCFA)

- ❖ Oxidation of VLCFA (more than 20 C) Occurs in Peroxisomes.
- ❖ Peroxisomes – Cell organelles containing Enzymes Peroxidase and Catalase.

**When? Why? How? Does Peroxisomal Oxidation of Fatty Acid Occurs?**

- ❖  $\beta$ -Oxidation of very long-chain fatty acids (>C22) occurs within Peroxisomes initially.
- ❖ Later undergoes Mitochondrial  $\beta$ -Oxidation.
- ❖ Carnitine is Involved in Transfer of VLCFA into and out of Peroxisomes.
- ❖ Peroxisomal Fatty acid oxidation is induced by a high Fat diet with VLCFAs to shorten VLCFAs into LCFA which are further degraded by  $\beta$ -Oxidation.
- ❖ Similar to Mitochondrial  $\beta$ -Oxidation, Initial double bond formation is catalyzed by FAD Dependant Acyl-CoA Oxidase.



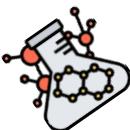


Acy CoA Oxidase – FAD transfers electrons to O<sub>2</sub> to yield H<sub>2</sub>O<sub>2</sub> Instead of ETC (No Energy)

- ❖ Once Very Long Chain Fatty acids are reduced in length within the Peroxisomes they may shift to Mitochondrial β-Oxidation for further Catabolism.
- ❖ No ATPs result from steps of Peroxisomal Oxidation of VLCFAs.
- ❖ Instead energy dissipated in the form of Heat.
- ❖ Many Drugs Commercially Available in Market for Reducing Obesity Stimulate Peroxisomal β-Oxidation Where Fatty acids are oxidized without much liberation of calories (ATPs).
- ❖ Peroxisomal Oxidation of Fatty acid efficiently takes place in:
  - Obese persons.
  - Persons taking Hypolipidemic drugs (Clofibrate).

### Peroxisomal Disorders Disorders Associated with Defective VLCFS Metabolism (Zellweger Syndrome)

- ❖ An Autosomal Recessive Disorder.
- ❖ Rare about 1 in 50,000 to 1 in 75,000 newborns.
- ❖ Symptoms:
  - Loss of hearing, vision and unable to eat.
  - Those with very underdeveloped muscles may not be able to move.
  - Breathing problems, liver failure or digestive tract bleeding.



## TUT 5 : Glycolipids Chemistry and Eicosanoids

# What are glycolipids?

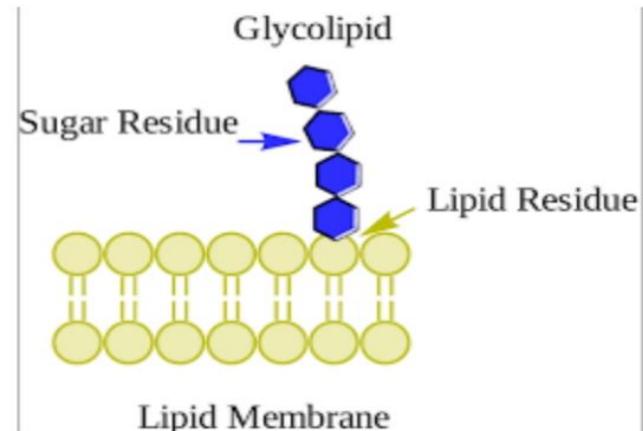
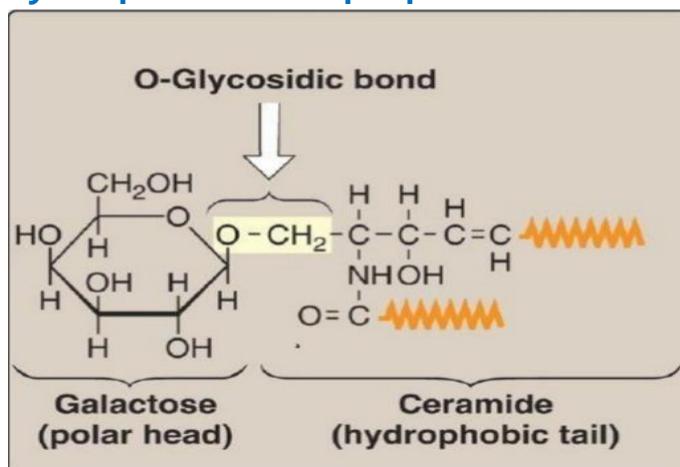
- Glycolipids are lipids with a carbohydrate attached by a glycosidic bond.
  - Glycolipids are complex (compound) lipids containing carbohydrate.

## Importance in diet

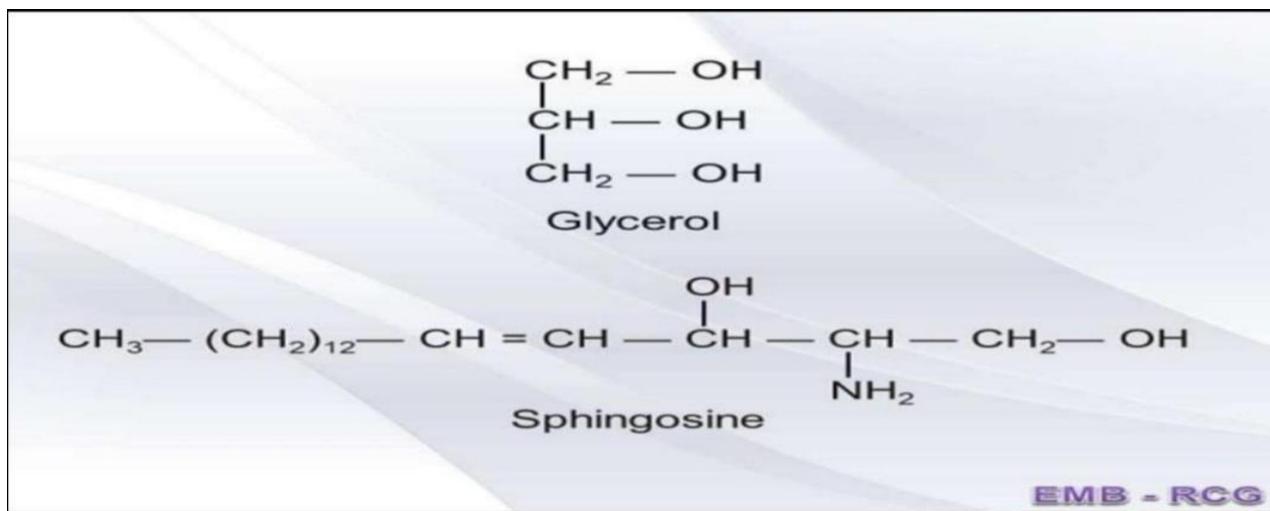
1-Glycolipids are present in high concentrations in brain, egg yolk, liver and kidney.

2-They are not essential dietary components.

**Glycolipids are amphipathic**



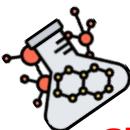
# Glycerol & sphingosine



## Types of glycolipids (based on Alcohol)

## 1-glycoglycerolipids: more in plants. Glycerol as Alcohol

2-glycosphingolipids: predominant in animal and humans. Sphingosine as Alcohol.



## Classification of glycolipids:

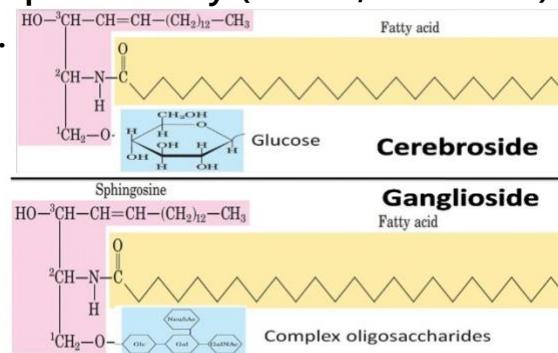
- Glycero-glycolipids: glycerole backbone with carbohydrates ( galactolipids-sulpholipids)
- Sphingo-glycolipids: sphingosine backbone with carbohydrates (cerebrosides-ganglioside-globosides)

### •Types of glycosphingolipids:

Neutral glycosphingolipids: cerebrosides & globosides.

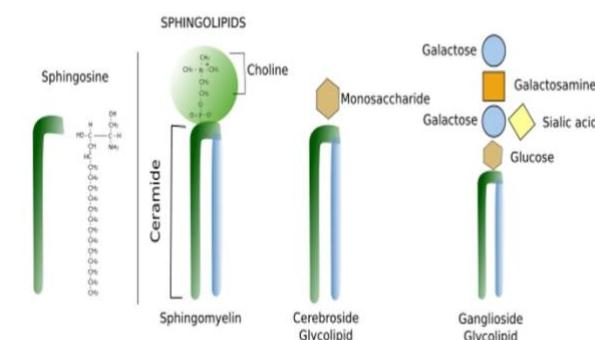
Acidic glycosphingolipids: They are negatively charged at physiologic PH.

This negative charge provided by (NANA, sialic acid) in gangliosides, or by sulfate groups in sulfatides.



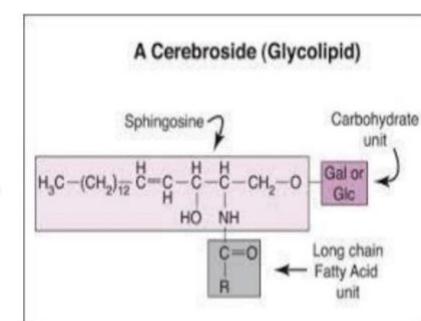
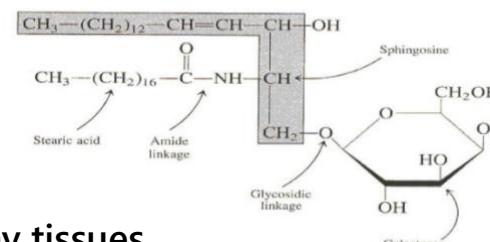
### 1-Cerebrosides

They are called simple glycolipids. Upon hydrolysis they give: Sphingosine, Fatty acid, Sugar (usually galactose or glucose). **Galactocerebroside**



### -Functions:

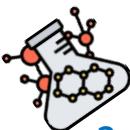
- 1) Cerebrosides are present in many tissues especially in the brain and myelin of nerve fibers.
- 2) Cerebrosides act as insulators of nerve impulses.



## Gaucher's disease (hereditary disease)

- 1) Accumulation cerebrosides in phagocytes due deficiency of  $\beta$ -glucocerebrosidase enzyme.
- 2) Manifestations: mental retardation, hepatomegaly and bone disorders.
- 3) symptoms: Distended abdomen, bone pain, Anemia, cognitive impairment.





## 2-Gangliosides

They are called complex glycolipids, because they contain in addition to hexose, one or more sialic acid molecules.

Upon hydrolysis they give:

1) Ceramide (sphingosine and fatty acid)

2) Hexoses (glucose and galactose)

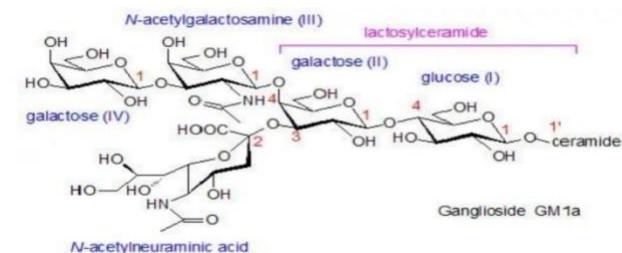
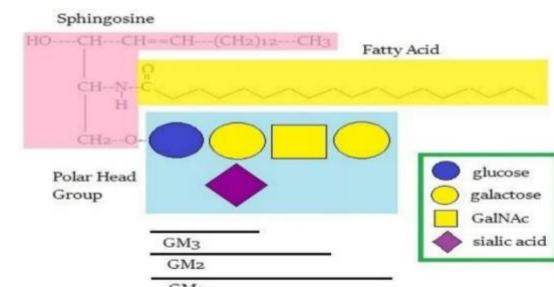
3) Hexosamines: Sialic acid (N-acetylneuraminic acid), N-acetylgalactosamine

-Functions:

1) They act as receptors at cell membrane.

2) They are present in high concentrations in brain.

Degradation by hexosaminidase enzyme.



## Tay Sachs disease:

1) Accumulation of gangliosides in brain and intestine due to deficiency of hexosaminidase enzyme.

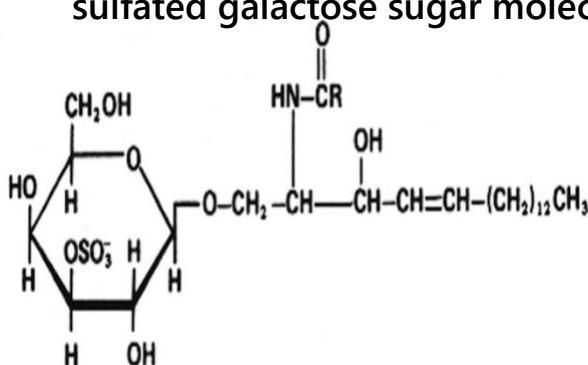
2) Manifestations: mental retardation, hepatomegaly, blindness and death in early life.

## 3-Globosides

They contain sphingosine base, fatty acid and many glucose and galactose units (ceramide oligosaccharides). They are present in heart and kidney.

## Sulfatides

Their structure consists of a ceramide backbone linked to a sulfated galactose sugar molecule.

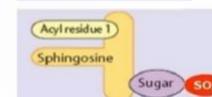


### Types of Glycolipids

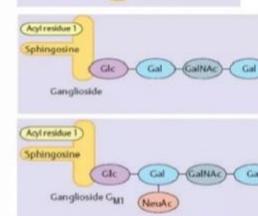
#### 1. Cerebrosides



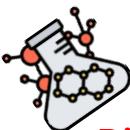
#### 2. Sulfatides



#### 3. Globosides



#### 4. Gangliosides



## Biosynthesis of Eicosanoids:

In humans, eicosanoids are synthesized from arachidonic acid.

**Site:** Except RBC eicosanoids are formed in all types of mammalian cells.

-**Biological actions of selected Eicosanoids:**

|               |                                                                            |
|---------------|----------------------------------------------------------------------------|
| PGD2          | Promotion to sleep                                                         |
| PGE2          | Smooth muscle contraction; inducing pain, heat, fever, bronchoconstriction |
| PGF2 $\alpha$ | Uterine contraction                                                        |
| PGI2          | Inhibition of platelet aggregation; vasodilation                           |
| TXA2          | Stimulation of platelet aggregation; vasoconstriction                      |
| Leukotriens   | Anaphylaxis; bronchial smooth muscle contraction                           |

### Medical importance

-Anti inflammatory drugs like corticosteroids work by inhibiting the action of phospholipase A2.

-Drugs like aspirin, indomethacin, ibuprofen and phenyl butazone work by inhibiting cyclooxygenase action.

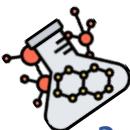
### -Questions

1-All of the following are constituents of ganglioside molecule except ?

- a. Glycerol      b. Sialic acid      c. Hexose sugar      d. SphingosineG2

2-Glycosphingolipids are a combination of.....

- a. Ceramide with one or more sugar residues      b. Glycerol with galactose
- c. Sphingosine with galactose      d. Sphingosine with phosphoric acid



3-Cerebrosides are composed of.....

- a. Sphingosine, fatty acid, glycerol and phosphoric acid
- b. Sphingosine, fatty acids, galactose
- c. Glycerol, fatty acids, galactose
- d. Glycerol, fatty acids, galactose, sphingol

4-Gangliosides derived from glucosyl ceramide contain in addition one or more molecules of....

- a. Sialic acid
- b. Glycerol
- c. Diacylglycerol
- d. Hyaluronic acid

5-Cerebrosides contain all of the following except ?

- a. Galactose
- b. Sulphate
- c. Sphingosine
- d. Fatty acid

6-Hexosaminidase A is deficient in

- a. Tay-Sachs disease
- b. Gaucher's disease
- c. Niemann-Pick disease
- d. Fabry's disease

7-Glycolipid are made up of which of the following?

- a. Proteins and lipids
- b. Carbohydrates and lipids
- c. Proteins and carbohydrates
- d. Lipids and nucleic acids

8-Gangliosides are complex glycosphingolipids found in...

- a. Liver
- b. Brain
- c. Kidney
- d. Muscles!

9-In cell membrane, carbohydrates in glycolipids are oriented

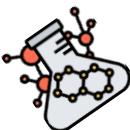
- a. Towards outside.
- b. Towards inside.
- c. Towards outside and inside.
- d. Randomly distributed.

10-Which of the following are included in the types of glycolipids?

- a. N-acetylgalactosamine.
- b. N-acetylglucosamine.
- c. Xylose.
- d. Cerebrosides.

### answers

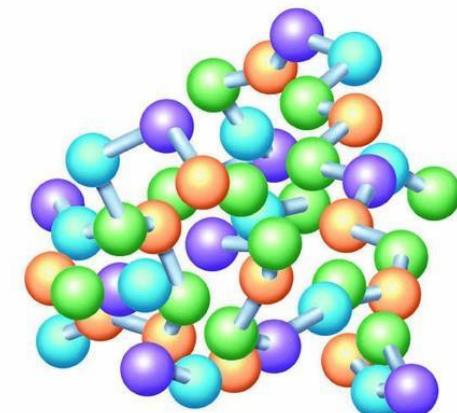
- 1-a
- 2-a
- 3-b
- 4-a
- 5-b
- 6-a
- 7-b
- 8-b
- 9-a
- 10-d



# Protein Chemistry

## Definition

- ❖ Proteins are organic **nitrogenous** compound of high molecular weight, consisting largely or entirely of **α, amino acids** united together by **peptide** linkages.
- ❖ They are composed of carbon, hydrogen, oxygen and nitrogen, many types of proteins may contain sulfur.
- ❖ **Nitrogen** is a characteristic component of proteins, forming about **16%** of their weight.



## Classification of proteins

- ❖ In general, proteins may be classified according to their **functions, composition and solubility**

### 1) Classification based on functions (Biological importance)

1. Catalytic proteins → enzymes.
2. Structural proteins → collagen, elastin.
3. Contractile proteins → actin, myosin.
4. Transport proteins → hemoglobin, albumin, transferrin, myoglobin.
5. Regulatory proteins or hormones → ACTH, insulin, growth hormone.
6. Genetic proteins → histones.
7. Protective proteins → immunoglobulins, clotting factors.

### 2) Classification based on the shape

#### 1. Fibrous or structural proteins

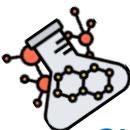
- ❖ The molecules are elongated or needle in shape.
- ❖ They resist digestion.
- ❖ Their solubility is minimum
- ❖ E.g. Keratin, elastin, Collagen, reticulin Muscle protein (Myosin and Actin), Protein of cell membrane , Protein of cytoplasm



#### 2. Globular or functional proteins

- ❖ They are spherical or oval in shape.
- ❖ They are easily soluble
- ❖ E.g. Enzyme ,Hormones , Receptors , Igs , Plasma protein, Hemoglobin





### 3) Classification based on nutritional value

1. According to their content of essential amino acids, proteins are classified into:

#### A. Proteins of high biological value

- ❖ These proteins are nutritionally rich proteins.
- ❖ They are also, called (complete proteins).
- ❖ They contain all the essential amino acids.
- ❖ Also, they are easily digested.
- ❖ On supplying these proteins in the diet, children will grow satisfactorily e.g. casein of milk.
- ❖ Proteins of animal origin are usually the first class, but also some plant proteins are considered of high biological value e.g. lentils proteins and beans proteins.

#### B. Incomplete proteins

- ❖ These proteins lack one essential amino acid.
- ❖ They cannot promote body growth in children, but may be able to sustain the body weight in adults.
- ❖ Protein from pulses is deficient in methionine, while proteins of cereals lack in lysine.
- ❖ If both of them are combined in diet, adequate growth may be obtained.

#### C. Proteins of low biological weight (Poor proteins)

- ❖ These proteins lack in many essential amino acids and a diet based on these will not even sustain the original body weight
- ❖ E.g. Zein from corn lacks tryptophan and lysine.

### 4) Classification based on composition and solubility

- ❖ Simple proteins.
- ❖ Compound or conjugated proteins.
- ❖ Derived proteins

#### 1. Simple Proteins

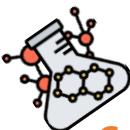
- ❖ These are formed of L- $\alpha$ -amino acids only.
- ❖ Simple protein can be classified according to their solubility in various solvents.

#### A. Albumin

- ❖ It is water soluble.
- ❖ It is coagulated by heat.
- ❖ It is the absolutely synthesized by the liver.

#### B. Globulins:

- ❖ It is water insoluble.
- ❖ It is coagulated by heat.

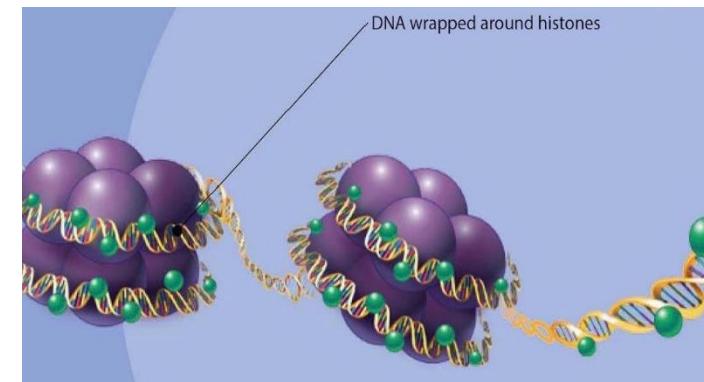


### C. Scleroproteins

- ❖ These are the fibrous proteins which support or protect the body
- ❖ They are insoluble in all protein solvents
- ❖ They are **resistant to digestion by proteolytic enzymes**

### D. Histones

- ❖ They are soluble in water.
- ❖ They are basic amino acids,
- ❖ Histones are usually found in animal tissue linked to nucleic acid forming nucleoprotein.



## 2. Compound or Conjugated proteins

- ❖ These are formed of protein part and non-protein part; sometimes called prosthetic group.

### A. According to the non-protein, compound proteins are divided into:

#### I. Phosphoproteins

- ❖ Phosphoproteins are formed of proteins with **phosphoric acid**.
- ❖ The combined phosphoric acid is esterified with the -OH group of the amino acid serine and threonine e.g. Casein of milk and vitellin of egg yolk.

#### II. Glycoproteins

- ❖ These are protein combined **carbohydrates**.
- ❖ Glycoproteins contain less than 5% of their structure carbohydrates.
- ❖ The chief glycoproteins are the mucins present in mucous membranes and saliva
- ❖ They acts as a lubricant and protective.
- ❖ ABO blood group antigens are glycoproteins.

#### III. Chromoproteins:

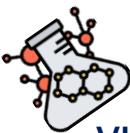
- ❖ They consist of **pigmented prosthetic groups** combined with proteins; the red iron containing hemoglobin.
- ❖ Other examples of chromoprotein are carotenoids, and flavoproteins.

#### IV. Lipoproteins:

- ❖ These are proteins conjugated to lipids.
- ❖ They constituted cell membranes, mitochondria membranes.
- ❖ Plasma lipoproteins are compound proteins.

#### V. Metalloproteins:

- ❖ These are proteins combined to a metal. e.g. ceruloplasmin in which copper is combined to proteins, ferritin in which iron is combined to protein and insulin in which zinc is combined to proteins.



## VI. Nucleoproteins:

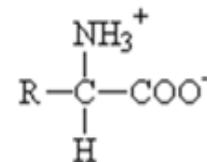
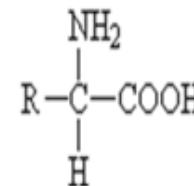
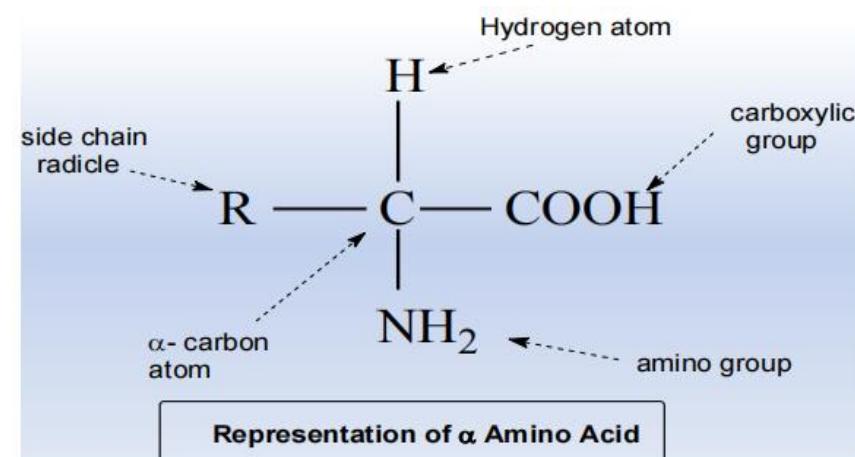
- ❖ These are proteins (usually basic proteins) combined to nucleic acids, RNA and DNA.

## 3. Derived proteins

- ❖ These are derived from the above-mentioned types by hydrolysis or denaturation, i.e. **Decomposition products of proteins.**
- ❖ Polypeptides → Short peptides as tri- and dipeptides.
- ❖ Protein → Peptones → Polypeptides → Short peptide → **α amino acids.**

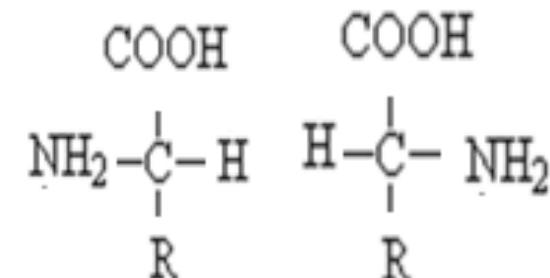
## Amino Acids

- ❖ Amino acid is an organic acid containing **one or more amino group** and carboxylic group (COOH)
- ❖ Carboxylic group (COOH) and amino group (NH<sub>2</sub>) are both attached to the **α-carbon**
- ❖ They are the **building units** of protein
- ❖ Amino acids found in proteins are of L-configuration, this means that the **amino group to the left** and the **hydrogen to the right** of the α-carbon



## Functions of amino acids (Question)

- ❖ Synthesis of structural and functional proteins.
- ❖ L-amino acid and their derivatives play a role in intracellular functions as nerve impulse transmission and regulation of cell growth.
- ❖ Biosynthesis of purines, pyrimidines, urea and porphyrins.
- ❖ L-and D-amino acids are present in polypeptide antibiotics secreted by microorganisms

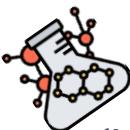


L-amino acid      D-amino acid

## Isomerism of amino acids

## Classifications of amino acids

- ❖ Chemical classification.
- ❖ Nutritional classification.
- ❖ Metabolic classification.
- ❖ Reaction classification (Charge properties)

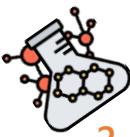


## 1) Chemical classification

### 1. Aliphatic amino acids:

- ❖ Have no ring
- ❖ These amino acids may be subclassified into:
  - A. Branched chain amino acids: as valine, leucine, and isoleucine.
  - B. Hydroxy amino acids: as serine and threonine.
  - C. Sulphur containing amino acids: as cysteine and methionine.
  - D. Amino acids with amide group: as asparagine and glutamine.

|            |         |                                                                                                                                                                                                                                                          |
|------------|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Glycine    | Gly-G   | $\text{H}-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$                                                                                                                                                                            |
| Alanine    | Ala-A   | $\text{CH}_3-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$                                                                                                                                                                         |
| Valine     | Val-V   | $\begin{matrix} \text{H}_3\text{C} & & \\ & \diagdown & \\ & \text{CH} & \diagup \\ & / & \backslash \\ \text{H}_3\text{C} & & \text{NH}_2 \end{matrix} \text{CH}-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$                    |
| Leucine    | Leu-L   | $\begin{matrix} \text{H}_3\text{C} & & \\ & \diagdown & \\ & \text{CH} & \diagup \\ & / & \backslash \\ \text{H}_3\text{C} & & \text{NH}_2 \end{matrix} \text{CH}-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$        |
| Isoleucine | Ile-I   | $\begin{matrix} \text{H}_3\text{C} & \text{H}_2\text{C} & \\ & \diagdown & \\ & \text{CH} & \diagup \\ & / & \backslash \\ \text{H}_3\text{C} & & \text{NH}_2 \end{matrix} \text{CH}-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$ |
| Serine     | Ser - S | $\text{HO}-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$                                                                                                                                                               |
| Threonine  | Thr - T | $\begin{matrix} \text{H}_3\text{C} & & \\ & \diagdown & \\ & \text{HO} & \diagup \\ & / & \backslash \\ \text{H}_3\text{C} & & \text{NH}_2 \end{matrix} \text{CH}-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$                    |
| Cysteine   | Cys - C | $\text{HS}-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$                                                                                                                                                               |
| Methionine | Met - M | $\text{H}_3\text{C}-\text{S}-(\text{CH}_2)_2-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH}$                                                                                                                                         |
| Cystine    | -       | $\begin{array}{c} \text{S}-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH} \\   \\ \text{S}-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{CH}}{\text{CH}}}-\text{COOH} \end{array}$                                   |



## 2. Aromatic amino acids:

- ❖ They have benzene ring in their side chains like

### A. Phenylalanine

### B. Tyrosine

### C. Tryptophan

|               |         |                                                                                     |
|---------------|---------|-------------------------------------------------------------------------------------|
| Phenylalanine | Phe - F |  |
| Tyrosine      | Tyr - Y |  |
| Tryptophan    | Trp - W |  |

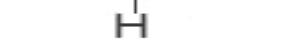
### **3. Heterocyclic amino acids:**

- ❖ They have heterocyclic ring as

### A. Histidine,

### B. Tryptophan,

### C. Proline and Hydroxyproline

|                 |         |                                                                                      |
|-----------------|---------|--------------------------------------------------------------------------------------|
| Proline         | Pro – P |   |
| Hydroxy proline | Hyp     |  |

## 2) Reaction classification (charge properties)

## 1. Nonpolar (Neutral)

- ❖ Having equal number of amino and carboxyl groups

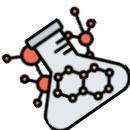
- ❖ E.g. Alanine, serine, valine

## 2. Polar Basic AA

- ❖ Having more than one amino group and one carboxyl group

- ❖ E.g. Arginine, lysine, histidine

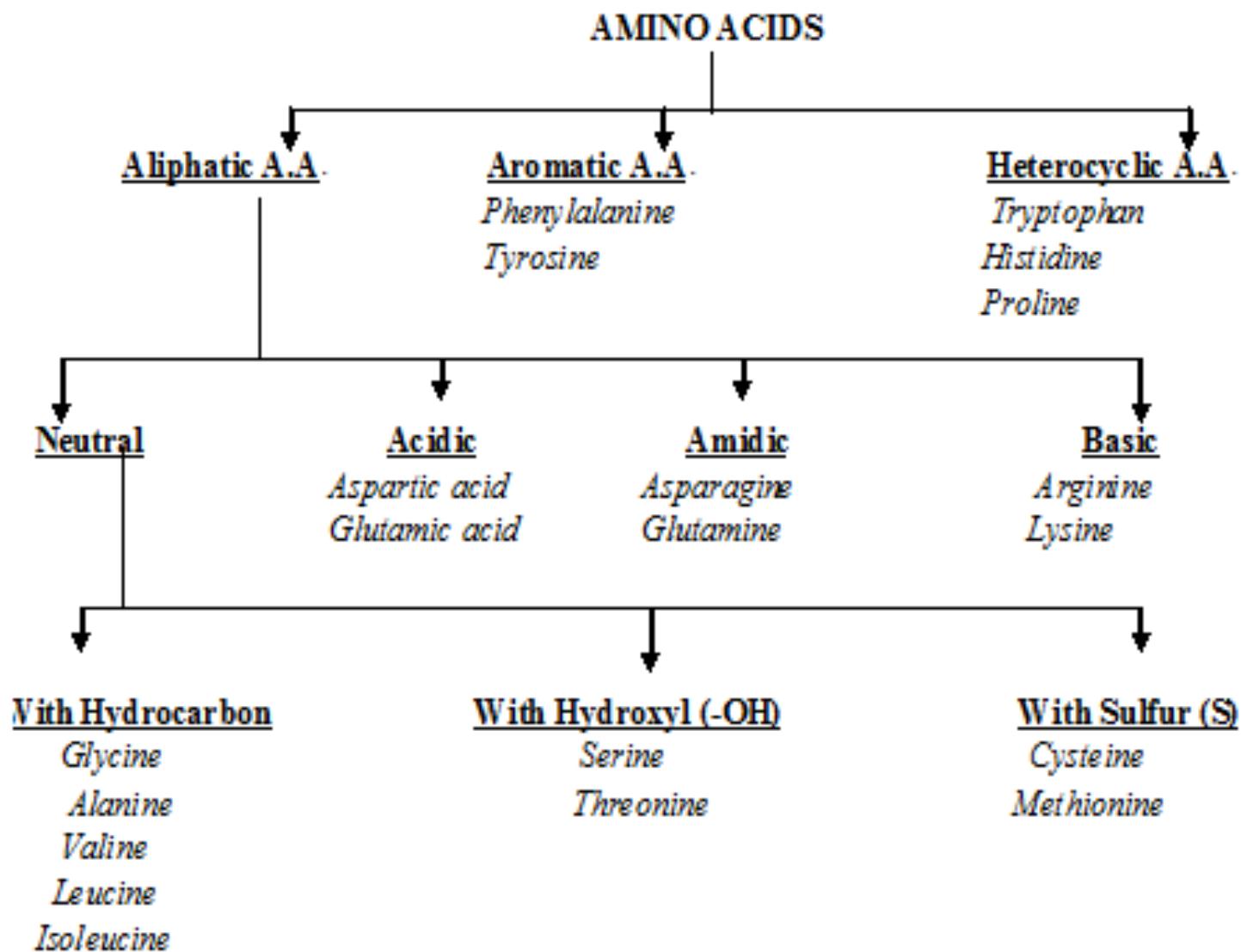
|           |         |                                                                                                                                                                         |
|-----------|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Arginine  | Arg - R | $\begin{array}{c} \text{HN}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\overset{\text{C}=\text{NH}}{\underset{\text{NH}_2}{\text{CH}}}-\text{COOH} \\ \text{NH}_2 \end{array}$ |
| Lysine    | Lys - K | $\text{H}_2\text{N}-\text{(CH}_2)_4-\overset{\text{CH}}{\underset{\text{NH}_2}{\text{CH}}}-\text{COOH}$                                                                 |
| Histidine | His - H | $\begin{array}{c} \text{CH}_2-\overset{\text{CH}}{\underset{\text{NH}_2}{\text{CH}}}-\text{COOH} \\ \text{HN}=\text{N:} \end{array}$                                    |

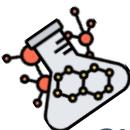


### 3. Polar (Acidic)

- ❖ Having one amino group and two carboxyl
- ❖ E.g. Glutamic acid and aspartic acid

|               |         |                                                                                                                                                                     |
|---------------|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Aspartic Acid | Asp - D | $\text{HOOC}-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{C}}{\text{CH}}}-\text{COOH}$                                                                         |
| Asparagine    | Asn - N | $\text{H}_2\text{N}-\overset{\text{O}}{\underset{\text{C}}{\text{CH}_2}}-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{C}}{\text{CH}}}-\text{COOH}$             |
| Glutamic Acid | Glu - E | $\text{HOOC}-\text{CH}_2-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{C}}{\text{CH}}}-\text{COOH}$                                                             |
| Glutamine     | Gln - Q | $\text{H}_2\text{N}-\overset{\text{O}}{\underset{\text{C}}{\text{CH}_2}}-\text{CH}_2-\text{CH}_2-\overset{\text{NH}_2}{\underset{\text{C}}{\text{CH}}}-\text{COOH}$ |



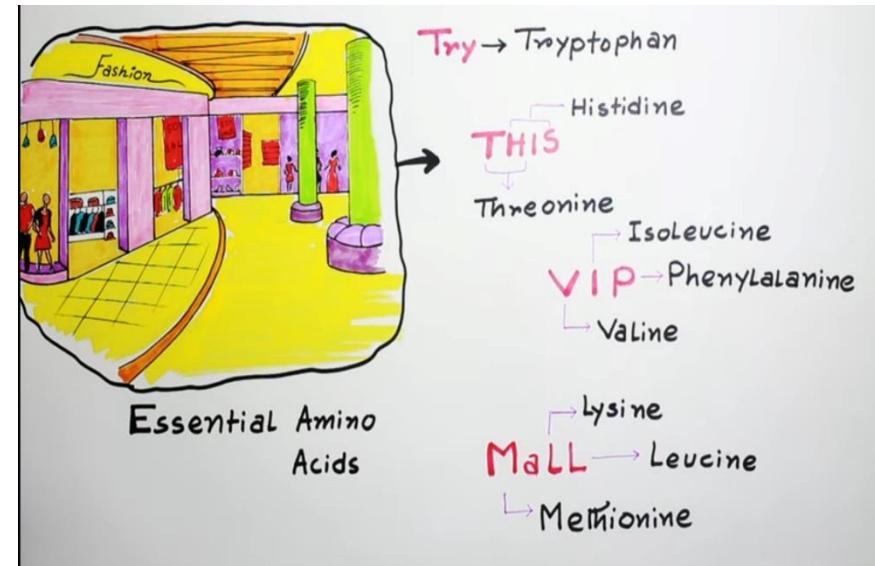


## 3) Nutritional classification

- They are classified into three groups:

### A. Essential amino acids

- They are amino acids which cannot be synthesized in the body and must be taken in diet
- Phenylalanine, methionine, isoleucine, leucine, lysine, valine, threonine and tryptophan.



### B. Semi essential amino acids:

- They are amino acids required in the food of growing children not in the food of adult as histidine and arginine.

### C. Non-essential amino acids

- Can be synthesized inside the body as glycine, alanine, serine, cysteine, cystine, aspartic, tyrosine, glutamic, proline and hydroxyproline

## 4) Metabolic classification

- According to their fate in the body they are classified into three groups

### A. Glucogenic amino acids:

- Give glucose inside the body as glycine, alanine, aspartic and glutamic.

### B. Ketogenic amino acid:

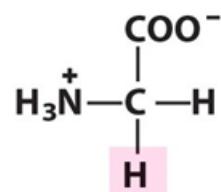
- Gives ketone bodies as leucine.

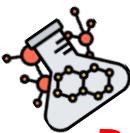
### C. Glucogenic and ketogenic amino acids:

- They give both glucose and ketone bodies as lysine, tryptophan, tyrosine, phenylalanine and isoleucine.

## Zwitterion

- At a particular pH, the amino acid carries no net charge and is called a zwitterion.
- Zwitterion .... dipolar ion
  - has 1 positive and 1 negative charge
- Amphoteric (ampholytes)



**Def.**

- pH at which the amino acid has a net charge of zero is called the **isoelectric point(PI)**
- At the isoelectric point (pl), the + and – charges are equal.

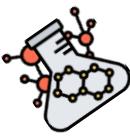
**Amino acid not found in proteins:**

Few amino acids are not found in proteins (**non-protein amino acids**):

- **L-Ornithine** is an intermediate of urea cycle. It is formed from arginine.
- **L- citrulline** is intermediate of urea cycle. It is formed from ornithine.
- **Homoserine** is an intermediate in methionine metabolism. It is formed from serine.
- **GABA**:  $\gamma$  amino butyric acid, is an inhibitor neurotransmitter.
- **DOPA**: Dihydroxyphenylalanine, Is formed from tyrosine and precursor for biosynthesis of epinephrine and norepinephrine.
- **$\beta$  alanine**: occurs in coenzyme A, Pantothenic acid and it is also formed during degeneration pyrimidine nucleotides.
- **Selenocysteine**:
  - is an amino acid containing selenium (trace element).
  - It is considered as the 21st amino acid since it is coded by the stop codon UGA.
  - Examples of proteins containing selenocysteine are glutathione peroxidase enzyme and 5'deiodinase.

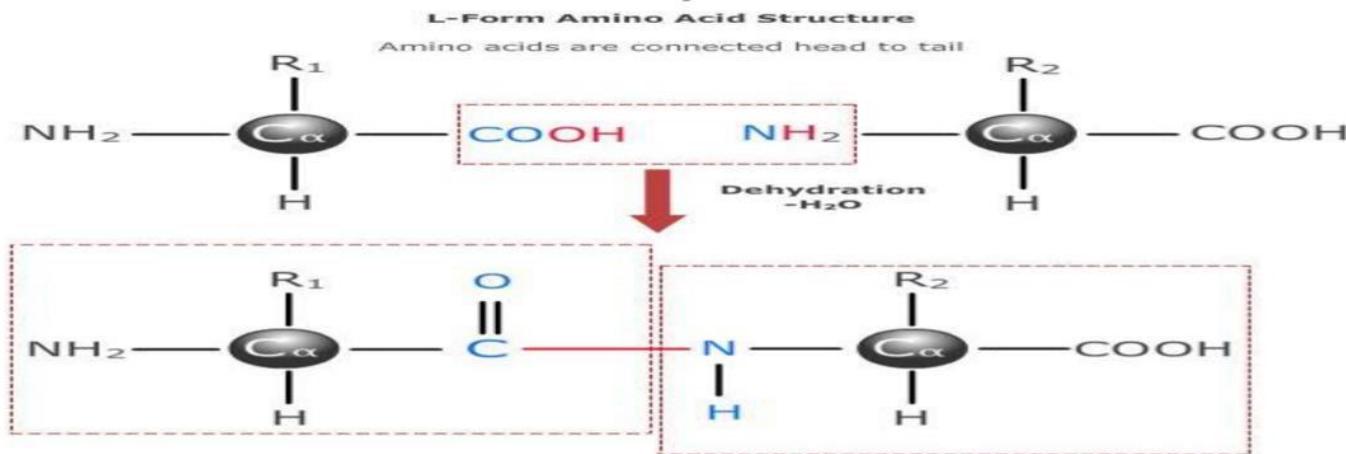
**Organization of proteins:**

- Protein - linear sequence of amino acids linked by **peptide bonds**.
- Peptide bond - **covalent bond** -  $\alpha$ -amino group of one amino acid and the  $\alpha$ -**carboxyl group** of another.
- dipeptide - two amino acids.
- oligopeptide - long, **unbranched chain** of amino acids (up to 25 amino acid residues)
- polypeptide - peptide chain **>25 amino acids residues**
- Way of writing: free  $\alpha$ -carboxyl group (**C-terminal**) on the right and free  $\alpha$ -amino (**N-terminal**) on the left and a hyphen between amino acids =>indicate **peptide bonds**.



- Tetrapeptide:
  - +H3N
  - Serine
  - Tyrosine
  - Phenylalanine
  - Leucine
  - COO
  - Ser
  - Tyr
  - Phe
  - Leu.

### Formation of peptide bond by dehydration:



### Four levels of structural organization of proteins:

#### 1. Primary structure:

- It is the number and sequence of amino acids along the polypeptide chain(s).
- The primary structure is maintained by the covalent bonds of the peptide linkages.

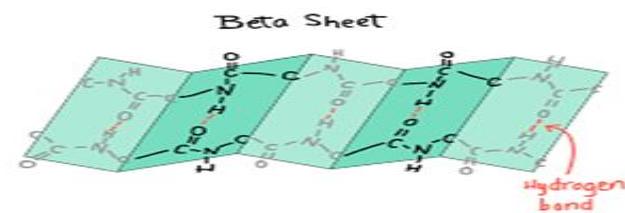
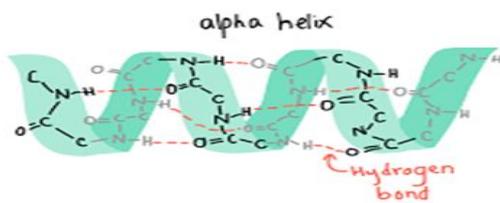
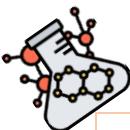
Amino acids link together like the cars of a train



#### 2. Secondary Structures:

- It is the twisting (folding) of the polypeptide chain into specific coiled structure held together by disulfide and hydrogen bonds.
- There are two main forms of secondary structure;  $\alpha$ -helix and  $\beta$ -pleated sheets.

| $\alpha$ -helix                                                                                                                                                                                                                                                                                     | $\beta$ -pleated sheets (extended structure of fibrous proteins)                                                                                                                                                                                                                               |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>- It is a coiled structure of fibrous protein.</li> <li>- The hydrogen bonds formed between peptide bonds in the same chain in the form of right-handed helix (clockwise).</li> <li>- It is formed in myosin and keratin in the unstretched hair.</li> </ul> | <ul style="list-style-type: none"> <li>- The hydrogen bonds form between peptide bonds in different chains which may run in the same direction parallel or in opposing directions (anti parallel).</li> <li>- It is formed in silk and <math>\beta</math>-Keratin in stretched hair</li> </ul> |

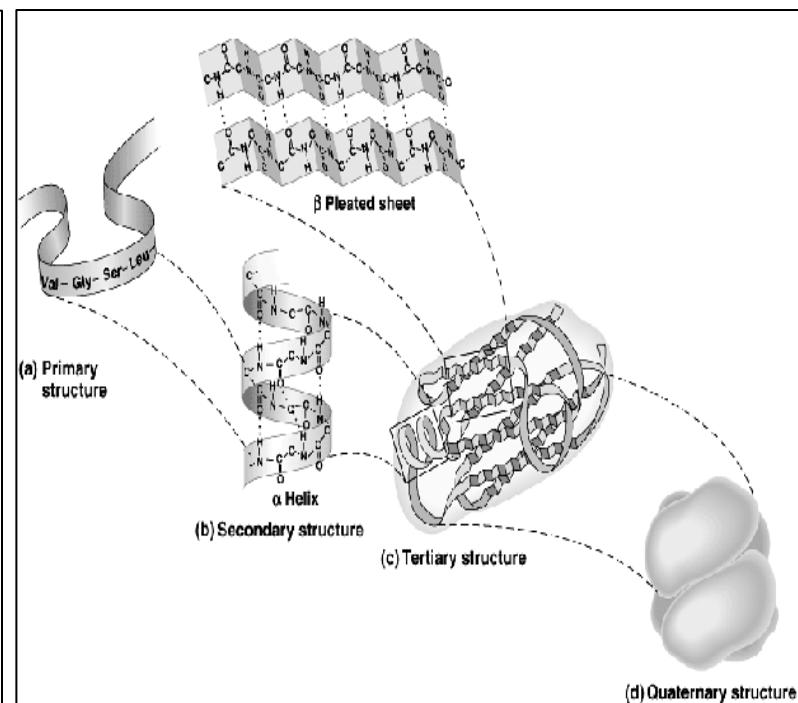
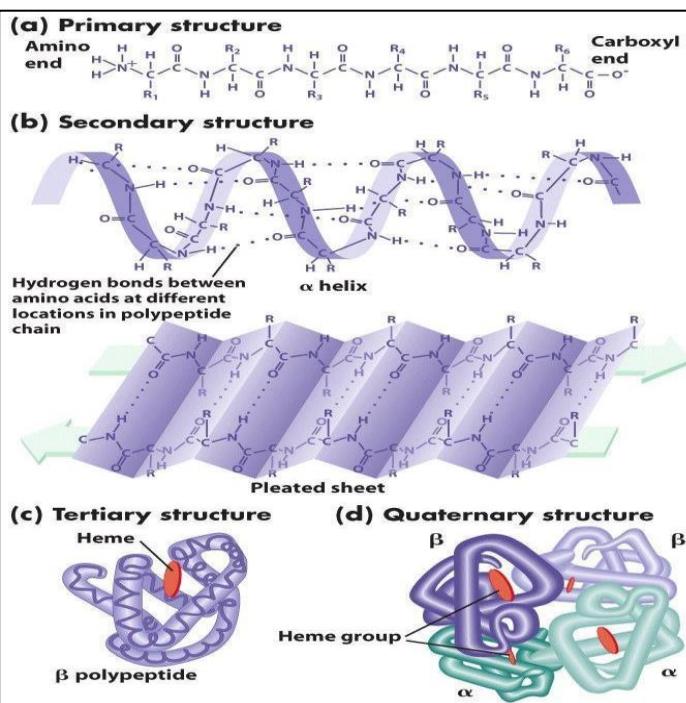
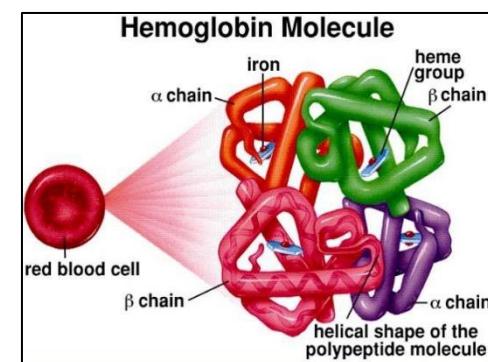


### 3. Tertiary Structure:

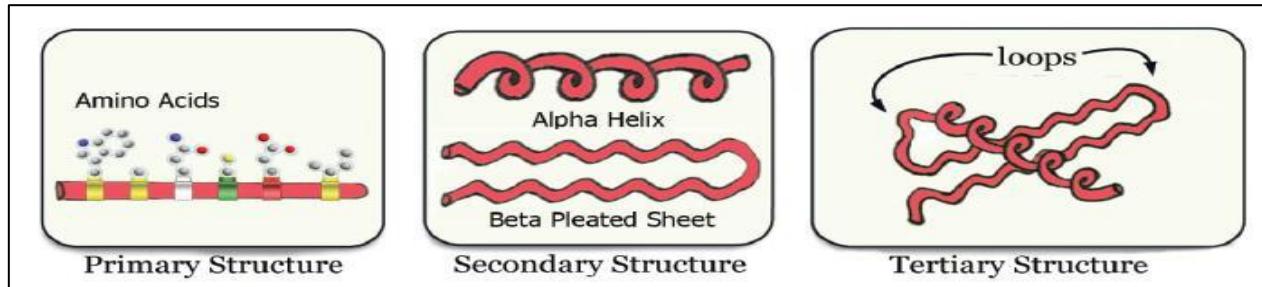
- It is the arrangement and inter-relationship of the twisted polypeptide chains.

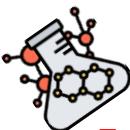
### 4. Quaternary structure:

- It is the aggregation of several polypeptide chains (subunits) to form a protein molecule.
- It describes the spatial relationships between the separate subunits, e.g. Insulin hormone is composed of 2 polypeptide chain (A and B) connected by disulfide bond.
- Globin of Hb formed by 4 chains (2  $\alpha$  and 2  $\beta$ ).
- It is a high level of organization which is essential for its function.



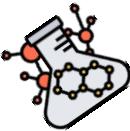
1ry, 2ry &  
3ry  
Structure:





### Forces stabilizing the 2ry, 3ry, 4ry structures

- **The Hydrogen bond (-NH----O=C-):**
  - This type of bond is between the imino H and carbonyl O of the adjacent extended regions of the polypeptide chain.
- **The disulfide bond (-S-S) bond:**
  - It is the oxidative union of the -SH groups of 2 cysteine residues, forming cystine.
- **The ionic bond: (-NH<sub>3</sub><sup>+</sup>----OOC-):**
  - It is formed between the positive and the negative side chain groups of basic and acidic amino acids; respectively.
  - For e.g. NH<sub>2</sub> of lysine and COOH of aspartic.
- **The hydrophobic bond: (-CH<sub>3</sub>----- CH<sub>3</sub>-):**
  - It is formed between the non polar, hydrophobic side chains of neutral amino acids; such as alanine and valine



# Bio TUT 1 : Classification of protein , glycoproteins , proteoglycans

|                                            |                                                        |                     |                              |                      |                  |                                                  |
|--------------------------------------------|--------------------------------------------------------|---------------------|------------------------------|----------------------|------------------|--------------------------------------------------|
| Classification of proteins                 | On the basis of nutritive value of proteins            | Complete proteins   |                              |                      |                  |                                                  |
|                                            |                                                        | Incomplete proteins |                              |                      |                  |                                                  |
|                                            | On the basis of composition and solubility of proteins | Conjugated proteins | Nucleo proteins              | Glyco/Muco-proteins  | Chromo-proteins  | Phospho-proteins Metallo- proteins Lipo-proteins |
|                                            |                                                        | Derived proteins    |                              |                      |                  |                                                  |
|                                            |                                                        | Simple proteins     |                              |                      |                  |                                                  |
| On the basis of shape and size of proteins |                                                        | Globular proteins   |                              |                      |                  |                                                  |
|                                            |                                                        | fibroud proteins    |                              |                      |                  |                                                  |
| On the basis of function of proteins       |                                                        | Structural proteins | Catalytic proteins           | Hormonal proteins    | Defence proteins |                                                  |
|                                            |                                                        | Transport proteins  | Blood clotting proteins      | Storage proteins     | Carrier proteins | Buffer proteins                                  |
|                                            |                                                        | Receptor proteins   | Signal transduction proteins | Contractile proteins |                  |                                                  |
|                                            |                                                        | Nucleoproteins      |                              |                      |                  |                                                  |

## According to the molecular shape

| Fibrous proteins (scleroproteins)                                                               | Globular proteins                                                                       |
|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| Proteins that have the arrangement of the 3ry structure in the form of fibers, sheets or layers | Proteins that have the arrangement of the 3ry structure in the form of rounded globules |
| Axial ratio (Length/width) > 10                                                                 | Axial ratio (Length/width) < 10                                                         |

### 1) Globular proteins:

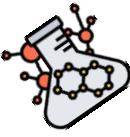
- Tightly Folded into spherical (globular) or ovoid shapes.
- Usually water soluble.
- Most of them have roles in metabolic reactions.

Examples: Albumin - Globulin - enzymes - haemoglobin

### 2) Fibrous proteins:

- Thread like fibrils or long fibers.
- Long parallel polypeptide chains.
- Cross linkages at intervals forming long fibres or sheets.
- Usually insoluble in water.
- Many have structural roles.

Examples: Collagen - Elastin - Keratin.



According to the composition

1) Simple proteins:

Formed of only L-amino acids.

Divided according to **solubility & heat coagulability** into:

| Protein                      | Solubility                                                                               | Heat Coaggulability  | Present in                                                                                                |
|------------------------------|------------------------------------------------------------------------------------------|----------------------|-----------------------------------------------------------------------------------------------------------|
| Albumin                      | Soluble in water                                                                         | Heat coaggulable     | Blood: serum albumin<br>Egg: Egg white albumin<br>Milk: Lactalbumin<br>Cereals                            |
| Globulins                    | Soluble in diluted salts solutions                                                       |                      | Blood: Serum globulin.<br>Muscle: myoglobin<br>Egg: Egg white globulins<br>Milk: lactoglobulin<br>Cereals |
| Glutelins                    | Soluble in dilute acids &alkalies                                                        |                      | Glutenin of wheat                                                                                         |
| Gliadins                     | Soluble in 70-80% alcohols                                                               | Non-heat coaggulable | Gliadin of wheat<br>Zein of maize: deficient in tryptophan a.a.                                           |
| Protamines                   | Soluble in water                                                                         |                      | Nucleic acids<br>Salmon fish                                                                              |
| Histones                     |                                                                                          |                      | Nucleic acids<br>Globin part of hemoglobin                                                                |
| Scleroproteins (albuminoids) | Insoluble in water, salt solutions or organic solvents. Soluble only in hot strong acids |                      | present only in animal tissues ,not in plants                                                             |

Glutelins & gliadins (prolamines) are rich in acidic a.as (Acidic proteins).

Protamines & histones are rich in basic a.as (Basic proteins).

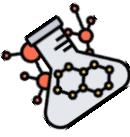


2) **Conjugated proteins:** Formed of simple protein combined with **Non protein part**

| Non protein part | Conjugated protein | Examples                                                                    |
|------------------|--------------------|-----------------------------------------------------------------------------|
| Carbohydrate     | Glycoprotein       | Immunoglobulins, ABO blood group.                                           |
| Lipids           | Lipoprotein        | Chylomicrons, LDL, HDL                                                      |
| Nucleic acid     | Nucleoprotein      | Histones associated with DNA &ribonucleoproteins associated with RNA        |
| Phosphoric acid  | Phosphoprotein     | Casein of milk                                                              |
| Metal            | Metalloprotein     | Ceruloplasmin containing copper<br>Hemoglobin or cytochrome containing iron |
| Heme             | Hemoprotein        | Hemoglobin & myoglobin                                                      |

3) **Derived proteins:**

|                                                          |                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                |
|----------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 <sup>ry</sup> derived proteins<br>(Denatured proteins) | are the derivatives produced by protein denaturation in which there is disruption of the 2 <sup>ry</sup> , 3 <sup>ry</sup> & 4 <sup>ry</sup> structures with preservation of the 1 <sup>ry</sup> structure (i.e. without hydrolytic cleavage of peptide bonds)<br>They are produced by the effect of heat, acids, alkalis...etc. | Include proteans & metaproteins                                                                                                                                                                                                                                |
| 2 <sup>ry</sup> derived proteins                         | are the hydrolytic products of proteins resulting from peptide bond cleavage                                                                                                                                                                                                                                                     | Include:<br><ul style="list-style-type: none"> <li>➤ <b>Proteoses:</b> result from partial hydrolysis of proteins.</li> <li>➤ <b>Peptones:</b> from further hydrolysis of proteoses</li> <li>➤ <b>Peptides:</b> from further hydrolysis of peptones</li> </ul> |



### According to the nutritional value:

- High biological value proteins
  - Are proteins rich in essential amino acids
  - Include animal proteins as meat, egg & milk proteins & some plant proteins as lentils
- Low biological value proteins
  - Are proteins poor in essential amino acids
  - As rice & wheat proteins

### According to the functions of proteins

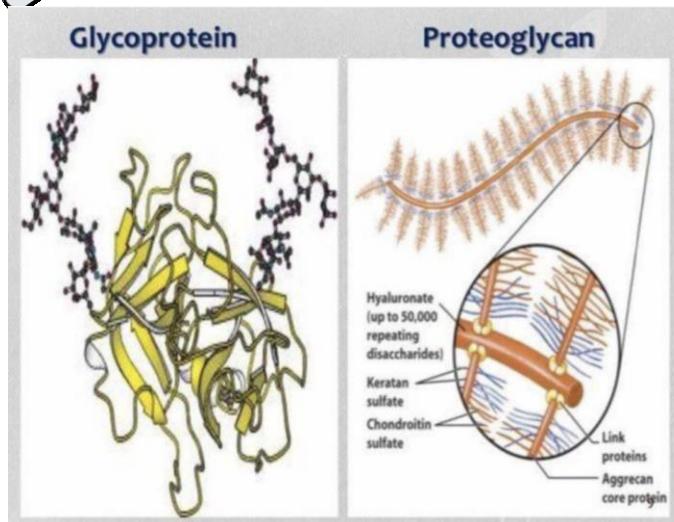
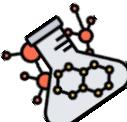
- ✓ Catalytic function: Enzymes
- ✓ Transport function:
  - Hemoglobin transports oxygen from lung to Tissues
  - Albumin: general carrier
- ✓ Storage function: Myoglobin stores oxygen in muscle cells
- ✓ Defense function: Immunoglobulins: protect against bacteria
- ✓ Regulatory function: Protein hormones and their receptors
- ✓ Contractile function: Actin and myosin in muscles
- ✓ Structural function:
  - Collagen: present in skin and bones
  - Elastin: present in lungs, wall of blood vessels
  - Keratin: present in hair, nails, skin

➤ **Glycoproteins** are proteins that contain oligosaccharide chains (glycans) covalently attached to polypeptide side-chains.

The attachment process is called **glycosylation** and is between

- the hydroxyl (-OH) group of the R group of serine or threonine - called "**O-linked**"
- the amino group (-NH<sub>2</sub>) in the R group of asparagine - called "**N-linked**".

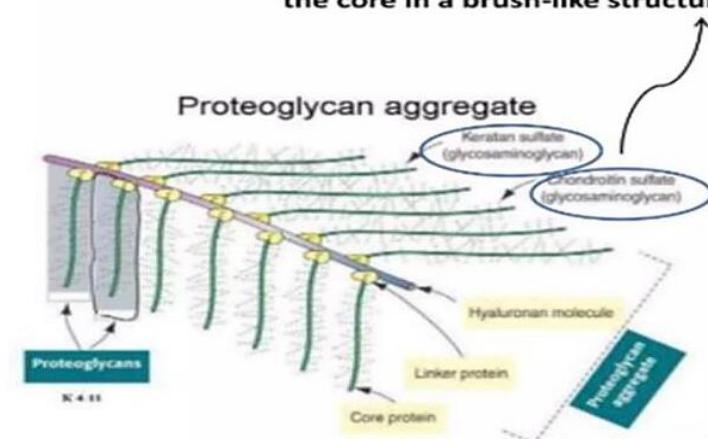
| Function                    | Glycoprotein                                     |
|-----------------------------|--------------------------------------------------|
| 1. Structural molecule      | Collagens Mucins e.g. Transferrin, Ceruloplasmin |
| 2. Lubricant                | Immunoglobulins, Histocompatibility antigens,    |
| 3. Transport molecule       | Blood group determinants                         |
| 4. Immune system            | e.g. HCG, TSH                                    |
| 5. Hormone                  | e.g. Alkaline phosphatase                        |
| 6. Enzymes                  | e.g. Fibrinogen                                  |
| 7. Blood clotting           | Lectins                                          |
| 8. Cell surface recognition |                                                  |



## STRUCTURE OF PROTEOGLYCAN

Proteoglycans = GAGs + Core proteins

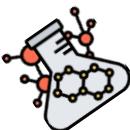
The GAGs extend perpendicularly from the core in a brush-like structure.



■ **Proteoglycans** are complex extracellular macromolecules consisting of a multidomain core protein to which is attached one or more glycosaminoglycan (GAG) chains.

| Glycoprotein                                                                                  | Proteoglycan                                                                                       |
|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| Primarily protein (Protein + some CHO)                                                        | Primarily carbohydrate                                                                             |
| CHO chains may be negatively charged                                                          | (CHO+ some protein) CHO chains always negatively charged                                           |
| Short chains Branched<br>Neutral or -ve No repeating disaccharide units<br>(Oligosaccharides) | Long chains Linear<br>Negatively charged repeating disaccharide units<br>(Glycosaminoglycans, GAG) |





## L27: Amino Acids Pool and Deamination

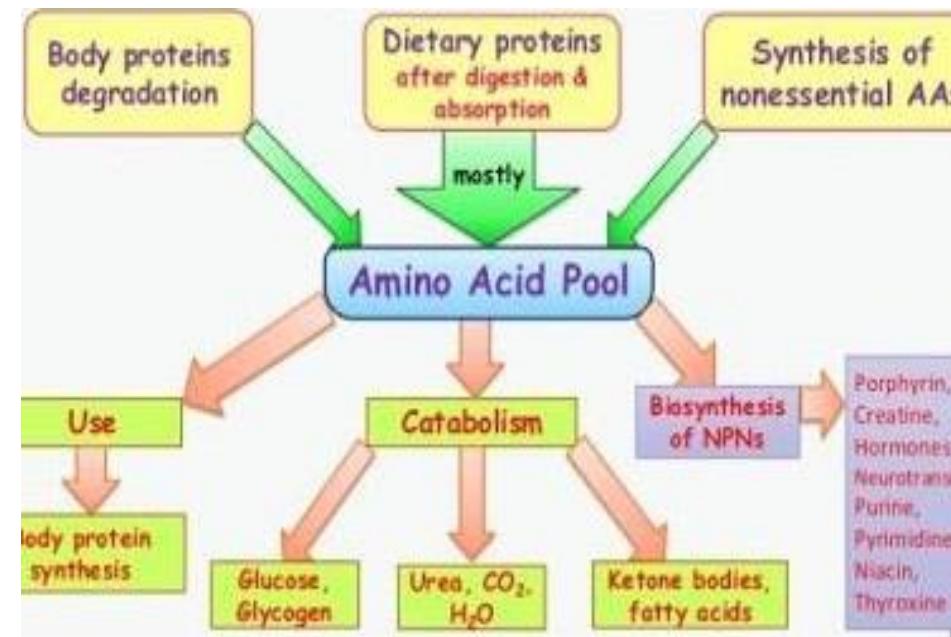
### Amino Acid Pool

#### Definition

- ❖ Free amino acids are present throughout the body, in cells, blood, and the extracellular fluids.

#### 1) This pool is supplied by three sources

- 1) Amino acids provided by the degradation of body proteins
- 2) Amino acids derived from dietary protein
- 3) Synthesis of nonessential amino acids from simple intermediates of metabolism.



#### 2) The amino pool is depleted by three routes:

- 1) Synthesis of body protein
  - 2) Amino acids consumed as precursors of essential nitrogen-containing small molecules (Non-protein nitrogenous compounds) NPNs
  - 3) Conversion of amino acids to glucose, glycogen, fatty acids, ketone bodies, CO<sub>2</sub> + H<sub>2</sub>O
- ❖ The amino acid pool is small (about 90-100 g of amino acids) in comparison with the amount of protein
  - ❖ in the body (about 12 kg in a 70-kg man), it is conceptually at the center of whole-body nitrogen metabolism

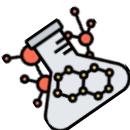
#### AA in blood

##### 1) Resorption phase

- ❖ Predominate Val, Leu, Ile
- ❖ Liver does not take them up from circulation (no specific aminotransferases in liver for Val, Leu, Ile)

##### 2) Post-resorption phase and fasting

- ❖ Predominate Gln and Ala
- ❖ Released from muscles (Gln + Ala) and liver (Gln)



## Site of AA pool

- ❖ 80 % in muscles
- ❖ 10 % in liver
- ❖ 5 % in kidney
- ❖ 5 % in blood

AA pool is not reserve !!

There is not a specific protein reserve in human body in contrast to saccharides (liver glycogen) and lipids (adipose tissue) !!

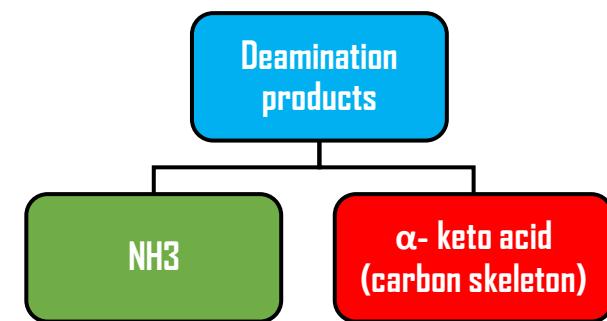
## Specific methods of deamination

- ❖ For certain a.a.s

## General methods of deamination

- ❖ Oxidative deamination
- ❖ Transamination

- ❖ Trans-deamination



### 1) Oxidative deamination

#### 1. Definition

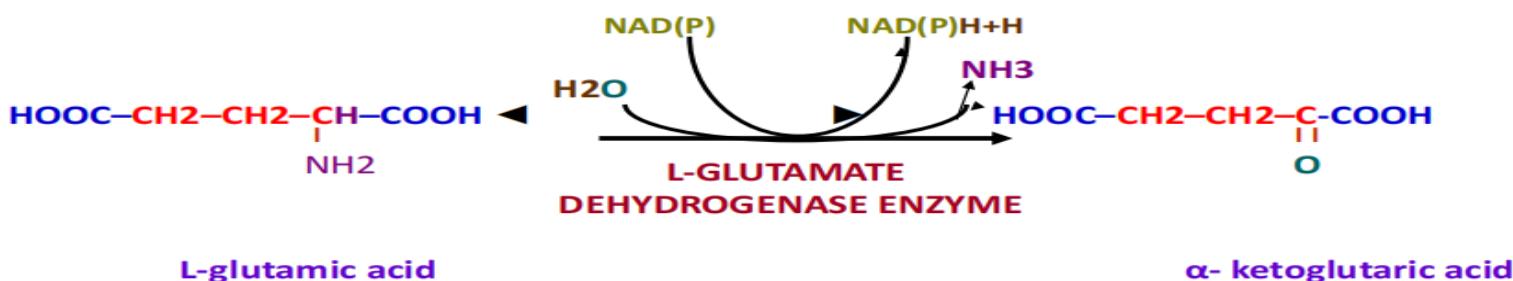
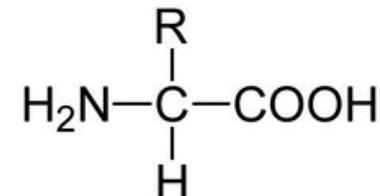
- ❖ Oxidation deamination is the removal of the amino group which is liberated as free ammonia.
- ❖ Oxidation deamination (reversible reactions) give  $\alpha$ - ketoacid (carbon skeleton) and ammonia
- ❖ It is the oxidation (removal of hydrogen) and deamination (removal of the amino group which is liberated as free ammonia) giving  $\alpha$ - ketoacid and ammonia (reversible reactions)

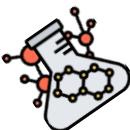
#### 2. Site

- ❖ In most tissues , mostly in the liver and kidney

#### 3. Enzymes involved

- ❖ L-glutamate dehydrogenase enzyme → Present in the cytosol & mitochondria of most tissues





## 2) Transamination

### 1. Definition

- ❖ It is the transfer of amino group from one  $\alpha$ - a.a. to  $\alpha$ - keto acid to form a new  $\alpha$ - a.a. & a new  $\alpha$ - keto acid (reversible reaction)

### 2. Site

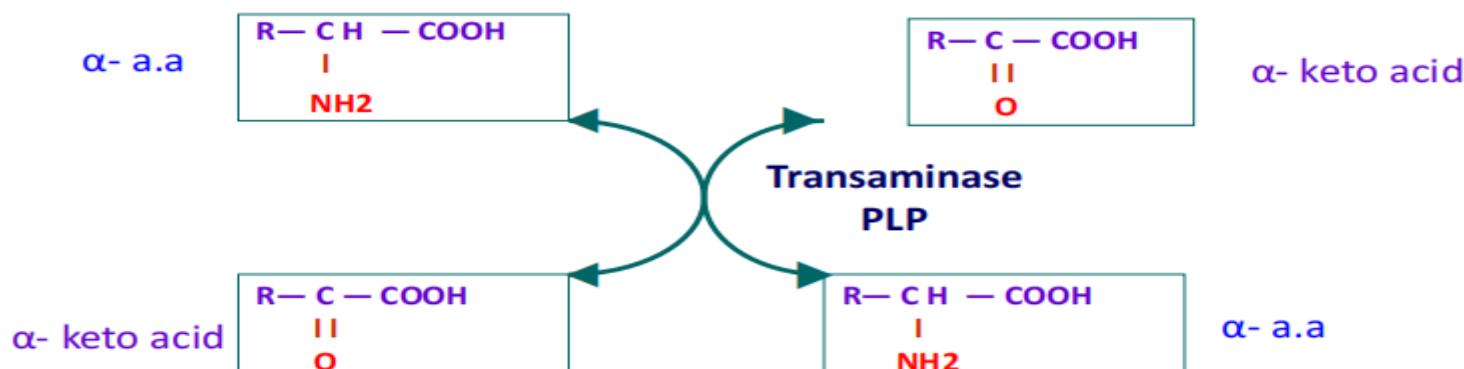
- ❖ In the cytosol or both the cytosol & the mitochondria of most cells especially the liver
- ❖ All a.as except threonine, lysine, proline & hydroxy proline

### 3. Enzymes involved

- ❖ Transaminases or aminotransferases

### 4. Coenzyme

- ❖ PLP (Pyridoxal phosphate)



| Alanine transaminase (ALT) or Glutamate pyruvate transaminase (GPT)                                                                                                                     | Aspartate transaminase (AST) or Glutamate oxaloacetate transaminase (GOT)                                                                                                              |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>Alanine</b>                          <math>\alpha</math>-Ketoglutarate</p> <p>PLP                                    ALT</p> <p>Pyruvate                            Glutamate</p> | <p>Oxaloacetate                          Glutamate</p> <p>PLP                                    AST</p> <p>Aspartate                            <math>\alpha</math>-Ketoglutarate</p> |

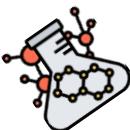
### 5. Value of transamination

#### A. Function:

- ❖ Degradation of a.as to form  $\alpha$ - keto acids.
- ❖ Synthesis of non-essential a.as.

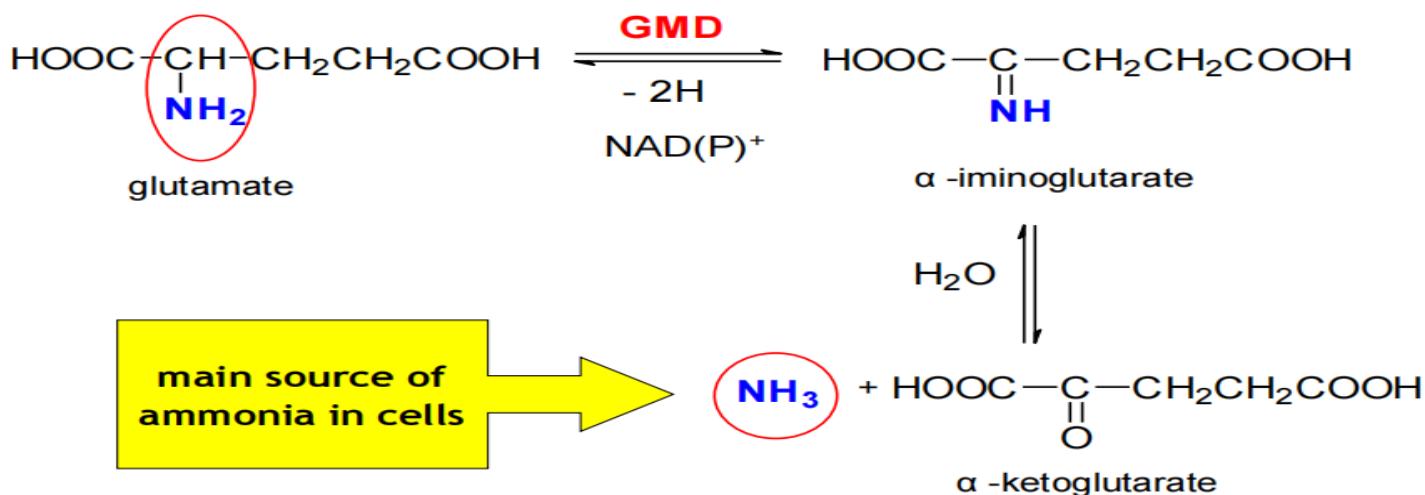
#### B. Diagnostic value

- ❖ Increase level of both ALT & AST indicates possible damage to the liver cells.
- ❖ Increase level of AST alone suggest damage to heart muscle ,skeletal muscle or kidney.



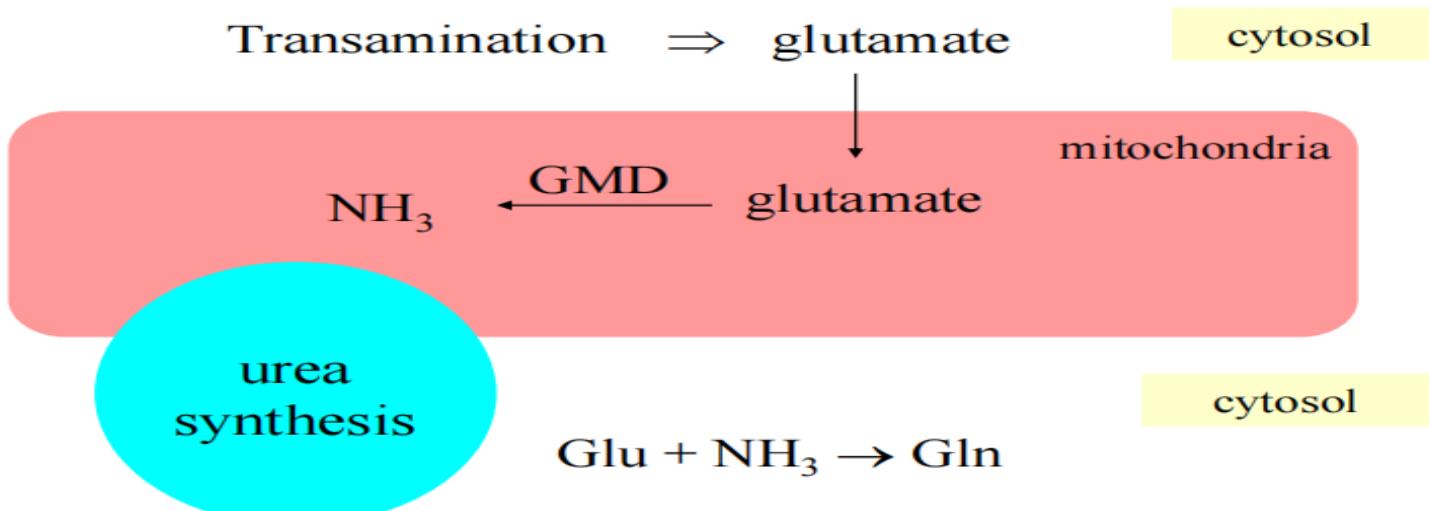
## 6. Notes

- In transamination, nitrogen of most AA is concentrated in glutamate
- Glutamate then undergoes dehydrogenation deamination and releases free ammonia NH<sub>3</sub>
- Dehydrogenation deamination of glutamate is a reversible reaction



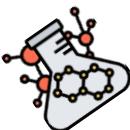
GMD = glutamate dehydrogenase

## 7. Intracellular localization



## Two main sources of ammonia in body

- Dehydrogenation deamination of glutamate in cells of most tissues
- Bacterial fermentation of proteins in large intestine, ammonia diffuses freely into portal blood → portal blood has high concentration of NH<sub>3</sub> → NH<sub>3</sub> is eliminated by liver (under normal cond.)



## Questions

# Written

i) Write a general equation of a reaction catalyzed by aminotransferases

**ANSWER**

## 2) Which cofactor is used by aminotransferases?

---

---

---

**3) What are two main sources of ammonia in human body?**

---

---

---

---

**MCQ**

1) Which of the following is the only amino acid which can be removed through oxidative deamination?

- A. Glycine
  - B. Alanine
  - C. Aspartate
  - D. L-glutamate
  - E. Valine

D

**2) What is the other name of alanine transaminase?**

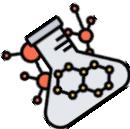
- A. Glutamate oxaloacetate transaminase
  - B. Glutamate lactate transaminase
  - C. Glutamate pyruvate transaminase
  - D. Glutamate ribulose transaminase
  - E. L glutamate dehydrogenase

6

3) What is the other name of aspartate transaminase?

- A. Glutamate oxaloacetate transaminase
  - B. Glutamate lactate transaminase
  - C. Glutamate pyruvate transaminase
  - D. Glutamate ribulose transaminase
  - E. L glutamate dehydrogenase

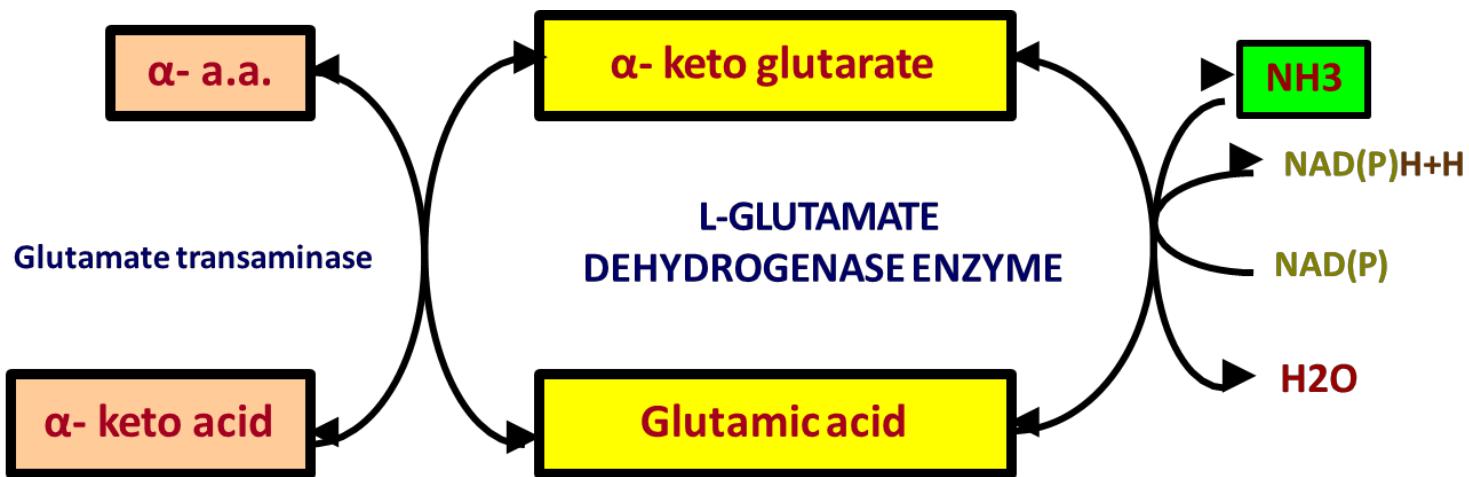
A



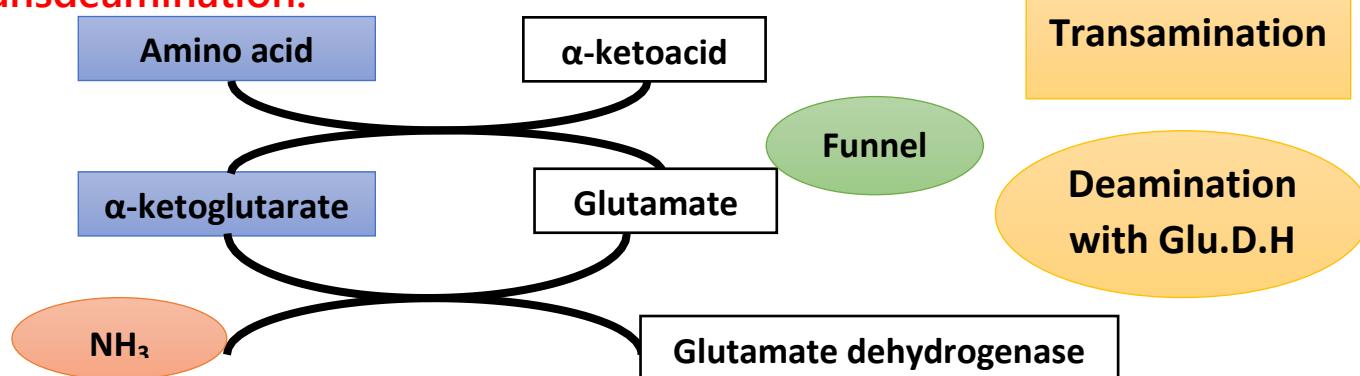
## L28 Bio\_Protein metabolism\_Transamination and transdeamination and specific methods of deamination

### C. Transdeamination

It is the combination of **transamination** & **oxidative deamination**. It includes the transamination of most **a.as** with  $\alpha$ - keto glutarate to form **glutamate** then the **glutamate** is **oxidatively deaminated** reforming  $\alpha$ - keto glutarate and giving **ammonia**. This provides a pathway by which the amino group of most **a.as** is released in the form of ammonia.



### Transdeamination:



Due to... L-amino acid oxidases, but not glutamate dehydrogenase, can sluggish (decrease) the rate of deamination of the amino acids.

So... the most important and rapid way to deamination of amino acids is **first** transamination with  $\alpha$ -ketoglutarate **followed** by deamination of glutamate.

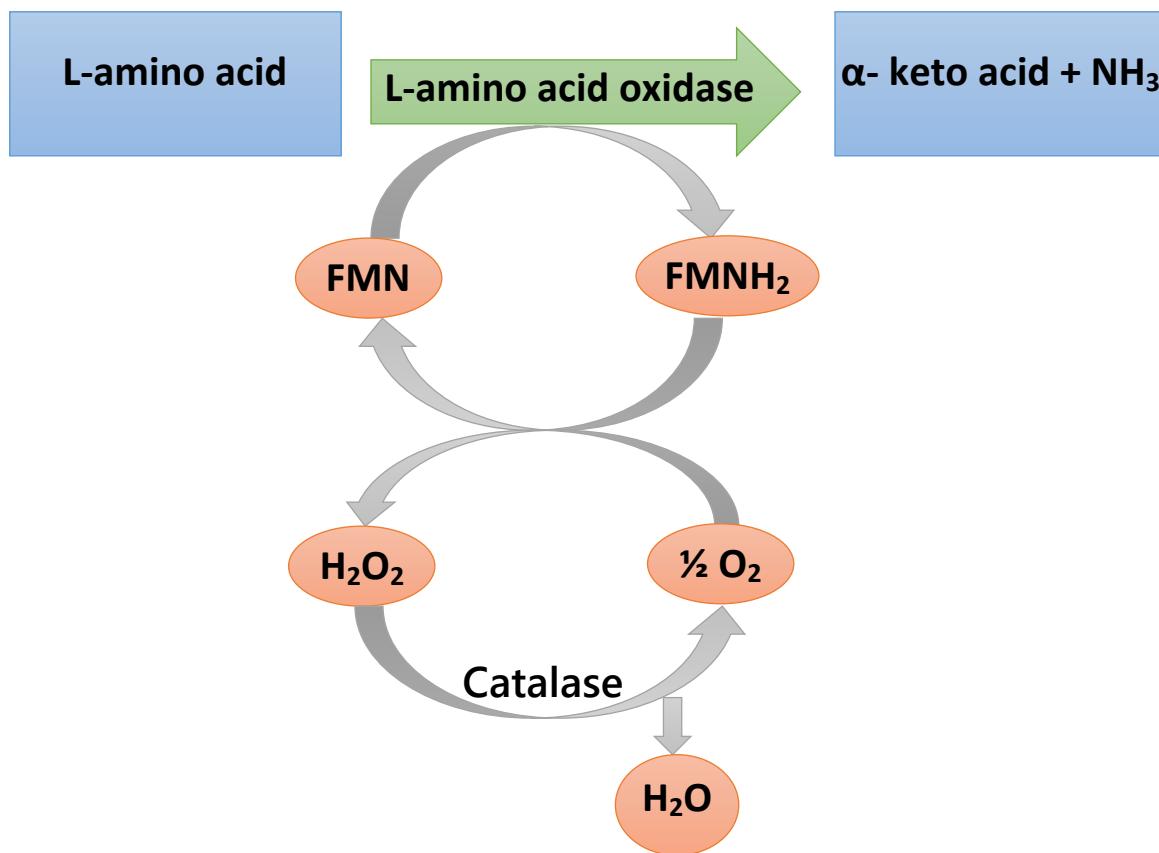
Therefore glutamate through transdeamination serves to a funnel ammonia from all amino acids.



### Fate of carbon skeletons of amino acids

1. Oxidation → TCA → Energy
2. Synthesis of Glucose
3. Formation of lipids ( fatty acids & ketone bodies )
4. Synthesis of non essential amino acids

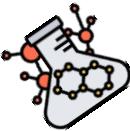
### Oxidative deamination of Amino acid oxidases



➤ Reduction of  $\text{O}_2$  to  $\text{H}_2\text{O}_2$  → hydrolysis to water.

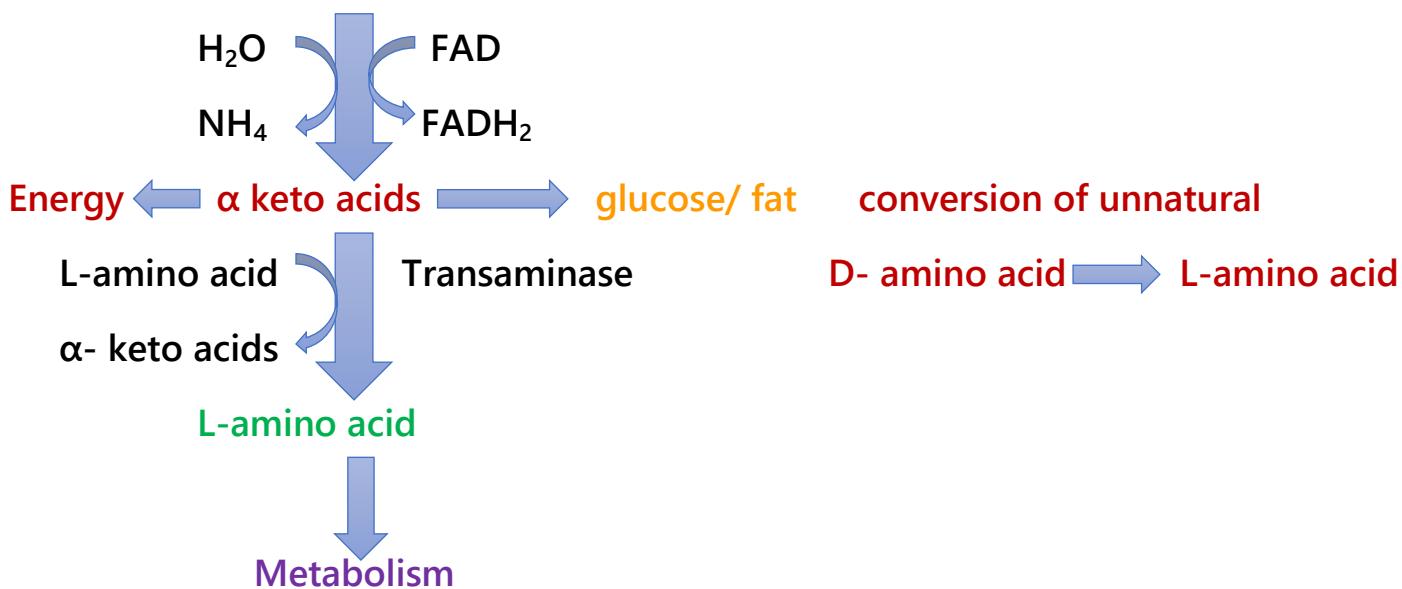
### Oxidative deamination by Amino acid oxidases

- Activity of L -amino acid oxidases low
- Activity of D amino acid oxidase high ( liver & kidney )
- L-amino acid oxidases dose not act on Glycine & di carboxylic acids
- L-amino acid oxidases dose appear to play significant role in amino acid metabolism.



## Oxidative deamination by D Amino acid oxidases

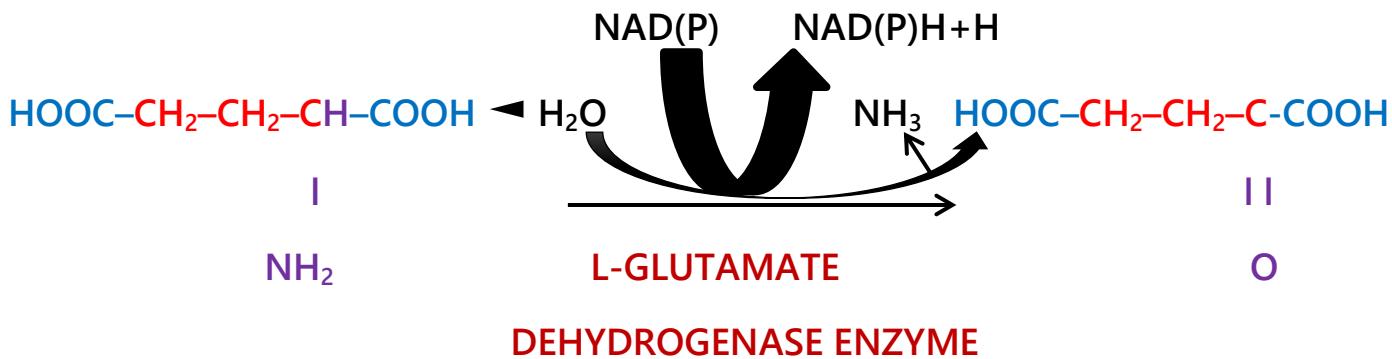
D-amino acids (dietary/ plants/ micro organisms/ mammals)



## Specific deamination of amino acids

Enzymes involved:

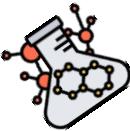
- 1) **L-glutamate dehydrogenase enzyme:** Present in the cytosol & mitochondria of most tissues.



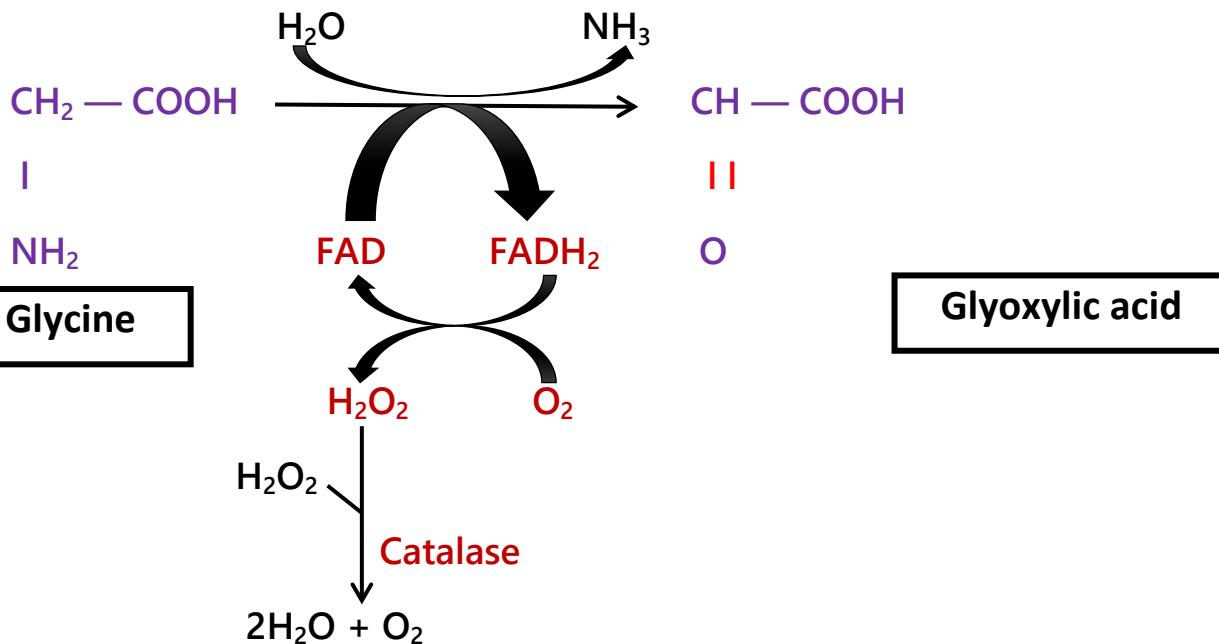
L- glutamic acid

$\alpha$ - ketoglutaric acid

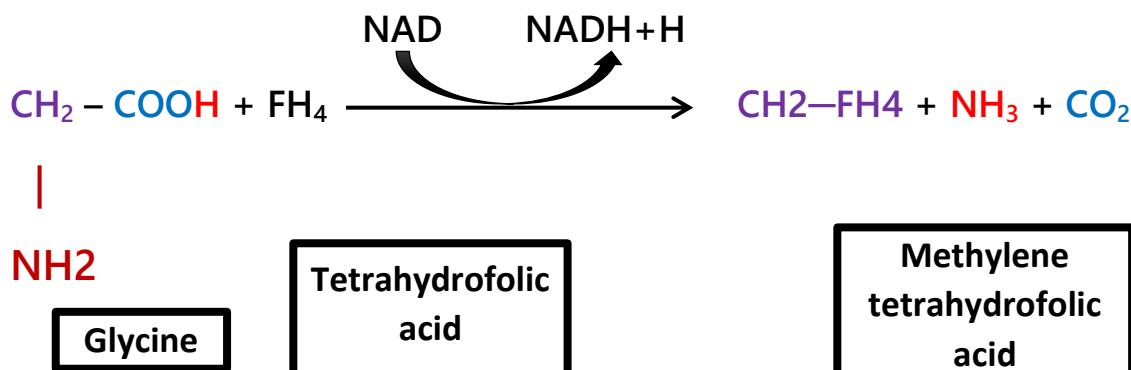
Coenzyme is NAD or NADP



## 2) Glycine oxidase: as the mechanism of action of D- amino acid oxidase

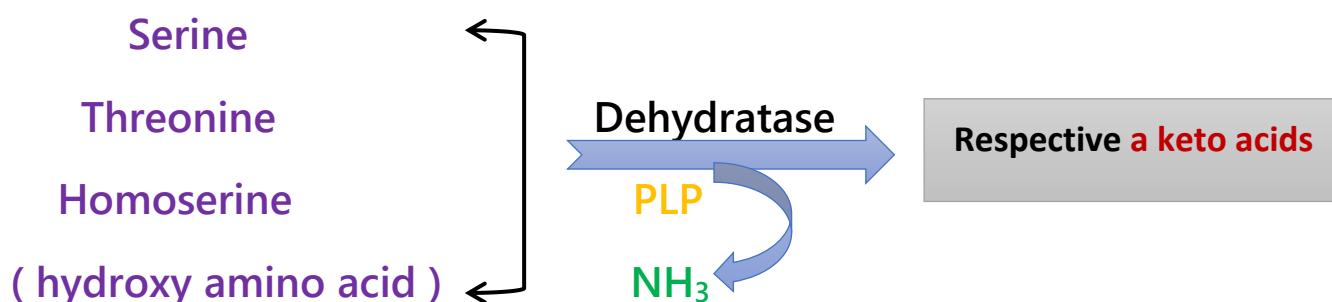


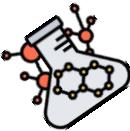
## 3) Glycine cleavage system:



Non- oxidative deamination

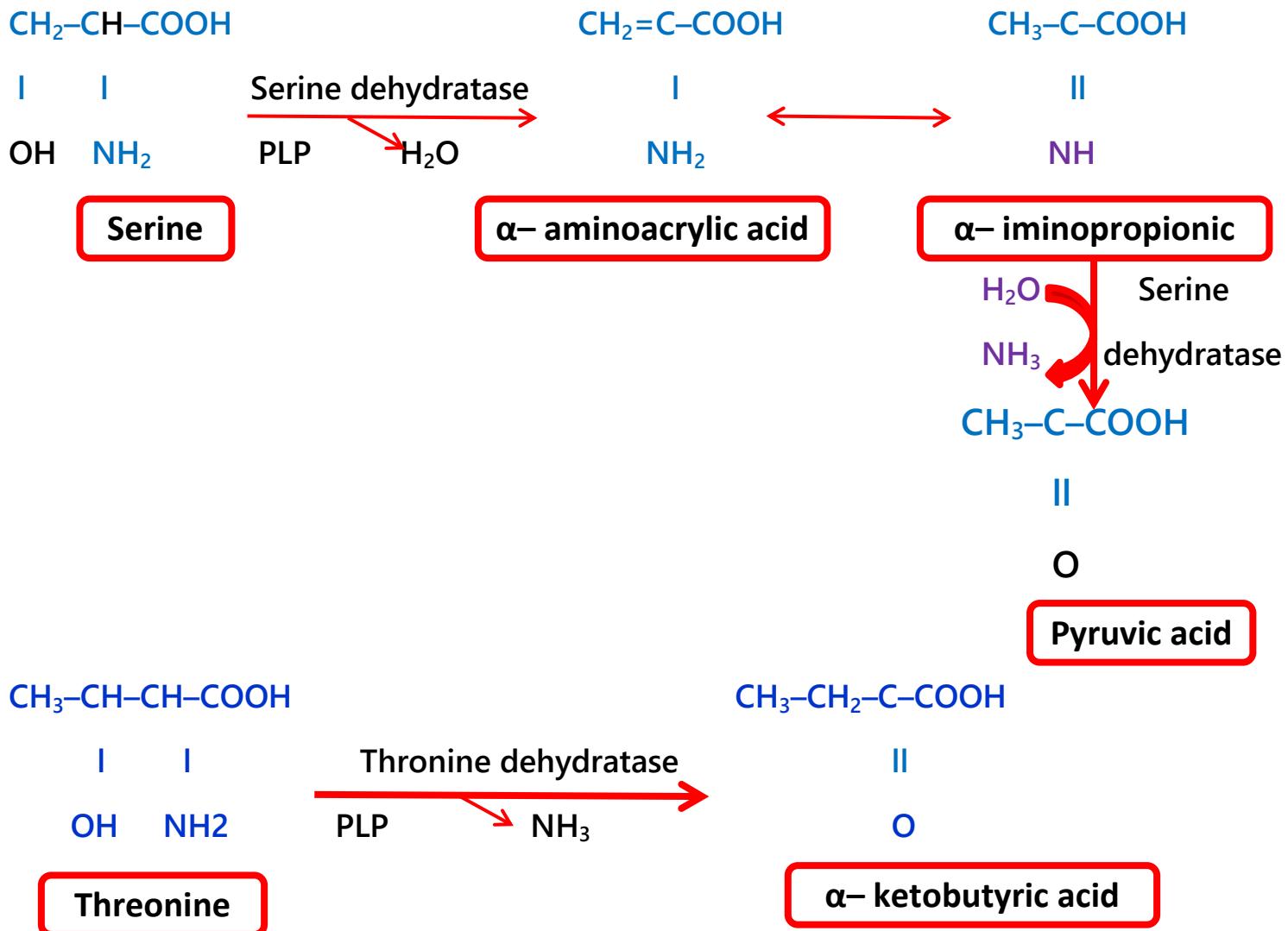
Amino acids deaminated to liberate ammonia without undergoing oxidation



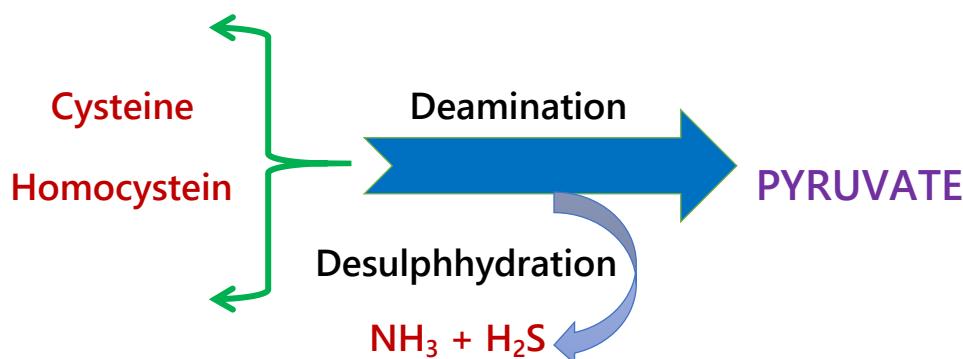


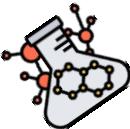
#### 4) Dehydratases: (Non oxidative deamination)

For hydroxy containing a.as (serine& threonine)



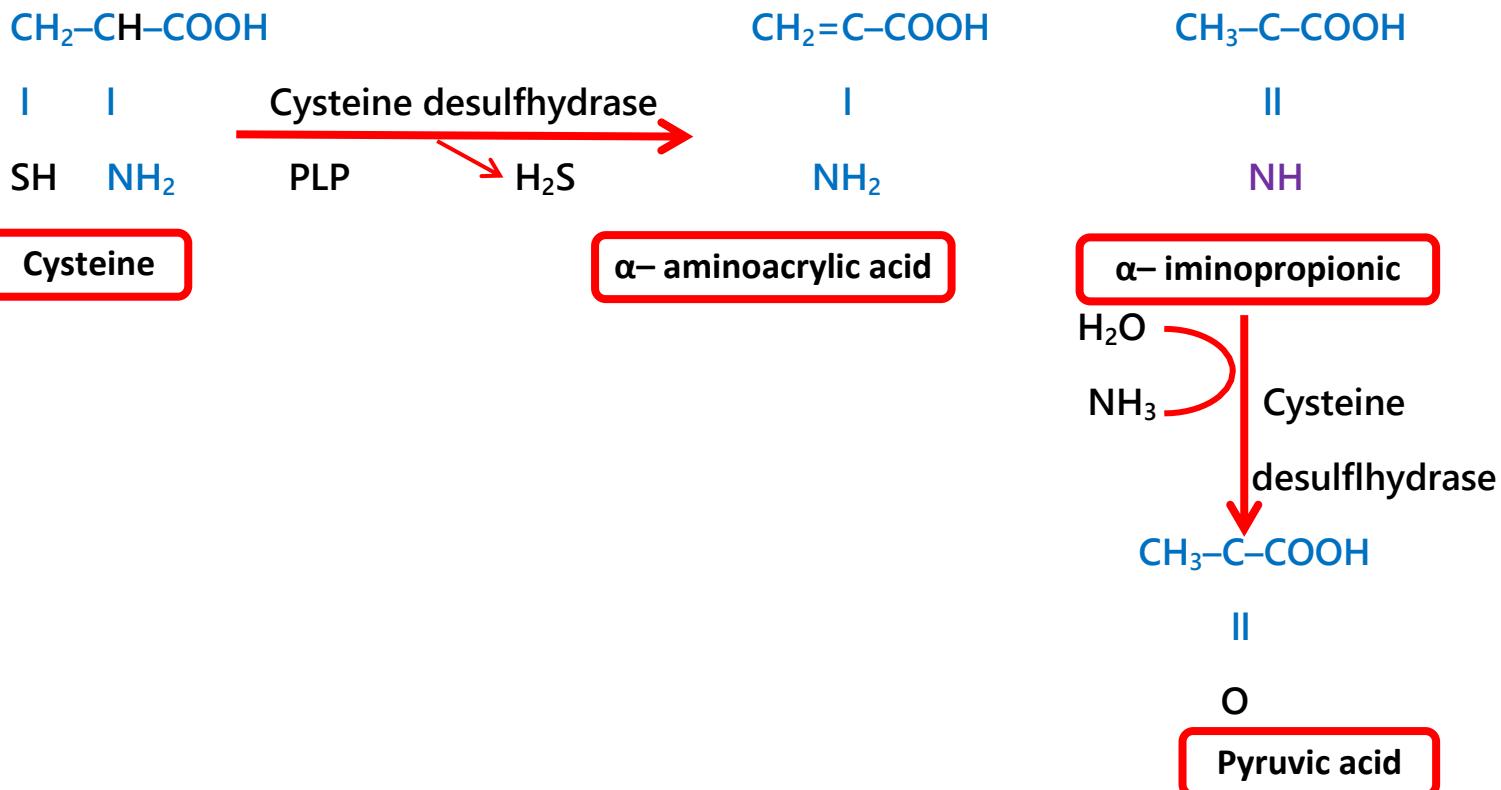
#### Non- oxidative deamination by De sulphhydratases



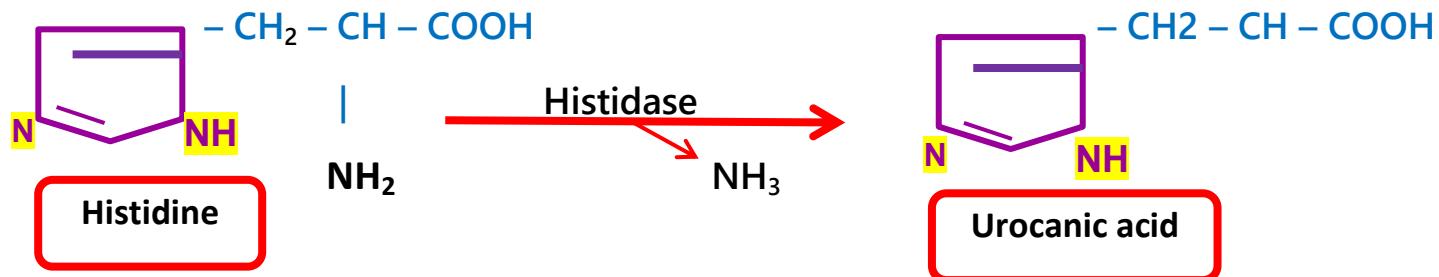


## 5) Desulphydases:

The a.a. cysteine is deaminated by cysteine desulphydase

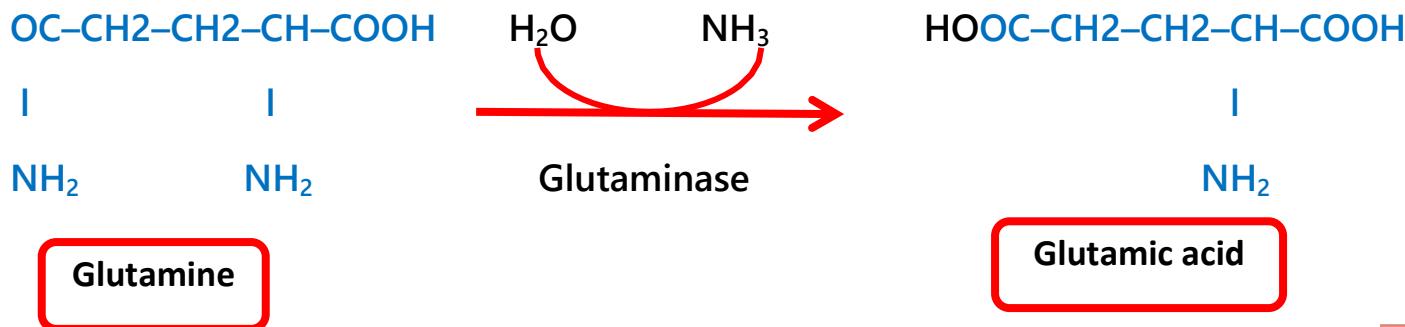


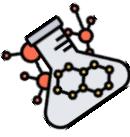
## 6) Histidase: ( Non oxidative deamination)



## 7) Hydrolytic deaminases: Deamination by H<sub>2</sub>O

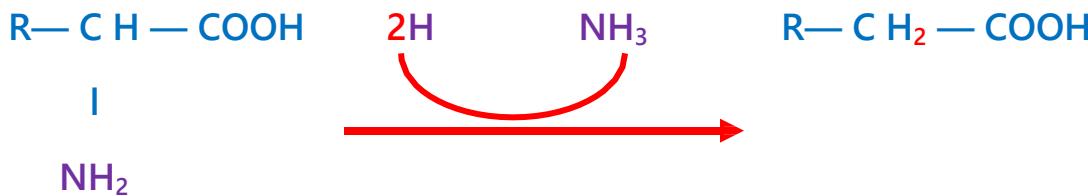
Glutaminase & asparaginase which catalyze the hydrolytic deamination of glutamine & asparagine respectively.





## 8) Reductive deaminases:

By the action of intestinal bacteria on the a.a.s (putrefaction) with the production of the corresponding organic acids.

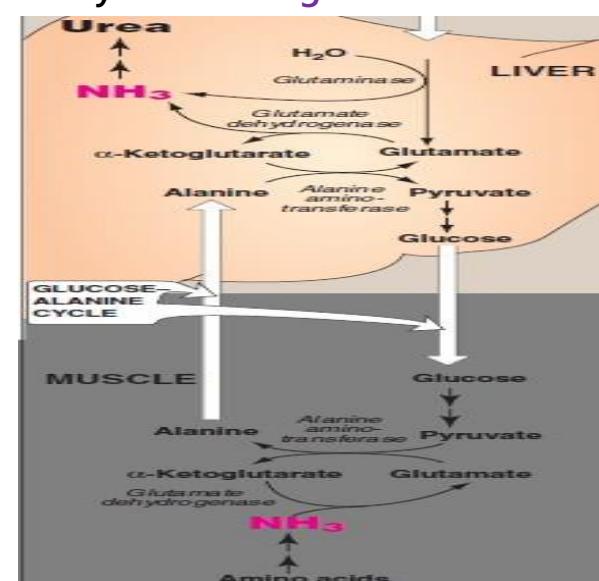
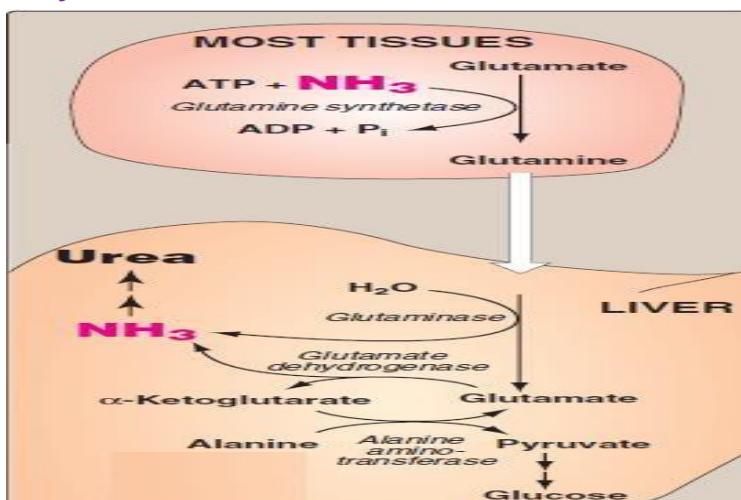


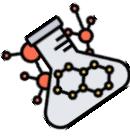
**a- a.a.**

**Corresponding organic acid**

### Transport of ammonia to the liver

- Two mechanisms are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate conversion to urea.
- The first (glutamine), found in most tissues, uses **glutamine synthetase** to combine ammonia ( $\text{NH}_3$ ) with glutamate to form glutamine a nontoxic transport form of ammonia. The glutamine is transported in the blood to the liver where it is cleaved by **glutaminase** to produce glutamate and free ammonia.
- The second (alanine), used primarily by muscle, involves transamination of pyruvate (the end product of aerobic glycolysis) to form **alanine**. Alanine is transported by the blood to the liver, where it is converted to pyruvate, again by transamination.
- In the liver, the pathway of gluconeogenesis can use the pyruvate to synthesize glucose, which can enter the blood and be used by muscle a pathway called the **glucose-alanine cycle**.





## What is the Role of pyridoxal phosphate in transamination?

- Pyridoxal phosphate acts as an intermediate carrier for amino group.
- Pyridoxal phosphate accepts the amino group from amino acid to form.

Pyridoxamine phosphate, which in turn gives the amino group to  $\alpha$ -keto acid.

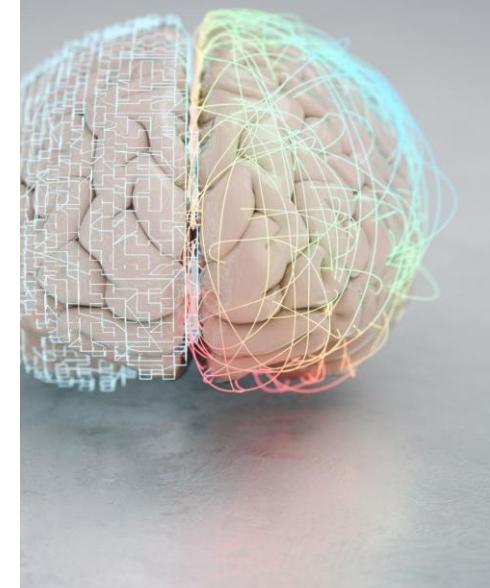
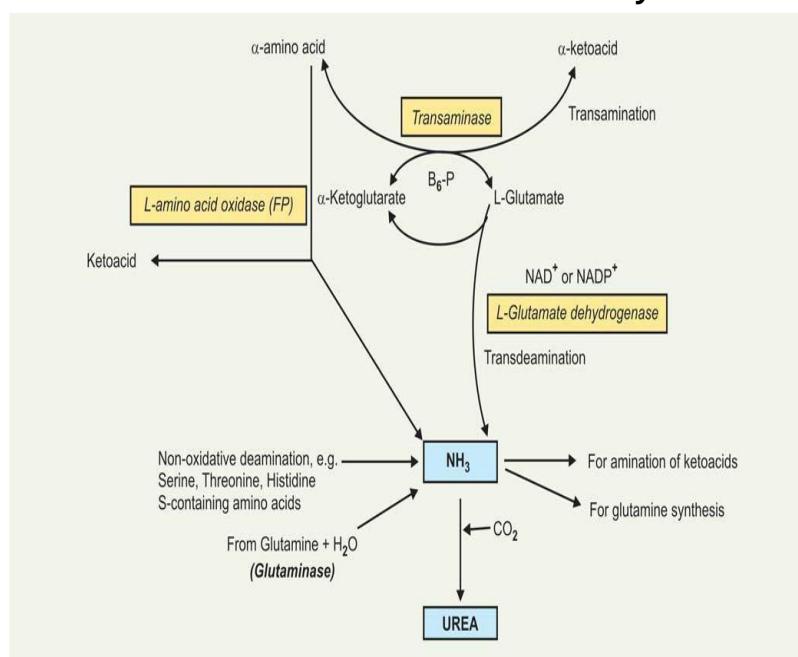
### ➤ Examples of transaminases

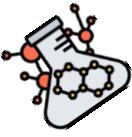
- Alanine transaminase
- Aspartate transaminase
- Glutamate transaminase

## Why NH<sub>3</sub> is toxic?

- Increased NH<sub>3</sub> concentration enhances amination of  $\alpha$ -ketoglutarate, an intermediate in TCA cycle to form glutamate in brain. This reduces mitochondrial pool of  $\alpha$ -ketoglutarate consequently depressing the TCA cycle, affecting the cellular respiration.
- Increased NH<sub>3</sub> concentration enhances "glutamine" formation from glutamate and thus reduces "brain-cell" pool of Glutamic acid. Hence there is decreased formation of inhibitory neurotransmitter "GABA" ( $\gamma$ - amino butyric acid).

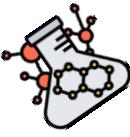
- Rise in brain glutamine level enhances the outflow of glutamine from brain cells.
- Glutamine is carried "out" by the same "transporter" which allows the entry of "tryptophan" into brain cells. Hence "tryptophan" concentration in brain cells increases which leads to abnormal increases in synthesis of "serotonin", a neurotransmitter.





### What are the Features of NH<sub>3</sub> intoxication?

- The symptoms of NH<sub>3</sub> intoxication include:
  - a unusual flapping tremor
  - slurring of speech
  - blurring of vision
  - and in severe cases follows to coma and death.
- These resemble those of syndrome of hepatic coma, where blood and brain NH<sub>3</sub> levels are elevated



## Tut 2 Enzyme 1

### Definition

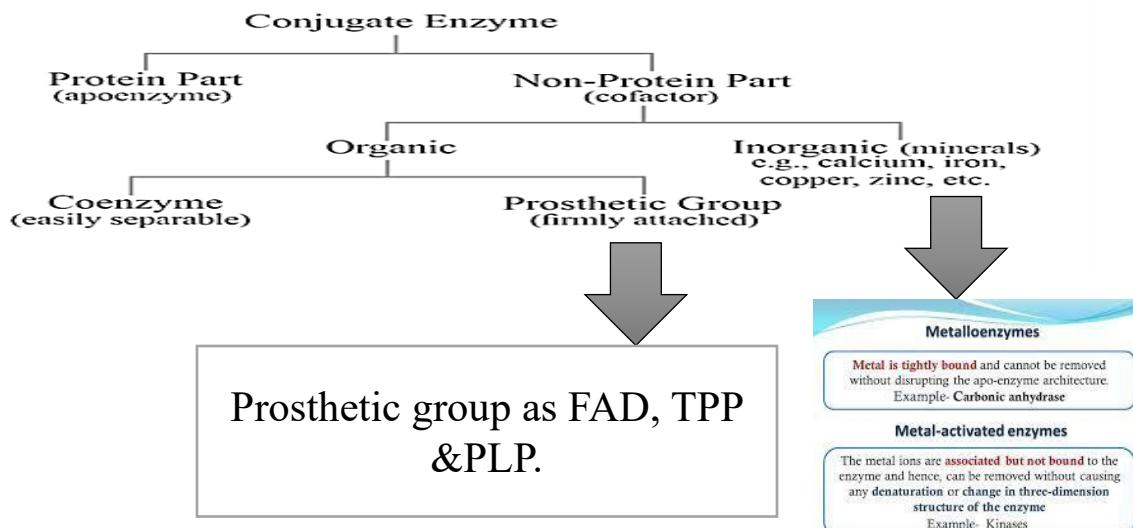
- Enzymes are biological catalysts.
- They increase the rate of chemical reactions (speeds it up)
- Enzymes enables a chemical reaction to proceed under different conditions (e.g., lower temperature),
- Substrates are changed into products
- Enzymes are not used up or changed in a reaction (they can be used over and over again)

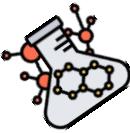
### Chemical nature

- ✓ Almost all enzymes are proteins:
- ✓ Small number are RNA in nature (ribozymes): They are involved in:
  1. RNA splicing: They catalyze cutting and formation of phosphodiester bond of other RNAs.
  2. Protein synthesis: They catalyze the formation of peptide bonds during protein synthesis.

Protein enzymes may be simple proteins or conjugated proteins

- Conjugated protein enzymes are called holoenzymes.
- Holoenzyme is formed of:
  1. Protein part called apoenzyme.
  2. Non protein part called cofactor.



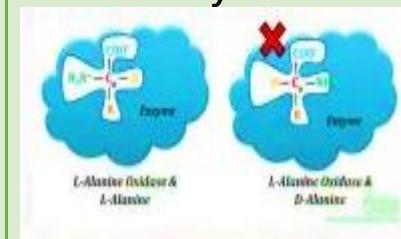


## Enzyme specificity

### Enzyme specificity

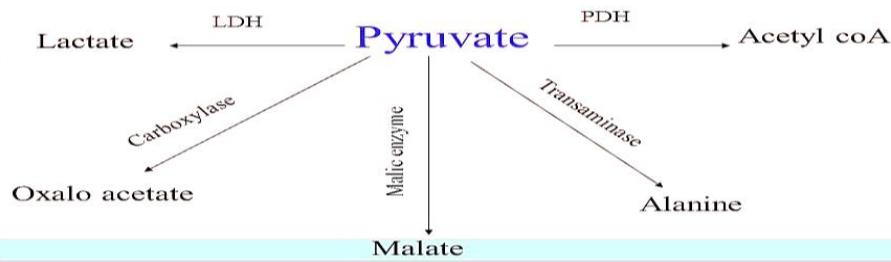
#### 1. Substrate specificity: 4 types

| Absolute specificity                               | Group (broad) specificity                                           | Bond specificity                                                              | Stereospecificity                                                                         |
|----------------------------------------------------|---------------------------------------------------------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Enzyme act only on one substrate                   | Enzyme act on a group of related substrates                         | Enzyme act only on certain bond                                               | Enzyme act only on one of the isomers of the substrate                                    |
| e.g.<br>Glucokinase<br>phosphorylates only glucose | e.g. Hexokinase<br>Phosphorylates glucose, fructose & other hexoses | e.g.<br>Proteolytic enzymes act on peptide bonds<br>Lipases act on ester bond | e.g. L-amino acid oxidase Acts on only L-a.as<br>D-amino acid oxidase Acts on only D-a.as |



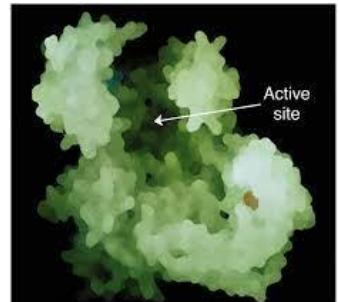
#### 2. Reaction specificity

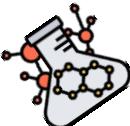
The same substrate can undergo different type of reaction. Each reaction is catalysed by a separate enzyme



## Mechanism of action

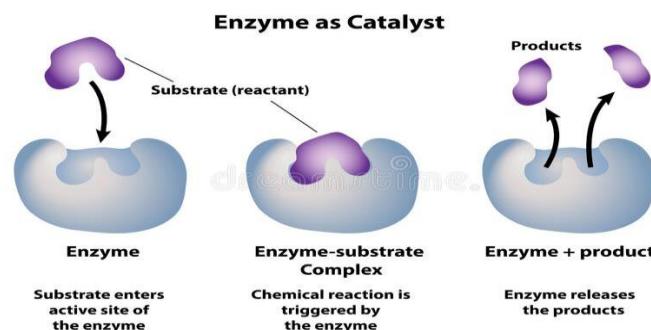
- ✓ Michaelis and Menton proposed a hypothesis for enzyme action.
- ✓ According to their hypothesis, the following steps occurs:
  1. Combination of enzyme with substrate on the active site of enzyme to form enzyme-substrate complex which is a transient complex
  2. This complex immediately dissociates to form products and enzyme.





**Active Site:** The site in the three-dimensional enzyme structure at which the substrate binds and is converted to product.

**Transition state:** It is the state in which the substrate is bound to the enzyme



### ➤ 2 theories to explain the substrate enzyme binding:

#### 1. Fischer's template theory (Key& lock theory):

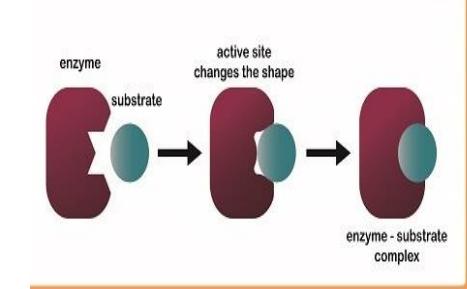
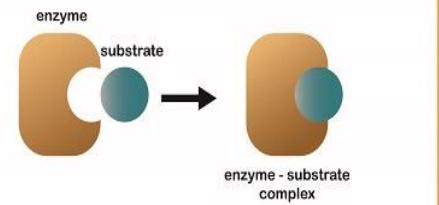
- The 3 dimensional structure of the active site of the enzyme is complementary to the substrate (even in the absence of substrate).
- The substrate fits in the active site of the enzyme as the key fits in its lock.

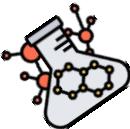
#### 2. Induced fit theory:

- The active site of the enzyme and the substrate are not complementary to each other in shape.
- Binding of the substrate to the active site, induces a change in shape of the active site so that complete binding occurs.

### ➤ Lowering of activation Energy:

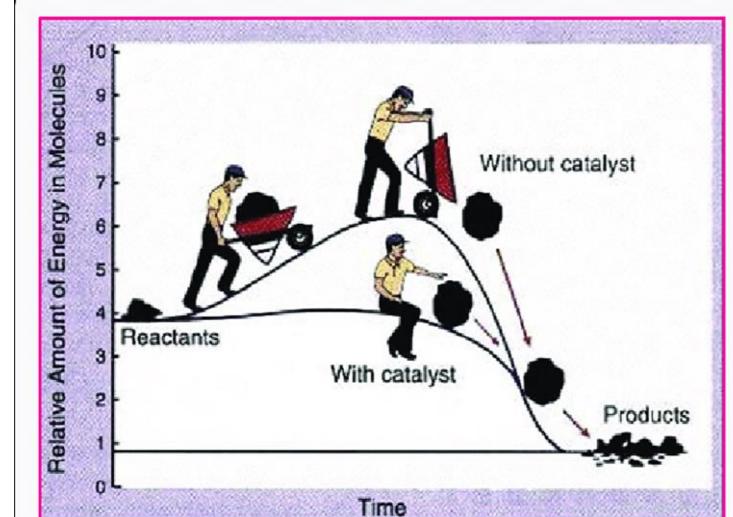
- All chemical reactions need energy to get started.
- Even reactions that release energy need a boost of energy in order to begin.





- The energy needed to start a chemical reaction is called activation energy. Enzyme works by reducing the amount of activation energy needed to start the reaction. Since less energy is required to start the reaction, the rate of the reaction is increased.

Activation energy: is the energy needed to convert all the molecules of reacting substances from the ground state (enzyme unbound) to the transition state (enzyme bound).

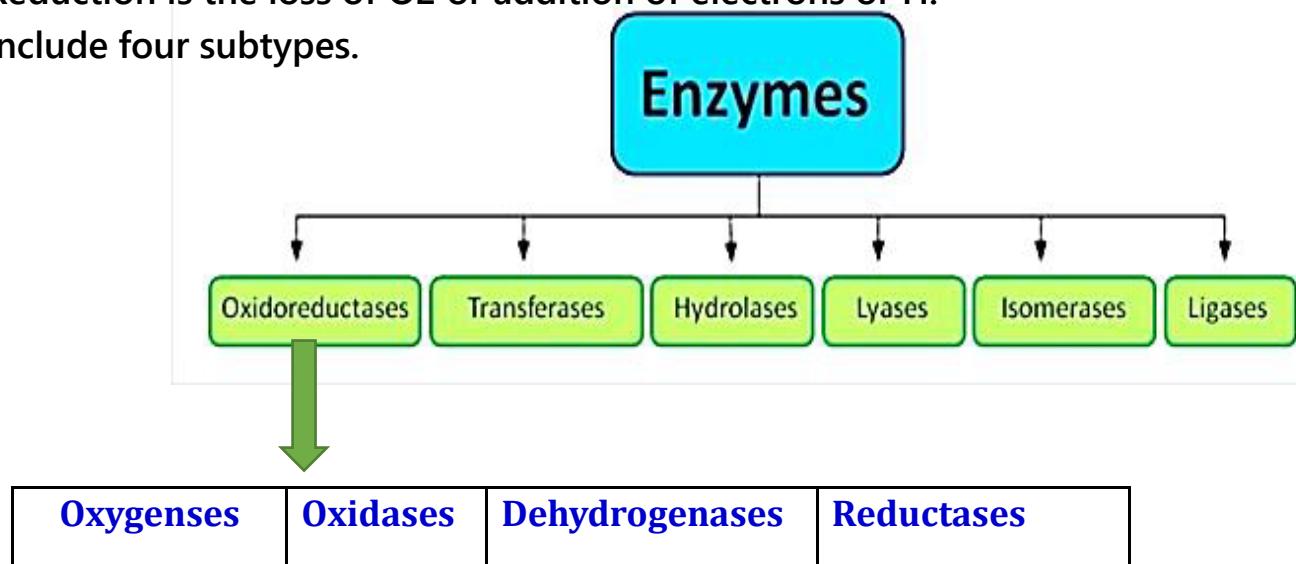


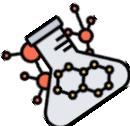
### Enzymes classification

Classification according the type of reactions catalyzed by the enzyme

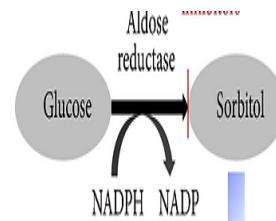
#### 1. Oxidoreductases:

- Catalyze oxidation reduction reactions.
- Oxidation is the addition of O<sub>2</sub> or loss of electrons or H.
- Reduction is the loss of O<sub>2</sub> or addition of electrons or H.
- Include four subtypes.





| Oxygenses                                                         |                                                     | Oxidases                                                                                                    | Dehydrogenases                                                                                                   | Reductases                                                      |
|-------------------------------------------------------------------|-----------------------------------------------------|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| Add one or two atoms of molecular O <sub>2</sub> to the substrate |                                                     | removes H or E from substrate to O <sub>2</sub> (that act as hydrogen acceptor)                             | Removes H from substrate to another hydrogen carrier. The hydrogen acceptor is usually NAD or NADP & FAD or FMN. | Add hydrogen to the substrate using NADPH+H as hydrogen carrier |
| Dioxygenase                                                       | Monooxygenase (Hydroxylase)                         |                                                                                                             |                                                                                                                  |                                                                 |
| Add both atoms of O <sub>2</sub>                                  | Add one atom of O <sub>2</sub> to create – OH group |                                                                                                             |                                                                                                                  |                                                                 |
| A+O <sub>2</sub> →A-O <sub>2</sub>                                | A-H+O <sub>2</sub> ----→AOH+H <sub>2</sub> O        | <p style="text-align: center;"><b>oxidases</b></p> <p style="text-align: center;"><b>dehydrogenases</b></p> |                                                                                                                  |                                                                 |



## 2. Transferases: Transfer groups between donor & acceptor molecules

e.g: Aminotransferases (Transaminases).

## 3. Hydrolases: Break a bond using water (hydrolytic cleavage of bonds).

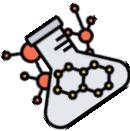
e.g: Glycosidases: hydrolyze glycosidic bond using H<sub>2</sub>O.

## 4. Lyase: Add or remove water, ammonia or CO<sub>2</sub> by cleavage of C-C,C-S & some C-N bonds

e.g:

1. Lyases as Citrate lyase: cleaves C-C bond.
2. Decarboxylase: removes CO<sub>2</sub> from the substrate.

|                           |  |                                                                                    |
|---------------------------|--|------------------------------------------------------------------------------------|
| 2 Transferases            |  | C-Transferases<br>Glycosyltransferases<br>Aminotransferases<br>Phosphotransferases |
| 3 Hydrolases              |  | Esterases<br>Glycosidases<br>Peptidases<br>Amidases                                |
| 4 Lyases ("synthases")    |  | C-C-Lyases<br>C-O-Lyases<br>C-N-Lyases<br>C-S-Lyases                               |
| 5 Isomerases              |  | Epimerases<br>cis trans isomerasers<br>Intramolecular transfersases                |
| 6 Ligases ("synthetases") |  | C-C-Ligases<br>C-O-Ligases<br>C-N-Ligases<br>C-S-Ligases                           |



## 5. Isomerases: Transfer groups within the same molecule

e.g: Epimerase: catalyze interconversion of epimers

## 6. Ligases

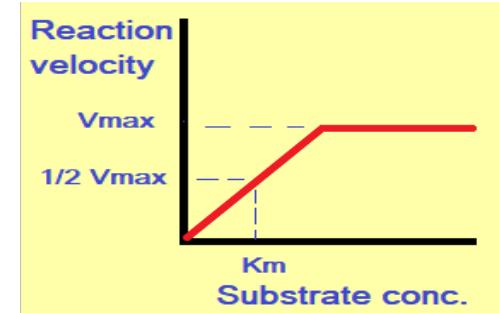
Form new covalent bond using high energy from ATP

e.g: Synthetase: Enzyme that link 2 molecules in ATP- dependant reaction.

## Factors affecting enzyme activity

### 1. Substrate concentration:

- ↑ Substrate conc. → ↑ reaction velocity (V), till the maximum velocity (V<sub>max</sub>) is reached, where any further ↑ in the substrate conc. Will not cause increase in the reaction velocity
- V<sub>max</sub> is reached when all the enzyme molecules are in the form of E-S complex.
- Michaelis Constant (k<sub>m</sub>):



It is the substrate conc. at which the reaction velocity is 1/2 the max. velocity.

- It is constant for each enzyme.
- It is: \*High → when the enzyme has lower affinity for the substrate  
\*Low → when the enzyme has higher affinity for the substrate

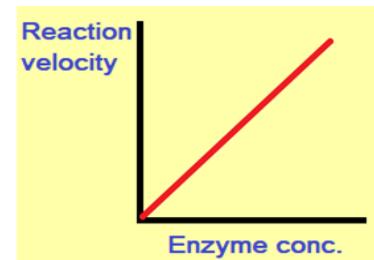
$[S] \propto V$  until V<sub>max</sub> is reached

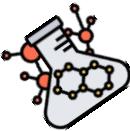
### 2. Products concentration [P]:

In reversible reaction, ↑ product conc. → slowing, stoppage or even reversal of the reaction occurs

### 3. Enzyme concentration [E]:

↑ Enzyme conc. → ↑ reaction velocity when enough substrate present  $[E] \propto V$

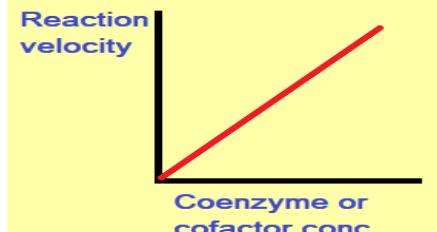




## 4. Coenzyme or activators concentration

$\uparrow$ Coenzyme conc.  $\rightarrow \uparrow$  reaction velocity

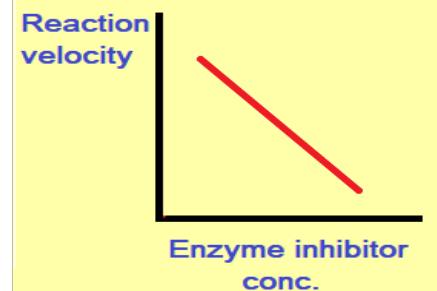
$$[C] \propto V$$



## 5. Enzyme inhibitors concentration

$\uparrow$ Enzyme inhibitor conc.  $\rightarrow \downarrow$  reaction velocity

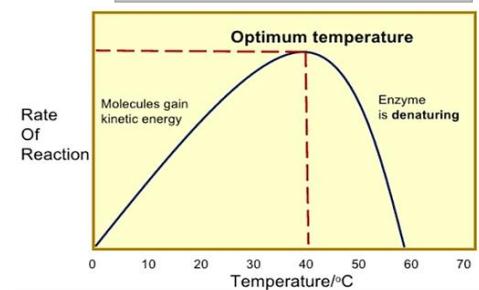
$$[I] \propto 1/V$$



## 6. Temperature:

- Optimum temperature is the temperature at which the enzyme is optimally active (enzyme velocity reaches  $V_{max}$ )..
- The optimum temp. of most enzymes is 37°C.
- Any  $\uparrow$  or  $\downarrow$  of temp. away from the optimal temp.  $\rightarrow \downarrow$  enzyme activity.
- At 0°C  $\rightarrow$  there is no activity of the enzyme
- Above the optimal temp, (usually above 56°C)  $\rightarrow$  Denaturation occurs with loss of enzyme activity

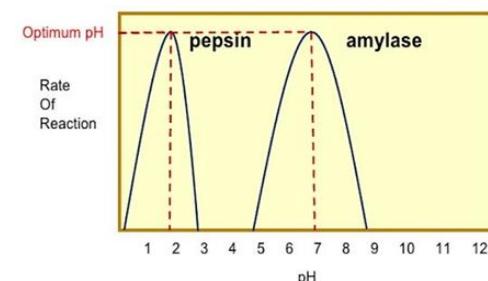
Bell shaped curve



## 7. PH:

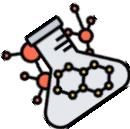
- Optimum PH is the PH at which the enzyme is optimally active.
- Each enzyme has optimal PH.
- Any  $\uparrow$  or  $\downarrow$  of PH away from the optimal PH.  $\rightarrow \downarrow$  enzyme activity.
- Exposure to strong acidic or alkaline conditions  $\rightarrow$  irreversible enzyme denaturation with loss of enzyme activity

Bell shaped curve

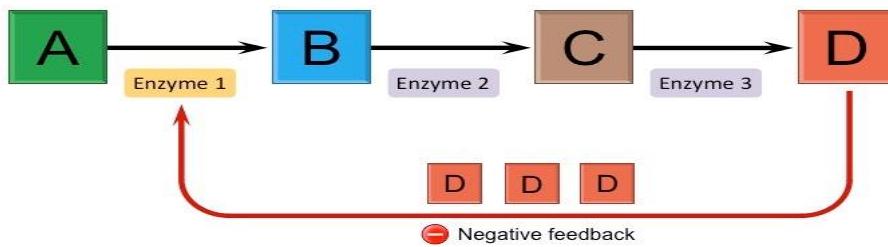


## 8. Allosteric regulation

- Some enzymes contain an allosteric site (other than the active site).
- Allosteric enzymes catalyze irreversible reactions
- Allosteric enzyme frequently catalyze the early rate limiting step (irreversible reaction) in a metabolic pathway (i.e. is the key enzyme or the rate limiting enzyme of the metabolic pathway)

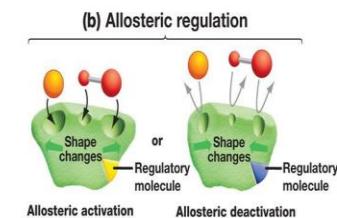


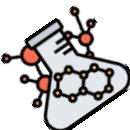
- Allosteric effectors are usually the end products of the pathway, that causes feed back inhibition of the allosteric



### Regulation of Enzyme Action

- Allosteric inhibition:** regulatory molecule binds at site other than active site (allosteric = different structure)
  - binding changes shape of active site





## Tut 3 Enzymes 2

### Enzymes: Factors affecting enzyme activity

#### 9. Covalent modification:

- It is ↑ or ↓ of the enzyme activity by "addition or removal of a group in the protein part of the enzyme" by other enzymes.
- This group is attached by covalent bond to a.a. residue of the enzyme.
- The addition or removal of this group → change in binding affinity to substrate.
- The most important groups:

phosphate, methyl, uridine, adenine and ADP-ribose

#### 1. Reversible protein phosphorylation:

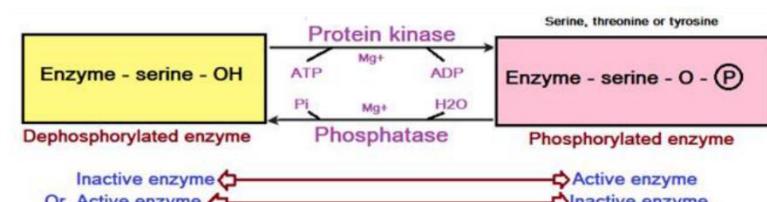
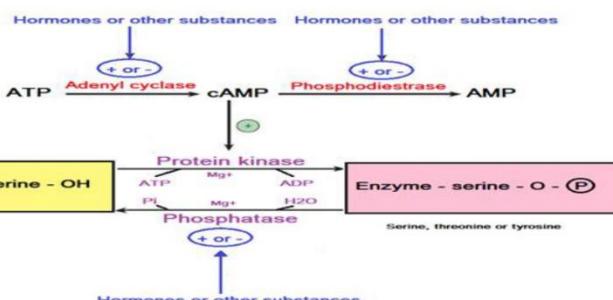
##### Most common type of covalent modification

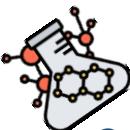
- Addition or removal of phosphate group to serine, threonine or tyrosine residues of the enzyme → ↑ or ↓ enzyme activity.
- Protein kinase produces phosphorylation of the enzyme
- Phosphatase produces dephosphorylation of the enzyme
- Phosphorylated enzyme may become active or inactive according to the enzyme..

#### Regulation of Phosphorylation-dephosphorylation of the enzyme :

Hormones or other substances stimulate or inhibit protein kinase or phosphatase.

- Hormones ++ adenyl cyclase → ↑ cAMP → ++ Protein kinase → phosphorylation of the enzyme.
- Hormones ++ phosphodiesterase → ↓ cAMP → -- protein kinase → dephosphorylation of the enzyme.
- Hormones ++ phosphatase → dephosphorylation of the enzyme.





## 2. Proenzyme (zymogen) activation by Limited proteolysis:

- Proenzyme or zymogen is **inactive** precursor of the enzyme → by **cutting** some specific peptide bonds change the conformation of the enzyme to become **active** enzyme that can bind the substrate.
- Proteolysis is **irreversible**.
- E.g. **Pepsinogen** in stomach  $\xrightarrow{\text{HCl}}$  Pepsin + peptide

(Inactive zymogen)

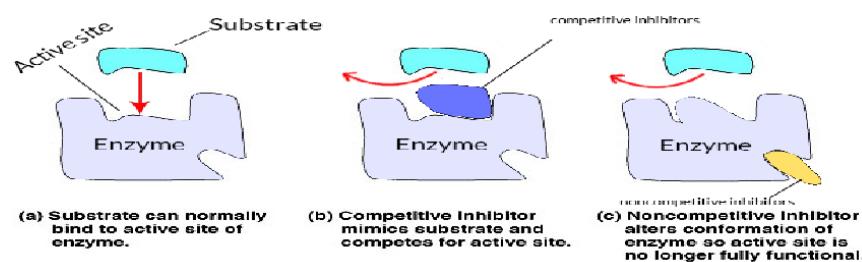
HCl

(Active enzyme)

### Enzyme inhibitors:

- ✓ Reversible inhibitors
- ✓ Irreversible inhibitors

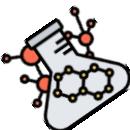
#### I- Reversible inhibitors:



They bind **non covalently** to the enzymes by hydrogen bonding or ionic interactions.

They include:

| 1. Competitive inhibitors                                                                                                                                                                        | 2. Non competitive inhibitors                                                                                                                                                                                                                                                                                                                                           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>They are <b>similar</b> in structure to substrate.<br/>So they <b>compete</b> with the substrate for binding to the active site of the enzyme.<br/>They are <b>reversible</b> inhibitors.</p> | <p>They are <b>not similar</b> in structure to substrate. So they <b>don't compete</b> with the substrate for binding to the active site of the enzyme.<br/>Instead they <b>reversibly</b> bind to the enzyme at a site <b>different</b> from the active site. They <b>change</b> the conformation of the enzyme, so the active site is no longer fully functional.</p> |
| Binds to the <b>free enzyme</b> only                                                                                                                                                             | Binds to the <b>Free enzyme or enzyme substrate complex</b>                                                                                                                                                                                                                                                                                                             |
| ↑ substrate <b>overcome</b> inhibition                                                                                                                                                           | ↑ substrate <b>doesn't overcome</b> inhibition.                                                                                                                                                                                                                                                                                                                         |
| <p>V<sub>max</sub> <b>unchanged</b><br/>K<sub>m</sub> <b>increased</b></p>                                                                                                                       | <p>V<sub>max</sub> <b>decreased</b><br/>K<sub>m</sub> <b>unchanged</b></p>                                                                                                                                                                                                                                                                                              |
| They can be used as drugs                                                                                                                                                                        | e.g.<br>Heavy metals at lower conc. are reversible non-competitive inhibitors.                                                                                                                                                                                                                                                                                          |



Competitive inhibitors can be used as drugs e.g.

| Inhibitor    | Structurally similar to       | Competitively inhibit                                                                       |
|--------------|-------------------------------|---------------------------------------------------------------------------------------------|
| Dicumarol    | Vitamin K                     | Vitamin K activity so used as anticoagulant                                                 |
| Methotrexate | Folic acid                    | Folate reductase needed for DNA synthesis & cell division so used as anticancer             |
| Sulfonamides | Paraamino benzoic acid (PABA) | Dihydrofolate reductase, thus blocking the conversion of PABA in the bacteria to folic acid |
| Allopurinol  | Hypo-xanthine                 | Xanthine oxidase. So allopurinol ↓ uric acid formation so used in gout treatment            |

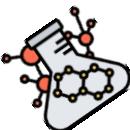
|              |                                         |          |                                         |           |
|--------------|-----------------------------------------|----------|-----------------------------------------|-----------|
| Hypoxanthine | $\xrightarrow{\text{Xanthine oxidase}}$ | Xanthine | $\xrightarrow{\text{Xanthine oxidase}}$ | Uric acid |
|--------------|-----------------------------------------|----------|-----------------------------------------|-----------|

## II- Irreversible inhibitors:

They bind **very tightly** to the enzyme through **covalent linkage**, so not easily dissociate from the enzyme.

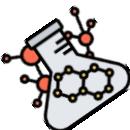
They bind the **active site** or **other binding** site of the enzyme.

↑ substrate **doesn't** overcome inhibition.



Important types include:

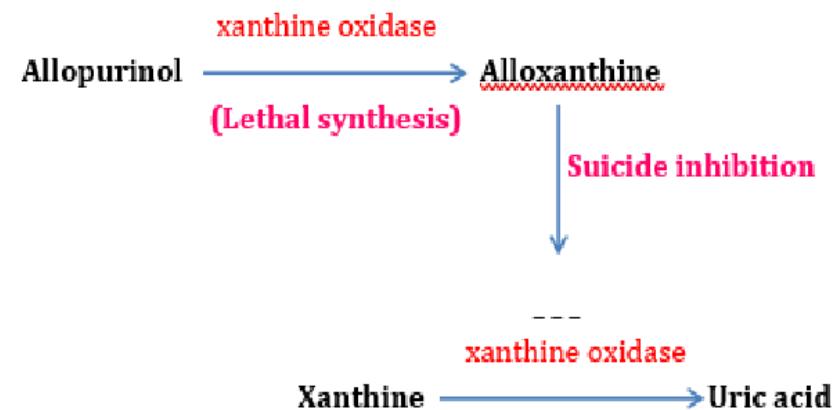
| 1. Group specific irreversible inhibitors                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 2. Suicide inhibitors                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>They react with specific R-groups of the a.a. residues of the enzyme, that play an important role in catalysis.</p>                                                                                                                                                                                                                                                                                                                                                                                                               | <p><u>2 steps:</u></p> <p>1. A substrate of enzyme synthesize inhibitor to that enzyme &amp; this process is called lethal synthesis. This enzyme normally induce another reaction in the body.</p> <p>2. The inhibitor binds to the active site of the enzyme which synthesized it, where it is modified by the enzyme to produce a reactive group that reacts by irreversible covalent bonding to the enzyme to form inhibitor enzyme complex. Thus the enzyme can not act on the normal substrate that it acts on normally in the body</p> |
| <p>e.g.</p> <p>1. <b>Diiisopropylfluorophosphate (DIFP)= Nerve gas poison:</b> one of the organophosphorous compounds that irreversibly binds the serine residues of the enzyme's active site as acetylcholine esterase enzyme → toxic accumulation of acetylcholine.</p> <p>2. <b>Heavy metals as Pb+2, Ag+, Hg+2 :</b> irreversibly bind the –SH group of cysteine residue of the enzymes. <b>BAL</b> (British Anti Lewisite) is –SH containing compound that interact with heavy metals, So used as <b>antidote</b> for them.</p> | <p>Normally the enzyme xanthine oxidase catalyze the following reactions in the body.</p> <p>Hypoxanthine <math>\xrightarrow{\text{xanthine oxidase}}</math> Xanthine<br/> <math>\xrightarrow{\text{xanthine oxidase}}</math> Uric acid</p> <p>The drug <b>Allopurinol</b> is a structural analog of hypoxanthine. It is converted in the body by xanthine oxidase to <b>Alloxanthine (=oxypurinol)</b> (lethal synthesis). Alloxanthine binds by covalent irreversible bond to xanthine oxidase forming enzyme inhibitor complex.</p>        |



So allopurinol inhibits uric acid formation by inhibition of xanthine oxidase by 2

Mechanisms:

1. Reversible competitive inhibition.
2. Irreversible suicide inhibition



| Enzyme regulation: Occurs by 2 major mechanisms          |                       |                                                              |                                |
|----------------------------------------------------------|-----------------------|--------------------------------------------------------------|--------------------------------|
| Regulation of enzyme activity<br>(Short term regulation) |                       | Regulation of enzyme concentration<br>(Long term regulation) |                                |
| Covalent modification                                    | Allosteric regulation | Induction of enzyme synthesis                                | Repression of enzyme synthesis |