# Biologic oxidation

By: Inaam A. Ameen

# Biological oxidation

- Biological oxidation is that oxidation which occurs in biological systems to produce energy.
- Oxidation can occur by:

1-Addition of oxygen (less common)

2-Removal of hydrogen (common)

3-Removal of electrons (most common)

- Electrons are not stable in the free state, so their removal form a substance (oxidation) must be accompanied by their acceptance by another substance (reduction) hence the reaction is called oxidation-reduction reaction or redox reaction and the involved enzymes are called oxido-reductases

# Biologic importance

- Oxidation:the removal of electrons and reduction as the gain of electrons. Thus, oxidation is always accompanied by reduction of an electron acceptor.
- The life of higher animals is absolutely dependent upon a supply of oxygen for respiration**,** the process by which cells derive energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water.
- molecular oxygen is incorporated into a variety of substrates by enzymes known as oxygenases; many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized by enzymes of this class, known as the cytochrome P450 system.
- **Oxidation** is the *loss* of electrons
- **Reduction** is the *gain* of electrons

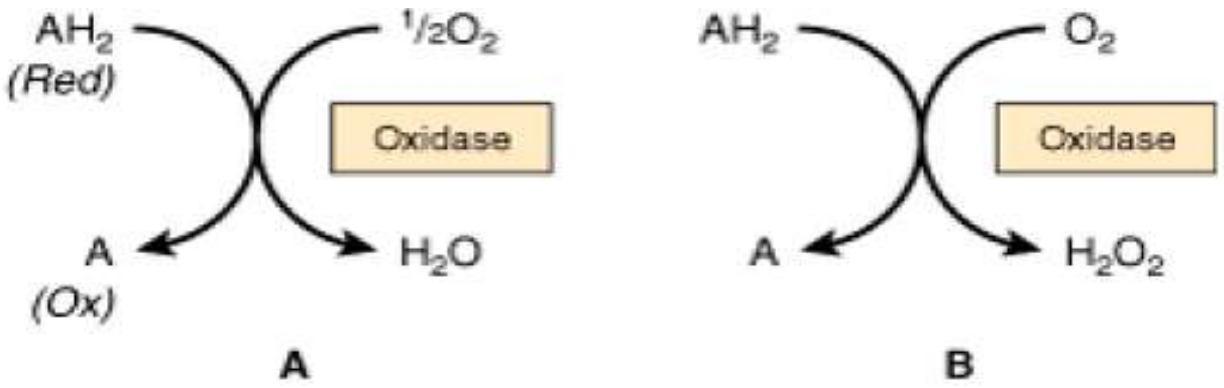
# REDOX POTENTIAL

- It is the affinity of a substance to accept electrons i.e. it is the potential for a substance to become reduced.
- Hydrogen has the lowest redox potential (-0.42 volt), while oxygen has the highest redox potential (+0.82 volt). The redox potentials of all other substances lie between that of hydrogen and oxygen.
- Electrons are transferred from substances with low redox potential to substances with higher redox potential.
- This transfer of electrons is an energy yielding process and the amount of energy liberated depends on the redox potential difference between the electron donor and acceptor.

# oxidoreductases

- Enzymes involved in oxidation and reduction are called oxidoreductases and are classified into four groups:
- oxidases, dehydrogenases, hydroperoxidases, and oxygenases.

1. Oxidases: (oxidases use oxygen as a hydrogen acceptor) Oxidases catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor. They form water or hydrogen peroxide as a reaction product.




Oxidation of a metabolite catalyzed by an oxidase **(A)** forming H2 O, **(B)** forming H2O2

# Some Oxidases Contain Copper & Other Oxidases Are Flavoproteins:

- Cytochrome oxidase is a hemoprotein widely distributed in many tissues, having the typical heme prosthetic group present in myoglobin, hemoglobin, and other cytochromes.
- The enzyme is poisoned by carbon monoxide, cyanide, and hydrogen sulfide.
- It contains two molecules of heme, each having one Fe atom that oscillates between Fe3+ and Fe2+ during oxidation and reduction. Furthermore, two atoms of Cu are present, each associated with a heme unit.
- Flavoprotein enzymes contain flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as prosthetic groups. FMN and FAD are formed in the body from the vitamin riboflavin (B2) and usually tightly—but not covalently—bound to their respective apoenzyme proteins.

# Examples of flavoprotein enzymes include:

- L-amino acid oxidase, an FMN-linked enzyme found in kidney with general specificity for the oxidative deamination of the naturally occurring L-amino acids.
- xanthine oxidase, which contains molybdenum and plays an important role in the conversion of purine bases to uric acid
- dehydrogenase, an FAD-linked enzyme present in mammalian livers, which contains molybdenum and non heme iron and acts upon aldehydes and N-heterocyclic substrates.
- The mechanisms of oxidation and reduction of these enzymes are complex. suggests a two-step reaction.

# DEHYDROGENASES

There are a large number of enzymes in this class. They perform two main functions:

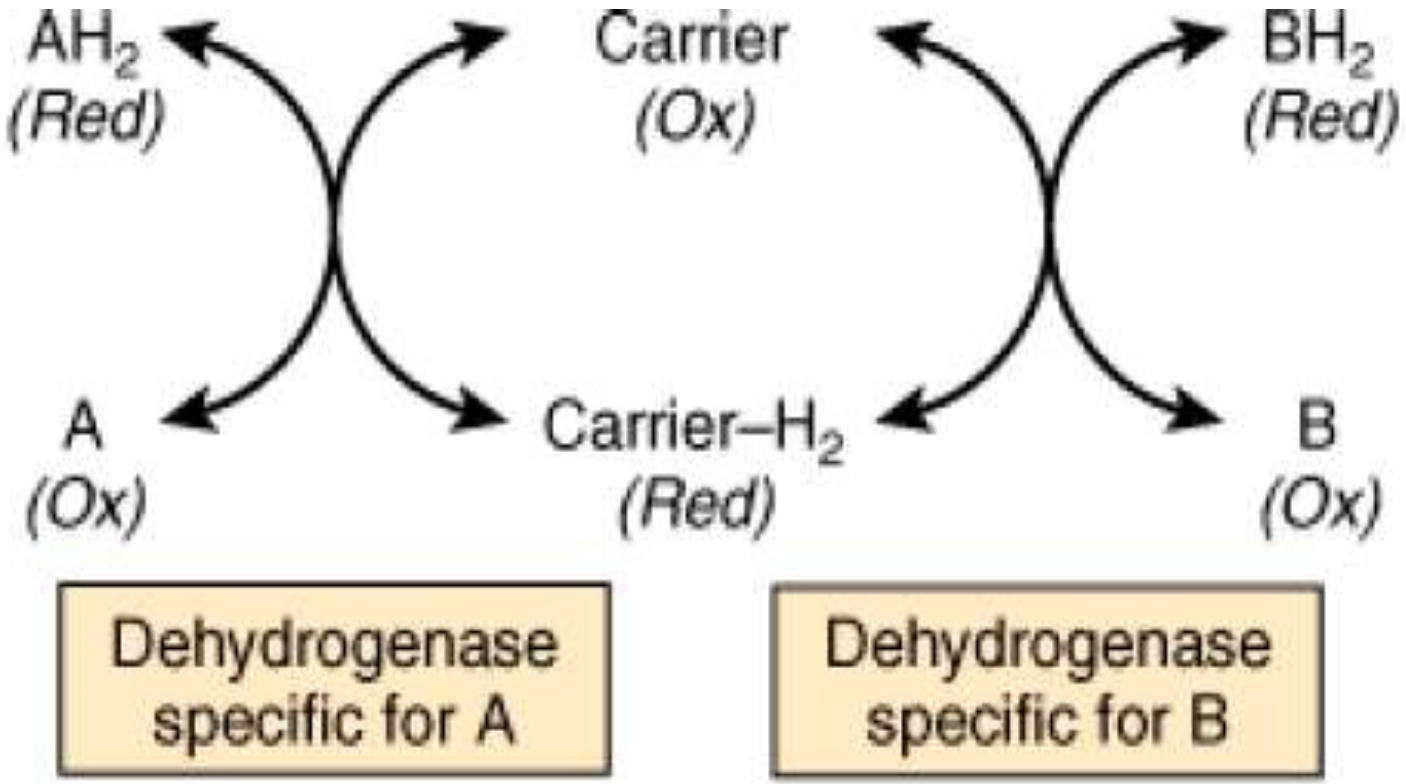
1-Transfer of hydrogen from one substrate to another in a coupled oxidation-reduction reaction.

These dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers, eg, NAD+ .

Since the reactions are reversible, these properties enable reducing equivalents to be freely transferred within the cell.

This type of reaction, which enables one substrate to be oxidized at the expense of another, is particularly useful for oxidation in the absence of oxygen, such as during the anaerobic phase of glycolysis.

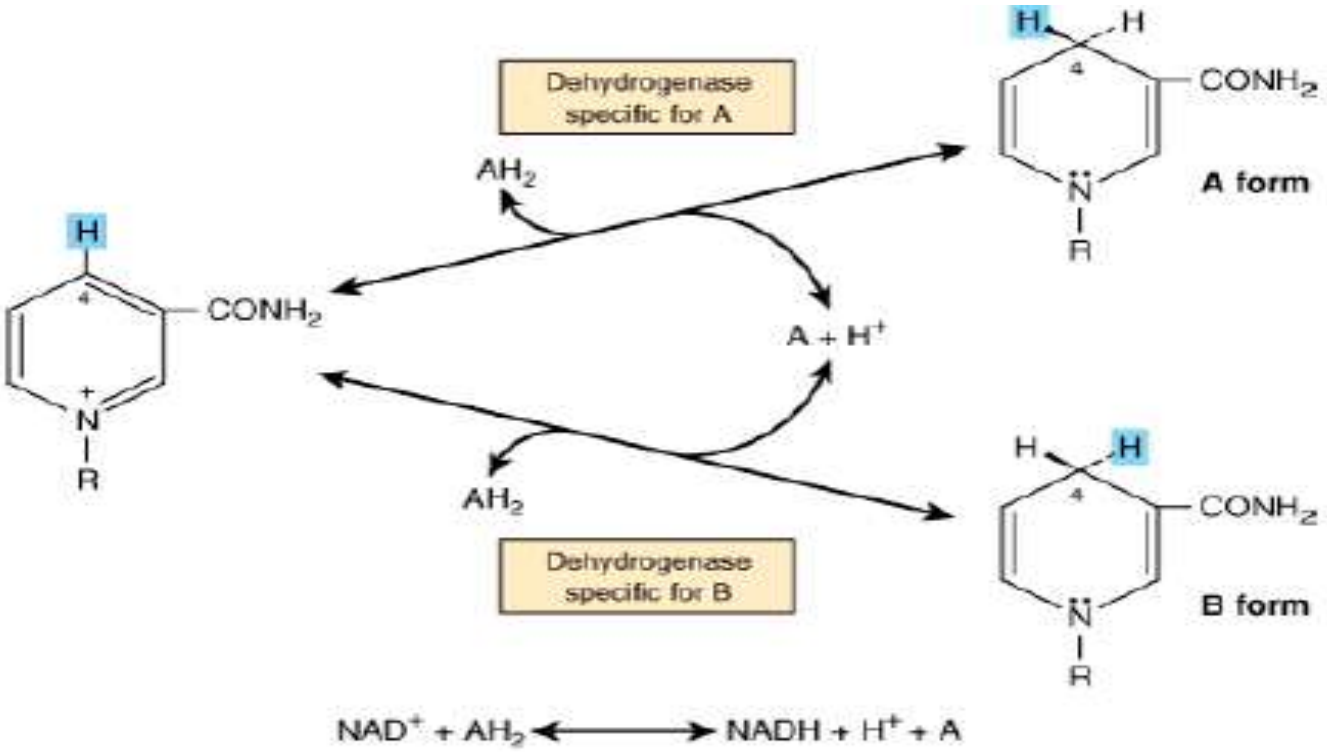
2-As components in the respiratory chain of electron transport from substrate to oxygen.




Oxidation of a metabolite catalyzed by coupled dehydrogenases

# Many Dehydrogenases Depend on Nicotinamide Coenzymes

- These dehydrogenases use nicotinamide adenine dinucleotide (NAD+ ) or nicotinamide adenine dinucleotide phosphate (NADP+ )—or both—which are formed in the body from the vitamin niacin(B3).
- The coenzymes are reduced by the specific substrate of the dehydrogenase and reoxidized by a suitable electron acceptor. They may freely and reversibly dissociate from their respective apoenzymes.

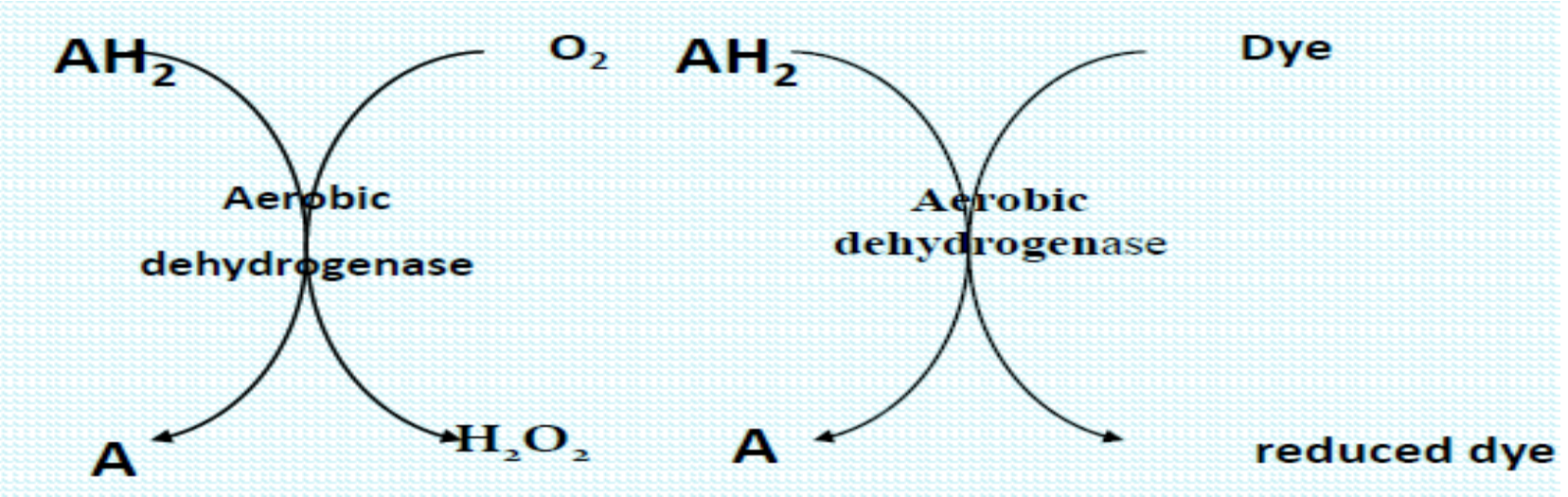


- Mechanism of oxidation and reduction of nicotinamide coenzymes:
- There is stereospecificity about position 4 of nicotinamide when it is reduced by a substrate AH2 .
- One of the hydrogen atoms is removed from the substrate as a hydrogen nucleus with two electrons (hydride ion, H– ) and is transferred to the 4 position, where it may be attached in either the A or the B form according to the specificity determined by the particular dehydrogenase catalyzing the reaction.
- The remaining hydrogen of the hydrogen pair removed from the substrate remains free as a hydrogen ion.
- Generally, NAD-linked dehydrogenases catalyze oxidoreduction reactions in the oxidative pathways of metabolism, mainly in glycolysis, in the citric acid cycle, and in the respiratory chain of mitochondria
- NADP-linked dehydrogenases are found normally in reductive syntheses, as in the extra mitochondrial pathway of fatty acid synthesis and steroid synthesis—and also in the pentose phosphate pathway.

# Dehydrogenases Depend on Riboflavin

- The flavin groups associated with these dehydrogenases are similar to FMN and FAD occurring in oxidases.
- Most of the riboflavin-linked dehydrogenases are related with electron transport in (or to) the respiratory chain
- NADH dehydrogenase acts as a carrier of electrons between NADH and the components of higher redox potential
- Other dehydrogenases such as succinate dehydrogenase, acyl-CoA dehydrogenase, and mitochondrial glycerol-3-phosphate dehydrogenase transfer reducing equivalents directly from the substrate to the respiratory chain.
- The electron-transferring flavoprotein (ETF) is an intermediary carrier of electrons between acyl-CoA dehydrogenase and the respiratory chain.

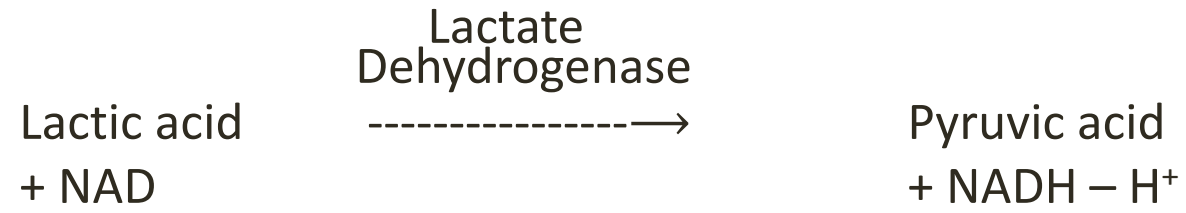
# *Aerobic Dehydrogenases(FlavoproteinLinked oxidases)*




- **The coenzyme of aerobic dehydrogenasesmay be:**
- •FMN (Flavin adenine mononucleotide) as in L-amino acid oxidase.
- •FAD (Flavin adenine dinucleotide) as in D-amino acid oxidase, xanthine oxidase, aldehyde dehydrogenase and glucose oxidase.

## *Anaerobic Dehydrogenases*

**Anaerobic Dehydrogenases.** Catalyzing oxidation of the substrate and coenzymes act as recipients of hydrogen e.g. Lactate Dehydrogenase with NAD and Glucose 6 phosphate dehydrogenase with NADP




- Anaerobic dehydrogenases are further classified according to their coenzymes into:
- **•NAD+linked anaerobic dehydrogenasese.g.**
- a)Cytoplasmicglycerol-3-phosphate dehydrogenase
- b)Isocitrate dehydrogenase.
- c)Malate dehydrogenase.
- d)β-Hydroxyacyl CoA dehydrogenase.
- e)β-Hydroxybutyrate dehydrogenase.

**NADP linked anaerobic dehydrogenases e.g.**

a)Glucose-6-phosphate dehydrogenase.

b)Malic enzyme.

c)Cytoplasmic isocitrate dehydrogenase

**FAD linked anaerobic dehydrogenases e.g.**

a)Succinate dehydrogenase.

b)Mitochondrial glycerol-3-phosphate dehydrogenase.

c)Acyl CoA dehydrogenase.

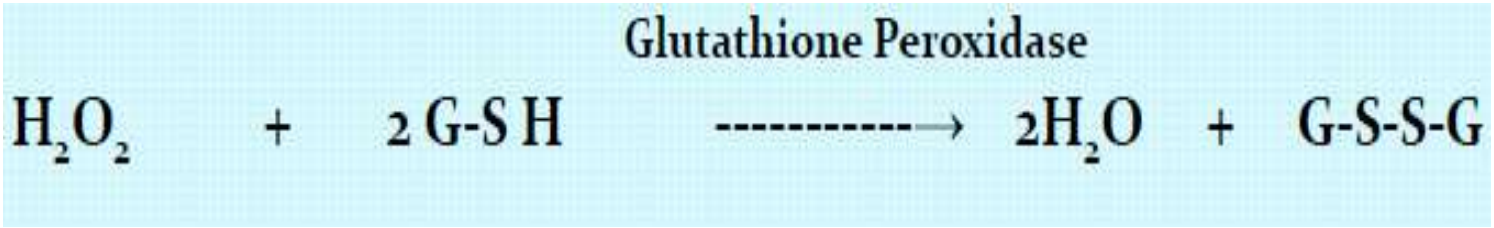
**Cytochromes:-**

All cytochromes are anaerobic dehydrogenases except cytochrome oxidase(cyta3), which is an oxidase and cytochromeP450 that is mono-oxygenase (hydroxylase). ***Ubiquinol(coenzyme Q) dehydrogenase,*** which is present in the respiratory chain.

# *Hydroperoxidases*

- These enzymes use hydrogen peroxide (H2O2) as substrate changing it into water to get rid of its harmful effects.
- They are further classified into peroxidases and catalases.
- **Peroxidases**: Peroxidases are found in milk and in leukocytes, platelets, and other tissues, In the reaction catalyzed by peroxidase, hydrogen peroxide is reduced at the expense of several substances that will act as electron acceptors, such as ascorbate, quinones, and cytochrome *c.* The reaction catalyzed by peroxidase is complex, but the overall reaction is as follows:


- Example :-Glutathione peroxidase gets rid of H2O2 from red cells to protect them from haemolysis, (containing selenium as a prosthetic group)



Catalases: These enzymes act on 2 molecules of hydrogen peroxide; one molecule is hydrogen donor & the other molecule is hydrogen accepetor.

$2H_2O_2 + \text{catalase} \longrightarrow 2H_2O + O_2$

Catalase is found in blood, bone marrow, mucous membranes, kidney, and liver. It functions to destroy of hydrogen peroxide formed by the action of oxidases.

## OXYGENASES

- These enzymes catalyze direct incorporation (addition) of oxygen into substrate.
- They catalyze the incorporation of oxygen into a substrate molecule in two steps:

(1) oxygen is bound to the enzyme at the active site and

(2) the bound oxygen is reduced or transferred to the substrate.

They are further classified into dioxygenases & monooxygenases.

- Dioxygenases Incorporate Both Atoms of Molecular Oxygen into the Substrate

The basic reaction is shown below:


- Examples include the liver enzymes
- dioxygenase (oxidase)
- 3-hydroxyanthranilate dioxygenase (oxidase), which contain iron;
- L-tryptophan dioxygenase (tryptophan pyrolase)

Monooxygenases (Mixed-Function Oxidases, Hydroxylases)

- Incorporate only one atom of molecular oxygen into the substrate, The other oxygen atom is reduced to water, an additional electron donor or co substrate (Z) being necessary for this purpose.

$$A—H + O_2 + ZH_2 \rightarrow A—OH + H_2O + Z$$

# CytochromeP450

- It is a group of hydroxylases which are collectively referred to as cytochromeP450.
- They are so called because their reduced forms show an intense absorption band at wavelength 450 nm when complexed to carbon monoxide.
- They are conjugated protein containing heme (hemoproteins).
- According to their intracellular localization they may be classified into:

•Microsomal cytochromeP450.

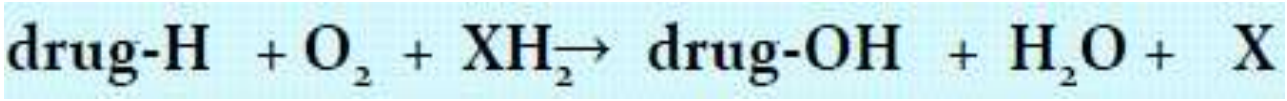
It is present mainly in the microsomes of liver cells. It represents about 14% of the microsomal fraction of liver cells.

•Mitochondrial cytochromeP450.

It is present in mitochondria of many tissues but it is mainly abundant in liver and steroidogenic tissues as adrenal cortex, testis, ovary, placenta and kidney.

## Fuctions of cytochrome P450

- Functions of microsomal cytochromeP450
- 1-It is important for detoxication of xenobiotics by hydroxylation. e.g. insecticides, carcinogens, mutagens and drugs.
- 2-It is also important for metabolism of some drugs by hydroxylation e.g. morphine, aminopyrine, benzpyrine and aniline.




- **Function of mitochondrial cytochromeP450**
- 1-It has a role in biosynthesis of steroid hormones from cholesterol in adrenal cortex, testis, ovary and placenta by hydroxylation
- 2-It has a role in biosynthesis of bile acids from cholesterol in the liver by hydroxylation at C26 by 26 hydroxylase.
- 3-It is important for activation of vitamin D

## Super oxide dismutase protects aerobic organisms against oxygen toxicity

- Transfer of a single electron to O2 generates the potentially damaging superoxide anion free radical O. , the destructive effects of which giving rise to free-radical chain reactions.
- The production of superoxide ($O_2^-$) leading to the generation of potent oxidants such as hydrogen peroxide ($H_2O_2$), hydroxyl radical ($HO^+$). Superoxide dismutase (SOD), by rapidly removing $O_2^-$, reduces the tissue concentration of $O_2^-$ and prevents the production of $HO^{+\cdot}$
- Superoxide dismutase is a metal containing (Cu, Zn) antioxidant enzyme that reduces harmful free radicals of oxygen formed during normal metabolic cell processes to oxygen and hydrogen peroxide
- Super oxide dismutase (SOD) enzymes deal with the superoxide radical by adding or removing an electron from the superoxide molecules it encounters, thus changing the $O_2^-$ in to one or two less damaging species: either molecular oxygen(O2) or hydrogen peroxide (H2O2) .
- This SOD-catalyzed dismutation of superoxide may be written, for Cu,Zn SOD, with the following reactions:
- $Cu^{+2} \cdot SOD + o^-_2 \longrightarrow Cu^+ \cdot SOD + O_2$
- $Cu^+ \cdot SOD + o^-_2 + 2H^+ \longrightarrow Cu^{+2} \cdot SOD + H_2O_2$

# The Respiratory Chain & Oxidative Phosphorylation

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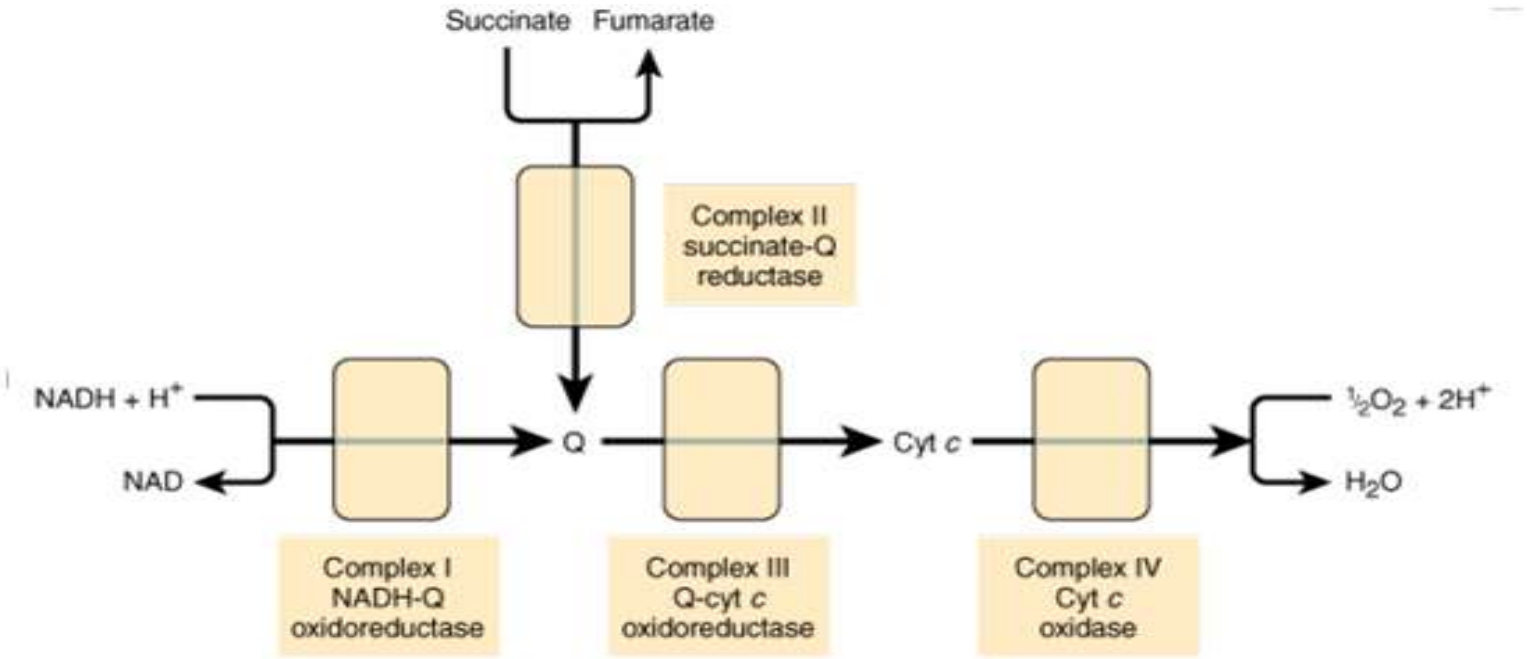
# The Respiratory Chain & Oxidative Phosphorylation

- Aerobic respiration involves four stages: glycolysis, a transition reaction that forms acetyl coenzyme A, the citric acid (Krebs) cycle, and an electron transport chain and chemiosmosis.
- During various steps in glycolysis and the citric acid cycle, the oxidation of certain intermediate precursor molecules causes the reduction of $NAD^+$ to NADH + $H^+$ and FAD to $FADH_2$. NADH and $FADH_2$ then transfer protons and electrons to the electron transport chain to produce additional ATPs by oxidative phosphorylation.
- The electron transport chain consists of a series of electron carriers that eventually transfer electrons from NADH and $FADH_2$ to oxygen.
- Most of this takes place inside mitochondria, which have been termed the "powerhouses" of the cell.
- Respiration is coupled to the generation of the high-energy intermediate, ATP, by oxidative phosphorylation.
- A number of drugs (eg, amobarbital ) and poisons (eg, cyanide, carbon monoxide ) inhibit oxidative phosphorylation, usually with lethal effects.

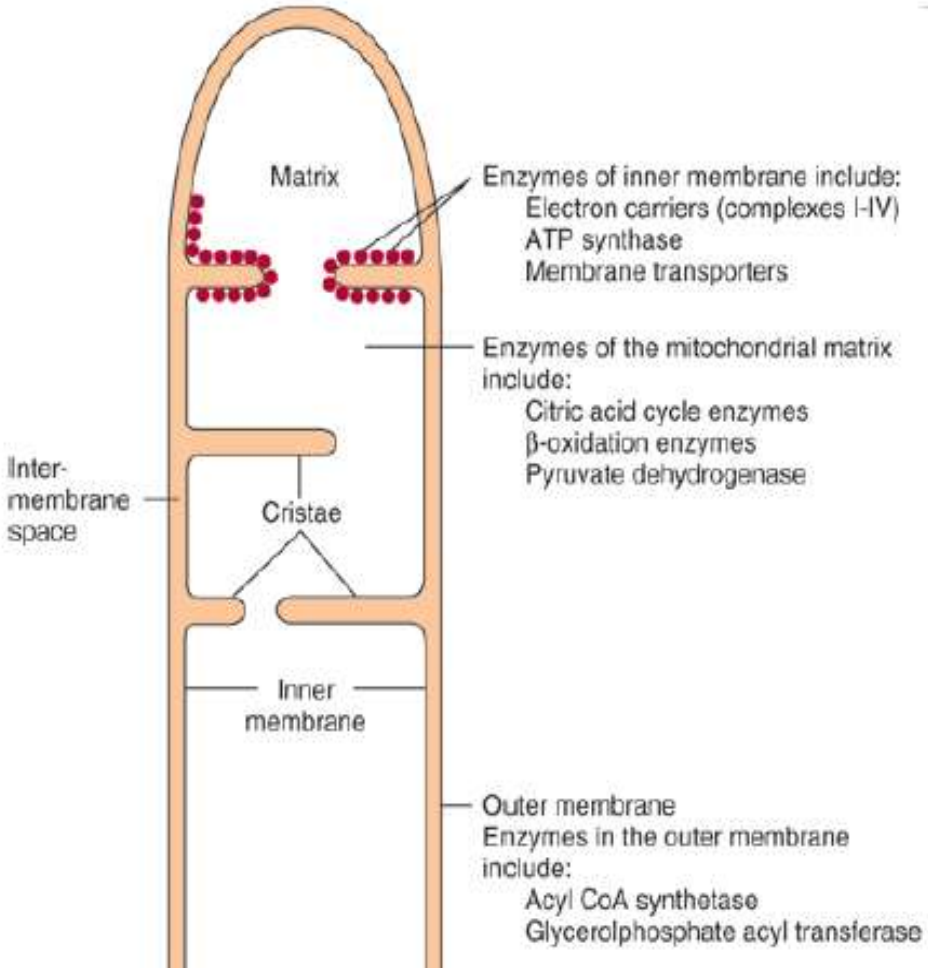
# Electron transport chain

- Electron transport chain:is a chain of catalysts(enzymes & coenzymes) arranged in stepwise of increasing redox potential. It collects reducing equivalents (hydrogen atoms and electrons) from substrates transferring it stepwise to be oxidized in a final reaction with oxygen to form water and energy. It is also known as redox chain or respiratory chain
- The electron carrier are found within four membrane-bound enzyme-complexes, which are imbedded in the inner mitochondrial membrane.




- Electron flow through the respiratory chain.
- (Q, coenzyme Q or ubiquinone; cyt, cytochrome)

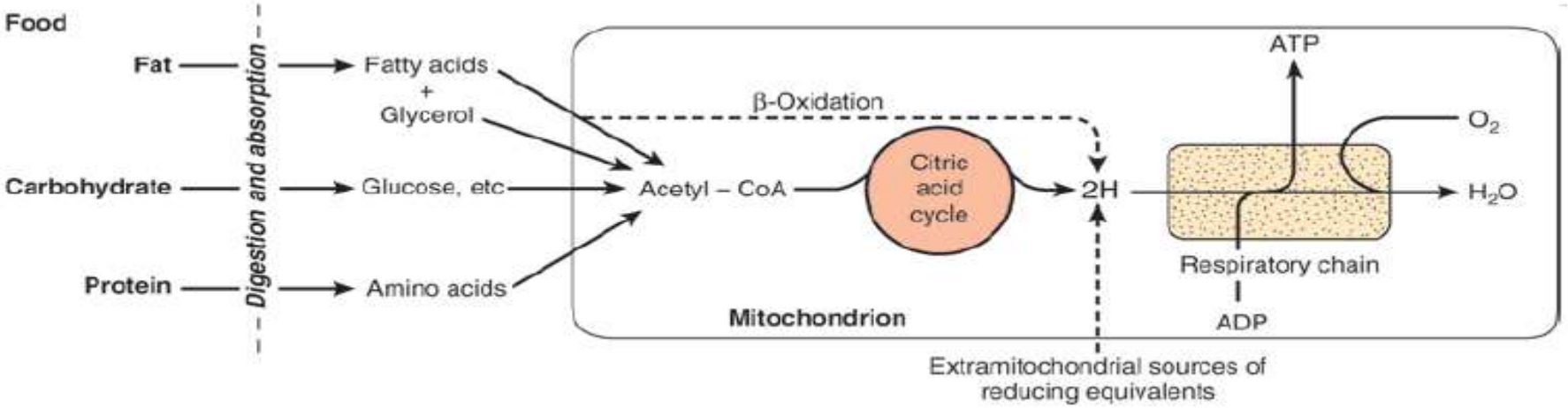
- Mitochondria have :
- outer membrane that is permeable to most metabolites
- inner membrane that is selectively permeable, enclosing a matrix within.
- The outer membrane is characterized by the presence of various enzymes, including acyl-CoA synthetase and glycerolphosphate acyltransferase.
- Adenylyl kinase and creatine kinase are found in the intermembrane space.
- The phospholipid cardiolipin is concentrated in the inner membrane together with the enzymes of the respiratory chain, ATP synthase and various membrane transporters.




Structure of the mitochondrial membranes, the inner membrane contains many folds, or cristae

# The respiratory chain oxidizes reducing equivalents & acts as a proton pump:

- Most of the energy liberated during the oxidation of carbohydrate, fatty acids, and amino acids is made available within mitochondria as reducing equivalents (—H or electrons).
- The enzymes of the citric acid cycle and -oxidation are contained in mitochondria, together with the respiratory chain, which collects and transports reducing equivalents, directing them to their final reaction with oxygen to form water, and the machinery for oxidative phosphorylation, the process by which the liberated free energy is trapped as high-energy phosphate.




- Role of the respiratory chain of mitochondria in the conversion of food energy to ATP. Oxidation of the major foodstuffs leads to the generation of reducing equivalents (2H) that are collected by the respiratory chain for oxidation and coupled generation of ATP.

# Components of the electron transport chain

The electron transport chain is formed of :-

- Hydrogen and electron carriers.
- Four membrane-bound enzyme complexes

## Hydrogen and electron carriers of the electron transport chain

1-NAD+ :-

It receives two hydrogen atoms (2H) from substractes as isocitrate, malate, β-hydroxyacy1 CoA and β-hydroxybutyrate.

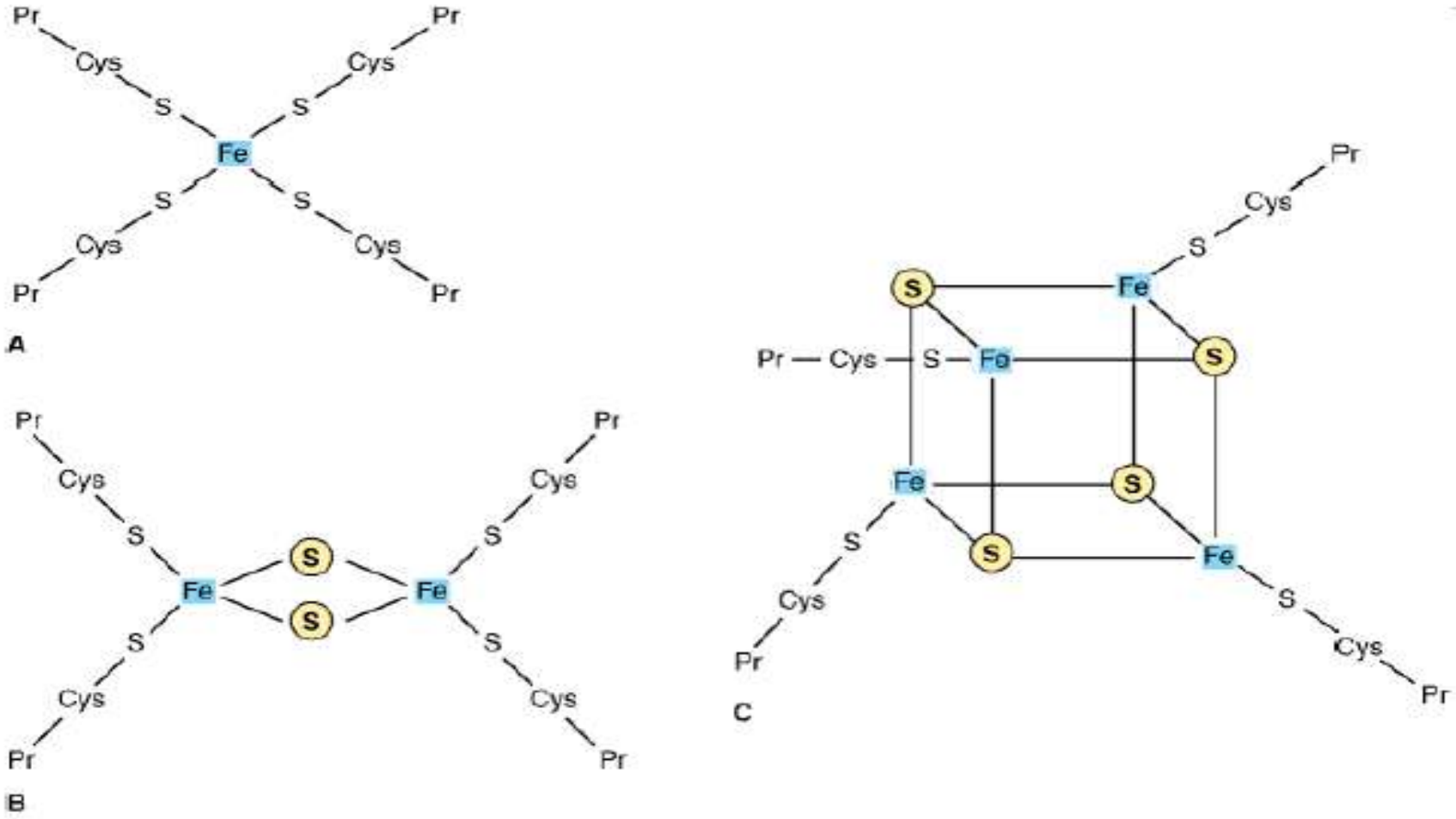
Its reduced form (NADH+H+) passes its hydrogen to flavoprotein containing FMN and iron sulfur protein (FeS).

- 2-Flavoproteins
- FAD and FMN serve as hydrogen carriers, which are tightly bound to flavoproteins in a manner that prevents its reduced form reacting with oxygen directly.
- There are many types of flavoproteins that have a role in electron transport chain FlavoproteinFp1 containing FMN receives two hydrogen atoms from reduced NAD+ passing them to coenzyme Q.
- FlavoproteinFp2 containing FAD receives two hydrogen atoms from substrates as succinate, acyl CoA passing them to coenzyme Q.

- 3-Ubiquinone(CoenzymeQ) :-
- Ubiquinones are a group of compounds containing quinine ring but vary according to number of isoprene units at the side chain.
- Ubiquinone can carry two hydrogen atoms forming ubiquinol(reduced coenzyme Q) or one hydrogen atom forming semiquinone. So, it forms a bridge between flavoproteins, which can carry 2 hydrogen atoms, and cytochrome b.
- Reduced coenzyme Q passes the electrons to cytochrome b and releases 2H+into the mitochondrial matrix.

- 4-Cytochromes:-
- They are electron carrier transferring electrons from coenzyme Q to oxygen. They have given letters a, b and c according to their order of discovery. All cytochromes are hemo proteins but they differ in redox potential.
- The hemein cytochromes differs from that of hemoglobin as the iron atom oscillates between oxidation and reduction during the physiological action of cytochromes, while the iron of hemoglobin remains in the reduced form during its physiological action.
- Cytochrome c is a water soluble, it is relatively mobile.
- Cytochrome a & cyt a3 contains copper in addition to the heme group.
- The mobile components of the electron transport chain include coenzyme Q and cytochrome c. they collect reducing equivalents from the other fixed components.

- 5-Iron sulfur protein :-
- It is an additional component found in the electron transport chain. It is also called FeS or non-heme iron. It consists of a cluster of cysteine residues which complex iron through covalent bonds with the sulfur of cysteine.
- It is associated with the flavoproteins (FAD &FMN)and cytochrome b.
- Both sulfur and iron take part in the oxidation-reduction mechanism between flavoprotein and coenzyme Q as the iron atom in these complexes oscillates between oxidation and reduction that allow them to either give up or accept electrons.
- Iron-sulfur proteins(Fe-S) are found in Complexes I, II, and III. These may contain one, two, or four Fe atoms linked to inorganic sulfur atoms and/or via cysteine-SH groups to the protein. The Fe-S take part in single electron transfer reactions in which one Fe atom undergoes oxido reduction between $Fe^{2+}$ and $Fe^{3+}$ .



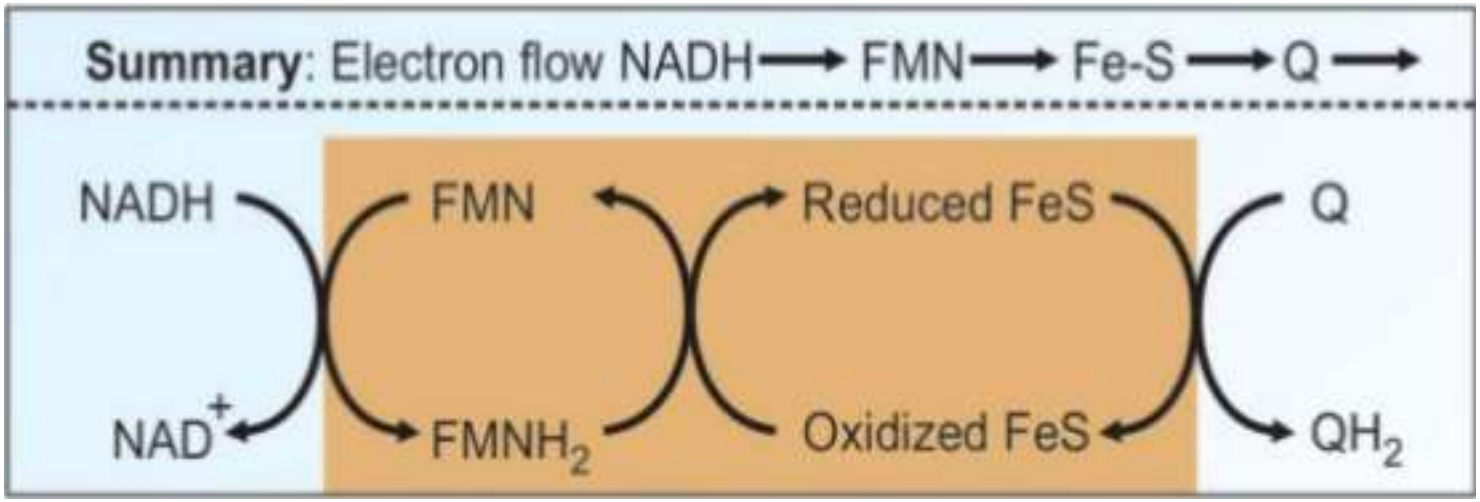

- Iron-sulfur proteins (Fe-S). **(A)** The simplest Fe-S with one Fe bound by four cysteines. **(B)** 2Fe-2S center. **(C)** 4Fe-4S center. (s) , Inorganic sulfur; Pr, apoprotein; Cys, cysteine.)

The enzymes of the electron transport chain are organized in the inner mitochondrial membrane in the form of four enzyme complexes.

The four enzyme complexes of the electron transport chain are:

**Complex I:** NADH dehydrogenase(NADH-uniquinone oxidoreductase)

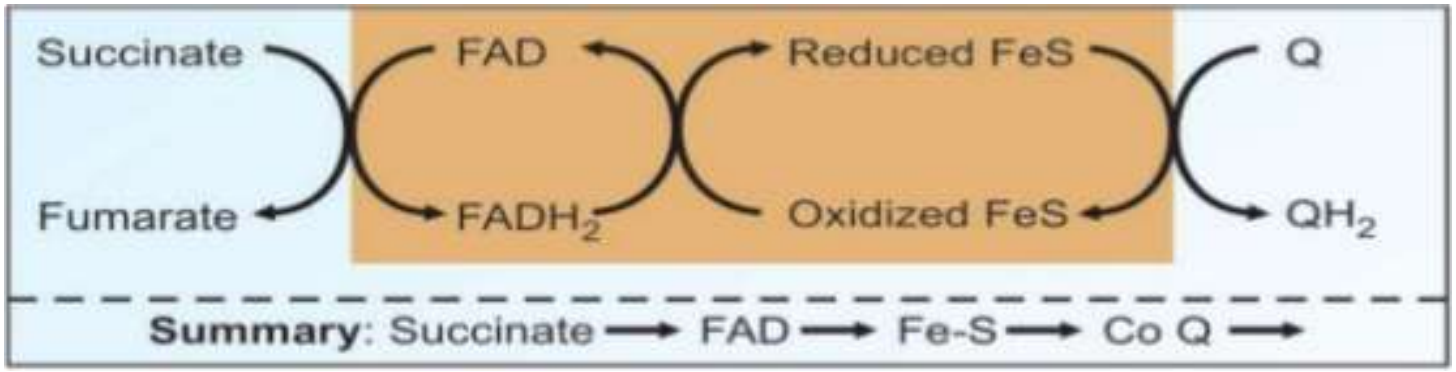
It is a flavoprotein that contains FMN as well as FeS protein as coenzymes. It transfers hydrogen atoms from NADH+H+ to uniquinone.




Complex I or NADH-CoQ reductase (NADH dehydrogenase complex)

**Complex II:** Succinate dehydrogenase(succinate-uniquinone oxidoreductase)

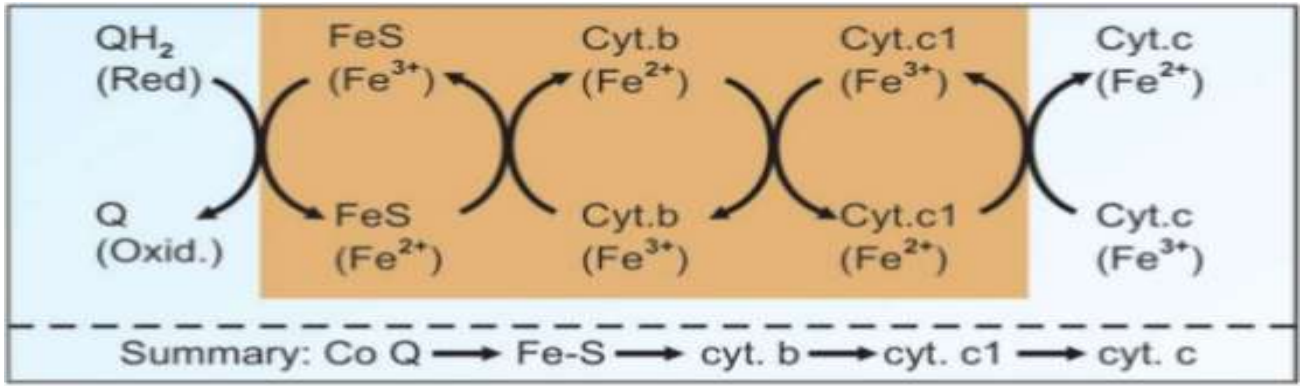
It is a flavoprotein that contains FAD as well as FeS protein as coenzymes. It transfers hydrogen atoms from succinate to uniquinone.




Complex II ; Succinate-Q- reductase

***ComplexIII:*** Ubiquinol dehydrogenase(ubiquinol-cytochrome c oxidoreductase).

It transfers electrons from ubiquinol to cytochrome c using cyt b and cyt c1 as coenzymes.



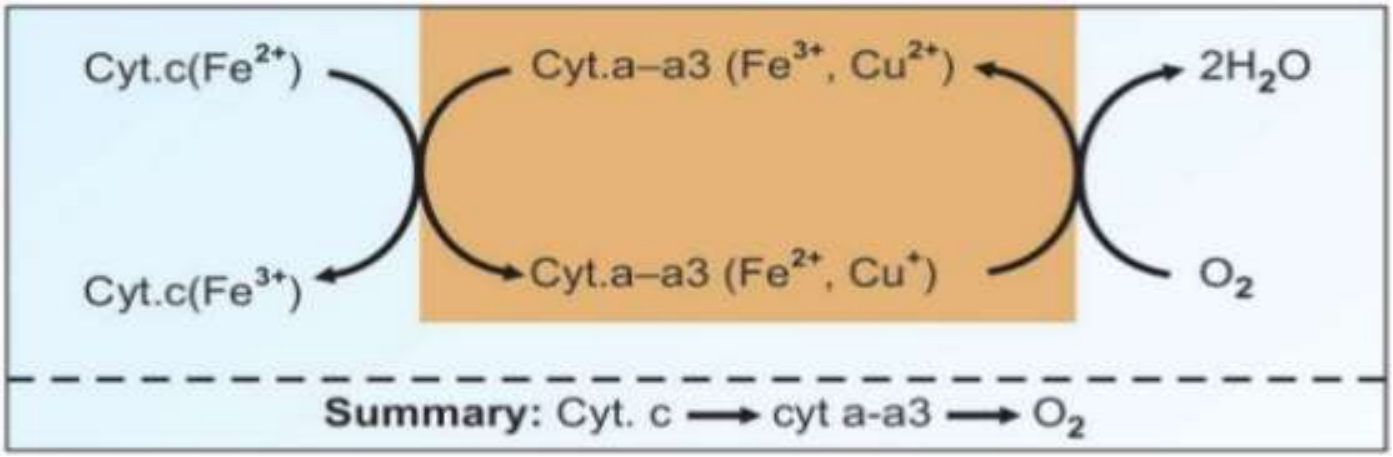

Complex III or cytochrome reductase (cytochrome b-$c_1$) of respiratory chain

- This complex is also known as Q-cytochrome c reductase because it passes the electrons from QH2 to cyt c through a very unique electron transport pathway called Q-cycle.

***Complex IV:*** Cytochrome oxidase(cytochrome-oxygen oxidoreductase)

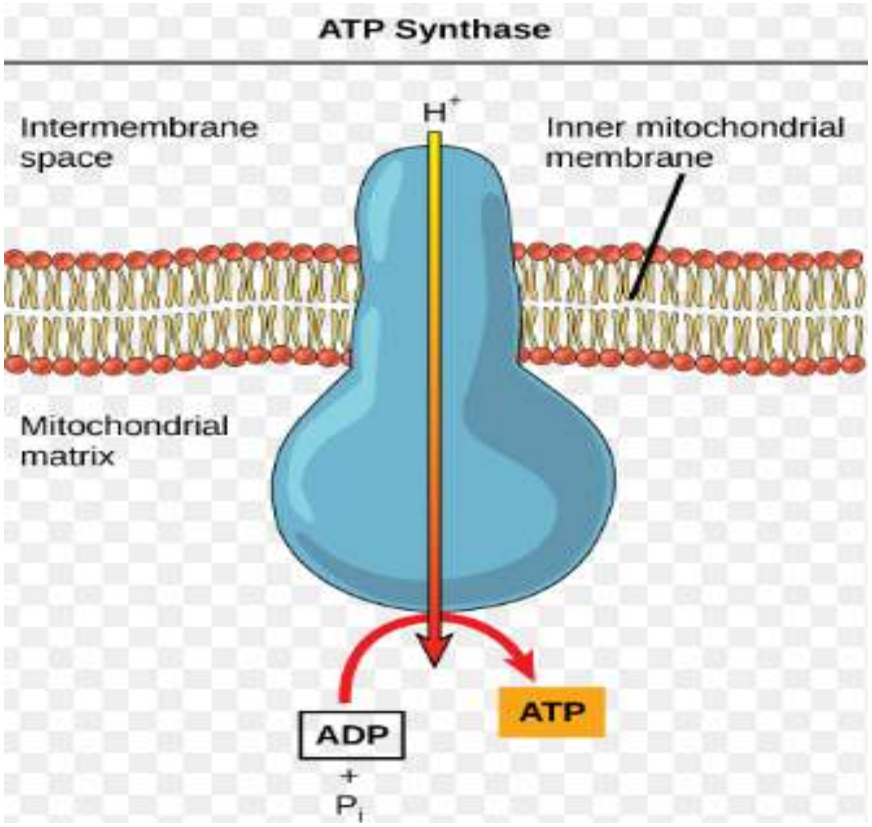
It transfers electrons from cytochrome c to oxygen.

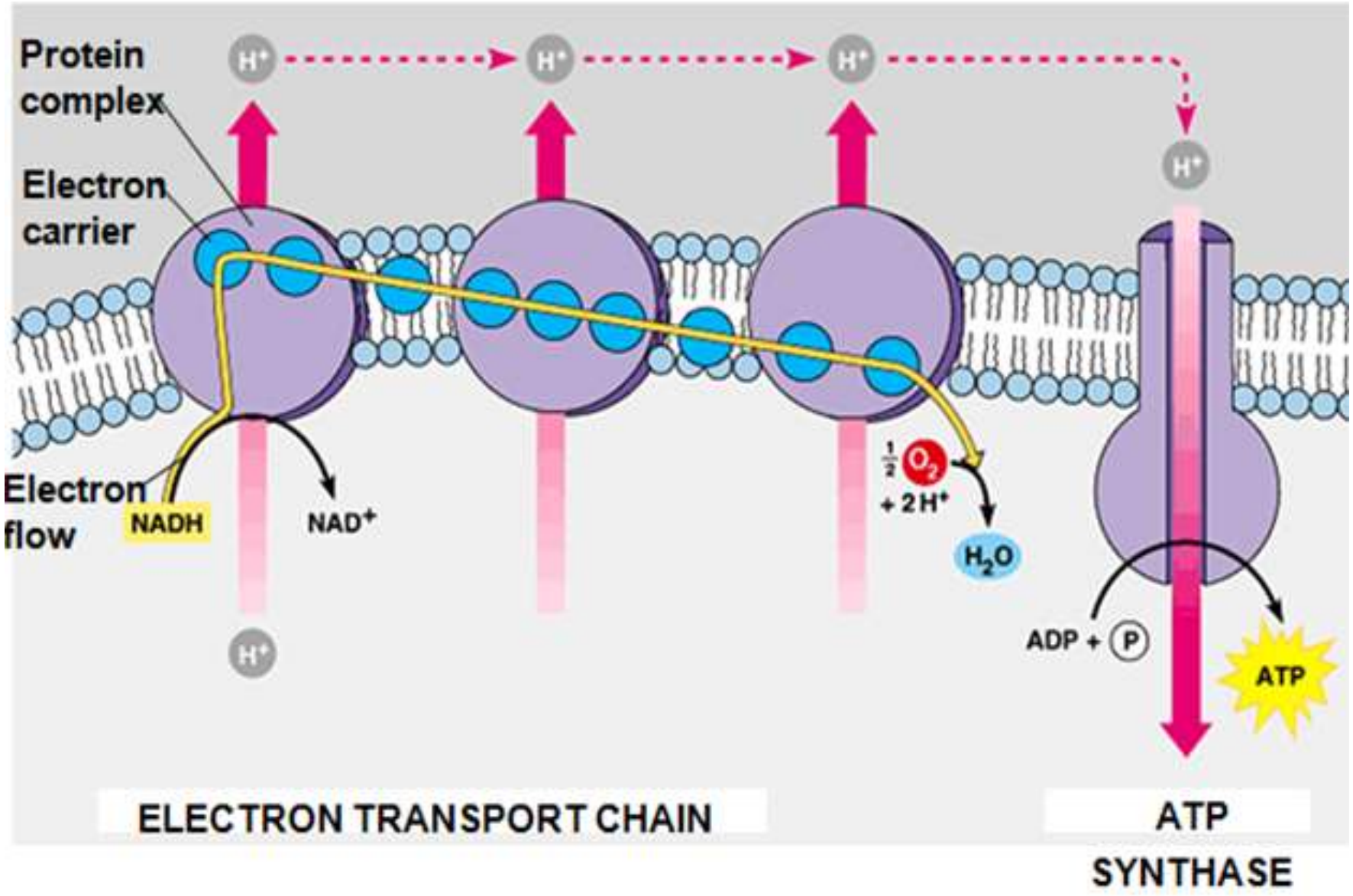
It needs cyt a and cyt a3 as coenzymes.




Complex IV (cytochrome oxidase) of respiratory chain

- In addition to these four enzyme complexes, there is fifth complex **(complex V)** which is the ATP synthase that responsible for biosynthesis of ATP from ADP and inorganic phosphate.
- Its structure consists of 2 domains:
- F1 unit (ATPsynthase) and F0 unit (transmembrane channel)




Protein complex
Electron carrier
H+
H+
H+
H+
Electron flow
NADH
NAD+
½ O2
+ 2H+
H2O
H+
ADP + P
ATP
ELECTRON TRANSPORT CHAIN
ATP SYNTHASE

| Enzyme Complex | Prosthetic Groups |
|---|---|
| Complex I (NADH dehydrogenase) | FMN, FeS |
| Complex II (succinate dehydrogenase) | FAD, FeS |
| Complex III (cytochrome $bc_1$ complex) | Hemes, FeS |
| Cytochrome c | Heme |
| Complex IV (cytochrome oxidase) | Hemes, Fe, Cu |

## Two mobile $e^-$ carriers in ETC

- **Ubiquinone (Co Q)**

  UQ is lipid-soluble can accept electrons from $FMNH_2$ / $FADH_2$ & transfer them to cytochrome.

- **Cytochrome C**

  Cytochrome C is a water-soluble mobile electron carrier of the outer face of the inner membrane

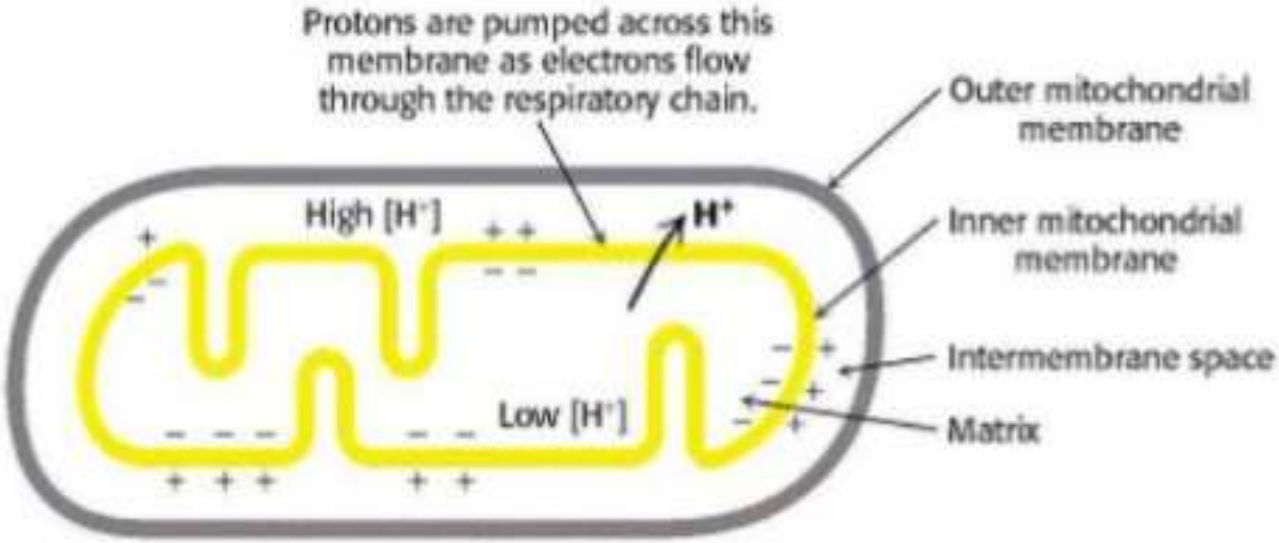
# Oxidative Phosphorylation

- It means coupling of the electron transport in respiratory chain with phosphorylation of ADP to form ATP.
- It is a process by which the energy of biological oxidation is finally converted to the chemical energy of ATP.
- There are 3 sites of the chain that can give enough energy for ATP synthesis. These sites are:
- Site I between FMN and coenzyme Q at enzyme complex I.
- Site II between cyt b and cyt c1 at enzyme complex III.
- Site III between cyt a and cyt a3 at enzyme complex IV.
- The number of ATP generated depends on the site at which the substrate is linked to the respiratory chain:

- If the substrate is linked to chain through FAD, 2 ATP are formed for each molecule oxidized. If the substrate is linked to chain through NAD+, 3 ATP are formed for each molecule oxidized
- P/O ratio
- It is ratio of the number of molecules of ADP converted to ATP to the number of oxygen atoms utilized by respiratory chain.
- *It is a measure to the efficiency of oxidative phosphorylation*.
- It is 3/1 if NADH+H+ is used and 2/1 if FADH2is used.

## Mechanism of oxidative phosphorylation

- There are 2 theories explaining this mechanism:-
- **1-Chemical theory :-**
- It suggests that there is a direct chemical coupling of oxidation and phosphorylation through high-energy intermediate compounds. This theory is not accepted, as postulated high-energy intermediate compounds were never found.
- **2-Chemiosomotic theory :-**
- It suggest that the transfer of electrons through the electrons transport chain causes protons to be translocated(pumped out) from the mitochondrial matrix to the intermembrane space at the three sites of ATP production (I , III, IV) (i.e. it acts as a proton pump) resulting in an electrochemical potential difference across the inner mitochondrial membrane. The electrical potential difference is due to accumulation of the positively charged hydrogen ions outside the membrane and the chemical potential difference is due to the difference in pH, being more acidic outside the membrane .




Electron transfer through the respiratory chain leads to pumping of protons from matrix to the cytosolic side of the inner mitochondrial membrane. The pH gradient and membrane potential constitute a proton-motive force that is used to drive ATP synthesis

As the hydrogen ions accumulate on one side of a membrane, the concentration of hydrogen ions creates an electrochemical gradient or potential difference (voltage) across the membrane (The fluid on the side of the membrane where the protons accumulate acquires a positive charge; the fluid on the opposite side of the membrane is left with a negative charge.)

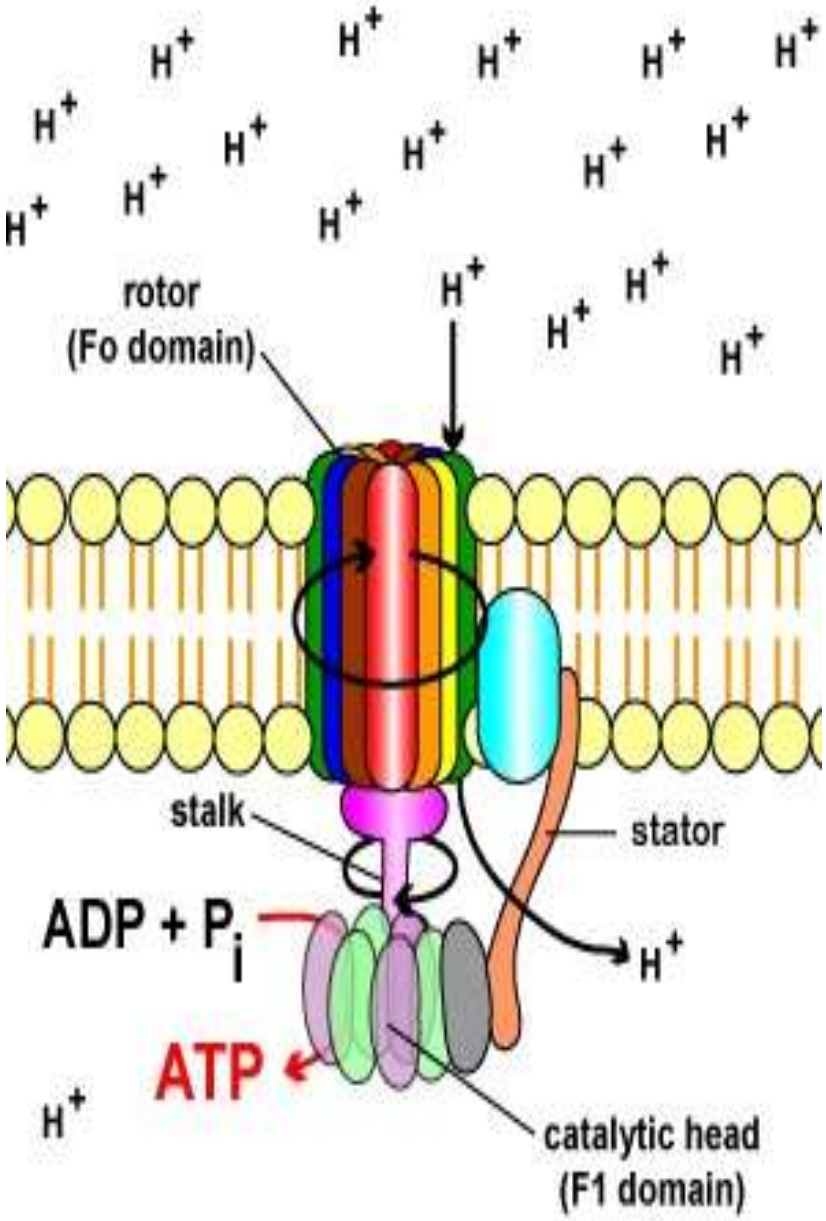
The energized state of the membrane as a result of this charge separation is called proton motive force or PMF.

This proton motive force provides the energy necessary for the enzymes ATP synthases, to catalyze the synthesis of ATP from ADP and phosphate.

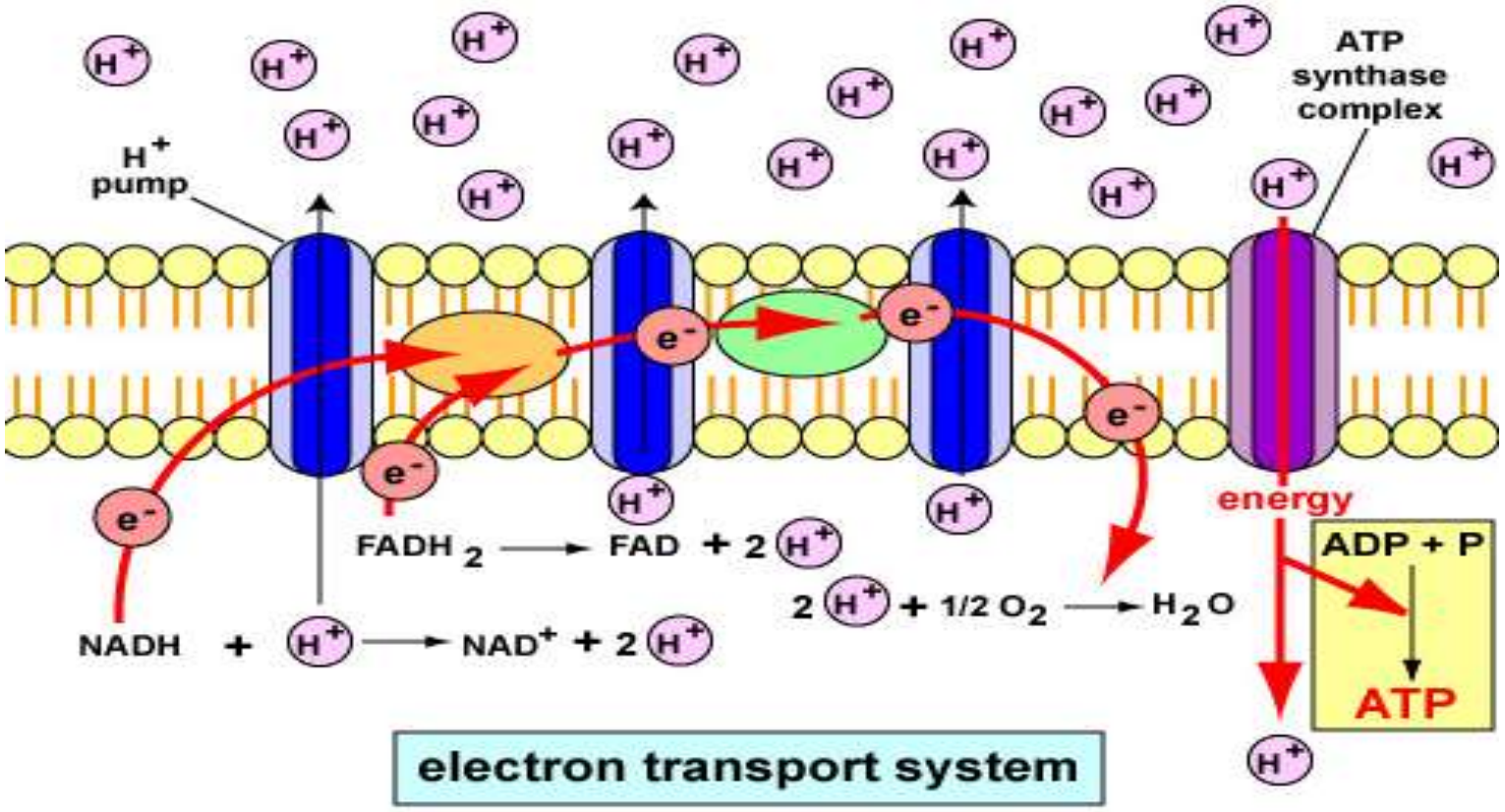
This generation of ATP occurs as the protons cross the membrane through the ATP synthase complexes and re-enter either the cytoplasm or the matrix of the mitochondria.

As the protons move down the concentration gradient through the ATP synthase, the energy released causes the rotor and rod of the ATP synthase to rotate.

The mechanical energy from this rotation is converted into chemical energy as phosphate is added to ADP to form ATP.



- At the end of the electron transport chain involved in aerobic respiration, the last electron carrier in the membrane transfers 2 electrons to half an oxygen molecule (an oxygen atom) that simultaneously combines with 2 protons from the surrounding medium to produce water as an end product.



# Inhibitors of oxidative phosphorylation

The inhibitors of oxidative phosphorylationare classified into:

I-Specific-site inhibitors that inhibit oxidation.

II-Non specific-site inhibitors that inhibit phosphorylation

## A-Specific-site inhibitors

They block the oxidation process at specific sites on the respiratory chain (at one of the 3 sites of ATP production).

**1.Site 1 inhibitors :-**These are substances that inhibit electron transport from reduced FMN to coenzyme Q.

They include:

1-Rotenone (insecticide and fish poisoning).

2-Chloropromazine(tranquilizer).

3-Barbiturates (hypnotic).

4-Alkyl guanidine (hypotensive).

**2-Site II inhibitors :-**

These are substances that inhibit electron transport from reduced cyt b to cyt c1. They include:

1-AntimycinA (antibiotic).

2-BAL (British Anti Lewisite). It is dithioglycerol, an antagonist for an old war gas.

3-Dimercaprol.

4-Phenformine(hypoglycemic).

5-Napthoquinone.

***3-Site III inhibitors :-***

These are substances that inhibit electron transport from reduced cyt a to cyt a3. They include:

1-Cyanide.

2-Carbon monoxide (CO).

3-Hydrogen sulphide(H2S).

4-Sodium azide.

## B-Non specific-site inhibitors :-

They function primarily by blocking phosphorylation, but they prevent the whole process of oxidative phosphorylation e.g. Oligomycin (antibiotic) that inhibits ATP synthase enzyme .

Atractyloside (herbicide) that inhibits ADP/ ATP transporter which is responsible for the transport of ADP into the mitochondria and the transport ATP out of the mitochondria.

## Uncouplers

They are substances that dissociate oxidation from phosphorylation leading to loss of the resulting energy as heat. There is normal oxygen consumption without ATP generation and P/O ratio becomes zero.

Uncoupling agents act as lipophilic weak acids, associating with protons on the exterior of mitochondria, passing through the membrane with the bound proton, and dissociating the proton on the interior of the mitochondrion. These agents cause maximum respiratory rates but the electron transport generates no ATP, since the translocated protons do not return to the interior through ATP synthase.

Example of the uncouplers include:

2,4 dinitrophenol.

Dinitrocrisol.

Pentachlorophenol.

Calcium injection.

Thyroid hormones.

# Overview of Metabolism & the Provision of Metabolic Fuels

By: Inaam A. Ameen

Metabolism: the sum of the physical and chemical processes in an organism by which its material substance is produced, provided, and destroyed, and by which energy is made available.

Metabolic pathways fall into three categories:

(1) Anabolic pathways, which are those involved in the synthesis of larger and more complex compounds from smaller precursors—eg, the synthesis of protein from amino acids and the synthesis of reserves of triacylglycerol and glycogen. Anabolic pathways are

endothermic.

(2) Catabolic pathways, which are involved in the breakdown of larger molecules, commonly involving oxidative reactions; they are exothermic, producing reducing equivalents, and, mainly via the respiratory chain, ATP.

(3) Amphibolic pathways, which occur at the "crossroads" of metabolism, acting as links between the anabolic and catabolic pathways, eg, the citric acid cycle.

## Biomedical importance:

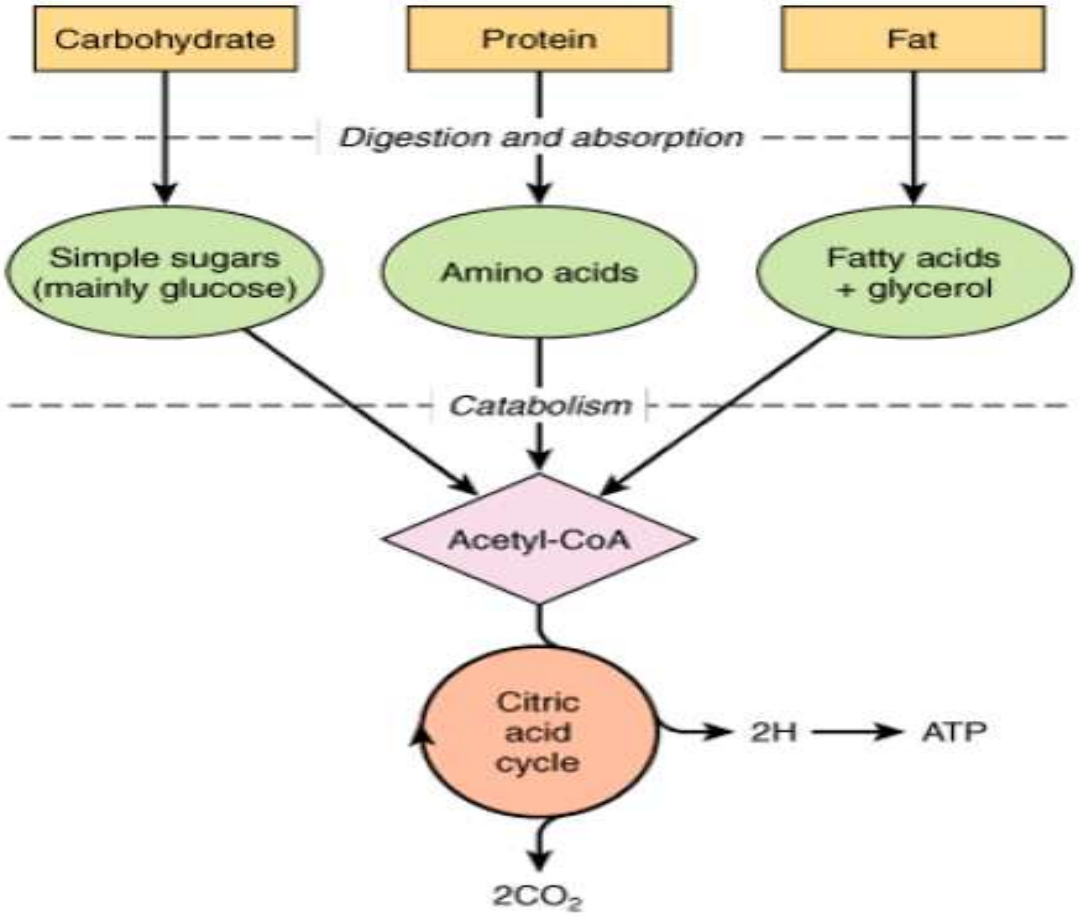
- Informations about the normal metabolism is essential for estimation of abnormalities that causing disease.
- Normal metabolism includes adaptation to periods of starvation, exercise, pregnancy, and lactation.
- Abnormal metabolism may result from nutritional deficiency, enzyme deficiency, abnormal secretion of hormones, or the actions of drugs and toxins.
- A 70-kg adult human being requires about 1920–2900 kcal from metabolic fuels each day, depending on physical activity.
- For human beings this requirement is met from carbohydrates (40–60%), lipids (mainly triacylglycerol, 30–40%), and protein (10–15%),
- The mix of carbohydrate, lipid, and protein being oxidized varies, depending on whether the subject is in the fed or fasting state, and on the duration and intensity of physical work.

- The requirement for metabolic fuels is relatively constant throughout the day, since average physical activity increases metabolic rate only by about 40–50% over the basal metabolic rate.
- However, most people consume their daily intake of metabolic fuels in two or three meals, so there is a need to form stores of carbohydrate (glycogen in liver and muscle) and lipid (triacylglycerol in adipose tissue) in the period following a meal, for use during the time in between meals.
- If the intake of metabolic fuels is greater than energy needed, the extra is stored, largely as triacylglycerol in adipose tissue, leading to the development of obesity and its associated health hazards.
- By contrast, if the intake of metabolic fuels is consistently lower than energy needed, there are insignificant stores of fat and carbohydrate, and amino acids arising from protein turnover are used for energy-yielding metabolism rather than replacement protein synthesis, leading to thinness**,** wasting, and, finally, death

- In the fed state, after a meal, there is an sufficient supply of carbohydrate, and the metabolic fuel for most tissues is glucose.
- In the fasting state glucose must be saved for use by the central nervous system (which is largely dependent on glucose) and the red blood cells (which depend on glucose).
- Therefore, tissues that can use fuels other than glucose do so; muscle and liver oxidize fatty acids and the liver produces ketone bodies from fatty acids to export to muscle and other tissues.
- As glycogen stores become depleted, amino acids arising from protein turnover are used for gluconeogenesis.
- The formation and utilization of stores of triacylglycerol and glycogen, and the extent to which tissues take up and oxidize glucose, are largely controlled by the hormones insulin and glucagon.
- In diabetes mellitus, there is either impaired synthesis and secretion of insulin (juvenile onset, or type I diabetes) or impaired sensitivity of tissues to insulin action (adult onset, or type II diabetes), leading to severe metabolic imbalance(disorder).

# Pathways that process the major products of digestion

The nature of the diet puts the basic design of metabolism. There is a need to process the products of digestion of dietary carbohydrate, lipid, and protein. These are mainly glucose, fatty acids and glycerol, and amino acids, respectively. All the products of digestion are metabolized to a common product, acetyl-CoA, which is then oxidized by the citric acid cycle

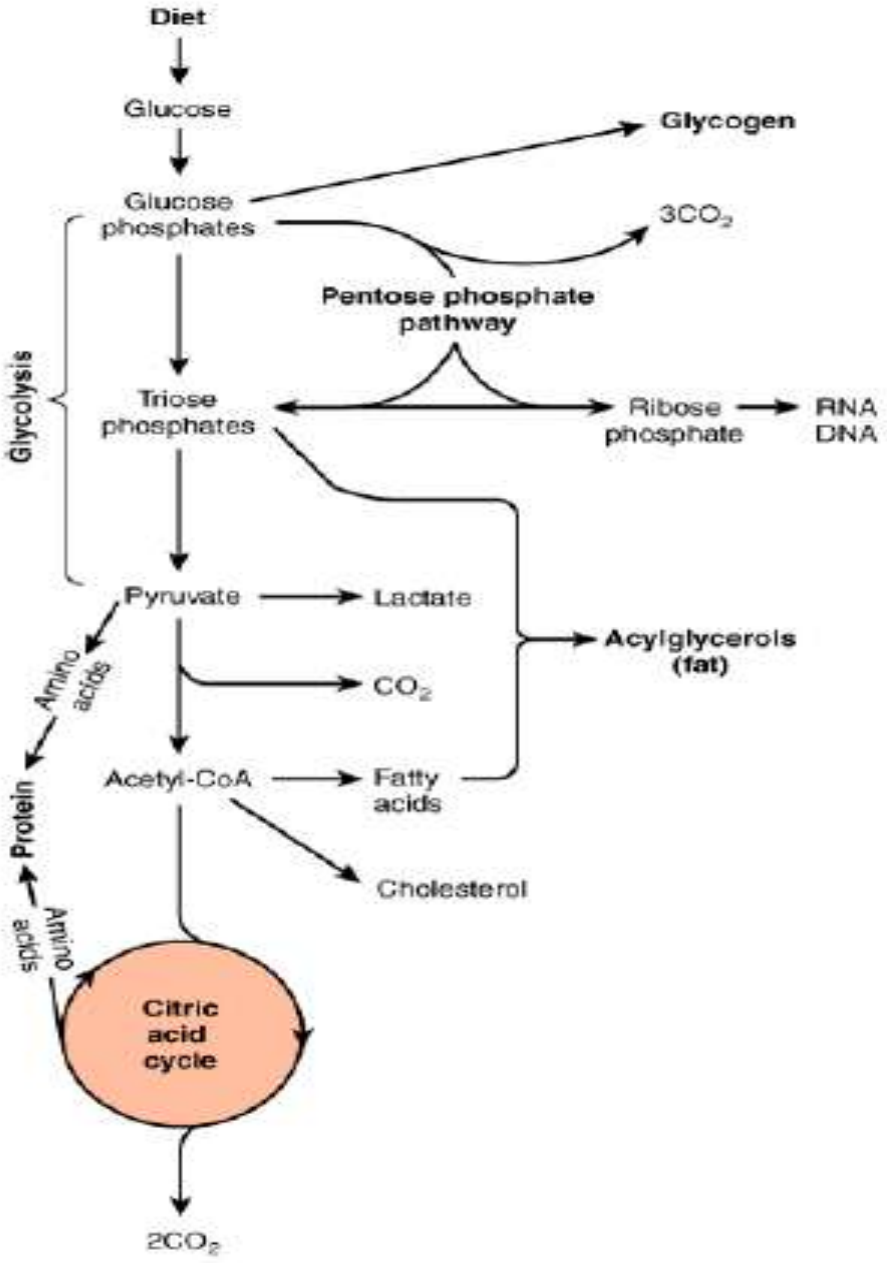


**Carbohydrate Metabolism:**

Glucose is the major fuel of most tissues. It is metabolized to pyruvate by the pathway of glycolysis.

Aerobic tissues metabolize pyruvate to acetyl-CoA, which can enter the citric acid cycle for complete oxidation to CO2 and H2O, linked to the formation of ATP in the process of oxidative phosphorylation.

Glycolysis can also occur anaerobically (in the absence of oxygen) when the end product is lactate.



# Glucose and its metabolites take part in other processes

(1) Synthesis of the storage polymer glycogen in skeletal muscle and liver.

(2) The pentose phosphate pathway, is replacement of a part of the pathway of glycolysis. It is a source of reducing equivalents (NADPH) for fatty acid synthesis and the source of ribose for nucleotide and nucleic acid synthesis.

(3) Triose phosphates gives rise to the glycerol moiety of triacylglycerols.

(4) Pyruvate and intermediates of the citric acid cycle provide the carbon skeletons for the synthesis of amino acids, and acetyl-CoA is the precursor of fatty acids and cholesterol (and hence of all steroids synthesized in the body). Gluconeogenesis is the process of forming glucose from noncarbohydrate precursors, eg, lactate, amino acids, and glycerol.

## Lipid Metabolism:

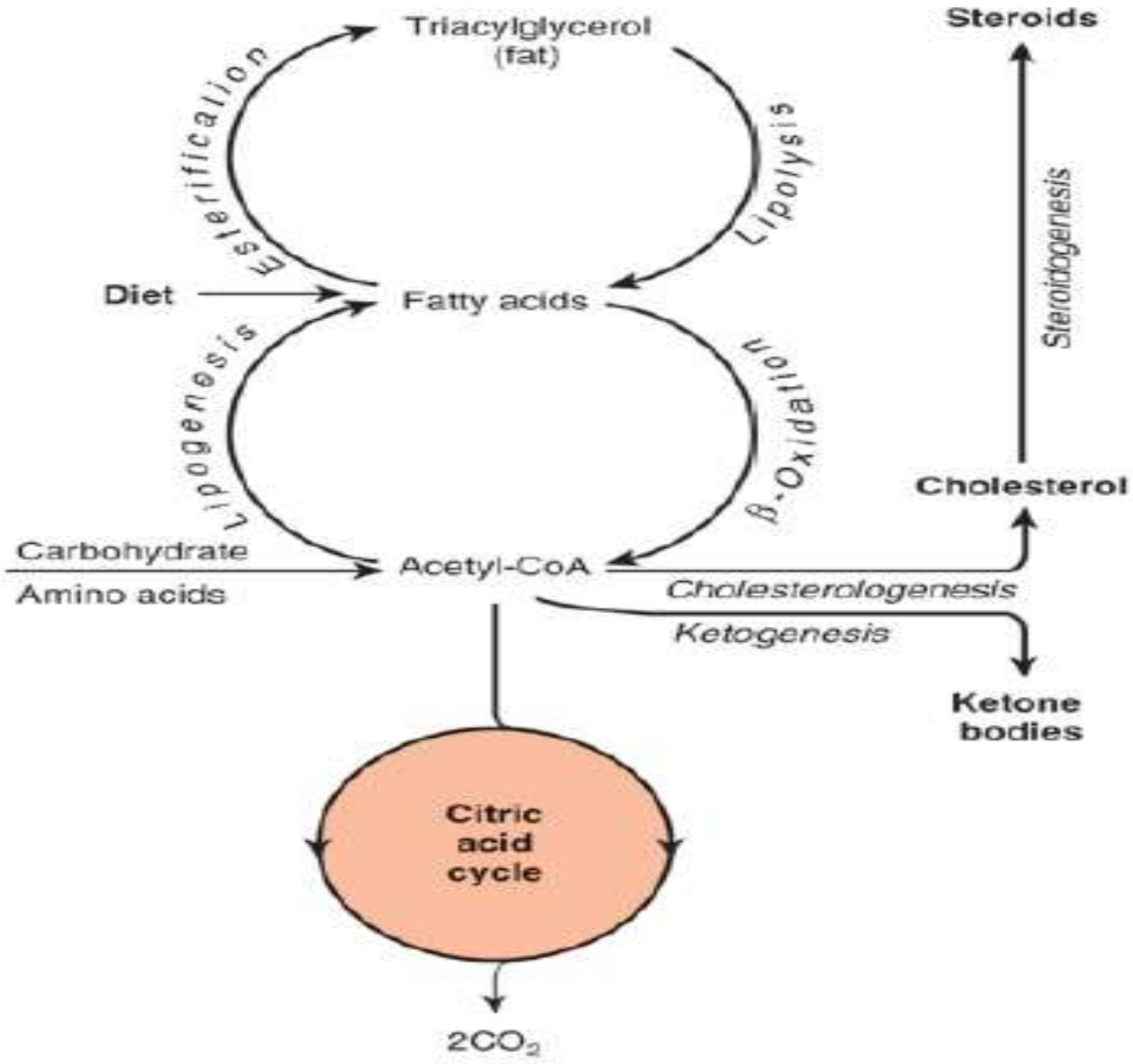
The source of long-chain fatty acids is either dietary lipid or de novo synthesis from acetyl-CoA derived from carbohydrate or amino acids. Fatty acids may be oxidized to acetyl-CoA ($\beta$-oxidation) or esterified with glycerol, forming triacylglycerol (fat) as the body's main fuel reserve.

Acetyl-CoA formed by $\beta$-oxidation may undergo three fates:

1. As with acetyl-CoA arising from glycolysis, it is oxidized to CO2 + H2 O via the citric acid cycle.

2. It is the precursor for synthesis of cholesterol and other steroids.

3-In the liver, it is used to form ketone bodies (acetoacetate and hydroxybutyrate) that are important fuels in prolonged fasting.

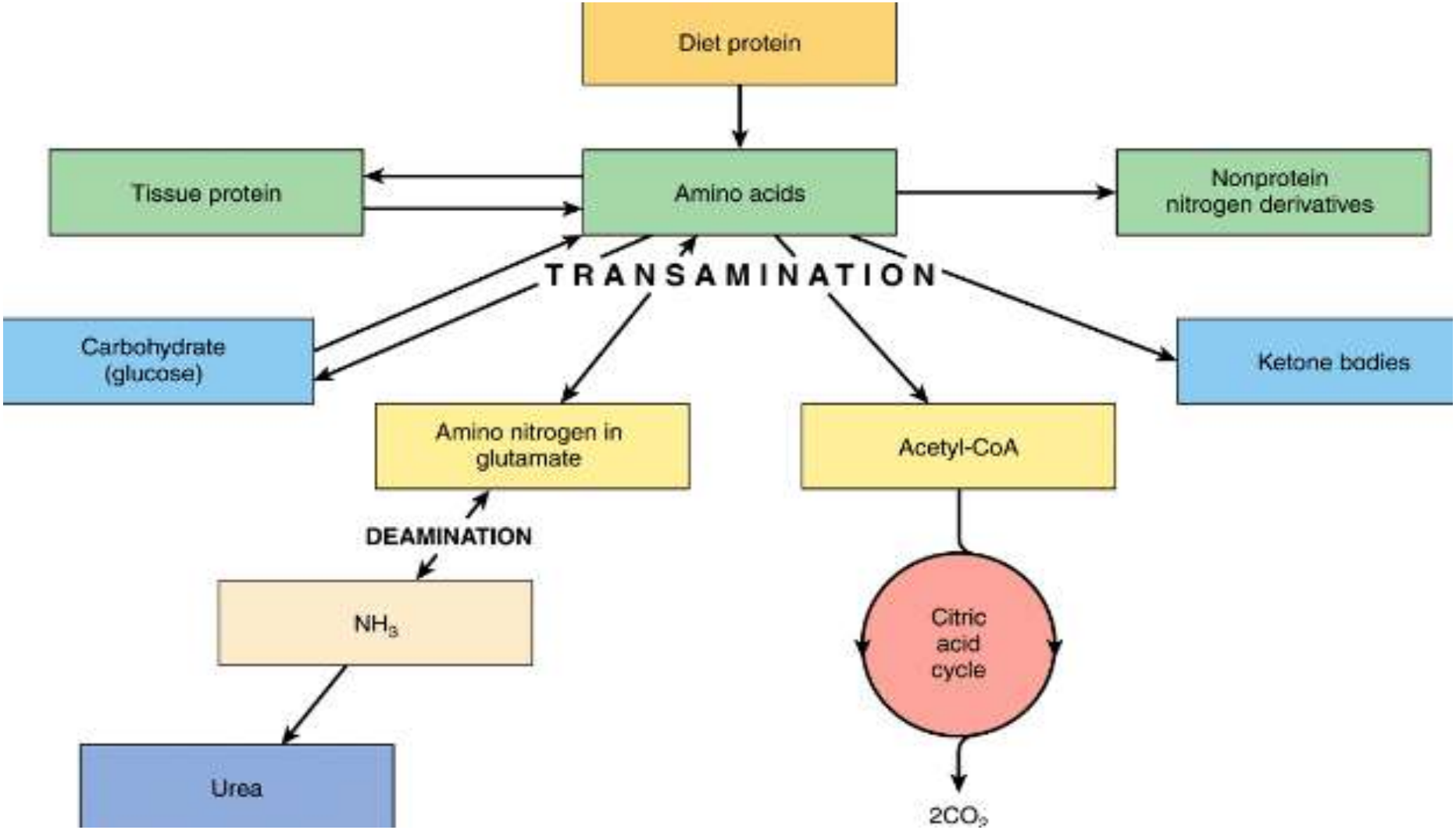



Overview of fatty acid metabolism showing the major pathways and end products. The ketone bodies are acetoacetate, 3-hydroxybutyrate, and acetone.

# Amino Acid Metabolism

- The amino acids are required for protein synthesis. Some must be supplied in the diet (the essential amino acids ), since they cannot be synthesized in the body. The remainder are nonessential amino acids, can be formed from metabolic intermediates by transamination using the amino nitrogen from other amino acids. After deamination, amino nitrogen is excreted as urea, and the carbon skeletons that remain after transamination may:
- (1) be oxidized to $CO_2$ via the citric acid cycle.
- (2) to be used for synthesis of glucose (gluconeogenesis), or
- (3) form ketone bodies, which may be oxidized or be used for synthesis of fatty acids.
- Several amino acids are also the precursors of other compounds, eg, purines, pyrimidines, hormones such as epinephrine and thyroxine, and neurotransmitters.

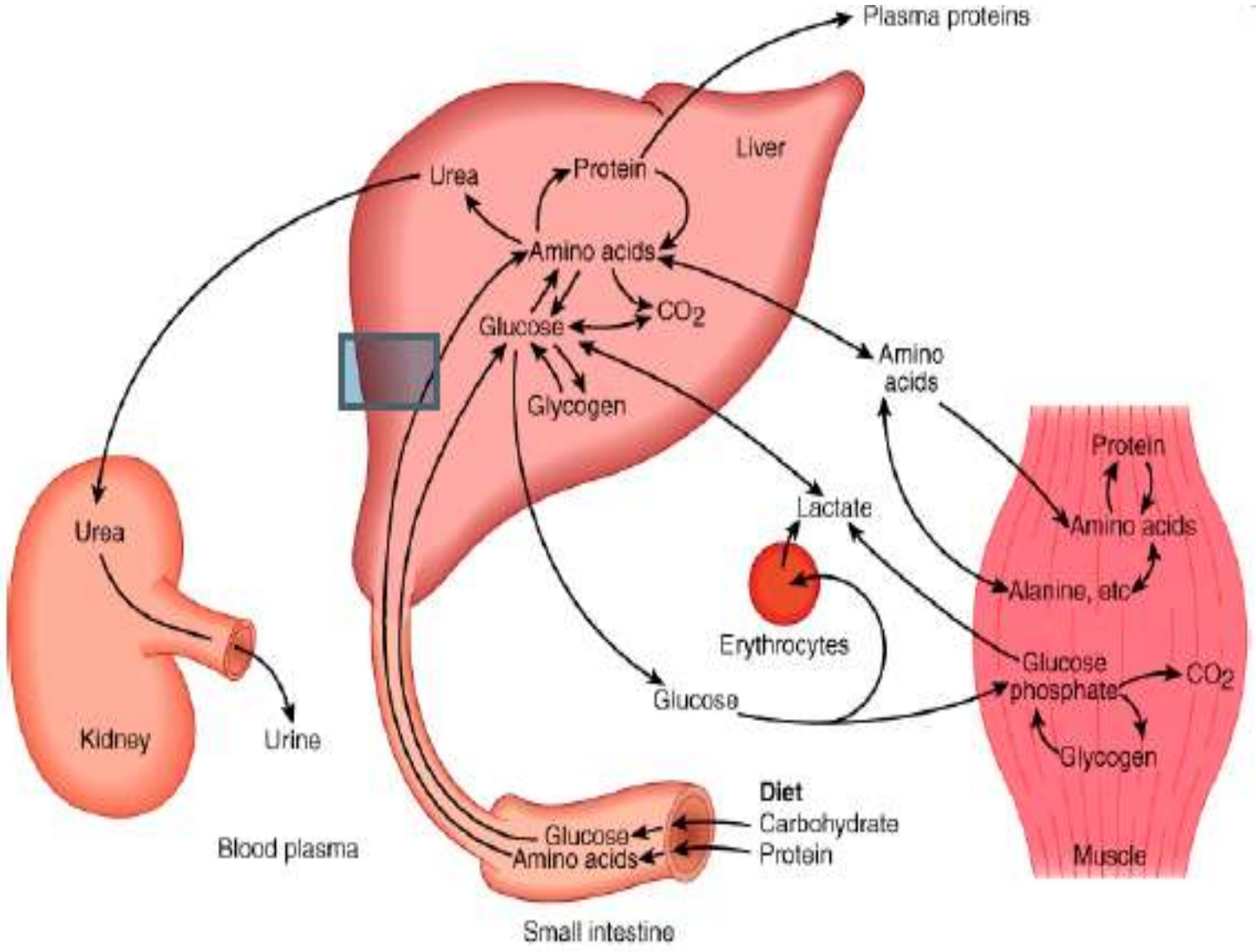
- Overview of amino acid metabolism showing the major pathways and end products



# Metabolic pathways at different levels of organization

- The location and combination of metabolic pathways is shown at several levels of organization.
- (1) At the tissue and organ level the nature of the substrates entering and metabolites leaving tissues and organs is defined.
- (2) At the subcellular level each cell organelle (eg, the mitochondrion) or compartment (eg, the cytosol) has specific roles that form part of a subcellular metabolic pathways.
- At the Tissue & Organ Level, the Blood Circulation Integrates Metabolism:
- Amino acids resulting from the digestion of dietary protein and glucose resulting from the digestion of carbohydrate are absorbed via the hepatic portal vein.
- The liver has the role of regulating the blood concentration of water-soluble metabolites . In the case of glucose, this is achieved by taking up glucose in excess of immediate requirements and converting it to glycogen (glycogenesis) or to fatty acids (lipogenesis).

- Between meals, the liver acts to maintain the blood glucose concentration by breaking down glycogen (glycogenolysis) and, together with the kidney, by converting non carbohydrate metabolites such as lactate, glycerol, and amino acids to glucose (gluconeogenesis).
- The maintenance of an adequate concentration of blood glucose is vital for those tissues in which it is the major fuel (the brain) or the only fuel (erythrocytes).
- The liver also synthesizes the major plasma proteins (eg, albumin) and deaminates amino acids that are in excess of requirements, forming urea, which is transported to the kidney and excreted.

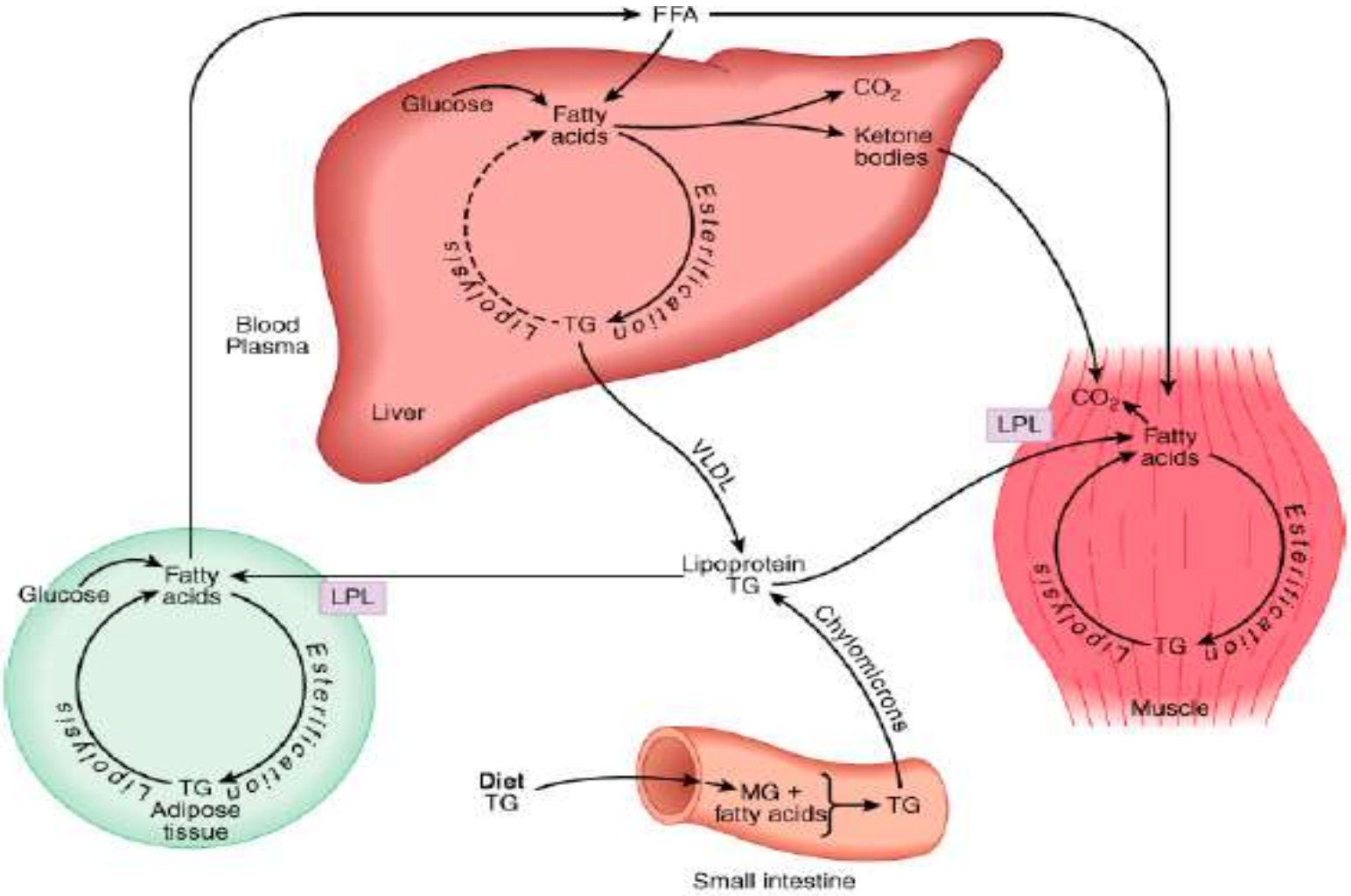



Transport and fate of major carbohydrate and amino acid substrates and metabolites. there is a little free glucose in muscle, since it is rapidly phosphorylated upon entry.

## Skeletal muscle

- Skeletal muscle utilizes glucose as a fuel, both aerobically, forming $CO_2$ , and anaerobically, forming lactate.
- It stores glycogen as a fuel for use in muscle contraction and synthesizes muscle protein from plasma amino acids.
- Muscle approximately 50% of body mass so represents a significant store of protein that can be drawn upon to supply amino acids for gluconeogenesis in starvation.

- Lipids in the diet are mainly triacylglycerol, and are hydrolyzed to monoacylglycerols and fatty acids in the gut, then re-esterified in the intestinal mucosa. Here they are packaged with protein and secreted into the lymphatic system and then into the bloodstream as chylomicrons, the largest of the plasma lipoproteins.
- Chylomicrons also contain other lipid-soluble nutrients. Unlike glucose and amino acids, chylomicron triacylglycerol is not taken up directly by the liver. It is first metabolized by tissues that have lipoprotein lipase, which hydrolyzes the triacylglycerol, releasing fatty acids that are incorporated into tissue lipids or oxidized as fuel.
- The chylomicron fragments are cleared by the liver. The other major source of long-chain fatty acids is synthesis (lipogenesis) from carbohydrate, in adipose tissue and the liver.

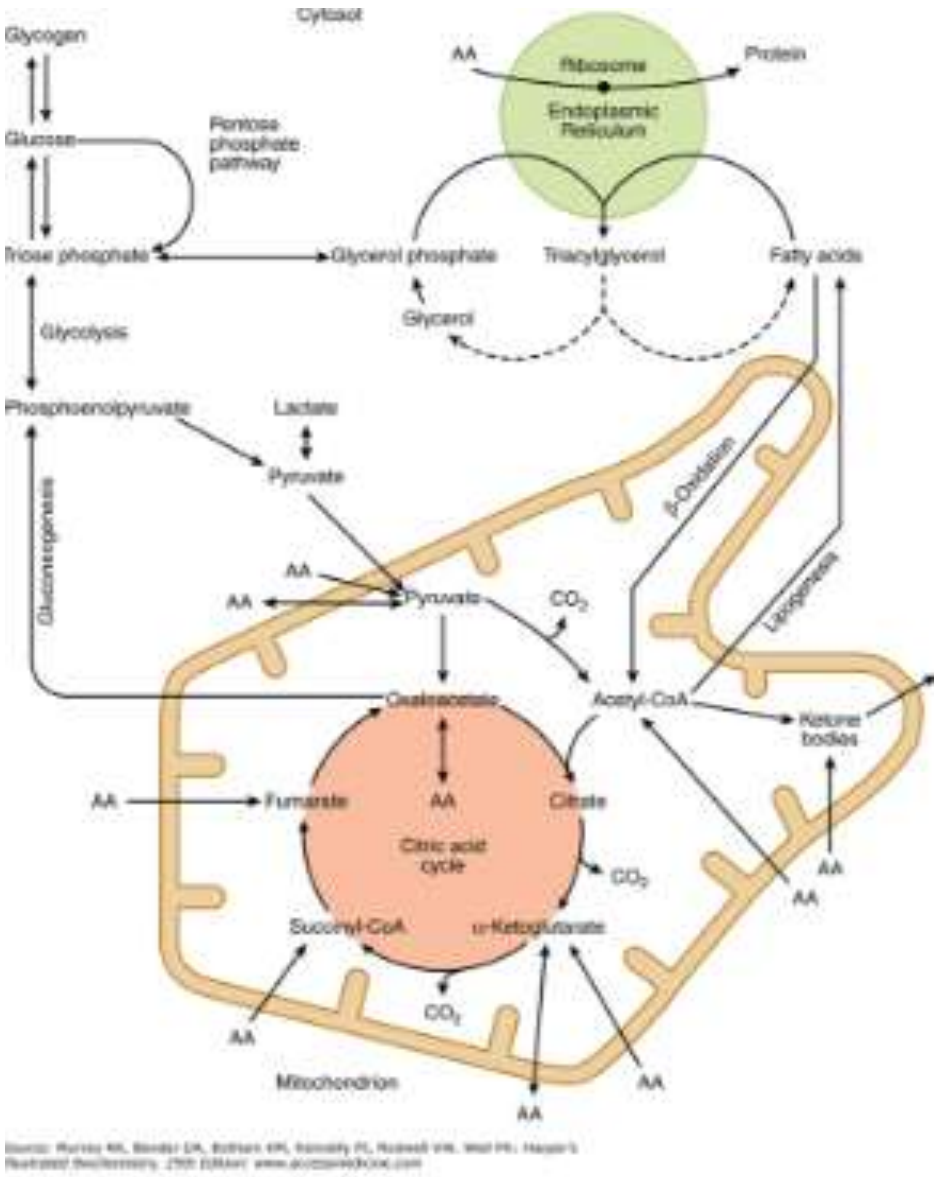



Transport and fate of major lipid substrates and metabolites. (FFA, free fatty acids; LPL, lipoprotein lipase; MG, monoacylglycerol; TG, triacylglycerol; VLDL, very low density lipoprotein.)

- Adipose tissue triacylglycerol is the main fuel reserve of the body. It is hydrolyzed (lipolysis) and glycerol and free fatty acids are released into the circulation.
- Glycerol is a substrate for gluconeogenesis.
- The fatty acids are transported bound to serum albumin; they are taken up by most tissues (but not brain or erythrocytes) and either esterified to triacylglycerols for storage or oxidized as a fuel.
- In the liver, triacylglycerol arising from lipogenesis, free fatty acids, and chylomicron remnants is secreted into the circulation in very low density lipoprotein (VLDL). This triacylglycerol undergoes a fate similar to that of chylomicrons.
- Partial oxidation of fatty acids in the liver leads to ketone body production (ketogenesis). Ketone bodies are transported to extrahepatic tissues, where they act as a fuel in prolonged fasting and starvation.

## At the Subcellular Level, Glycolysis Occurs in the Cytosol & the Citric Acid Cycle in the Mitochondria

- Compartmentation of pathways in separate subcellular compartments or organelles permits integration and regulation of metabolism. Not all pathways are of equal importance in all cells.
- Intracellular location and overview of major metabolic pathways in a liver parenchymal cell. (AA , metabolism of one or more essential amino acids; AA , metabolism of one or more nonessential amino acids.)



- The central role of the mitochondrion is shown directly, since it acts as the center of carbohydrate, lipid, and amino acid metabolism.
- It contains the enzymes of the citric acid cycle , -oxidation of fatty acids and ketogenesis, as well as the respiratory chain and ATP synthase.
- Glycolysis, the pentose phosphate pathway, and fatty acid synthesis all occur in the cytosol.
- In gluconeogenesis, substrates such as lactate and pyruvate, which are formed in the cytosol, enter the mitochondrion to yield oxaloacetate as a precursor for the synthesis of glucose in the cytosol.
- The membranes of the endoplasmic reticulum contain the enzyme system for triacylglycerol synthesis, and the ribosomes are responsible for protein synthesis.

**Allosteric&hormonal mechanisms are important in the metabolic control of enzyme catalyzed reactions**

- Metabolic pathways are coordinated series of reactions, catalyzed by enzymes and designed to make specific products. As a general rule, catabolic pathways provide energy and reducing power to the cell, while anabolic pathways consume energy and reducing power.
- Not every metabolic pathway that occurs in an organism occurs in every cell. Some pathways take place only in specific cellular locations or in specific organs or tissues.
- In a living cell, molecules flow through each metabolic pathway at some rate, called the **flux**. For the cell to function efficiently, it must be able to change the flux of molecules through each pathway. Control of flux is achieved by altering enzymatic activity, and usually several different enzymes contribute to control of the overall flux through a pathway. In addition, flux through a pathway can be influenced by the concentrations of metabolites in that pathway, such as the starting material.

# Glucose Is Always Required by the Central Nervous System and Erythrocytes

- Erythrocytes lack mitochondria and hence are only dependent on (anaerobic) glycolysis and the pentose phosphate pathway at all times. The brain can metabolize ketone bodies to meet about 20% of its energy requirements; the remainder must be supplied by glucose.
- The metabolic changes that occur in the fasting state and starvation results of the need to preserve glucose and the limited stores of glycogen in liver and muscle for use by the brain and red blood cells, and to ensure the delivery of alternative metabolic fuels for other tissues.
- In pregnancy the fetus requires a significant amount of glucose, as ensures the synthesis of lactose in lactation.

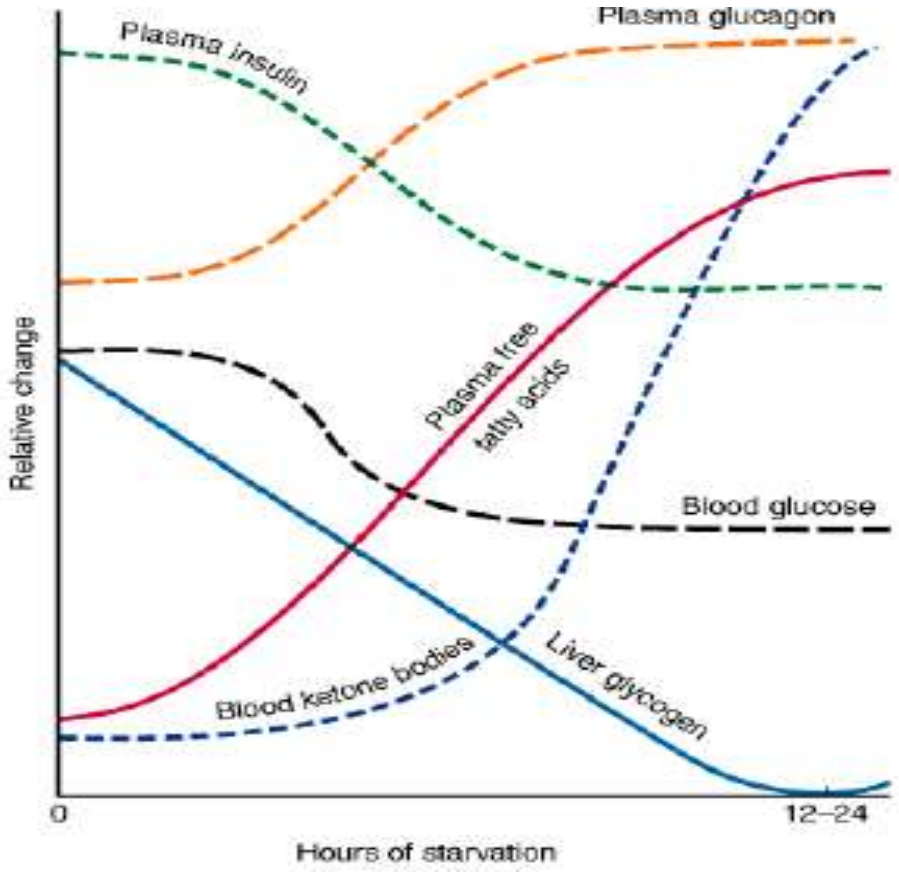
# Metabolic Fuel Reserves Are Mobilized in the Fasting State

There is a small fall in plasma glucose in the fasting state, and then little change as fasting is prolonged into starvation.

Plasma free fatty acids increase in fasting, but then rise little more in starvation; as fasting is prolonged, so the plasma concentration of ketone bodies (acetoacetate and 3-hydroxybutyrate) increases markedly.

In the fasting state, as the concentration of glucose in the portal blood falls, so insulin secretion decreases, and skeletal muscle and adipose tissue take up less glucose.

The increase in secretion of glucagon by The $\alpha$ cells of the pancreas inhibits glycogen synthetase, and activates glycogen phosphorylase in the liver. The resulting glucose 6-phosphate is hydrolyzed by glucose 6-phosphatase, and glucose is released into the bloodstream for use by the brain and erythrocytes.



- Muscle glycogen cannot provided directly to plasma glucose, since muscle lacks glucose 6-phosphatase, and the primary purpose of muscle glycogen is to provide a source of glucose 6-phosphate for energy-yielding metabolism in the muscle itself.
- However, acetyl-CoA formed by oxidation of fatty acids in muscle inhibits pyruvate dehydrogenase, leading to an accumulation of pyruvate. Most of this is transaminated to alanine, at the expense of amino acids arising from breakdown of protein stores that produced in the fed state.
- The alanine, and much of the keto acids resulting from this transamination are exported from muscle, and taken up by the liver, where the alanine is transaminated to yield pyruvate.
- The resultant amino acids are largely exported back to muscle, to provide amino groups for formation of more alanine, while the pyruvate is a major substrate for gluconeogenesis in the liver.

- In adipose tissue the decrease in insulin and increase in glucagon results in inhibition of lipogenesis, inactivation of lipoprotein lipase, and activation of intracellular hormone-sensitive lipase.
- This leads to release from adipose tissue of increased amounts of glycerol (which is a substrate for gluconeogenesis in the liver) and free fatty acids, which are used by liver, heart, and skeletal muscle as their preferred metabolic fuel, therefore supplies glucose.
- Although muscle takes up and metabolizes free fatty acids in the fasting state, it cannot meet all of its energy requirements by -oxidation.
- By contrast, the liver has a greater capacity for $\beta$-oxidation than it requires to meet its own energy needs, and as fasting becomes more prolonged, it forms more acetyl-CoA than can be oxidized.
- This acetyl-CoA is used to synthesize the ketone bodies , which are major metabolic fuels for skeletal and heart muscle and can meet some of the brain's energy needs.

- In prolonged starvation, glucose may represent less than 10% of whole body energy-yielding metabolism.
- Were there no other source of glucose, liver and muscle glycogen would be consumed after about 18 h fasting. As fasting becomes more prolonged, so an increasing amount of the amino acids released as a result of protein catabolism is utilized in the liver and kidneys for gluconeogenesis .

# Clinical significance

- In prolonged starvation, as adipose tissue reserves are depleted, there is a very considerable increase in the net rate of protein catabolism to provide amino acids, not only as substrates for gluconeogenesis, but also as the main metabolic fuel of all tissues.
- Death results when essential tissue proteins are catabolized and not replaced.
- In patients with cachexia as a result of release of cytokines in response to tumors and a number of other pathologic conditions, there is an increase in the rate of tissue protein catabolism, as well as a considerably increased metabolic rate, so they are in a state of advanced starvation. Again, death results when essential tissue proteins are catabolized and not replaced.
- The high demand for glucose by the fetus, and for lactose synthesis in lactation, can lead to ketosis.

- In poorly controlled type 1 diabetes mellitus, patients may become hyperglycemic, partly as a result of lack of insulin to stimulate uptake and utilization of glucose, and partly because in the absence of insulin there is increased gluconeogenesis from amino acids in the liver.
- At the same time, the lack of insulin results in increased lipolysis in adipose tissue, and the resultant free fatty acids are substrates for ketogenesis in the liver.

# Citric Acid Cycle

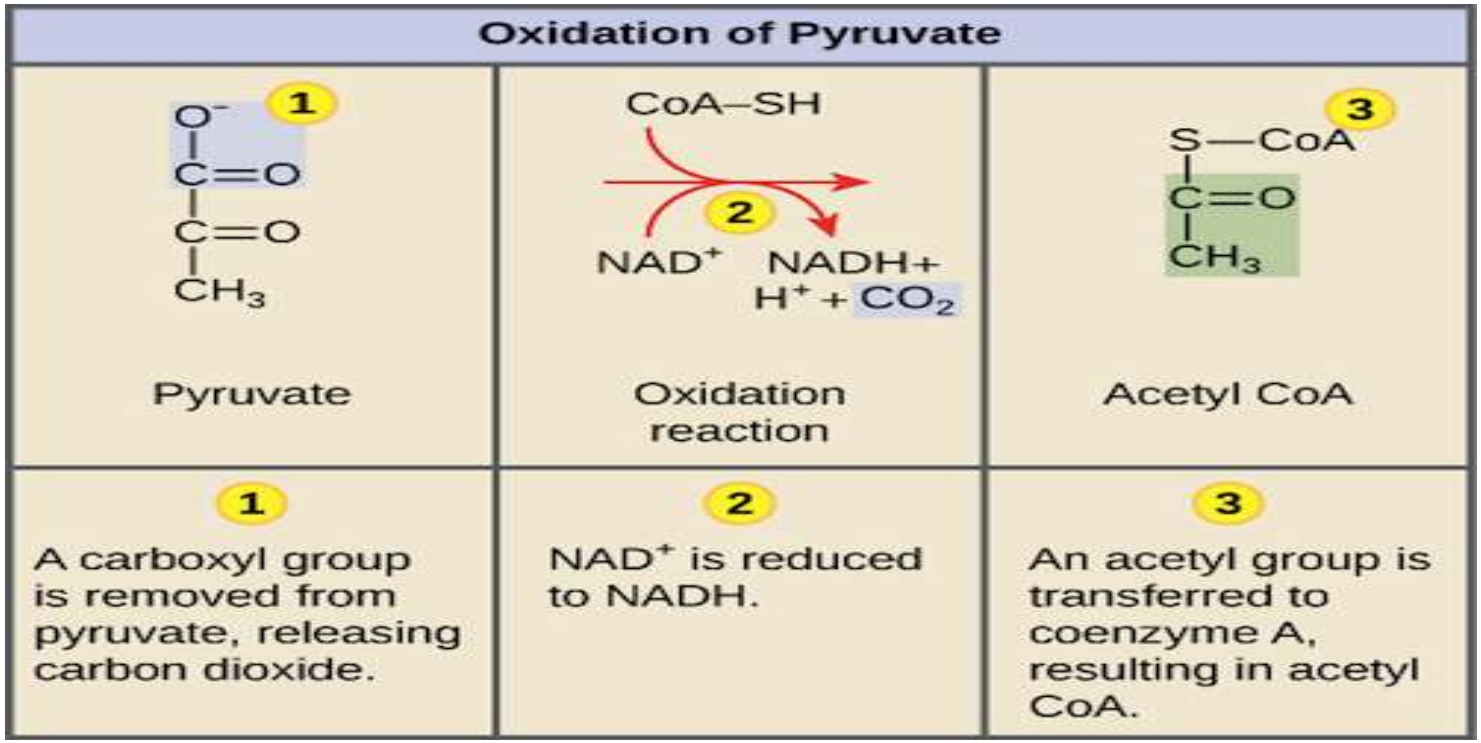
By: Inaam A. Ameen

# citric acid cycle

- Also known as Krebs cycle or tricarboxylic acid cycle: is a sequence of reactions in mitochondria that oxidizes the acetyl moiety of acetyl-CoA and reduces coenzymes that are reoxidized through the electron transport chain, linked to the formation of ATP.
- The citric acid cycle aid in releasing the stored energy in the foods.
- The usable energy found in the carbohydrates, proteins, and fats is released mainly through the citric acid cycle. Although the citric acid cycle does not use oxygen directly, it works only when oxygen is present.
- The CAC is the final pathway for the oxidation of carbohydrate, lipid, and protein because glucose, fatty acids, and most amino acids are metabolized to acetyl-CoA or intermediates of the cycle.
- In CAC the oxidation of acetic acid or acetyl equivalent provides energy for storage in phosphate bonds (as in ATP)
- It also has a central role in gluconeogenesis, lipogenesis, and interconversion of amino acids. Many of these processes occur in most tissues, but the liver is the only tissue in which all occur to a significant extent.

# Citric Acid Cycle Steps

- The first phase of cellular respiration called glycolysis takes place in the cytosol of the cell's cytoplasm. The citric acid cycle, however, occurs in the matrix of cell mitocondria. Prior to the beginning of the citric acid cycle, pyruvic acid generated in glycolysis crosses the mitochondrial membrane and is used to form acetyl coenzyme A (acetyl CoA). Acetyl CoA is then used in the first step of the citric acid cycle. Each step in the cycle is catalyzed by a specific enzyme.



# Steps of Citric acid cycle

- **CAC essentially involves the oxidation of acetyl CoA to CO2 and H2O**
- **Step 1** - The two-carbon acetyl group of acetyl CoA is added to the four-carbon **oxaloacetate** to form the six-carbon citrate. The conjugate acid of citrate is citric acid, hence the name citric acid cycle. Oxaloacetate is regenerated at the end of the cycle so that the cycle may continue. Enzyme: citrate synthase.
- **Step 2** - **Citrate** loses a molecule of water forming cis-Aconitate.
- **Step3**- Another water molecule is added. In the process, citric acid is converted to its isomer isocitrate. Enzyme: aconitase.
- **Step 4** - **Isocitrate** loses a molecule of carbon dioxide ($CO_2$) and is oxidized forming the five-carbon alpha ketoglutarate. Nicotinamide adenine dinucleotide ($NAD^+$) is reduced to NADH + $H^+$ in the process. Enzyme: isocitrate dehydrogenase.
- **Step 5** - **Alpha ketoglutarate** is converted to the 4-carbon succinyl CoA. A molecule of $CO_2$ is removed and $NAD^+$ is reduced to NADH + $H^+$ in the process. Enzyme: alpha ketoglutarate dehydrogenase.

- **Step 6** - CoA is removed from the **succinyl CoA** molecule and is replaced by a phosphate group. The phosphate group is then removed and attached to guanosine diphosphate (GDP) thereby forming guanosine triphosphate (GTP). Like ATP, GTP is an energy-yielding molecule and is used to generate ATP when it donates a phosphate group to ADP. The final product from the removal of CoA from succinyl CoA is **succinate**. Enzyme: succinyl-CoA synthetase.
- **Step 7** - Succinate is oxidized and **fumarate** is formed. Flavin adenine dinucleotide (FAD) is reduced and forms $FADH_2$ in the process. Enzyme: succinate dehydrogenase.
- **Step 8** - A water molecule is added and bonds between the carbons in fumarate are rearranged forming **malate**. Enzyme: fumarase.
- **Step 9** - Malate is oxidized forming **oxaloacetate**, the beginning substrate in the cycle. $NAD^+$ is reduced to NADH + $H^+$ in the process. Enzyme: malate dehydrogenase.

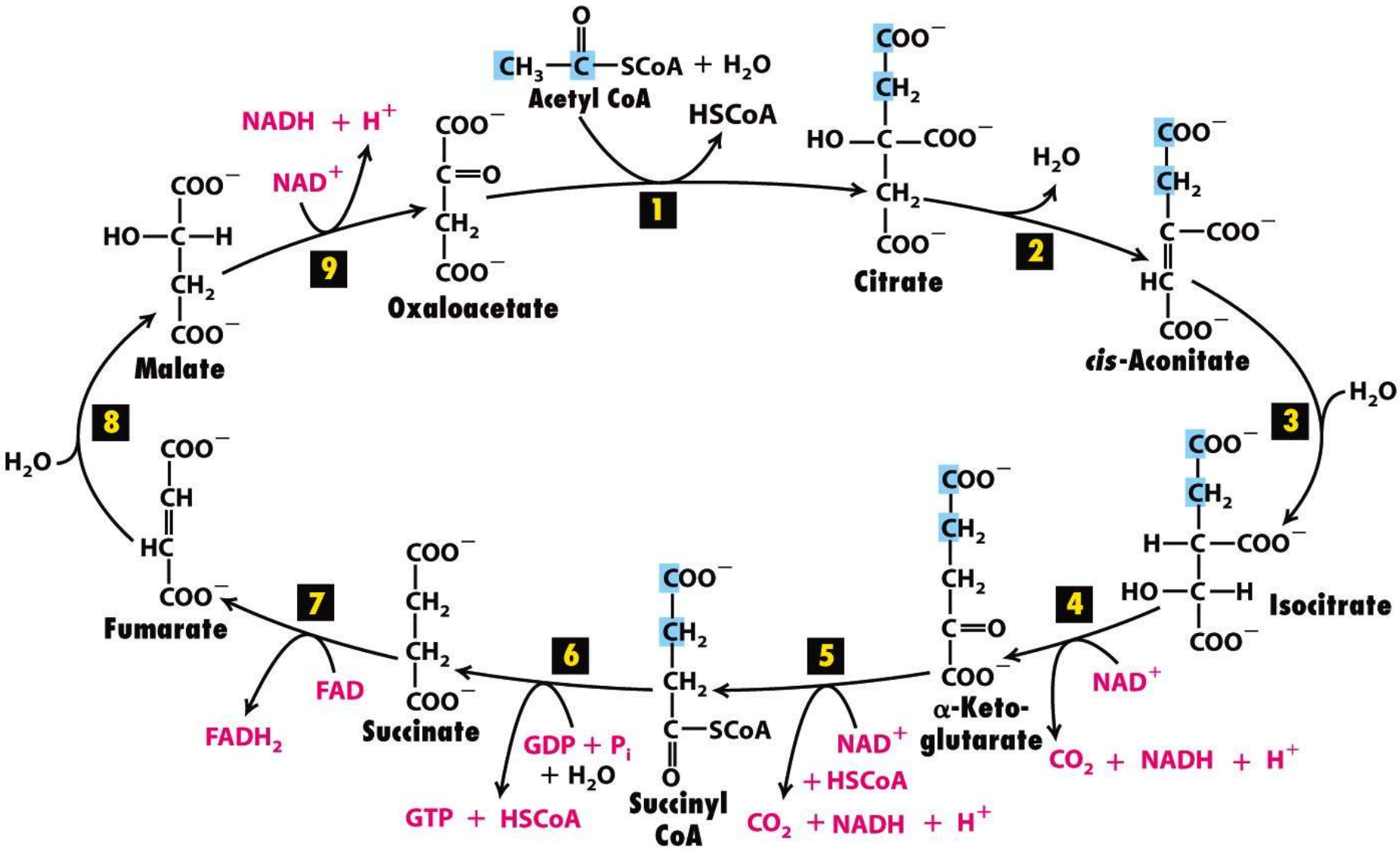
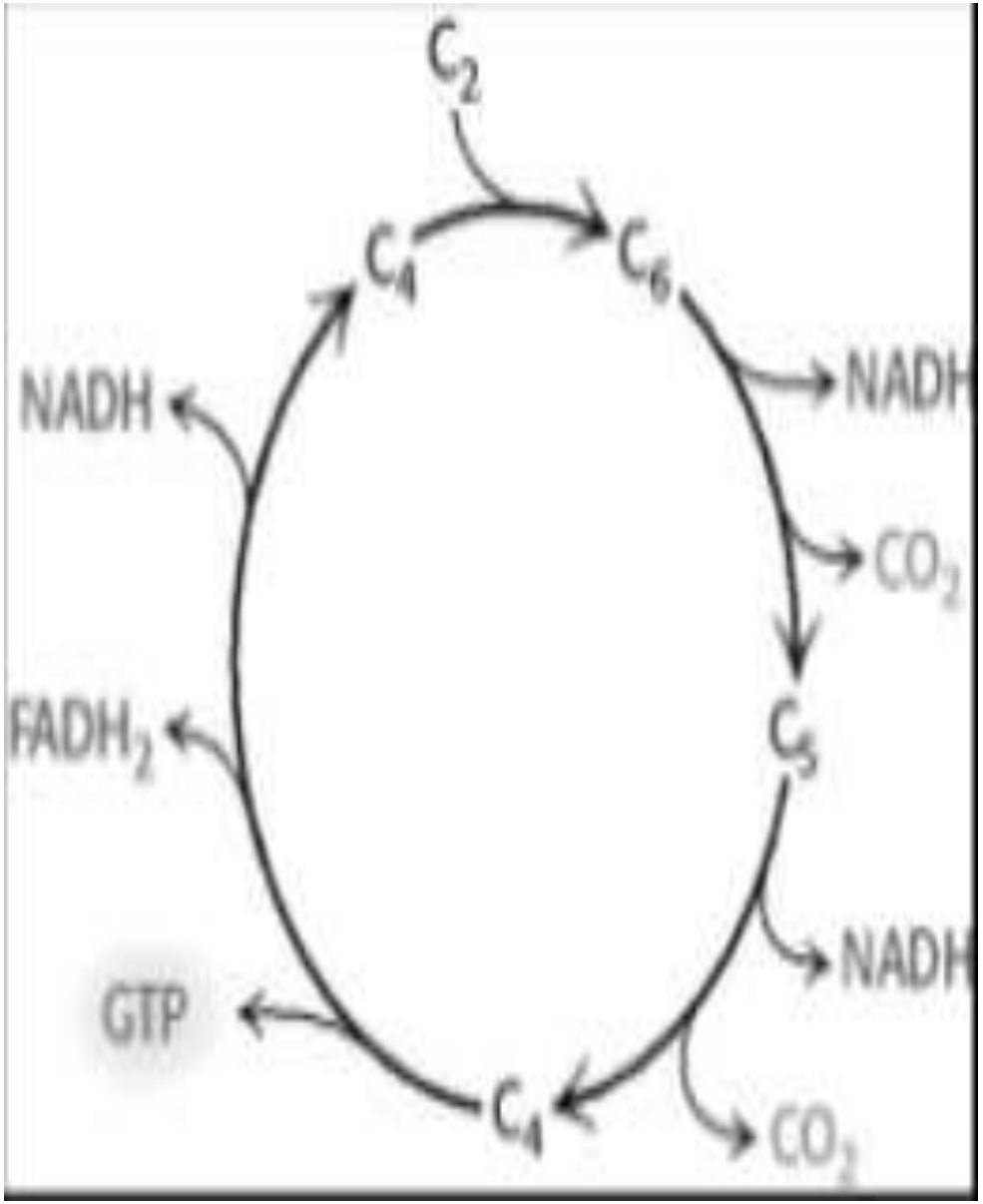



**Figure 12-10**
***Molecular Cell Biology, Sixth Edition***

# Citric Acid Cycle Summary

- A cycle of enzyme- catalyzed reactions in living cells that is the final series of reactions of aerobic metabolism of carbohydrates, proteins, and fatty acids, and by which carbon dioxide is produced, oxygen is reduced, and ATP is formed.
- In eukaryotes, the reactions of the CAC take place inside mitocondria, in contrast with those of glycolysis, which take place in the cytosol.
- In eukaryotes the citric acid cycle uses one molecule of acetyl CoA to generate 1 ATP, 3 NADH, 1 $FADH_2$, 2 $CO_2$, and 3 $H^+$.
- Since two acetyl CoA molecules are generated from the two pyruvic acid molecules produced in glycolysis, the total number of these molecules yielded in the citric acid cycle is doubled to 2 ATP, 6 NADH, 2 $FADH_2$, 4 $CO_2$, and 6 $H^+$.
- Two additional NADH molecules are also generated in the conversion of pyruvic acid to acetyl CoA prior to the start of the cycle.
- The NADH and $FADH_2$ molecules produced in the citric acid cycle are passed along to the final phase of cellular respiration called the electron transport chain. Here NADH and $FADH_2$ undergo oxidative phosphorylation to generate more ATP.




Three hydride ions (hence, six electrons) are transferred to three molecules of NAD, whereas one pair of hydrogen atoms( hence, two electrons) are transferred to one molecule of FAD.

The function of the CAC is the harvesting of high-energy electrons from carbon fuels.

# ATP THAT FORMED PER TURN OF THE CITRIC ACID CYCLE

- As a result of oxidations catalyzed by the dehydrogenases of the citric acid cycle, three molecules of NADH and one of FADH2 are produced for each molecule of acetyl-CoA catabolized in one turn of the cycle.

- These reducing equivalents are transferred to the respiratory chain, where reoxidation of each NADH results in formation of 2.5 ATP, and of FADH2 , 1.5 ATP.

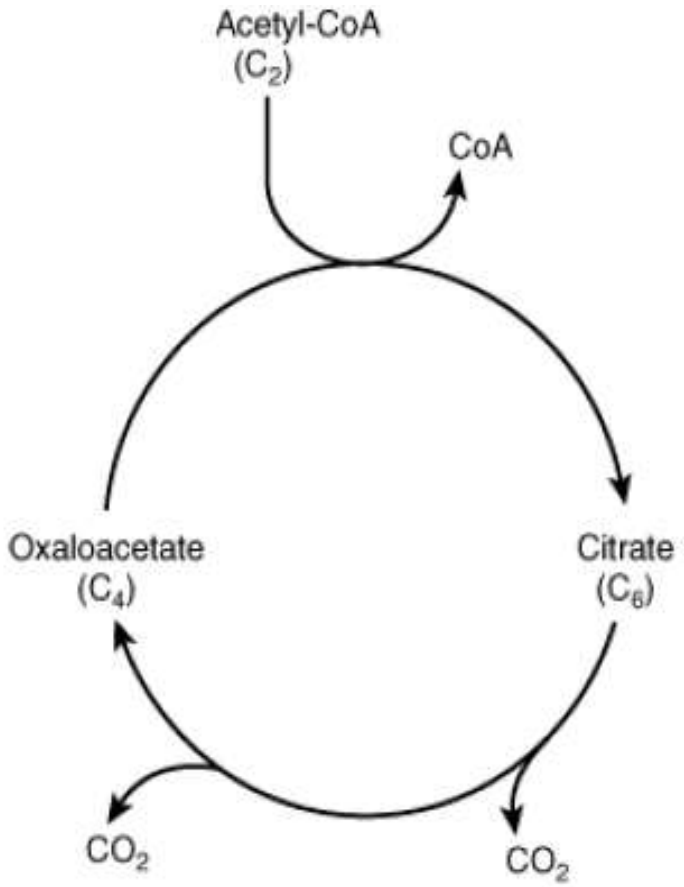
- In addition, 1 ATP (or GTP) is formed by substrate-level phosphorylation catalyzed by succinate thiokinase.
- Ten ATP are formed per turn of the citric acid cycle:
- Nine ATP are generated via oxidative phosphorylation and one ATP (or GTP) arises at substrate level from the conversion of succinyl-CoA to succinate.

# THE CITRIC ACID CYCLE PROVIDES SUBSTRATE FOR THE RESPIRATORY CHAIN

- The cycle starts with reaction between the acetyl moiety of acetyl-CoA and the four-carbon dicarboxylic acid oxaloacetate, forming a six-carbon tricarboxylic acid, citrate.
- In the subsequent reactions, two molecules of $CO_2$ are released and oxaloacetate is regenerated.
- Only a small quantity of oxaloacetate is needed for the oxidation of a large quantity of acetyl-CoA (catalytic role).
- The citric acid cycle is an essential part of the process by which much of the free energy liberated during the oxidation of fuels is made available

# Role of oxaloacetate in citric acid cycle

- The four-carbon molecule, oxaloacetate that initiates the first step in the CAC is regenerated at the end of one passage through the cycle.
- The oxaloacetate acts catalytically: it participates in the oxidation of the acetyl group but is itself regenerated.
- Thus, one molecule of oxaloacetate is capable of participating in the oxidation of many acetyl molecules.

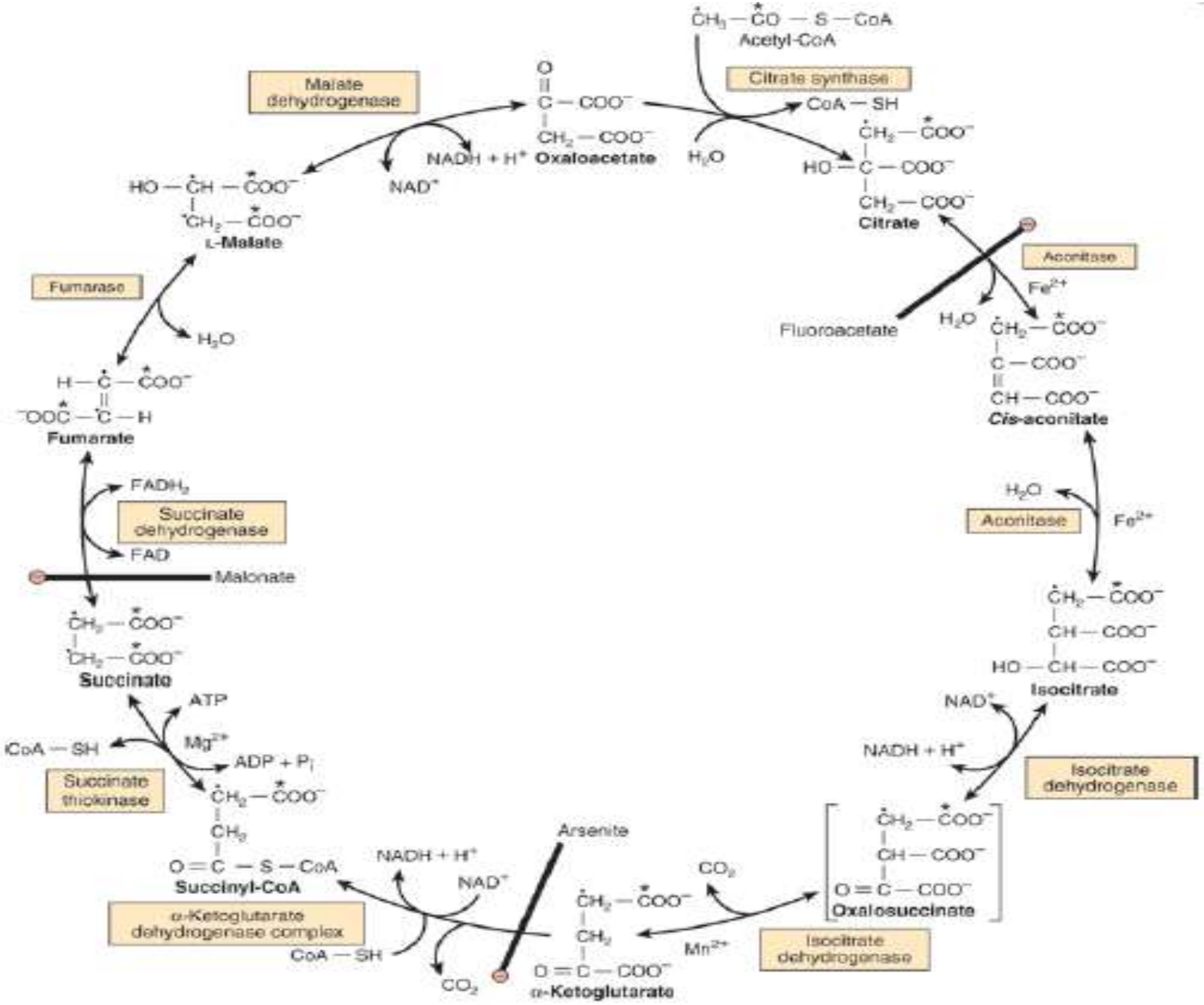


# Oxidative phosphorylation

- During the oxidation of acetyl-CoA, coenzymes are reduced and subsequently reoxidized in the respiratory chain, linked to the formation of ATP (oxidative phosphorylation)
- This process is aerobic, requiring oxygen as the final oxidant of the reduced coenzymes.
- The enzymes of the citric acid cycle are located in the mitochondrial matrix, either free or attached to the inner mitochondrial membrane and the crista membrane, where the enzymes and coenzymes of the respiratory chain are also found

# The citric acid cycle:

- The major catabolic pathway for acetyl-CoA in aerobic organisms.
- Acetyl-CoA, the product of carbohydrate, protein, and lipid catabolism, is taken into the cycle and oxidized to CO2 with the release of reducing equivalents (2H).
- Subsequent oxidation of 2H in the respiratory chain leads to phosphorylation of ADP to ATP.
- For one turn of the cycle, nine ATP are generated via oxidative phosphorylation and one ATP (or GTP) arises at substrate level from the conversion of succinyl-CoA to succinate.


Acetyl-CoA
Citrate synthase
CoA — SH
Oxaloacetate
$H_2O$
Citrate
Malate dehydrogenase
$NADH + H^+$
$NAD^+$
L-Malate
Fumarase
$H_2O$
Fumarate
Aconitase
$Fe^{2+}$
Fluoroacetate
$H_2O$
Cis-aconitate
$H_2O$
Aconitase
$Fe^{2+}$
Isocitrate
$NAD^+$
$NADH + H^+$
Isocitrate dehydrogenase
Oxalosuccinate
$CO_2$
$Mn^{2+}$
Isocitrate dehydrogenase
α-Ketoglutarate
Arsenite
$NADH + H^+$
$NAD^+$
$CO_2$
α-Ketoglutarate dehydrogenase complex
CoA — SH
Succinyl-CoA
ATP
$Mg^{2+}$
CoA — SH
$ADP + P_i$
Succinate thiokinase
Succinate
$FADH_2$
Succinate dehydrogenase
FAD
Malonate

# Role of vitamins

Four of the B vitamins are essential in the citric acid cycle and hence energy-yielding metabolism:

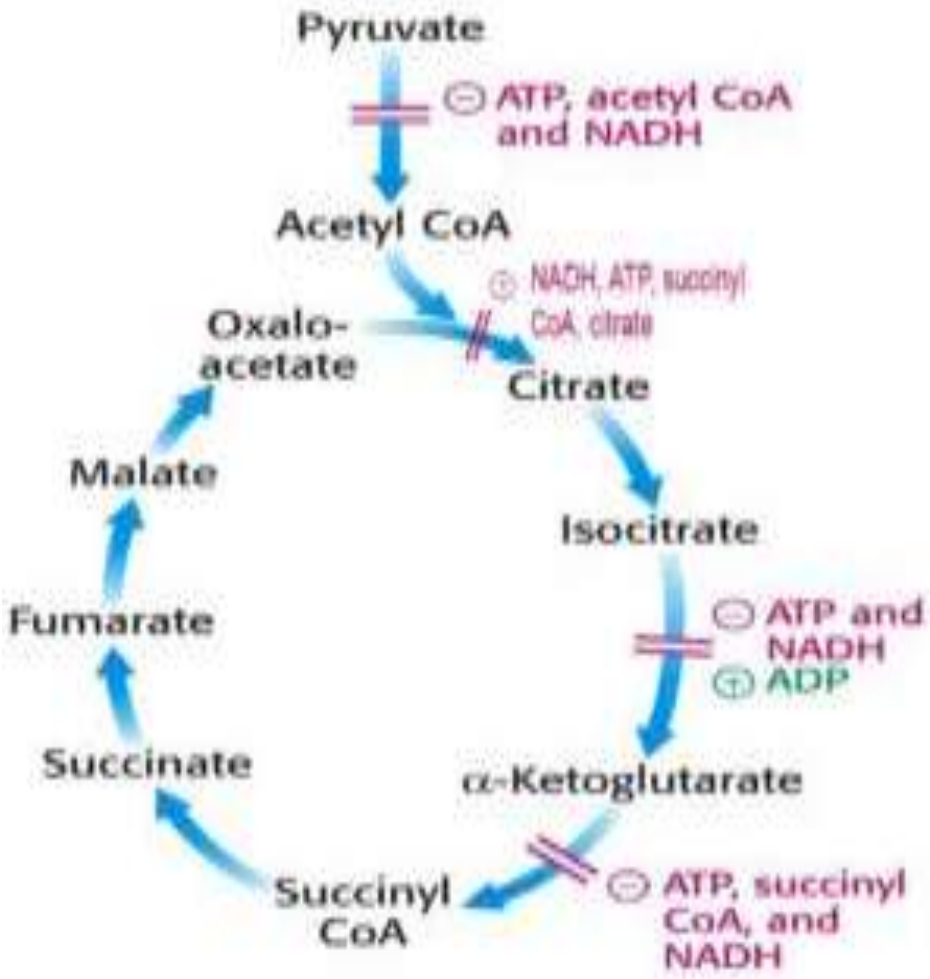
(1)riboflavin, in the form of flavin adenine dinucleotide (FAD), a cofactor for succinate dehydrogenase;

(2) niacin, in he form of nicotinamide adenine dinucleotide (NAD), the electron acceptor for isocitrate dehydrogenase, -ketoglutarate dehydrogenase, and malate dehydrogenase;

(3) thiamin (vitamin B1 ), as thiamin diphosphate, the coenzyme for decarboxylation in the $\alpha$-ketoglutarate dehydrogenase reaction; and

(4) pantothenic acid, as part of coenzyme A, the cofactor attached to "active" carboxylic acid residues such as acetyl-CoA and succinyl-CoA.

# Summary for regulation of CAC




Excess of ATP shows energy rich state of the cell, hence CAC is inhibited while reverse occurs when the cell is in a low energy state with excess of ADP

## Regulation of citric acid cycle

- Citrate synthase is inhibited by ATP, NADH, acyl CoA& succinyl CoA.
- Isocitrate dehydrogenase is activated by ADP & inhibited by ATP and NADH
- $\alpha$-ketoglutarate dehydrogenase is inhibited by succinyl CoA & NADH.
- Availability of ADP is very important for CAC to proceed.

# Function of citric acid cycle

Amphibolic Function:

- The citric acid cycle is amphibolic, since in addition to oxidation it is important in the provision of carbon skeletons for gluconeogenesis, fatty acid synthesis, and interconversion of amino acids.

Citric acid cycle has both anabolic and catabolic functions

A- Catabolic role:

It is the final common pathway for oxidation of carbohydrates, lipids and proteins with energy production

B- Anabolic role:

Source of the intermediates used in biosynthesis like

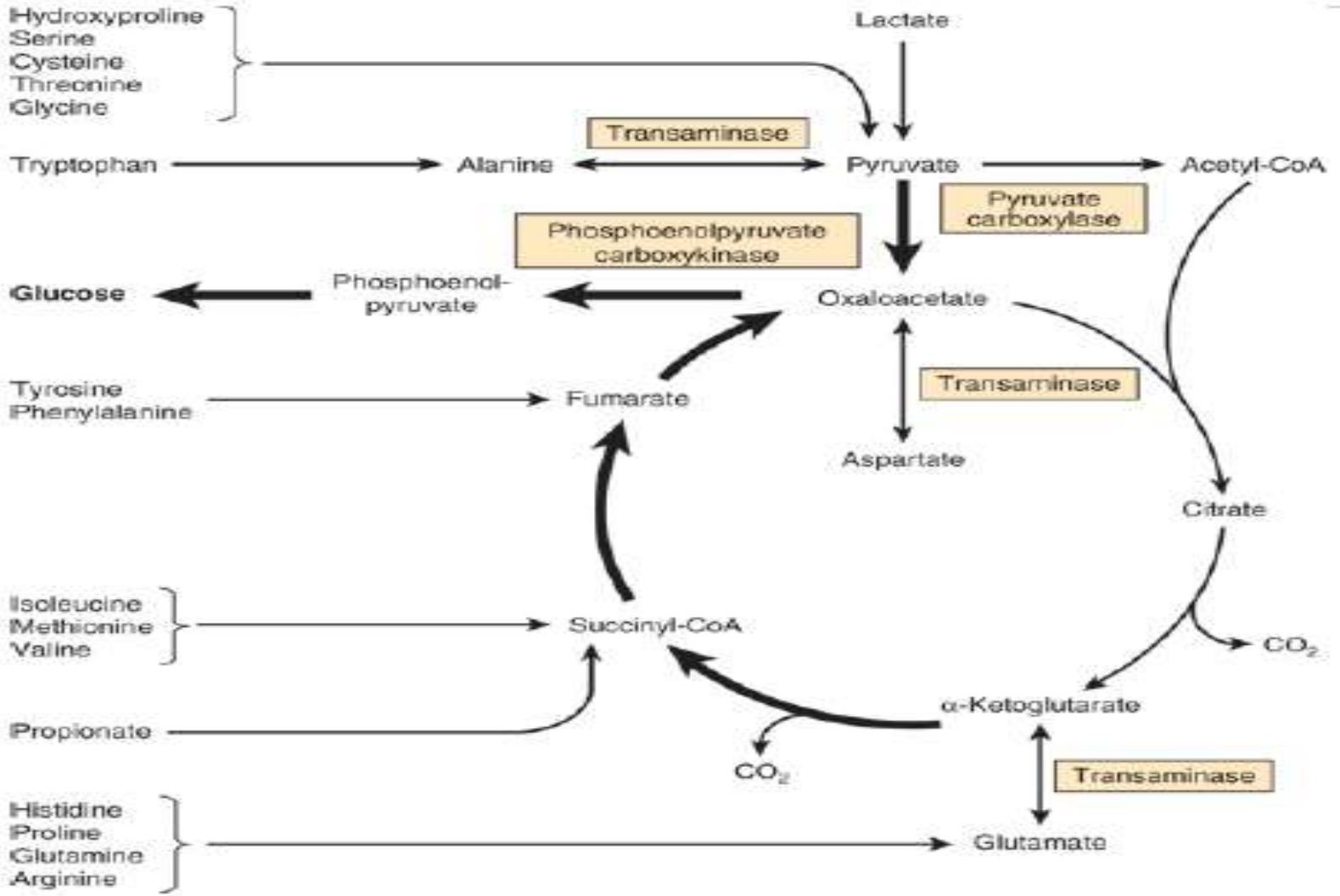
1-Oxaloacetic acid is used in gluconeogenesis.

2-$\alpha$-ketoglutarate is used for synthesis of some non essential amino acids.

3- Succinyl CoA is used in heme synthesis

## Amphibolic Function

- The citric acid cycle is not only a pathway for oxidation of two-carbon units, but is also a major pathway for:
- interconversion of metabolites arising from transamination and deamination of amino acids and providing the substrates for amino acid synthesis by transamination,
- as well as for gluconeogenesis and fatty acid synthesis
- Because it functions in both oxidative and synthetic processes, it is amphibolic.



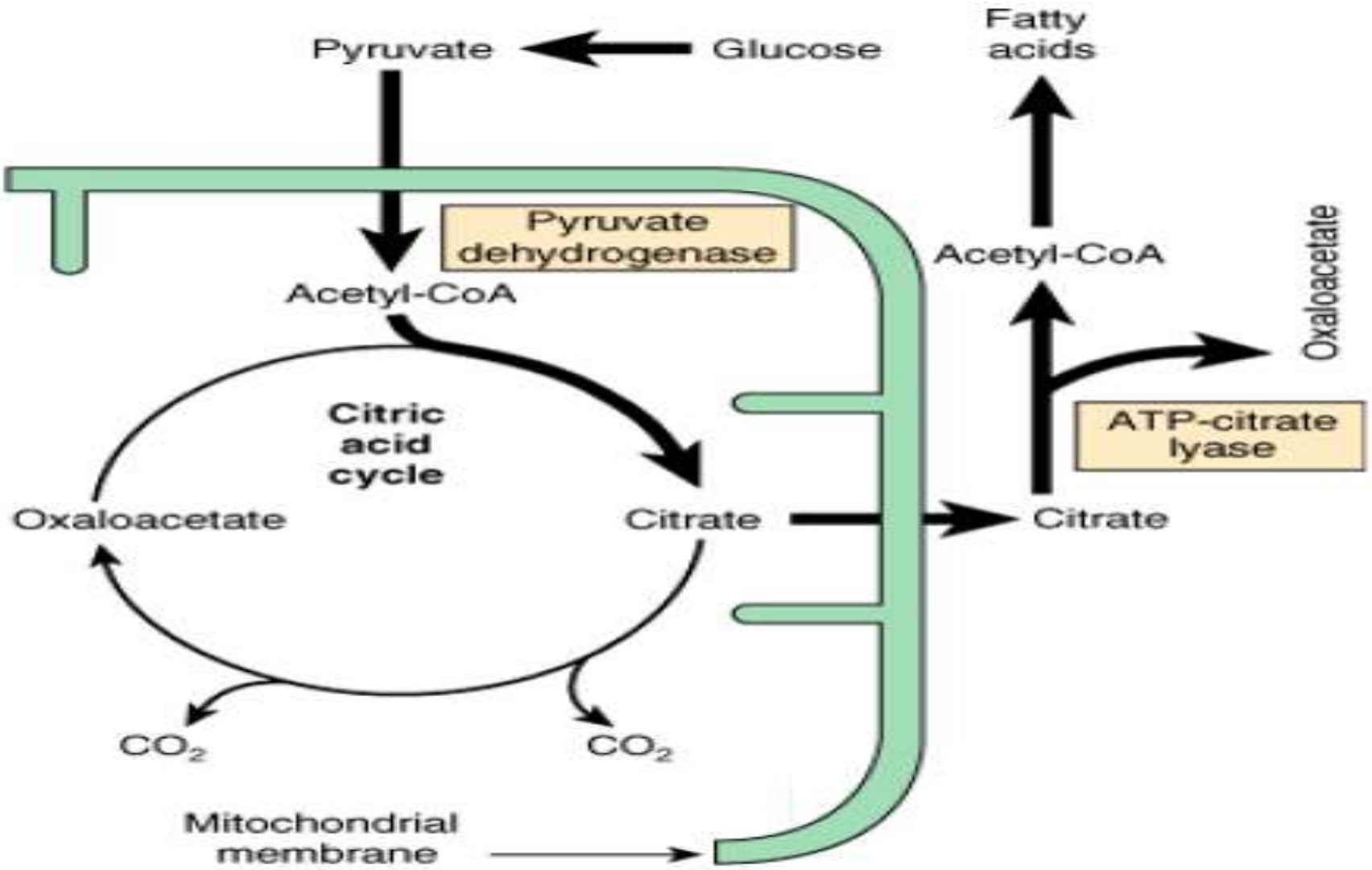

Involvement of the citric acid cycle in transamination and gluconeogenesis. The bold arrows indicate the main pathway of gluconeogenesis

## The Citric Acid Cycle Takes Part in Gluconeogenesis, Transamination, & Deamination

- All the intermediates of the cycle are potentially **glucogenic,** since they can give rise to oxaloacetate, and hence net production of glucose (in the liver and kidney, the organs that carry out gluconeogenesis).
- The key enzyme that catalyzes net transfer out of the cycle into gluconeogenesis is **phosphoenolpyruvate carboxykinase,** which catalyzes the decarboxylation of oxaloacetate to phosphoenolpyruvate with GTP acting as the phosphate donor.
- **Aminotransferase** (transaminase) reactions form pyruvate from alanine, oxaloacetate from aspartate, and $\alpha$-ketoglutarate from glutamate.
- Because these reactions are reversible, the cycle also serves as a source of carbon skeletons for the synthesis of these amino acids.
- Other amino acids contribute to gluconeogenesis because their carbon skeletons give rise to citric acid cycle intermediates.
- Alanine, cysteine, glycine, hydroxyproline, serine,, histidine, glutamine, and proline yield $\alpha$-ketoglutarate;
- isoleucine, methionine, and valine yield succinyl-CoA; tyrosine and phenylalanine yield fumarate

## The Citric Acid Cycle Takes Part in Fatty Acid Synthesis

- Acetyl-CoA, formed from pyruvate by the action of pyruvate dehydrogenase, is the major substrate for long-chain fatty acid synthesis.
- Pyruvate dehydrogenase is a mitochondrial enzyme, and fatty acid synthesis is a cytosolic pathway; the mitochondrial membrane is impermeable to acetyl-CoA. Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol, and cleaved in a reaction catalyzed by **ATP-citrate lyase.**
- Citrate is only available for transport out of the mitochondrion when aconitase is saturated with its substrate, and citrate cannot be channeled directly from citrate synthase onto aconitase.
- This ensures that citrate is used for fatty acid synthesis only when there is an adequate amount to ensure continued activity of the cycle.




- Participation of the citric acid cycle in fatty acid synthesis from glucose

## Regulation of the Citric Acid Cycle Depends Primarily on a Supply of Oxidized Cofactors

- **Respiratory control** via the respiratory chain and oxidative phosphorylation regulates citric acid cycle activity
- Thus, activity is immediately dependent on the supply of NAD+ , which in turn, because of the tight coupling between oxidation and phosphorylation, is dependent on the availability of ADP and hence, ultimately on the rate of utilization of ATP in chemical and physical work.
- In addition, individual enzymes of the cycle are regulated.
- The most likely sites for regulation are the nonequilibrium reactions catalyzed by pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and $\alpha$-ketoglutarate dehydrogenase.
- The dehydrogenases are activated by Ca2+ , which increases in concentration during muscular contraction and secretion, when there is increased energy demand.
- In a tissue such as brain, which is largely dependent on carbohydrate to supply acetyl-CoA, control of the citric acid cycle may occur at pyruvate dehydrogenase.

- Several enzymes are responsive to the energy status as shown by the [ATP]/[ADP] and [NADH]/[NAD+ ] ratios.
- Thus, there is allosteric inhibition of citrate synthase by ATP and long-chain fatty acyl-CoA.
- Allosteric activation of mitochondrial NAD-dependent isocitrate dehydrogenase by ADP is counteracted by ATP and NADH.
- The $\alpha$ ketoglutarate dehydrogenase complex is regulated in the same way as is pyruvate dehydrogenase.
- Succinate dehydrogenase is inhibited by oxaloacetate, and the availability of oxaloacetate, as controlled by malate dehydrogenase, depends on the [NADH]/[NAD+ ] ratio.
- The concentration of oxaloacetate controls the rate of citrate formation.

# Glycolysis & the Oxidation of Pyruvate

By: Inaam A. Ameen

# Glycolysis

- It is a universal catabolic pathway in the living cells.
- Glycolysis can be defined as the sequence of reactions for the breakdown of Glucose (6-carbon molecule) to two molecules of pyruvic acid (3-carbon molecule) under aerobic conditions; or lactate under anaerobic conditions along with the production of small amount of energy.
- This pathway was described by Embden, Meyerhof and Parnas. Hence, it is also called as **Embden-Meyerhof pathway** (EM pathway)
- Pyruvate is oxidized to acetyl-CoA by a multienzyme complex, pyruvate dehydrogenase, which is dependent on the vitamin-derived cofactor thiamin diphosphate.
- In erythrocytes, the first site in glycolysis for generation of ATP may be bypassed, leading to the formation of 2,3-bisphosphoglycerate, which is important in decreasing the affinity of hemoglobin for O2 .

# BIOMEDICAL IMPORTANCE

- Most tissues have at least some requirement for glucose.
- In the brain, the requirement is significant.
- Glycolysis, the major pathway for glucose metabolism, occurs in the cytosol of all cells.
- It can function either aerobically or anaerobically, depending on the availability of oxygen and the electron transport chain.
- Erythrocytes, which lack mitochondria, are completely dependent on glucose as their metabolic fuel, and metabolize it by anaerobic glycolysis.
- However, to oxidize glucose forming pyruvate (the end product of glycolysis) requires both oxygen and mitochondrial enzyme systems: the pyruvate dehydrogenase complex, the citric acid cycle, and the respiratory chain.
- Glycolysis is both the principal route for glucose metabolism and also the main pathway for the metabolism of fructose, galactose, and other carbohydrates derived from the diet.

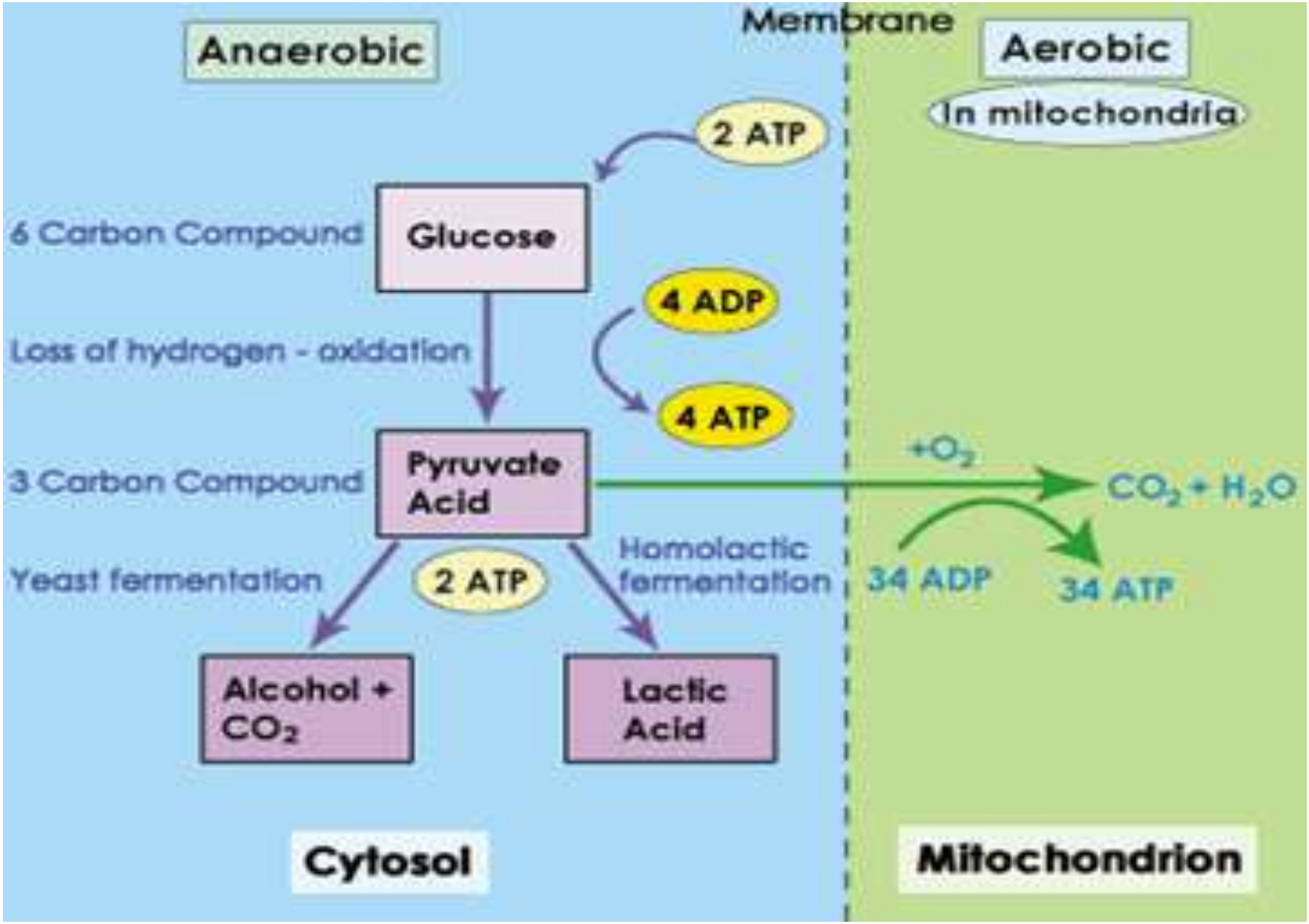
- Heart muscle, which is adapted for aerobic performance, has relatively low glycolytic activity and poor survival under conditions of **ischemia.**
- Diseases in which enzymes of glycolysis (eg, pyruvate kinase) are deficient are mainly seen as **hemolytic anemias** or, if the defect affects skeletal muscle (eg, phosphofructokinase), as **fatigue.**
- In fast growing cancer cells, glycolysis proceeds at a high rate, forming large amounts of pyruvate, which is reduced to lactate and exported.
- This produces a relatively acidic local environment in the tumor, which may have effects for cancer therapy.

# Aerobic & Anaerobic Glycolysis

- Aerobic glycolysis is the glycolytic pathway which occurs in the cytosol in the presence of oxygen. When compared to anaerobic glycolysis, this pathway is much more efficient and produces more ATP per glucose molecule. In aerobic glycolysis, the end product, pyruvate is transferred to mitochondria for the initiation of Citric acid cycle. Therefore, the ultimate products of aerobic glycolysis are 34 ATP molecules, water, and carbon dioxide.
- Anaerobic glycolysis takes place in the cytoplasm when a cell lacks oxygenated environment or lacks mitochondria. In this case, NADH is oxidized to NAD+ in the cytosol by converting pyruvate into lactate. Anaerobic glycolysis produces (2 lactate + 2 ATP + 2 $H_2O$ + 2 $H^+$) from one glucose molecule. Unlike the aerobic glycolysis, anaerobic glycolysis produces lactate, which reduces the pH and inactivates the enzymes.

# Difference between Aerobic and Anaerobic Glycolysis

- Aerobic glycolysis occurs in oxygen rich environments, whereas anaerobic glycolysis occurs in oxygen lack environments.
- Aerobic glycolysis is more efficient than anaerobic glycolysis; hence it produces a large amount of ATP than anaerobic glycolysis.
- Aerobic glycolysis occurs only in eukaryotes while anaerobic glycolysis occurs in both prokaryotes and eukaryotes.
- Unlike in anaerobic glycolysis, the end product of Aerobic glycolysis (pyruvate) is used to initiate other pathways in mitochondria.
- Anaerobic glycolysis produces 2ATPs per glucose molecule while aerobic glycolysis produces 36 to 38 ATPs per glucose molecule.
- Ultimate end product of anaerobic glycolysis is lactate, which may be harmful to the cell itself, whereas that of aerobic glycolysis is water and carbon dioxide, which are not harmful to cells.
- Unlike in anaerobic glycolysis, NADH + H+ undergo oxidative phosphorylation in the presence of oxygen in aerobic glycolysis.
- Pyruvate is reduced to lactate during anaerobic glycolysis whereas, during aerobic glycolysis, pyruvate is oxidation to acetyl coenzyme A (acetyl- CoA).


Membrane
Anaerobic
Aerobic
In mitochondria
2 ATP
6 Carbon Compound
Glucose
4 ADP
Loss of hydrogen - oxidation
4 ATP
3 Carbon Compound
Pyruvate Acid
+O2
CO2 + H2O
Yeast fermentation
2 ATP
Homolactic fermentation
34 ADP
34 ATP
Alcohol + CO2
Lactic Acid
Cytosol
Mitochondrion

- Fates of pyruvate under anaerobic conditions:
- Pyruvate is the terminal electron acceptor in lactic acid fermentation
  When sufficient oxygen is not present in the muscle cells for further oxidation of pyruvate and NADH produced in glycolysis, NAD+ is regenerated from NADH by reduction of pyruvate to lactate. **Pyruvate** is converted to **lactate** by the enzyme lactate dehydrogenase
- Ethanol fermentation
  Yeast and other anaerobic microorganisms convert glucose to ethanol and CO2 rather than pyruvate. **Pyruvate** is first converted to **acetaldehyde** by enzyme **pyruvate decarboxylase** in the presence of Thiamine pyrophosphate and Mg++. Carbon-dioxide is released during this reaction. **Acetaldehyde** is then converted to **ethanol** by the enzyme **alcohol dehydrogenase**. NADH is oxidized to NAD+ during this reaction.

(a)

Glucose

first priming reaction ① ATP → ADP

Glucose 6-phosphate

②

Fructose 6-phosphate

second priming reaction ③ ATP → ADP

Fructose 1,6-bisphosphate

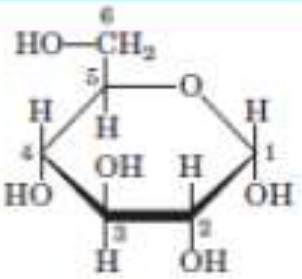
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates ④

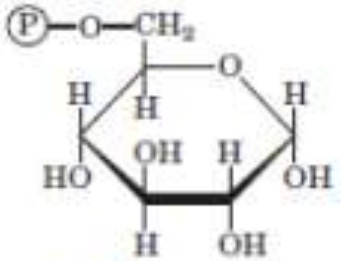
Glyceraldehyde 3-phosphate

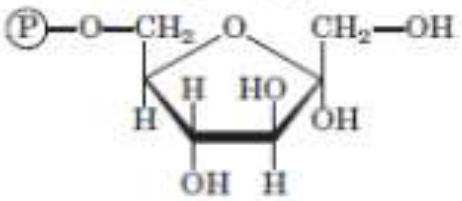
+

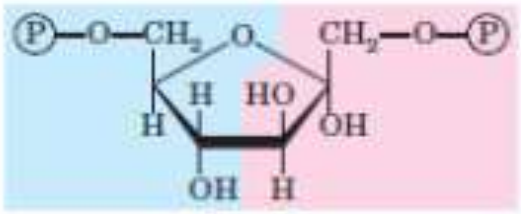
Dihydroxyacetone phosphate

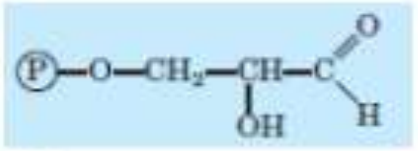
⑤











**Preparatory phase**

Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate

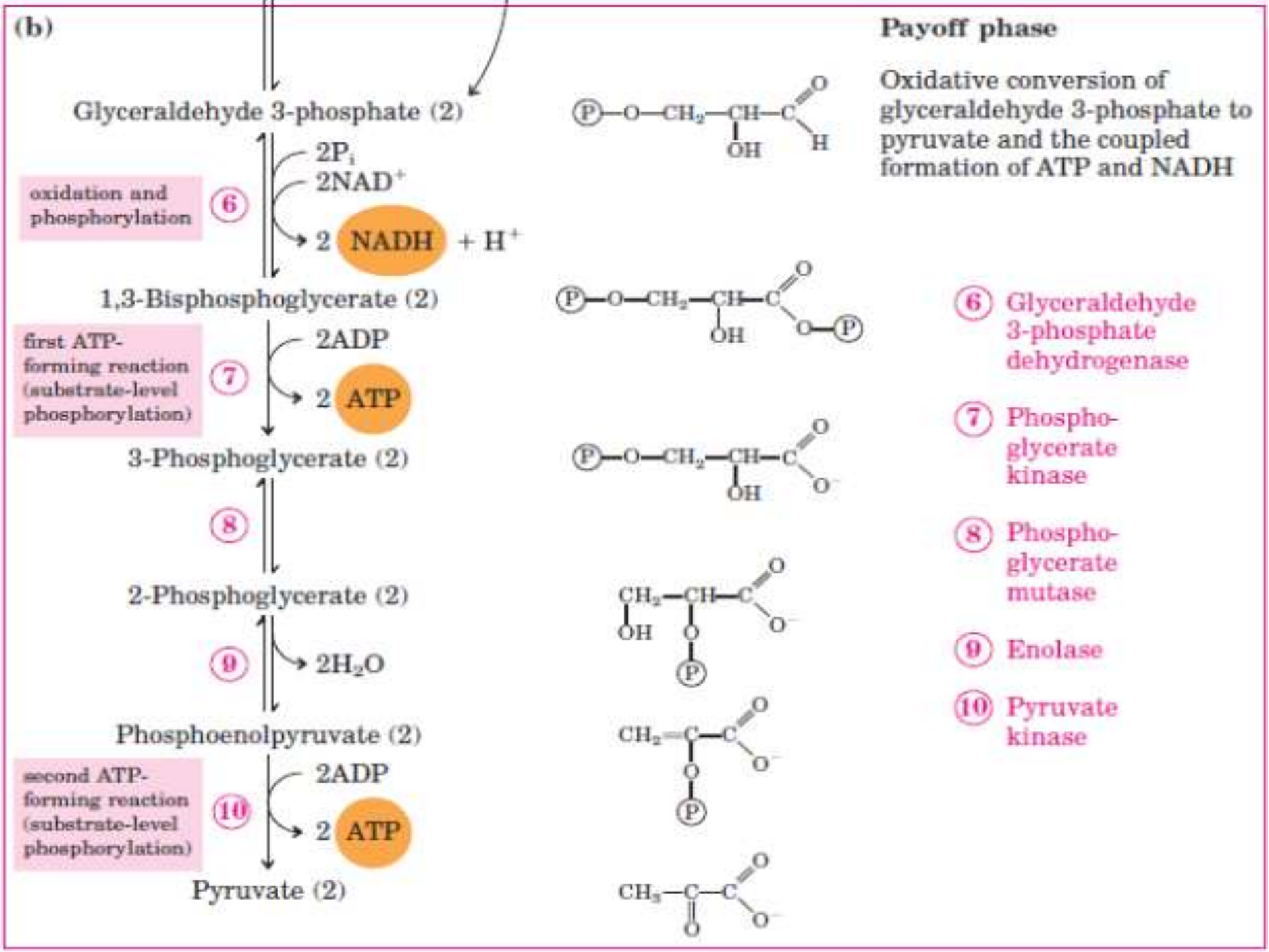
① Hexokinase

② Phosphohexose isomerase

③ Phospho-fructokinase-1

④ Aldolase

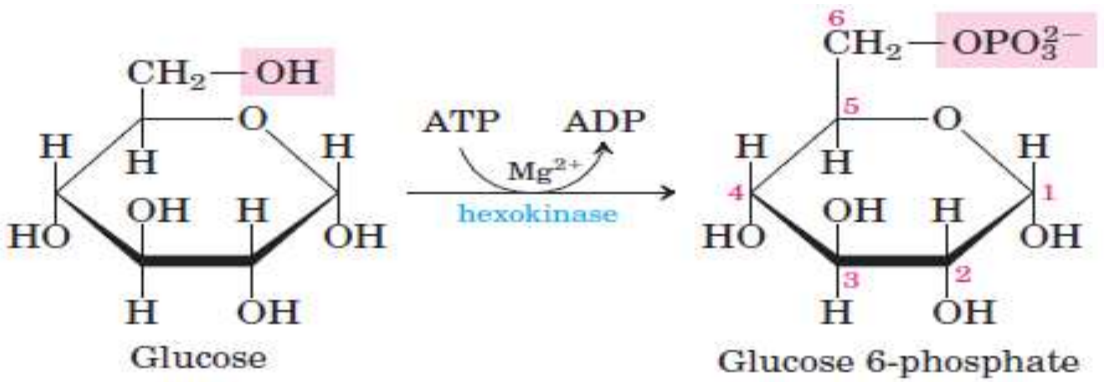
⑤ Triose phosphate isomerase


(b)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
Glyceraldehyde 3-phosphate (2)
2P$_i$
2NAD$^+$
oxidation and phosphorylation
6
2 NADH + H$^+$
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H$_2$O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase

# Steps of Glycolysis

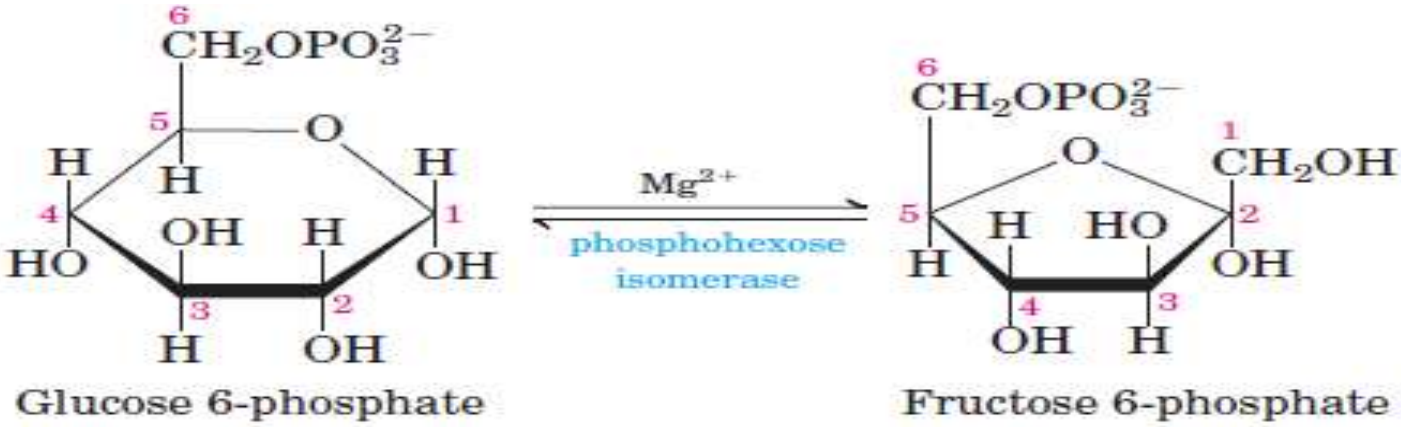
- Glycolysis is an extramitochondrial pathway and is carried by a group of eleven enzymes. Glucose is converted to pyruvate in 10 steps by glycolysis. The glycolytic patway can be divided into two phases:
- **Preparatory Phase :**
- This phase is also called **glucose activation phase**. In the preparatory phase of glycolysis, two molecules of ATP are spent and the hexose chain is cleaved into two triose phosphates. During this, phosphorylation of glucose and it's conversion to glyceraldehyde-3-phosphate take place. The steps 1, 2, 3, 4 and 5 together are called as the preparatory phase.
- **Payoff Phase :**
- This phase is also called **energy extraction phase**. During this phase, conversion of glyceraldehyde-3-phophate to pyruvate and the coupled formation of ATP take place.
  Because Glucose is split to yield two molecules of D-Glyceraldehyde-3-phosphate, each step in the payoff phase occurs twice per molecule of glucose. The steps after 5 constitute payoff phase.

- **Step 1 : Uptake and Phosphorylation of Glucose**



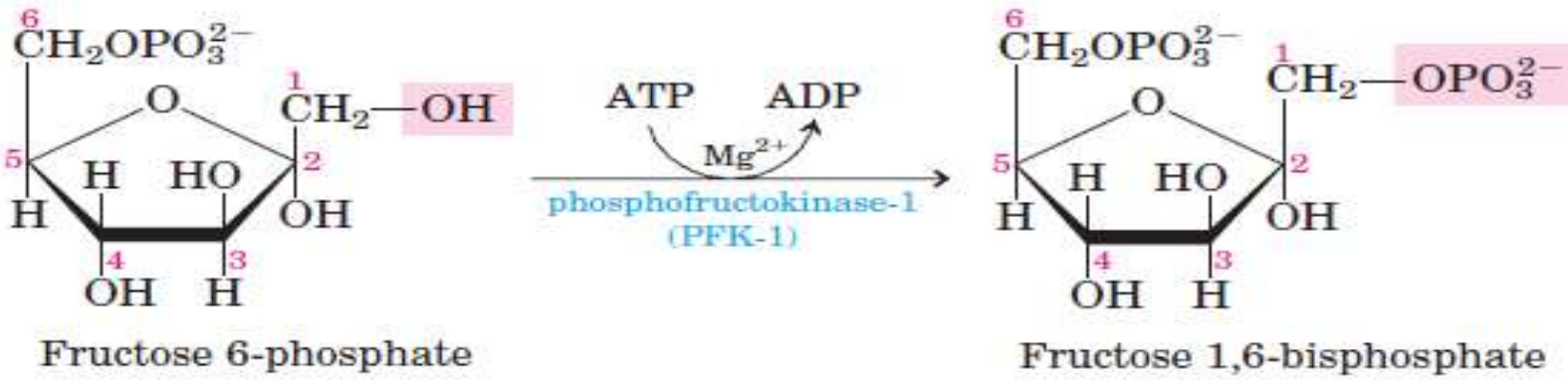

- Glucose is phosphorylated to form glucose-6-phosphate.
- The reaction is catalysed by the specific enzyme **glucokinase** in liver cells and by non specific enzyme **hexokinase** in liver and extrahepatic tissue. The enzyme splits the ATP into ADP, and the Pi is added onto the glucose.
- Hexokinase is a **key glycolytic enzyme**. Hexokinase catalyses a regulatory step in glycolysis that is irreversible.
- Hexokinase, like many other kinases, requires Mg2+ for its activity.

- **Step 2 : Isomerization of Glucose-6-Phsphate to Fructose-6-Phosphate**



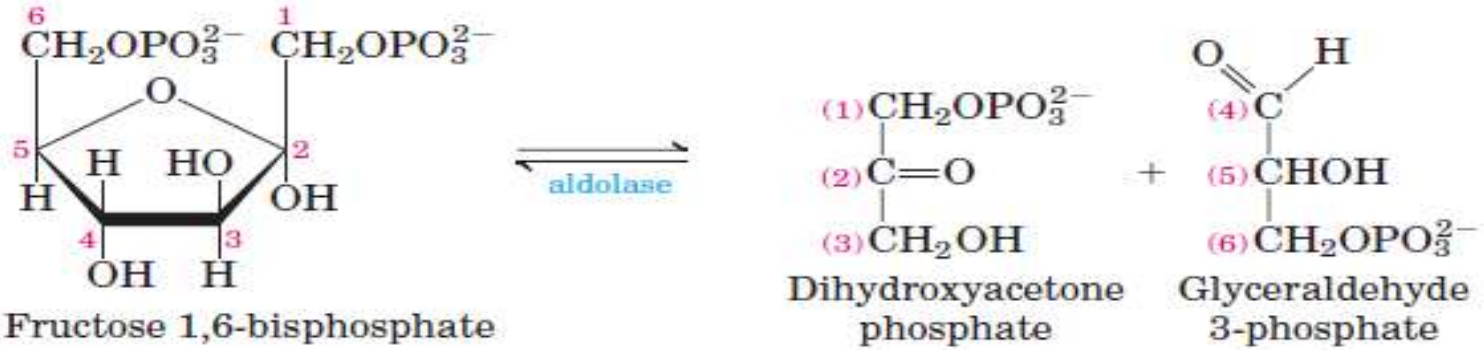

- Glucose-6-phosphate is isomerised to fructose-6-phosphate by **phosphohexose isomerase**.
- This reaction involves an aldose-ketose isomerisastion catalysed by phosphohexose isomerase. The reaction involves the rearrangement of the carbon-oxygen bond to transform the six-membered ring into a five-membered ring. To rearrangement takes place when the six-membered ring opens and then closes in such a way that the first carbon becomes now external to the ring.

- **Step 3 : Phosphorylation of F-6-P to Fructose 1,6-Biphosphate**



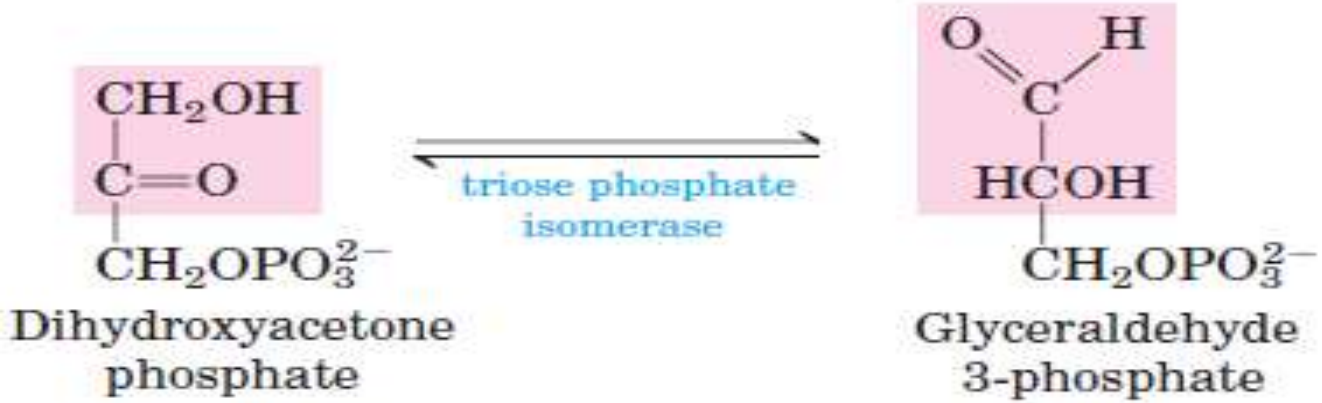

- Fructose-6-phosphate is further phosphorylated to fructose 1,6-bisphosphate.
- The enzyme is **phosphofructokinase-1**. It catalyses the transfer of a phosphate group from ATP to fructose-6-phosphate.
- The reaction is irreversible.
- One ATP is utilized for phosphorylation.
- Phosphofructokinase-1 is the **key enzyme** in glycolysis which regulates breakdown of glucose.

- **Step 4 : Cleavage of Fructose 1,6-Biphosphate**



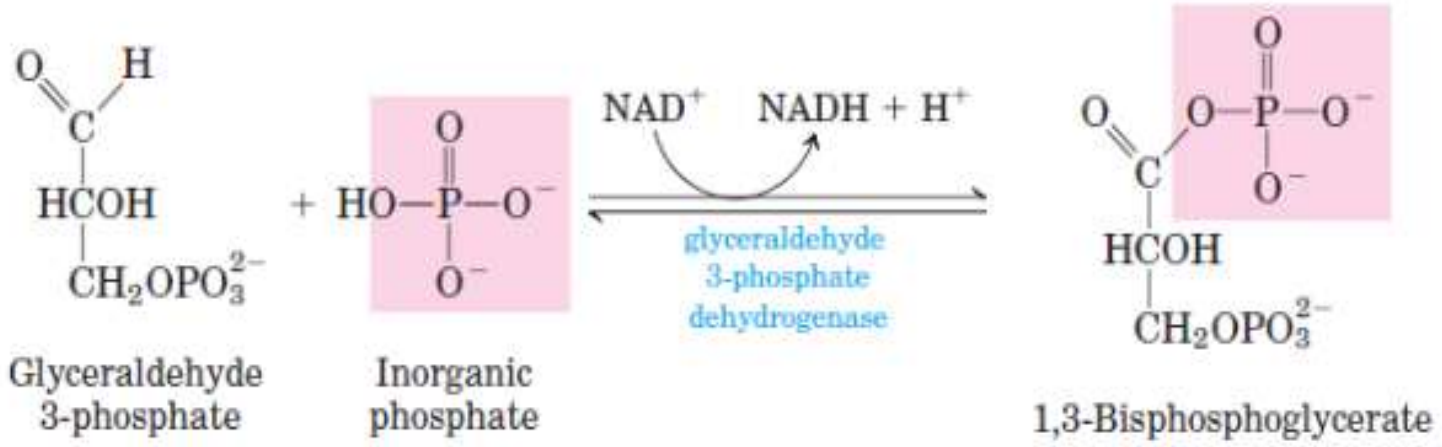

- The 6 carbon fructose-1,6-bisphosphate is cleaved into two 3 carbon units; one glyceraldehyde-3-phosphate (GAP) and another molecule of dihydroxy acetone phosphate (DHAP).
- The enzyme which catalyses the reaction is **aldolase**. Since the backward reaction is an aldol condensation, the enzyme is called aldolase.
- The reaction is reversible.

- **Step 5 : Interchange of the Triose Phosphates**



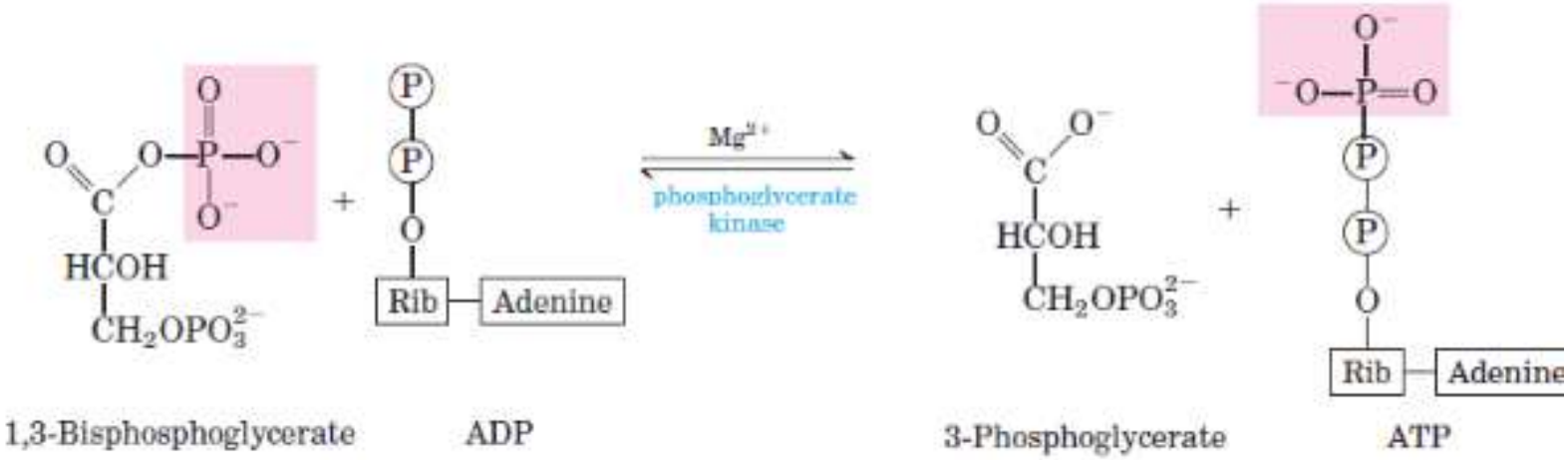

- GAP is on the direct pathway of glycolysis, whereas DHAP is not. Hence **Triose-phosphate isomerase** converts DHAP into GAP useful for generating ATP.
- Thus net result is that glucose is now cleaved into 2 molecules of glyceraldehyde-3-phosphate.
- This reaction is rapid and reversible.

- **Step 6 : Oxidative phosphorylation of GAP to 1,3-Bisphosphoglycerate**




- The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate.
- This reaction is catalyzed by **glyceraldehyde 3-phosphate dehydrogenase**.
- It is the energy-yielding reaction. Reactions of this type in which an aldehyde group is oxidized to an acid are accompanied by liberation of large amounts of potentially useful energy.
- two main events take place: 1) glyceraldehyde-3-phosphate is oxidized by the coenzyme nicotinamide adenine dinucleotide (NAD); 2) the molecule is phosphorylated by the addition of a free phosphate group. During this reaction, NAD+ is reduced to NADH.
- This is a reversible reaction.

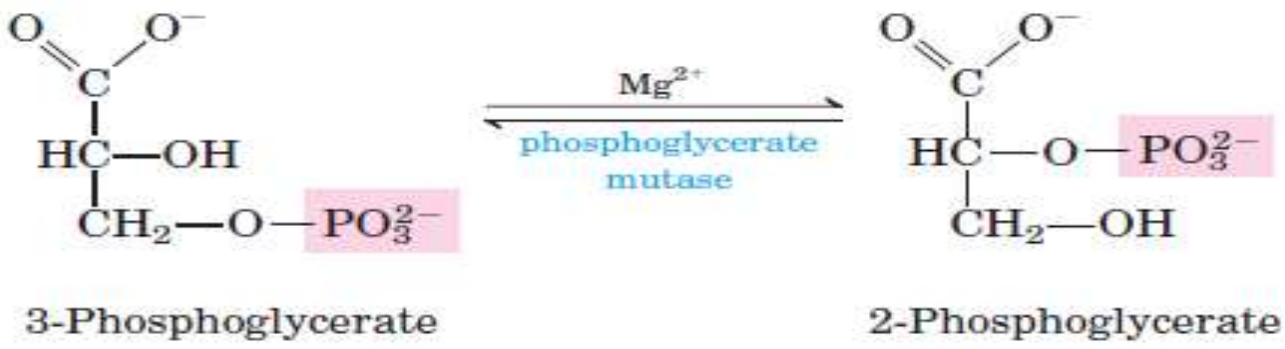
- **Step 7 : Conversion of 1,3-Biphosphoglycerate to 3-Phosphoglycerate**




1,3-Bisphosphoglycerate ADP 3-Phosphoglycerate ATP

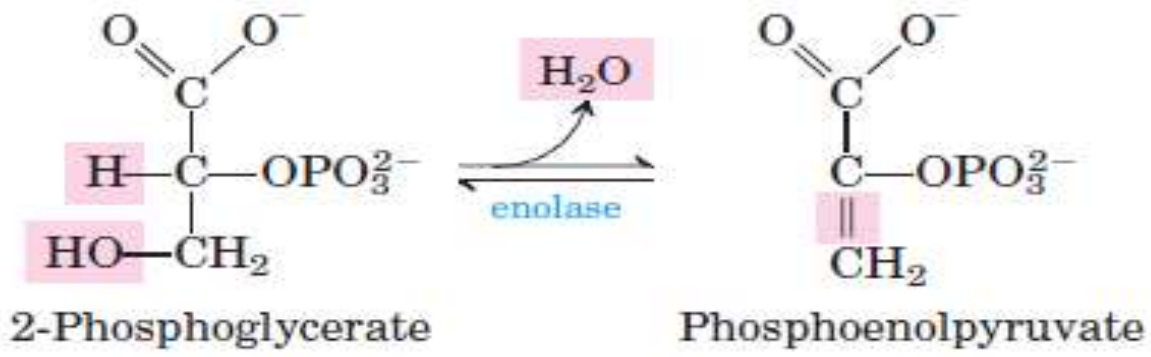
- The enzyme **phosphoglycerate kinase** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate.
- This is a unique example where ATP can be produced at substrate level without participating in electron transport chain. This type of reaction where ATP is formed at substrate level is called as Substrate level phosphorylation.
- This reaction involves the loss of a phosphate group from the starting material. The phosphate is transferred to a molecule of ADP that yields the first molecule of ATP.

- **Step 8 : Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate**



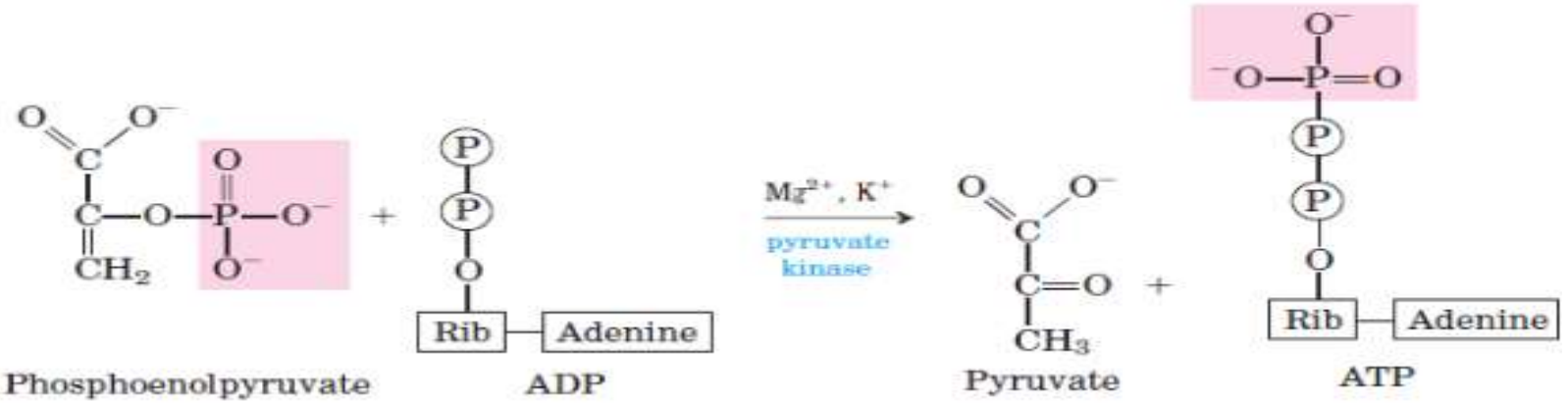

- 3-phospho glycerate is isomerized to 2-phospho glycerate by shifting the phosphate group from 3rd to 2nd carbon atom.
- The enzyme is **phosphoglycerate mutase**.
- This is a readily reversible reaction.
- Mg2+ is essential for this reaction.

- **Step 9 : Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate**



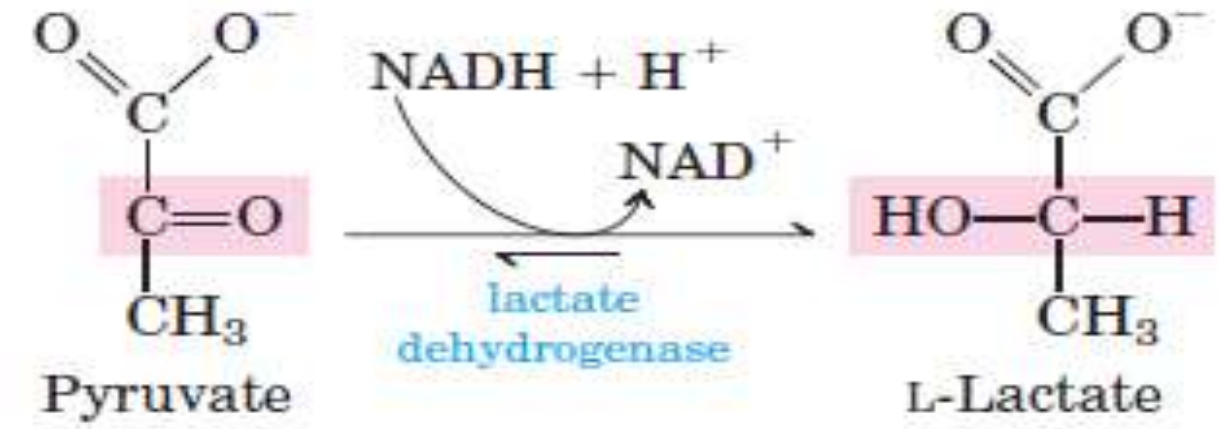

- 2-phosphoglycerate is converted to phosphoenol pyruvate by the enzyme **enolase**.
- One water molecule is removed.
- A high energy phosphate bond is produced. The reaction is reversible.
- Enolase requires Mg++.

- **Step 10 : Conversion of Phosphoenol Pyruvate to Pyruvate**




- Phosphoenol pyruvate (PEP) is dephosphorylated to pyruvate, by **pyruvate kinase**.
- First PEP is made into a transient intermediary of enol pyruvate; which is isomerized into keto pyruvate, the stable form of pyruvate.
- One mole of ATP is generated during this reaction. This is again an example of substrate level phosphorylation.
- The pyruvate kinase is a **key glycolytic enzyme**. This step is irreversible.

- **Additional Step in Anaerobic Condition**
- When the tissues cannot be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAD+ is regenerated from NADH by the reduction of pyruvate to lactate. Some tissues and cell types (such as erythrocytes, which have no mitochondria and thus cannot oxidize pyruvate to CO2) produce lactate from glucose even under aerobic conditions. The reduction of pyruvate is catalyzed by **lactate dehydrogenase**.



# Net energy (ATP) yield per molecule of Glucose in Glycolysis

## Energy Yield in Aerobic Glycolysis

| Step | Enzyme | Source | No. of ATP |
|---|---|---|---|
| 1 | Hexokinase | – | -1 |
| 3 | Phosphofructokinase | – | -1 |
| 6 | Glyceraldehyde-3- phosphate dehydrogenase | NADH | (+3) x 2 = +6 |
| 7 | Phosphoglycerate kinase | ATP | (+1) x 2 = +2 |
| 10 | Pyruvate kinase | ATP | (+1) x 2 = +2 |
| **Net Yield** | | | **8 ATPs** |

## Energy Yield in Anaerobic Glycolysis

| Step | Enzyme | Source | No. of ATP Formed/consumed |
|---|---|---|---|
| 1 | Hexokinase | – | -1 |
| 3 | Phosphofructokinase | – | -1 |
| 7 | Phosphoglycerate kinase | ATP | (+1) x 2 = +2 |
| 10 | Pyruvate kinase | ATP | (+1) x 2 = +2 |
| **Net Yield** | | | **2 ATPs** |

# GLYCOLYSIS IS REGULATED AT THREE STEPS INVOLVING NONEQUILIBRIUM REACTIONS

- The rate of conversion of glucose into pyruvate is regulated to meet two major cellular needs: (1) the production of ATP, generated by the degradation of glucose, and (2) the provision of building blocks for synthetic reactions, such as the formation of fatty acids.
- In metabolic pathways, enzymes catalyzing essentially irreversible reactions are potential sites of control.
- In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are virtually irreversible; hence, these enzymes would be expected to have regulatory as well as catalytic roles. In fact, each of them serves as a control site. Their activities are regulated by the reversible binding of allosteric effectors or by covalent modification.
- Phosphofructokinase is significantly inhibited at normal intracellular concentrations of ATP; this inhibition can be rapidly relieved by 5'AMP that is formed as ADP begins to accumulate, signaling the need for an increased rate of glycolysis.

- **Fructose** enters glycolysis by phosphorylation to fructose 1-phosphate, and bypasses the main regulatory steps, so resulting in formation of more pyruvate (and acetyl-CoA) than is required for ATP formation.
- In the liver and adipose tissue, this leads to increased lipogenesis, and a high intake of fructose may be a factor in the development of obesity

# Inhibition of Pyruvate Metabolism Leads to Lactic Acidosis

- Arsenite and mercuric ions react with the —SH groups of lipoic acid and inhibit pyruvate dehydrogenase, as does a **dietary deficiency of thiamin,** allowing pyruvate to accumulate.
- Many alcoholics are thiamin-deficient (both because of a poor diet and also because alcohol inhibits thiamin absorption), and may develop potentially fatal pyruvic and lactic acidosis.
- Patients with **inherited pyruvate dehydrogenase deficiency,** which can be the result of defects in one or more of the components of the enzyme complex, also present with lactic acidosis, particularly after a glucose load.
- Because of the dependence of the brain on glucose as a fuel, these metabolic defects commonly cause neurologic disturbances.

# Significance of the Glycolysis Pathway

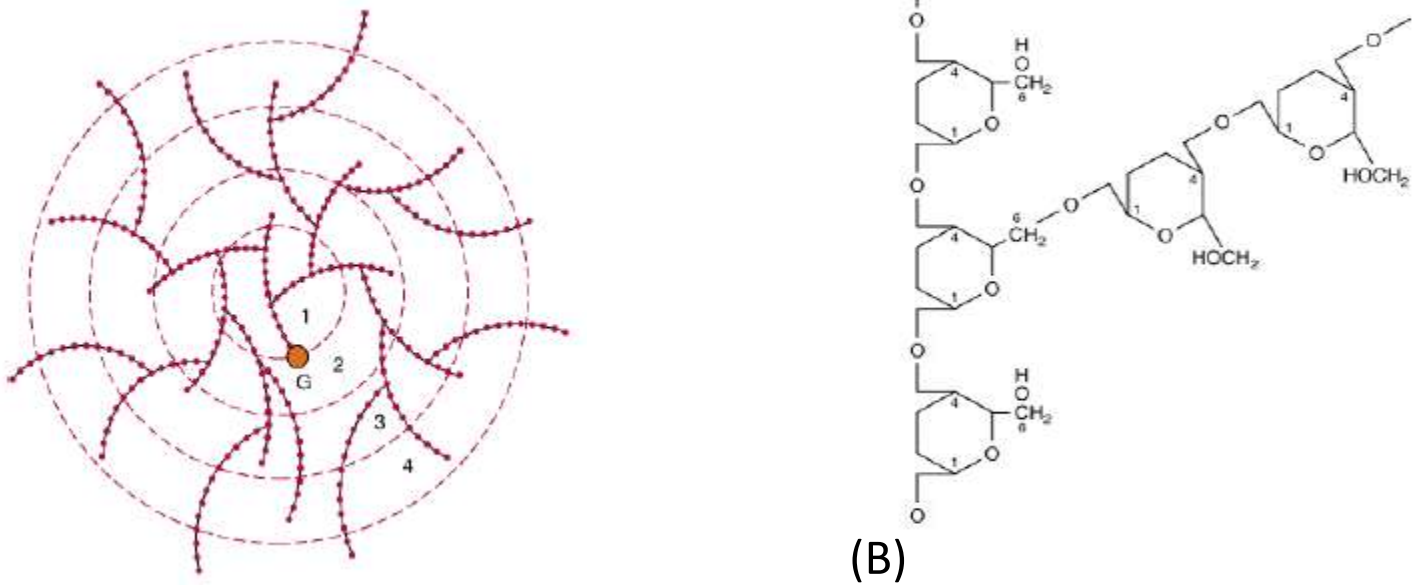
1. Glycolysis is the only pathway that is taking place in all the cells of the body.
2. Glycolysis is the only source of energy in erythrocytes.
3. In strenuous exercise, when muscle tissue lacks enough oxygen, anaerobic glycolysis forms the major source of energy for muscles.
4. The glycolytic pathway may be considered as the preliminary step before complete oxidation.
5. The glycolytic pathway provides carbon skeletons for synthesis of non-essential amino acids as well as glycerol part of fat.
6. Most of the reactions of the glycolytic pathway are reversible, which are also used for gluconeogenesis.

# Metabolism of glycogen

By: Inaam A. Ameen

# BIOMEDICAL IMPORTANCE 329

- Glycogen is the major storage carbohydrate in animals, corresponding to starch in plants; it is a branched polymer of $\alpha$-D -glucose.
- It occurs mainly in liver and muscle. Although the liver content of glycogen is greater than that of muscle, because the muscle mass of the body is considerably greater than that of the liver, about three-quarters of total body glycogen is in muscle.

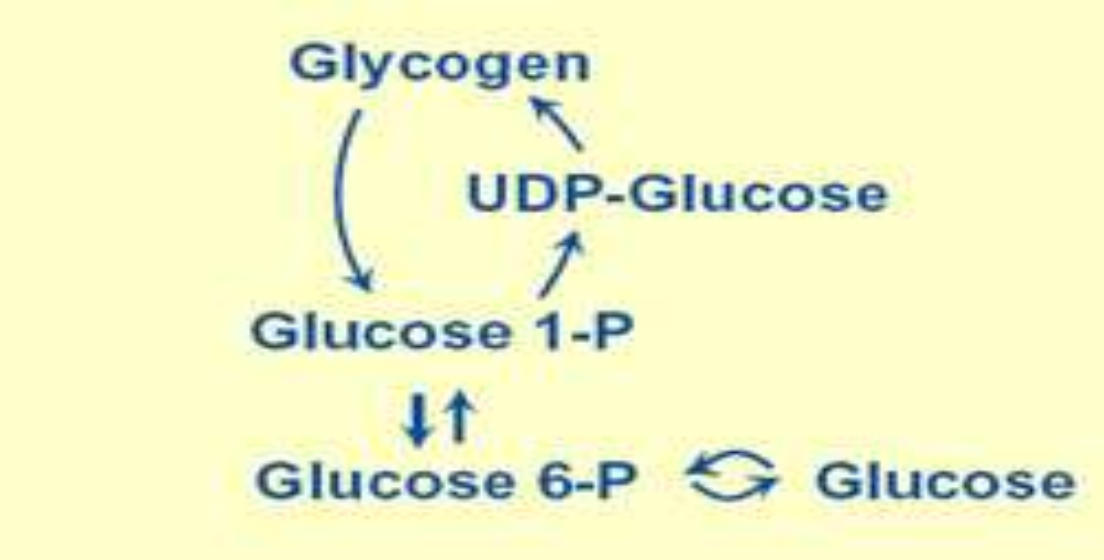



(A) (B)

- The glycogen molecule. **(A)** General structure. **(B)** Enlargement of structure at a branch point consists of polysaccharide chains, each containing about 13 glucose residues. The chains are either branched or unbranched and are arranged in 12 concentric layers (only four are shown in the figure). The branched chains (each has two branches) are found in the inner layers and the unbranched chains in the outer layer. (G, glycogenin, the primer molecule for glycogen synthesis.)

- Muscle glycogen provides a readily available source of glucose for glycolysis within the muscle itself.
- Liver glycogen functions to store and export glucose to maintain blood glucose between meals.
- Although muscle glycogen does not directly yield free glucose (because muscle lacks glucose 6-phosphatase), pyruvate formed by glycolysis in muscle can undergo transamination to alanine, which is exported from muscle and used for gluconeogenesis in the liver.
- The highly branched structure of glycogen provides a large number of sites for glycogenolysis, permitting rapid release of glucose 1-phosphate for muscle activity.
- **Glycogen storage diseases** are a group of inherited disorders characterized by deficient mobilization of glycogen or deposition of abnormal forms of glycogen, leading to muscle weakness; some glycogen storage diseases result in early death.

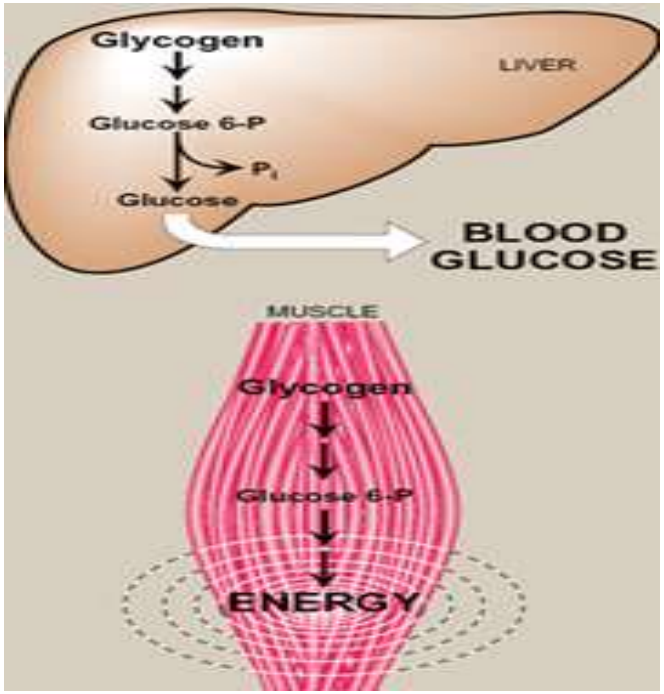
- Blood glucose can be obtained from 3 primary sources: diet, degradation of glycogen, & gluconeogenesis
- In absence of a dietary source of gluc, this cpd is rapidly released from liver & kidney glycogen. Similarly, muscle glycogen is extensively degraded in exercising muscle to provide that tissue with an important energy source
- When glycogen stores are depleted, specific tissues synthesize gluc de novo, using aa's from body's proteins as primary source of carbons for gluconeogenic pathway.




- Glycogen synthesis and degradation shown as a part of the essential reactions of energy metabolism

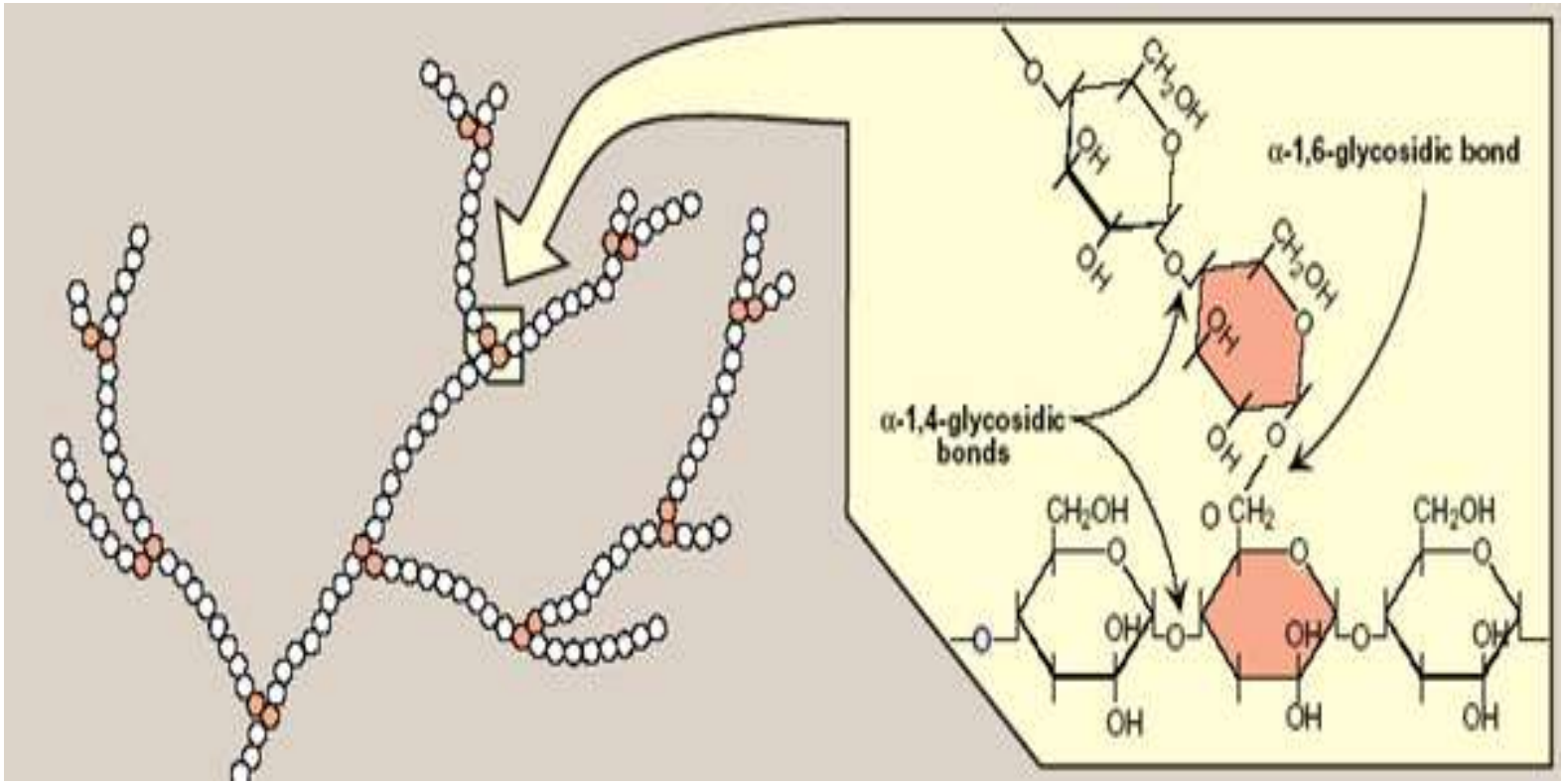
# function and Structure of glycogen

- The main stores of glycogen in the body are found in skeletal muscle & liver, although most other cells store small amounts of glycogen for their own use.
- Function of muscle glycogen is to serve as a fuel reserve for synthesis of ATP during muscle contraction.
- That of liver glycogen is  to maintain blood glucose conc., particularly during early stages of fast.



Structure of glycogen

- Glycogen is a branched-chain homo-polysaccharide made entirely from α-glucose
- The primary glycosidic bond is an α(1→4) linkage.
- After an av. of 8-10 glucosyl residues, there is a branch containing an α(1→6) linkage.
- A single molecule of glycogen can have a molecular mass of up to $10^8$ daltons.
- These molecules exist in separated cytoplasmic granules that contain most of the enz's necessary for glycogen synthesis & degradation




- Branched structure of glycogen, showing a-1,4 and a-1,6 linkages.

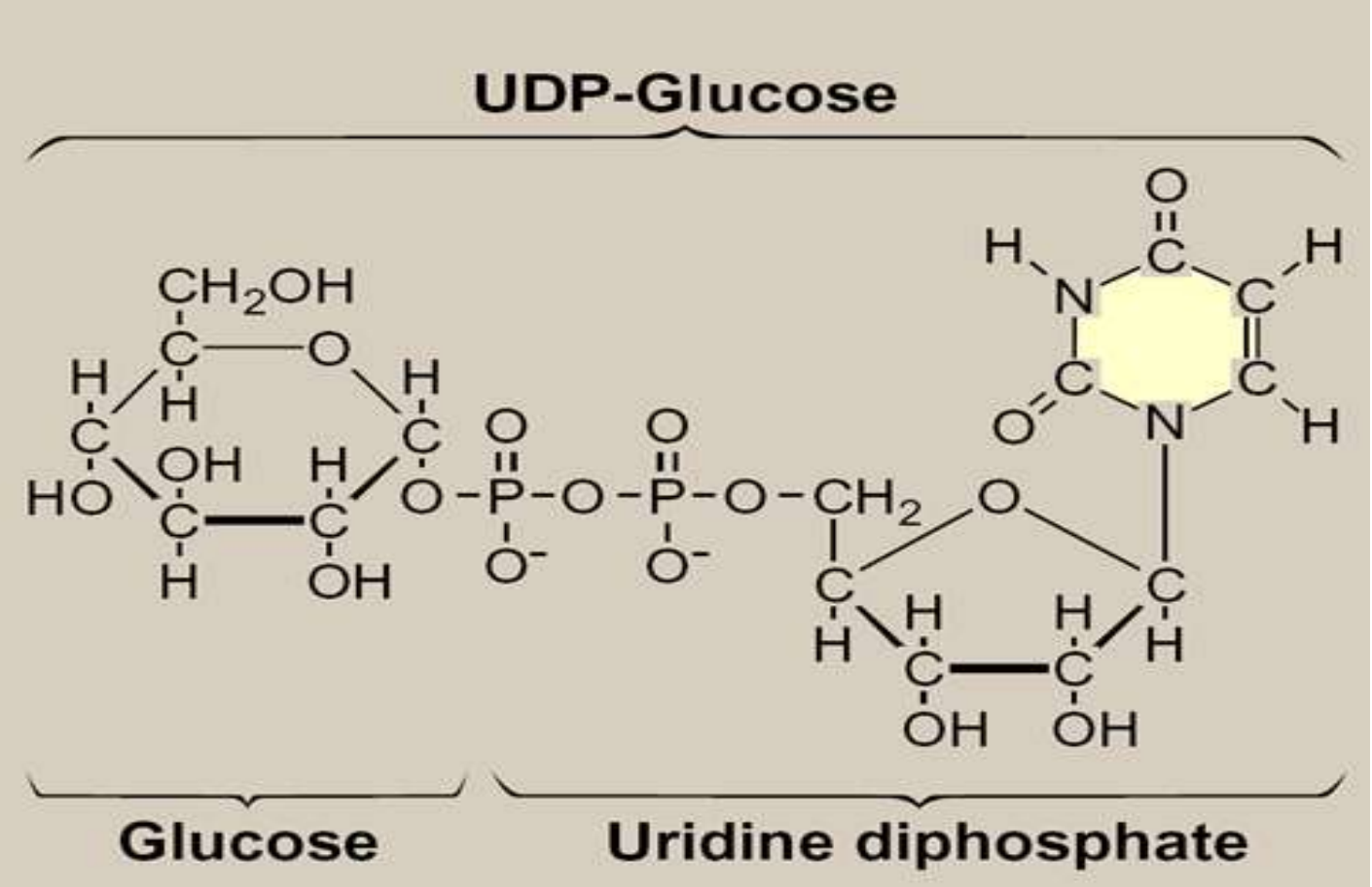
# Synthesis of glycogen (glycogenesis)

- Glycogen is synthesized from molecules of α-D-glucose. The process occurs in cytosol, and requires energy supplied by ATP (for phosphorylation of gluc) & uridine triphosphate (UTP)

A. Synthesis of UDP-glucose:

- α-D-gluc attached to UDP is the source of all of glucosyl residues that are added to the growing glycogen molecule.
- UDP-gluc is synthesized from glucose-1-P & UTP by *UDP-glucose pyrophosphorylase*
- Glycogen synthase:
- catalyzes the formation of a glycoside bond between C-1 of the glucose of UDPGlc and C-4 of a terminal glucose residue of glycogen, liberating uridine diphosphate (UDP).

Note: G-6-P is converted to G-1-P by phosphoglucomutase. G-1,6-BP is an intermediate in this reaction

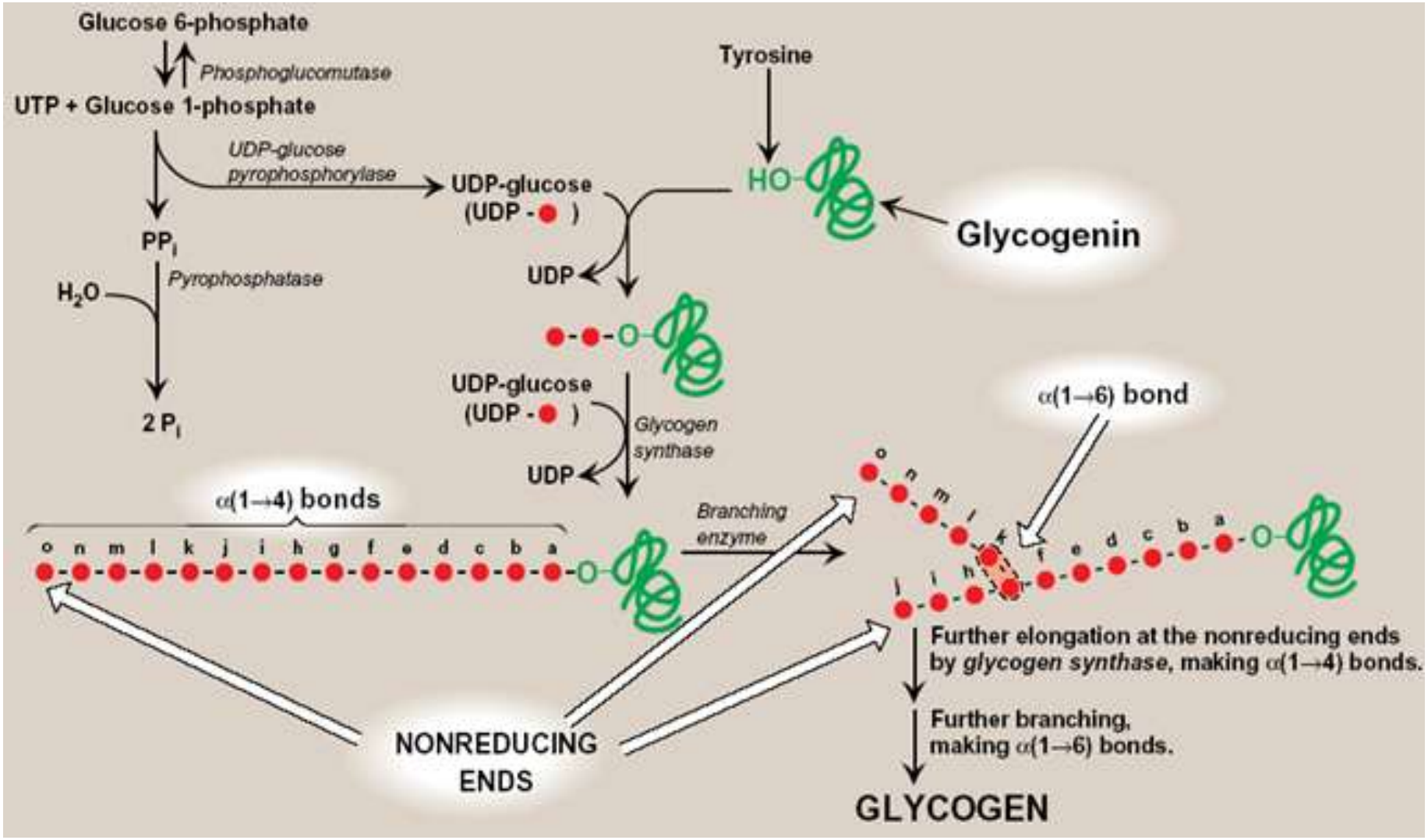



The structure of UDP-glucose

B. Synthesis of a primer to initiate glycogen synthesis

- Glycogen synthase is responsible for making α (1→4) linkages in glycogen. This enz can't initiate chain synthesis using free gluc as an acceptor of a molecule of gluc from UDP-gluc. Instead, it can only elongate already existing chains of gluc
- Therefore, a fragment of glycogen can serve as a primer in cells whose glycogen stores are not totally depleted
- In the absence of a glycogen fragment, a protein, called glycogenin, can serve as an acceptor of gluc residues.
- the first step in glycogen synthesis is the transfer of a glucose unit from UDP-glucose to a tyrosine in glycogenin.
- Transfer of first few molecules of gluc from UDP-gluc to glycogenin is catalyzed by glycogenin itself, which can then transfer additional glucosyl units to the growing α (1→4)-linked glucosyl chain
- This short chain serves as an acceptor of future gluc residues

Note: glycogenin stays associated with & is found in center of completed glycogen molecule




Glycogen synthesis

C. Elongation of glycogen chain by *glycogen synthase*

- Elongation of glycogen chain involves transfer of gluc from UDP-gluc to the non-reducing end of growing chain, forming a new glycosidic bond b/w the hydroxyl of C-1 of activated gluc & C-4 of accepting glucosyl residue.

D. Formation of branches in glycogen

- If no other synthetic enz's acted on the chain, resulting structure would be a linear molecule of glucosyl residues attached by α (1→4) linkages.
- Such a cpd is found in plant tissues, & is called amylose. In contrast, glycogen has branches located, on av., 8 glucosyl residues apart, resulting in a highly branched, tree-like structure that is far more soluble than unbranched amylose
- Branching also increases of non-reducing ends to which new glucosyl residues can be added (and also, from which these residues can be removed), thereby greatly accelerating the rate at which glycogen synthesis & degradation can occur, & dramatically increasing the size of the molecule

1. Synthesis of branches:

- Branches are made by action of “branching enzyme”, *amylo-α (1→4) → α (1→6)-transglucosidase*. This enz transfers a chain of 5 to 8 glucosyl residues from non-reducing end of glycogen chain [breaking α (1→4) bond] to another residue on the chain and attaches it by an α (1→6) linkage
- Resulting new, non-reducing end, as well as the old non-reducing end from which the 5 to 8 residues were removed, can now be elongated by glycogen synthase

2. Synthesis of additional branches:

- After elongation of these two ends has been accomplished by glycogen synthase, their terminal 5 to 8 glucosyl residues can be removed & used to make further branches

# Degradation of glycogen (glycogenolysis)

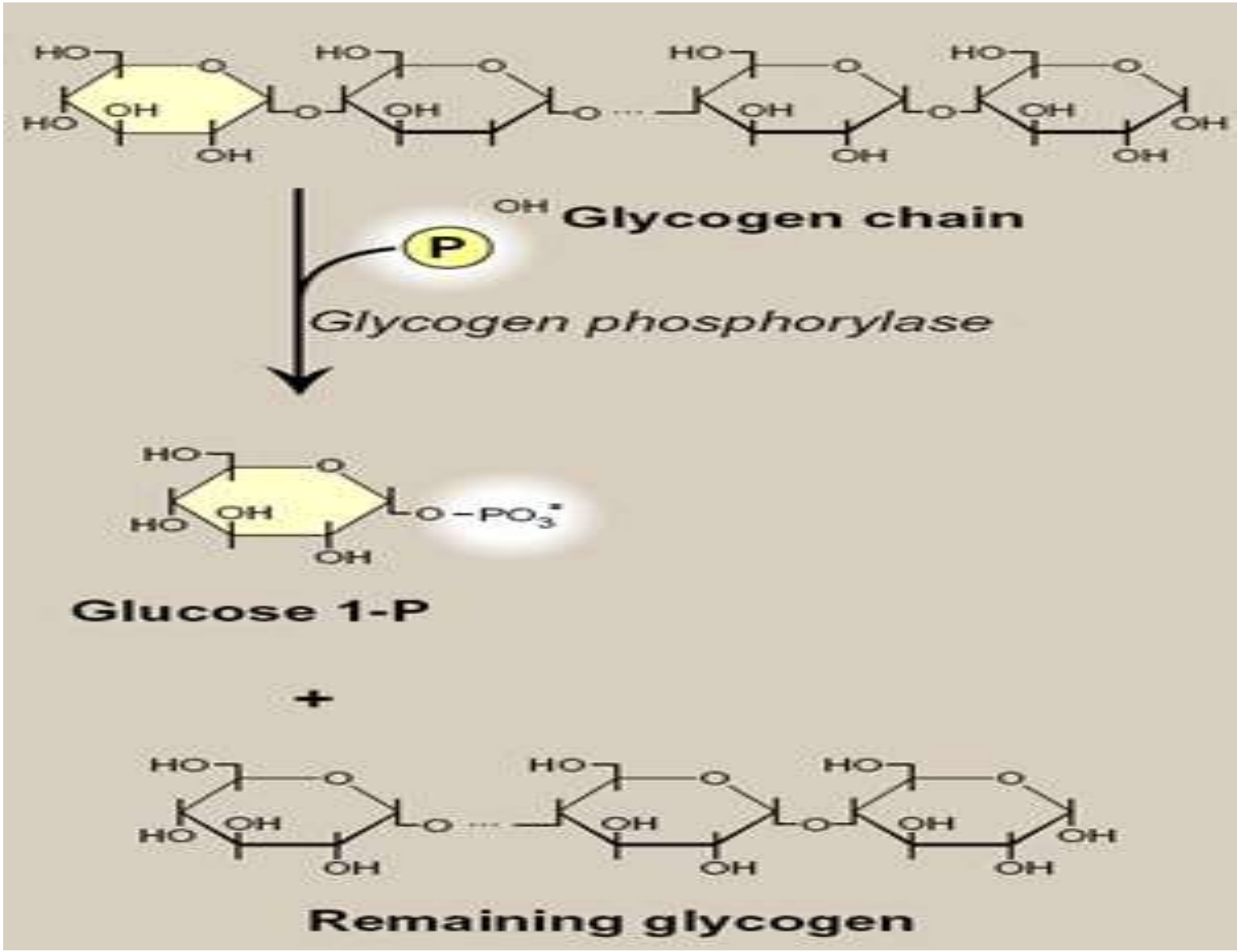
- The degradative pathway that mobilizes stored glycogen in liver & skeletal muscle is not a reversal of the synthetic reactions. Instead a separate set of cytosolic enz's is required.
- When glycogen is degraded, the primary product is G-1-P, obtained by breaking α (1→4) glycosidic bonds. In addition, free gluc is released from each α (1→6)-linked glucosyl residue

A. Shortening of chains

- *Glycogen phosphorylase* sequentially cleaves the α (1→4) glycosidic bonds b/w the glucosyl residues at the non-reducing ends of glycogen chains by simple phosphorolysis until 4 glucosyl units remain on each chain before a branch point

Note: this enz contains a molecule of covalently bound pyridoxal phosphate that is required as a coenzyme

- Resulting structure is called a limit dextrin, & phosphorylase can't degrade it any further




Cleavage of an α **(1→ 4)**-glycosidic bond

B. Removal of branches

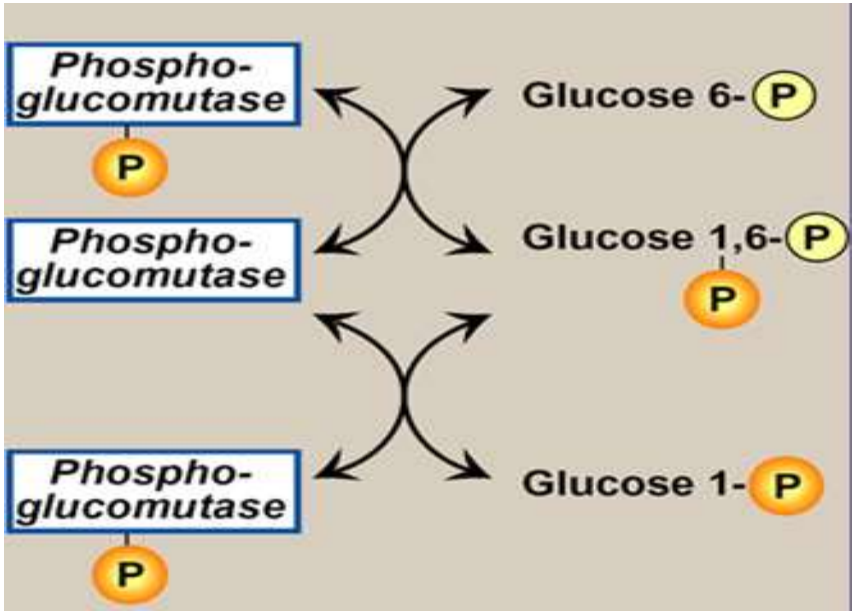
- Branches are removed by 2 enzymatic activities. 1st *oligo-α(1→4)→α (1→4)-glucan transferase* removes the outer 3 of the 4 glucosyl residues attached at a branch. It next transfers them to the non-reducing end of another chain, lengthening it. Thus, an α(1→4) bond is broken and an α(1→4) bond is made.
- Next, the remaining single gluc residue attached in an α(1→6) linkage is removed hydrollytically by *amylo- α(1→6)-glucosidase* activity, releasing free gluc.

Note: both the transferase & glucosidase are domains of a single polyp molecule, the 'debranching enzyme".

- The glucosyl chain is now available for degradation by glycogen phosphorylase until 4 glucosyl units from next branch are reached

C. Conversion of G-1-P to G-6-P

- G-1-P, produced by *glycogen phosphorylase*, is converted in the cytosol to G-6-P by *phosphoglucomutase*, a reaction that produces G-1,6-BP as a temporary but essential intermediate.




- In liver, G-6-P is translocated into ER by *glucose 6-phosphate translocase*. There it is converted to glucose by *glucose 6-phosphatase*, the same enz used in last step of gluconeogenesis
- Resulting glu is then transported out of ER to cytosol. Hepatocytes release glycogen-derived gluc into blood to help maintain blood gluc levels until gluconogenic pathway is actively producing gluc

Note: in muscle, G-6-P can't be dephosphorylated because of a lack of glucose-6-phosphatase. Instead, it enters glycolysis, providing energy needed for muscle contraction

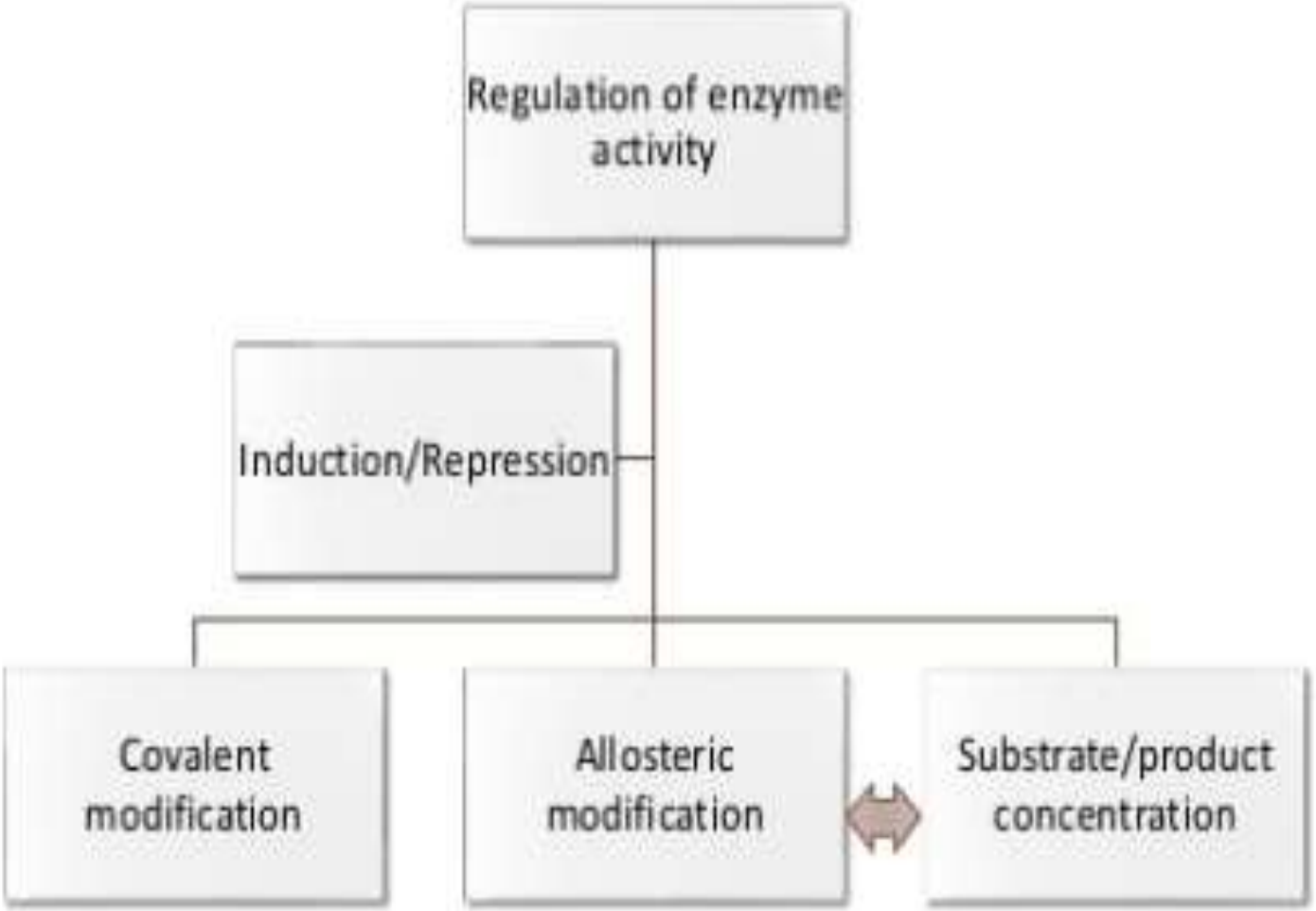
D. Lysosomal degradation of glycogen

- A small amount of glycogen is continuously degraded by lysosomal enz, *α(1→4)-glucosidase* (acid maltase).
- However, a deficiency of this enz causes accumulation of glycogen in vacuoles in the cytosol, resulting in the serious glycogen storage disease type II (Pompe disease)

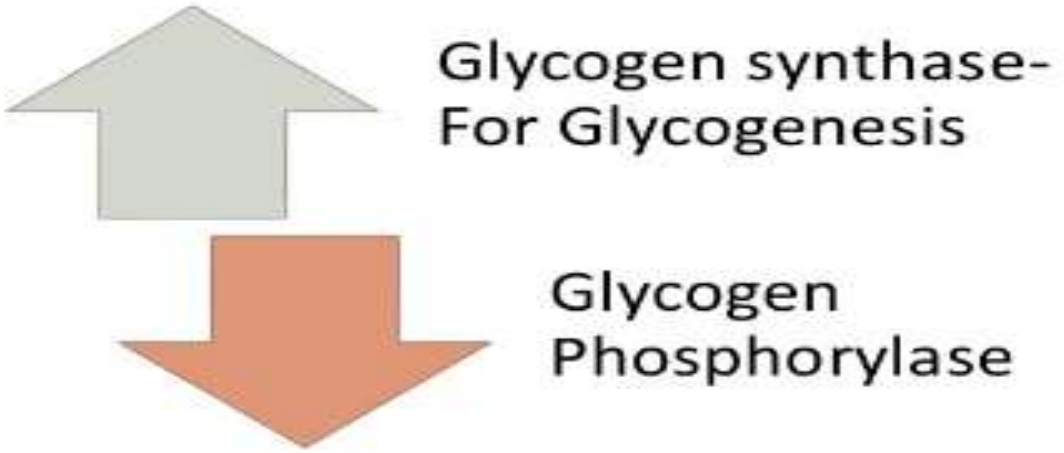
## Regulation of glycogen synthesis & degradation

- Because of importance of maintaining blood gluc levels, synthesis & degradation of its glycogen storage form are tightly regulated
- In liver, glycogen synthesis accelerates during periods when the body has been well fed, whereas degradation accelerates during periods of fasting.
- In skeletal muscle, glycogen degradation occurs during active exercise, & synthesis begins as soon as the muscle is again at rest
- Regulation of glycogen synthesis & degradation is accomplished on two levels

# General mechanisms involved in the regulation of enzyme activities



# Key enzymes involved in the regulation of glycogen metabolism

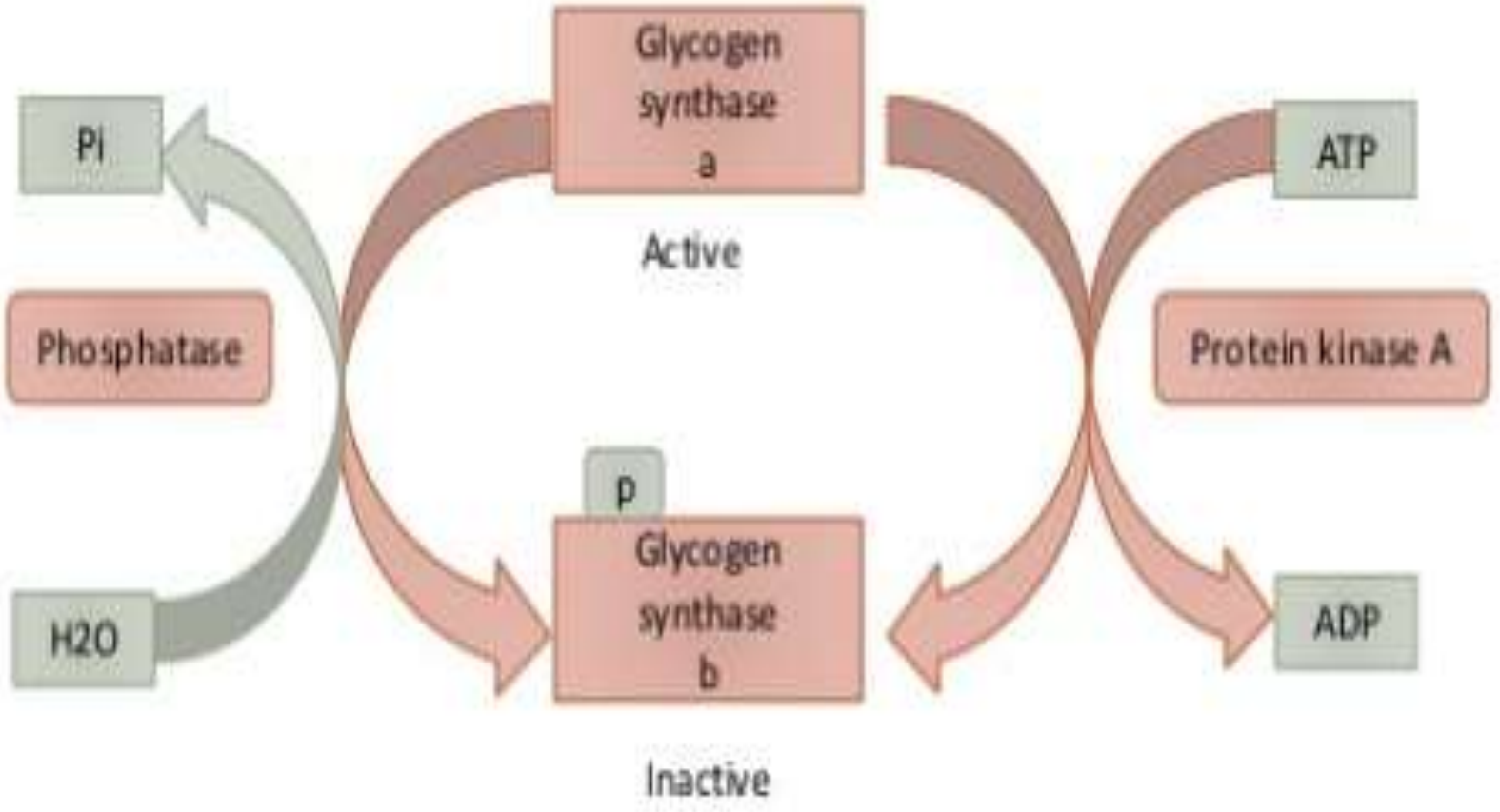



Both these enzymes are reciprocally regulated.

Reciprocal regulation of glycogenesis & glycogenolysis:

- Glycogen synthase &phosphorylase activity are reciprocally regulated
- At the same time as phosphorylase is activated by a rise in concentration of cAMP(via phosphorylase kinase), glycogen synthase is converted to the inactive form.
- Thus,inhibition of glycogenolysis enhance net glycogenesis, and inhibition of glycogenesis enhances net glycogenolysis.
- Both processes do not occur at the sametime.

# Covalent modification of glycogen synthase



## Phosphorylation of phosphorylase

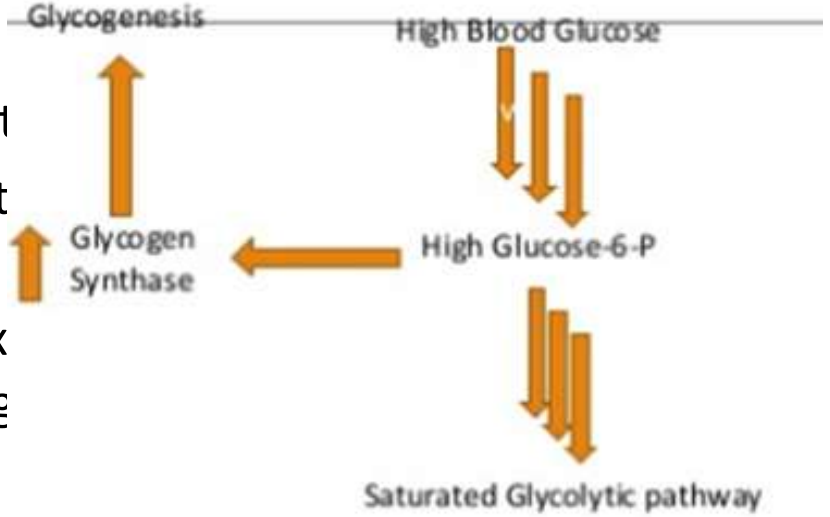
- The enzyme phosphorylase is activated by phosphorylase kinase to yield phosphorylase a (active)
- Inactivated by dephosphorylation catalyzed by phosphoprotein phospha

to yield phosphorylase b(inactive)


Substrate conc. & allosteric modification:

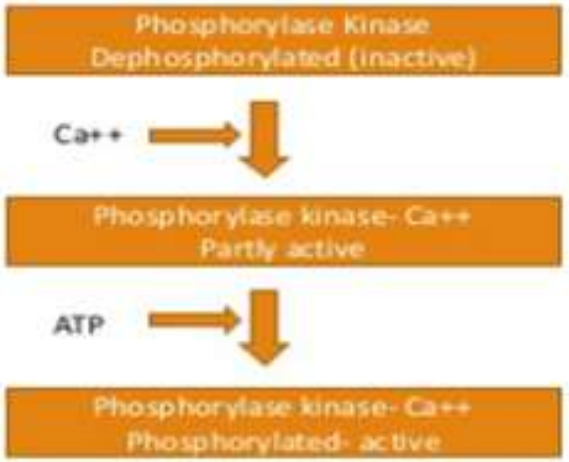
Substrate Glucose-6-P

- Glycogen synthase is allosterically activat
- High blood glucose concentration leads t P
- When glycolytic pathway is saturated, ex glycogen synthase and thus is stored as g



# Role of calcium in muscle degradation

- Phosphorylase kinase is partly activated by binding of Ca ++, further activation is by Phosphorylation

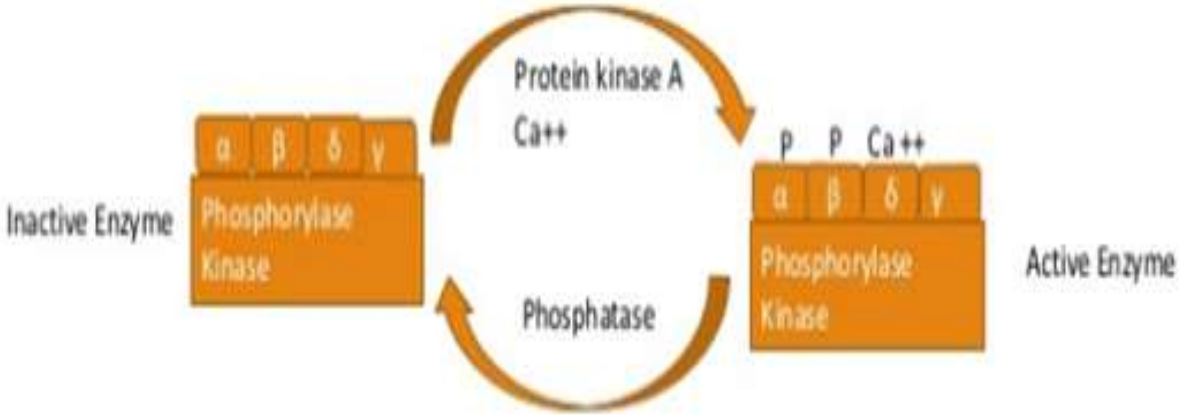



## Role of calcium in the activation of phosphorylation kinase:

Muscle phosphorylase kinase, which activates glycogen phosphorylase is a tetramer of four different subunits.

The $\alpha$ $and$ $\beta$ subunits contain serine residues that are phosphorylated by cAMP-dependent protein kinase.

The binding of Ca++ activates the catalytic site of the subunit even while the enzyme is in the dephosphorylated b state, the phosphorylated a form is only fully activated in the presence of Ca++.



# Biological significance

- When the blood glucose is low as in fasting or starvation, the predominant hormones such as Glucagon and epinephrine trigger the cAMP mediated phosphorylation cascade.
- In the phosphorylated state glycogen synthase becomes inactive while phosphorylase becomes active
- Liver glycogen breakdown restores the lowered blood glucose conc. Back to normal.
- When the blood glucose conc. Is high: insulin, the main hormone, promotes the dephosphorylated forms of the enzymes by disrupting the cAMP mediated phosphorylation cascade and by stimulating phosphatase activities.
- Phosphorylase is dephosphorylate becomes inactivewhile glycogen synthase becomes active.
- Hence extra glucose is used for glycogen synthesis and blood glucose conc. Is restored back to normal.

- Glycogen is a highly-branched polymer of glucose that can be quickly degraded to yield glucose-1-P, which is isomerized to glucose-6-P for use in glycolysis by muscle cells, or it is dephosphorylated in liver cells and exported.
- Glycogen  phosphorylase removes one glucose at a time from the nonreducing ends using inorganic phosphate(Pi) which makes glucose release a free reaction. Glycogen phosphorylase is activated by phosphorylation in responseto glucagon and epinephrine and allosterically regulated by energy.
- Glycogen synthase adds glucose residues to nonreducing ends in a reaction involving UDP-glucose,a nucleotide form of glucose. Since the UDP that is released  following glucose addition needs to be phosphorylated to regenerate UTP, the cost of glycogen synthesis is 1 ATP / glucose residue.

# Glycogen storage diseases

- is a metabolic disorder caused by enzyme deficiencies affecting either glycogen synthesis, glycogen breakdown or glycolysis (glucose breakdown), typically within muscles and/or liver cells.
- GSD has two classes of cause: genetic and acquired. Genetic GSD is caused by any inborn error of metabolism (genetically defective enzymes) involved in these processes. In livestock, acquired GSD is caused by intoxication.
- Different types of GSD (more than ten types):

<u>Glycogen storage disease type I</u>

- Glycogen storage disease (GSD) type I, also known as von Gierke disease, is a group of inherited autosomal recessive metabolic disorders of the glucose-6-phosphatase system which helps maintain glucose homeostasis.

Glycogen storage disease type II

- GSD type II, also known as alpha glucosidase deficiency (GAA, acid maltase deficiency) or Pompe disease, is a prototypic lysosomal disease. it is caused by the deficiency of the lysosomal enzyme, alpha-1,4-glucosidase, leading to the pathologic accumulation of normally structured glycogen within the lysosomes of most tissues

Glycogen storage disease type III

- GSD type III is also known as Forbes-Cori disease or limit dextrinosis. It is an autosomal recessive disorder, which causes deficiency in glycogen debranchinging enzyme and limited storage of dextrin. The disease presents with variable cardiac muscle, skeletal muscle and liver involvement and has different subtypes. Glycogen deposited in these organs has an abnormal structure.

Glycogen storage disease type IV

- GSD type IV, also known as amylopectinosis, Glycogen Branching enzyme deficiency (GBE) or Andersen disease, is a rare disease that leads to early death. with progressive hepatosplenomegaly and accumulation of abnormal polysaccharides. The main clinical features are liver insufficiency and abnormalities of the heart and nervous system.

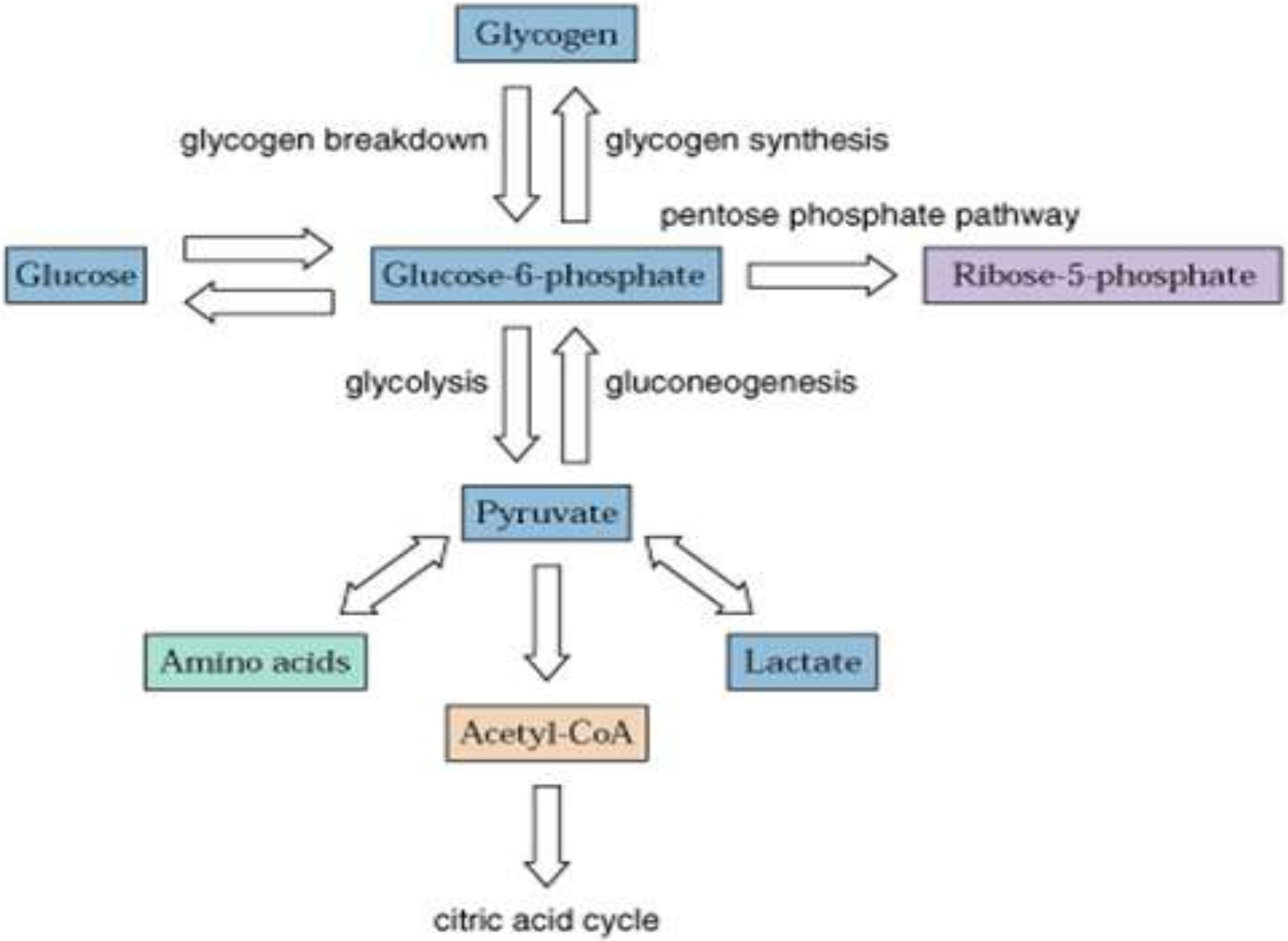
Glycogen storage disease type V

- GSD type V, also known as McArdle disease, affects the skeletal muscles. It is an autosomal recessive disorder in which there is a deficiency of glycogen phosphorylase.

# Gluconeogenesis

By: Inaam Ahmed Ameen

# Overview of Glucose Metabolism



# Gluconeogenesis:

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

The need for gluconeogenesis:

1. To maintain the blood glucose levels within the normal range, 3.3 to 5.5 mmol/L (60 and 99 mg/dL), (prevent hypoglycemia).

2. Shift sugar/energy to important body parts (brain and muscles).

Brain is more important:

1-Needs large amounts of energy .

2- Cannot store energy (very little glycogen storage) .

3- Not sensitive to insulin regulation

4- Must have glucose for energy, Will not adapt to using ketone bodies (fat) for energy until severe conditions arise (weeks of fasting)

# Substrates of Gluconeogenesis

Glucose is made from non-carbohydrates

1-Lactate (Lactate is produced as a byproduct of glycolysis in muscles, red blood cells etc)

2. Pyruvate

3. Amino acids(Glucogenic amino acids like alanine and glutamine)

4. Glycerol (from triacylglyceride hydrolysis) enters as dihydroxyacetone phosphate

5. Acetate(Citric acid cycle intermediates through oxaloacetic acid)

Oxaloacetate is the starting material for gluconeogenesis

- Major sites of gluconeogenesis:

1-Liver (90%)

2. Kidney (10%)

Net reaction:

2 pyruvate + 2 NADH + 4 ATP + 2GTP + 6 $H_2O$ + 2 $H^+$ ----------→

Glucose + 2 $NAD^+$ + 4 ADP + 2 GDP + 6 Pi

# Gluconeogenesis Pathway

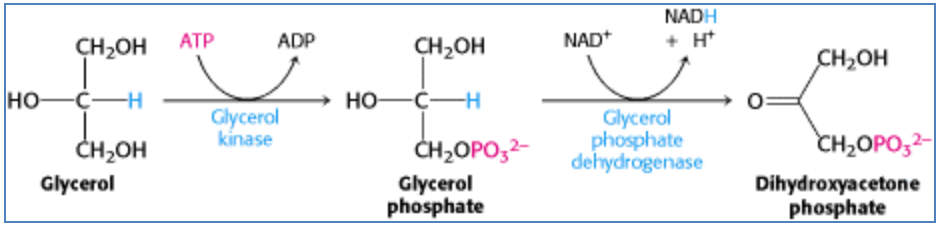
- Gluconeogenesis begins in either the mitochondria or cytoplasm of the liver or kidney. First, two pyruvate molecules are carboxylated to form oxaloacetate. One ATP (energy) molecule is needed for this.
- Oxaloacetate is reduced to malate by NADH so that it can be transported out of the mitochondria.
- Malate is oxidized back to oxaloacetate once it is out of the mitochondria.
- Oxaloacetate forms phosphoenolpyruvate using the enzyme PEPCK.
- Phosphoenolpyruvate is changed to fructose-1,6-biphosphate, and then to fructose-6-phosphate. ATP is also used during this process, which is essentially glycolysis in reverse.
- Fructose-6-phosphate becomes glucose-6-phosphate with the enzyme phosphoglucoisomerase.
- Glucose is formed from glucose-6-phosphate in the cell's endoplasmic reticulum via the enzyme glucose-6-phosphatase. To form glucose, a phosphate group is removed, and glucose-6-phosphate and ATP becomes glucose and ADP.

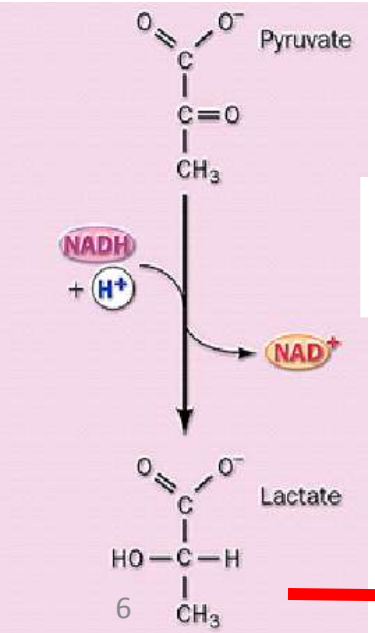
# Noncarbohydrate precursors of glucose

Triglycerols

Fatty acids

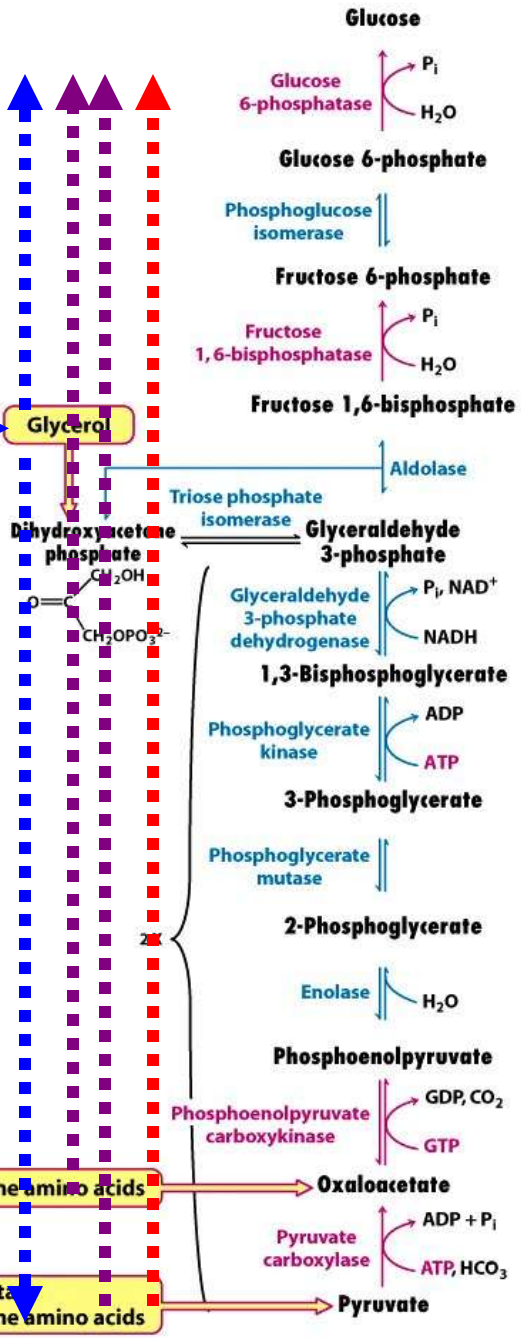
glycerol

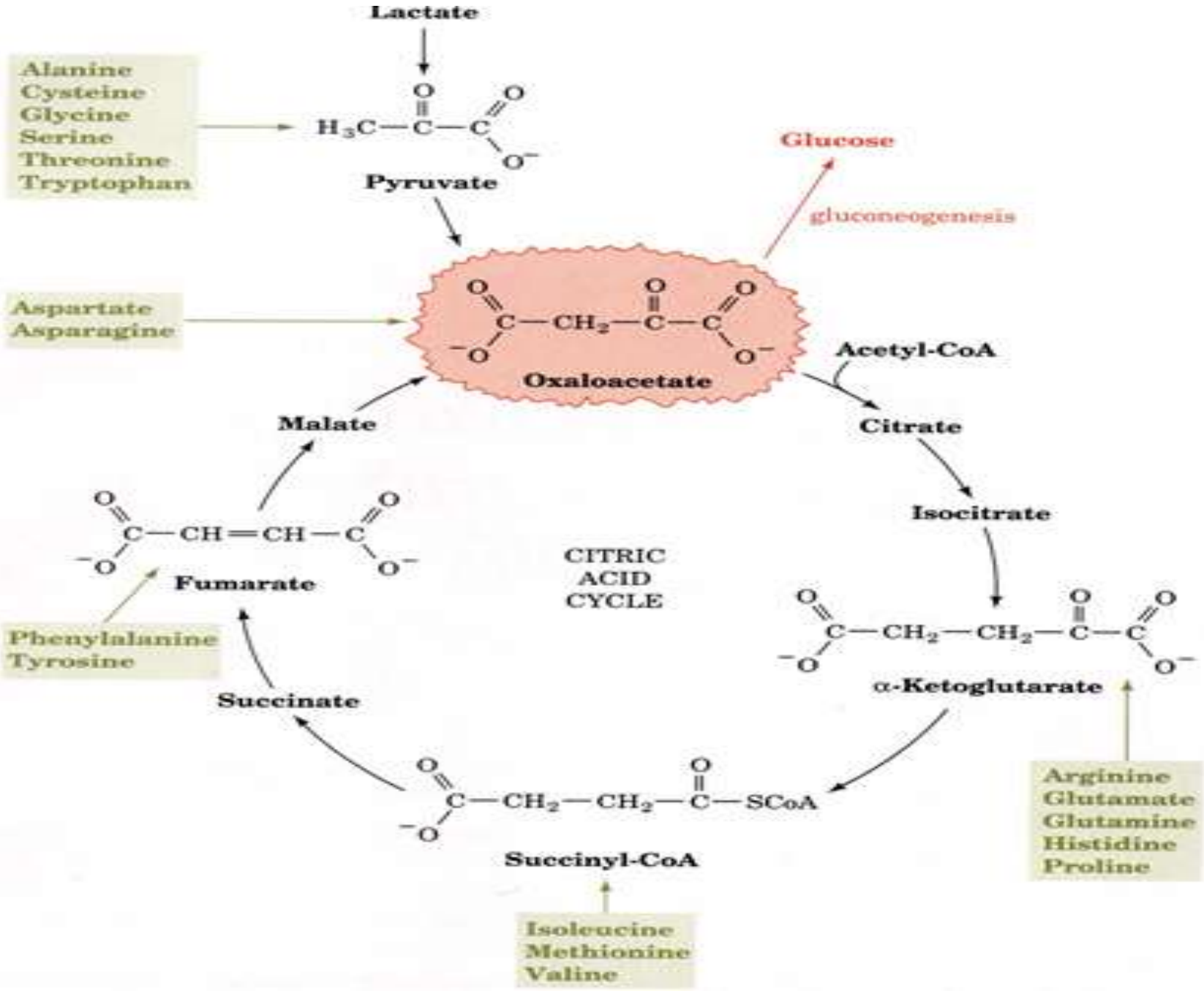



Dietary & muscle proteins

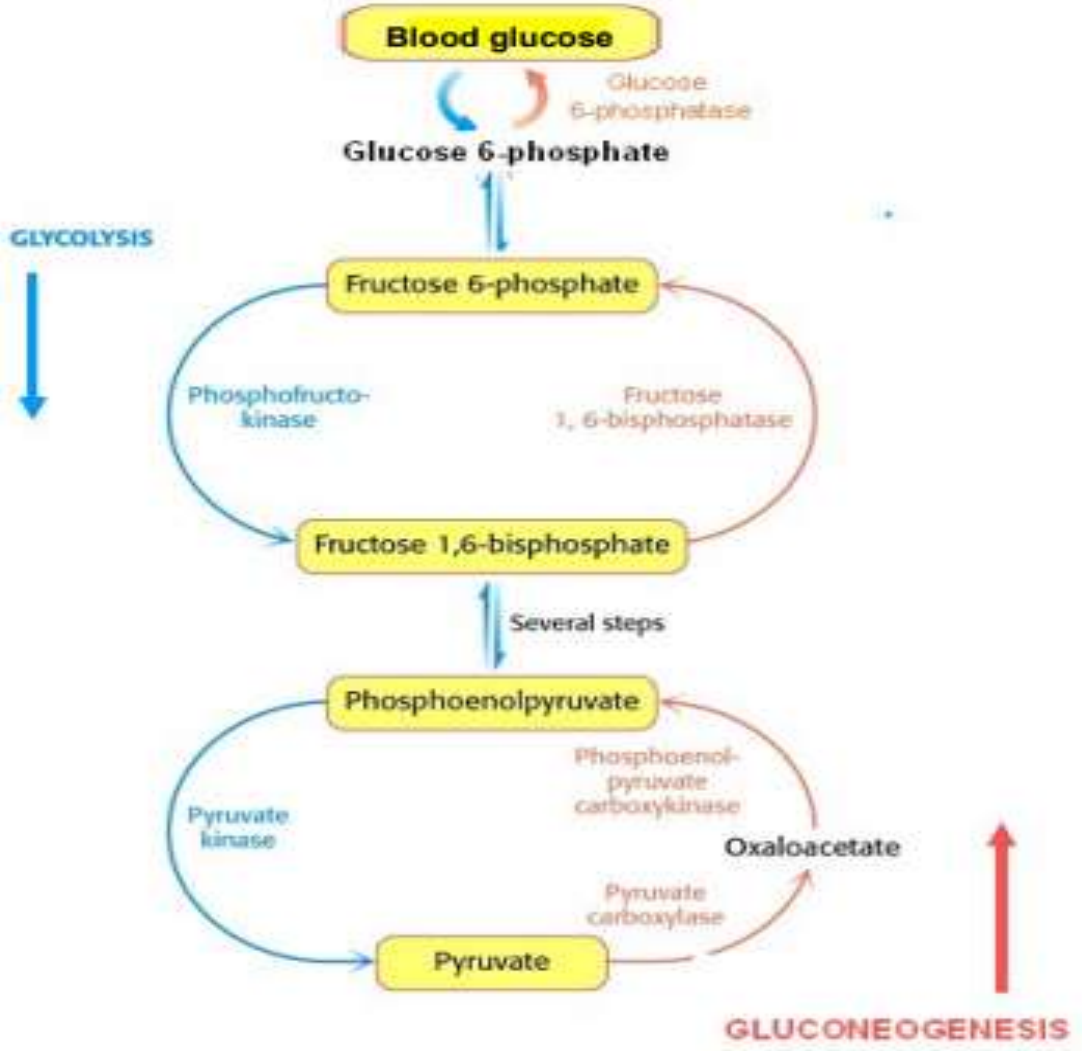
Amino acids

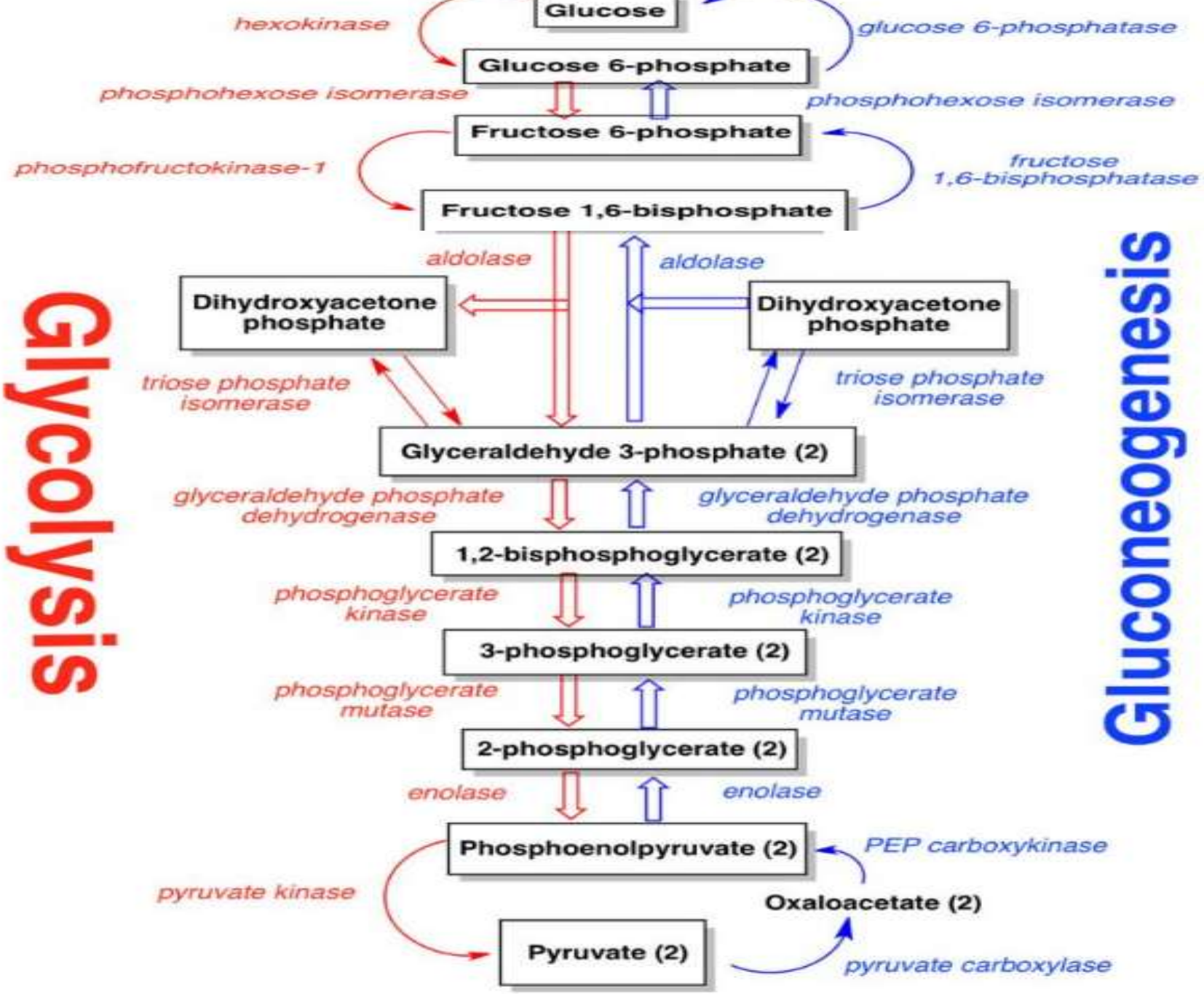



Lactate
Alanine
Cysteine
Glycine
Serine
Threonine
Tryptophan
Pyruvate
Glucose
gluconeogenesis
Aspartate
Asparagine
Oxaloacetate
Acetyl-CoA
Citrate
Malate
Isocitrate
CITRIC
ACID
CYCLE
Fumarate
Phenylalanine
Tyrosine
α-Ketoglutarate
Succinate
Arginine
Glutamate
Glutamine
Histidine
Proline
Succinyl-CoA
Isoleucine
Methionine
Valine

# Gluconeogenesis is not a reversal of glycolysis

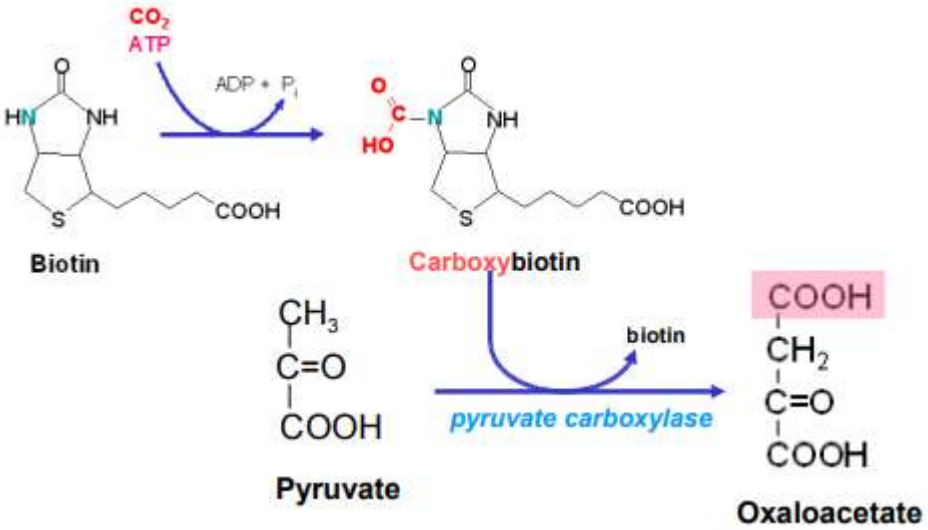
- because there are three irreversible steps in glycolysis.
- In gluconeogenesis, four alternate reactions bypass these irreversible steps of glycolysis.

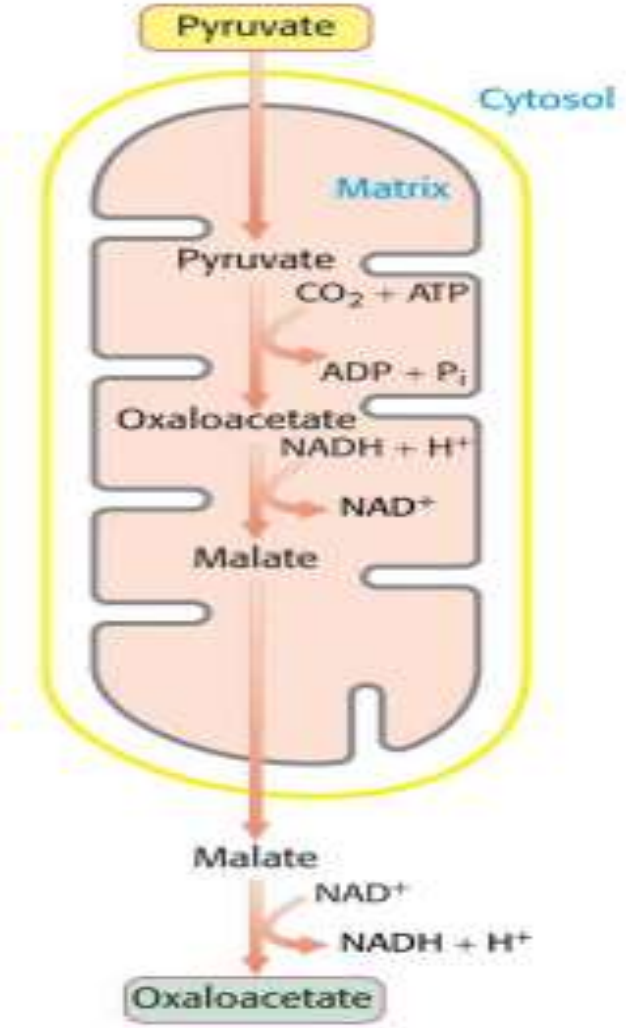



Glycolysis
Gluconeogenesis
Glucose
hexokinase
glucose 6-phosphatase
Glucose 6-phosphate
phosphohexose isomerase
phosphohexose isomerase
Fructose 6-phosphate
phosphofructokinase-1
fructose 1,6-bisphosphatase
Fructose 1,6-bisphosphate
aldolase
aldolase
Dihydroxyacetone phosphate
Dihydroxyacetone phosphate
triose phosphate isomerase
triose phosphate isomerase
Glyceraldehyde 3-phosphate (2)
glyceraldehyde phosphate dehydrogenase
glyceraldehyde phosphate dehydrogenase
1,2-bisphosphoglycerate (2)
phosphoglycerate kinase
phosphoglycerate kinase
3-phosphoglycerate (2)
phosphoglycerate mutase
phosphoglycerate mutase
2-phosphoglycerate (2)
enolase
enolase
Phosphoenolpyruvate (2)
PEP carboxykinase
pyruvate kinase
Oxaloacetate (2)
Pyruvate (2)
pyruvate carboxylase

# 1- Carboxylation of pyruvate to oxaloacetate

- In the mitochondria of liver and kidney cells, pyruvate is carboxylated. Carboxybiotin is the donor of carboxyl group:

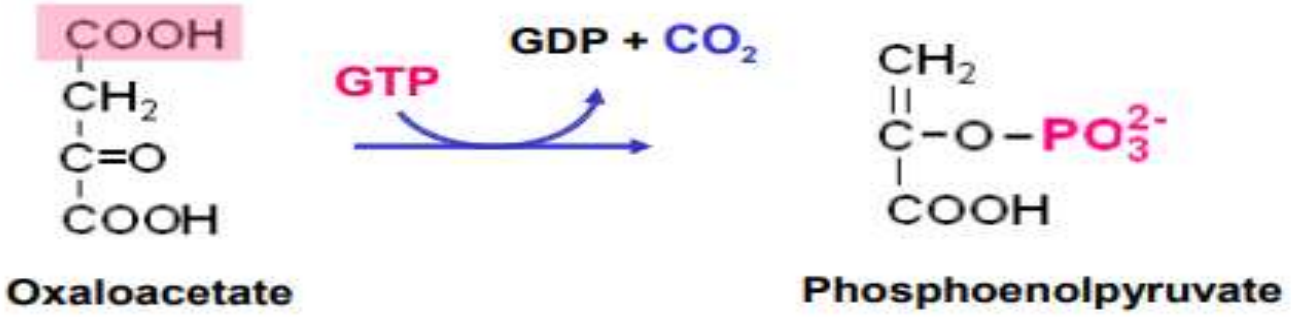



- The activity of pyruvate carboxylase depends on the presence of an allosteric activator - acetyl-CoA.
- Oxaloacetate is transported into the cytosol in the form of malate, which is then reoxidized to oxaloacetate.

# 2- Conversion of oxaloacetate to phosphoenolpyruvate (PEP)

- Oxaloacetate at the same time decarboxylated and phosphorylated by phosphoenolpyruvate carboxykinase in the cytosol:




- The two-step pathway for the formation of phosphoenolpyruvate (the sum of reactions 1 and 2)

Pyruvate + ATP + GTP + H2O ↔ phosphoenolpyruvate + ADP + GDP + Pi + 2 H+ is much more favourable than the reaction catalyzed by pyruvate kinase, because of the use of a molecule of ATP in the carboxylation reaction 1. The added molecule of CO2 is then removed to power the endergonic formation of PEP in the decarboxylation step.

# 3- Dephosphorylation of fructose 1,6-bisphosphate

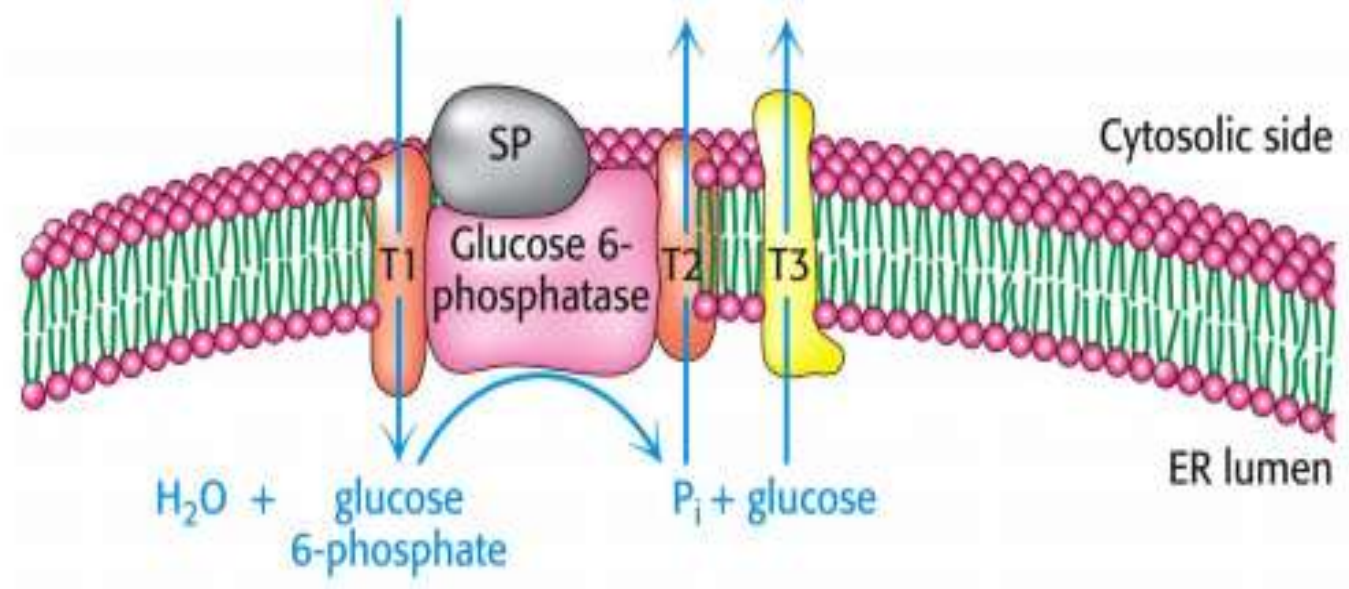
- The hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate is catalyzed by fructose 1,6-bisphosphatase.
- Fructose 1,6-bisphosphate + $H_2O$ ------→ fructose 6-phosphate + Pi

- Fructose 1,6-bisphosphatase is an allosteric enzyme. Like its glycolytic corresponding phosphofructokinase-1, it participates in the regulation of gluconeogenesis. Both enzymes are reciprocally controlled by fructose 2,6-bisphosphate in the liver. Fructose 2,6-bisphosphate strongly stimulates phosphofructokinase-1 and inhibits fructose 1,6-bisphosphatase.

- In most tissues, gluconeogenesis (if there is any) ends at glucose 6-phosphate, free glucose is not generated.

- 4- Glucose 6-phosphatase is present only in the liver cells and to a lesser extent in the kidney, only these tissues can release free glucose into the blood.

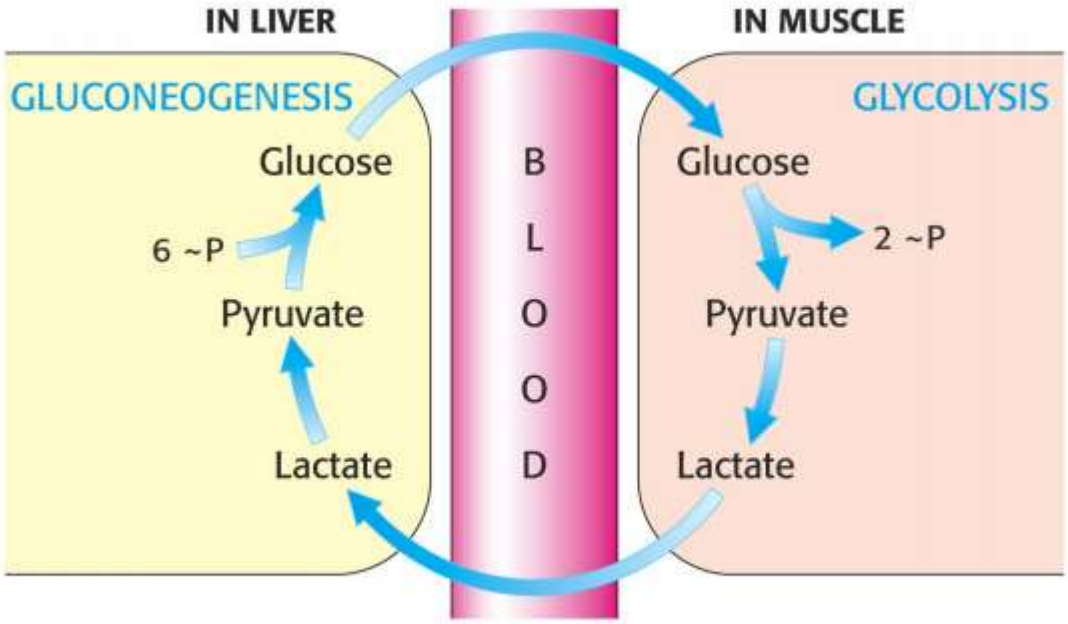
- The dephosphorylation of glucose 6-phosphate takes place within the lumen of endoplasmic reticulum.




- SP – Ca2+-binding stabilizing protein is essential for Glu-6-phosphatase activity

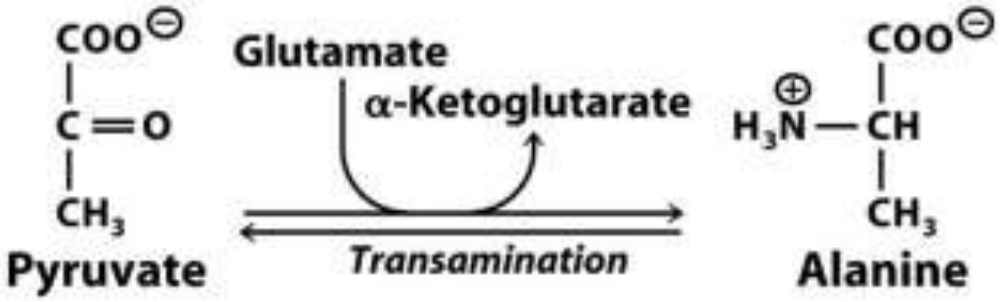
# The Cori cycle

- Gluconeogenesis in the liver transforms part of the lactate formed by active skeletal muscle to glucose:



## Glucogenic amino acids

Glucogenic amino acid undergoes transamination which causes change in the carbon skeleton and directly gets converted to pyruvate. Some Glucogenic amino acids form oxaloacetic acid or other intermediates of Citric acid cycle. alanine is preferred in liver & glutamine is preferred in kidney.




**Lactate:** Muscular activities and anaerobic glycolysis in red blood cells produce a large amount of lactate. This lactate is taken up by the liver and gets converted to pyruvate by the enzyme lactate dehydrogenase. Pyruvate then gets converted to glucose by hepatic Gluconeogenesis which is then sent back to muscles for reuse (Cori cycle).

## Glycerol

Glycerol is formed by breaking down of triacylglecerol in the fatty tissue. It is then carried to the liver where it gets converted to pyruvate and enters Gluconeogenesis

# Gluconeogenesis Enzymes

**The enzymes that are same as that of glycolysis are**

Phosphoglucoisomerase
Enolase
Phosphoglycerate mutase
Phosphoglycerate kinase
G3P dehydrogenase
Triosephosphate isomerase
Fructose 1,6 bisphosphate aldolase

**The key enzymes of Gluconeogenesis are**

Pyruvate carboxylase
Phosphoenol pyruvate carboxykinase
Fructose 1,6 bisphosphatase
Glucose 6 phosphatase

# Gluconeogenesis Regulation

As Gluconeogenesis is the reversed process of glycolysis, both are regulated reciprocally. The factors which increase Glycolysis will decrease Gluconeogenesis and vice versa. This regulation is needed to control the blood glucose level which will be either too low or too high in an unregulated condition. The regulation of this process is brought about by availability of substrates and through hormones.

There are **3 types of regulation which takes place at different speed.** They are

1. Change in the rate of enzyme synthesis – **occurs over several hours**

**Availability of substrate** – Increased availability of Glucogenic amino acid stimulates Gluconeogenesis. High glucose increases the synthesis of enzymes of Glycolysis so that the glucose level is brought down. The opposite happens to the enzymes of Gluconeogenesis. The synthesis of gluconeogenic enzymes are decreased so that there is less formation of new glucose.

**Through hormones** – Gluconeogenesis is increased by the Glucogenic hormones like glucagon, epinephrine and glucocorticoids. Glucagon stimulates phosphoenolpyruvate carboxykinase while insulin reduces the synthesis of these enzymes.

2. Covalent modification by reversible phosphorylation – **rapid**

Phosphorylation is the process of addition of a phosphate group to an enzyme. On phosphorylation, some enzymes are activated while some are inactivated. The gluconeogenic hormones like glucagon and epinephrine leads to the phosphorylation of a key enzyme of glucose breakdown, pyruvate kinase which gets inactivated.

This leads to inhibition of glycolysis and stimulation of Gluconeogenesis. This type of regulation acts as a rapid response to the presence of low glucose.

3. Allosteric modification – **instant**

In addition to the enzymes that speeds up a reaction, other substances called allosteric activators further increase the rate of reaction. Acetyl coA is one such allosteric activator.

# Important Hormones of Gluconeogenesis

The important hormones that regulate the blood sugar level and thus Gluconeogenesis are glucagon, insulin and glucocorticoids. The hormones which stimulate Gluconeogenesis and other mechanisms which increase the blood glucose level are called diabetogenic hormones.

**Gluconeogenesis and Glucagon:**

Glucagon is synthesized by alpha cells of pancreas. F-2,6-BP levels are regulated by glucagon, with high glucagon (low blood sugar) favoring conversion of F-2,6-BP back into F6P.

In addition, glucagon activates lipases is adipose tissue, promoting release of fatty acids into the bloodstream. These fatty acids are broken down in the mitochondria of liver, resulting in high concentrations of acetyl CoA. Acetyl CoA acts as an allosteric activator of pyruvate carboxylase.

**They stimulate the Gluconeogenesis by 3 mechanisms**.

They are

1-During fasting, glucagon inhibits the enzyme catalyzing the synthesis of fructose 2, 6 bisphosphate. Thus it acts as a counter hormone to insulin.

2-It causes conversion of pyruvate kinase to its inactive form by phosphorylation thus inhibiting Glycolysis and favoring Gluconeogenesis.

3-It increases the transcription of Phosphoenolpyruvate carboxykinase gene there by increasing the availability of the enzyme.

## Gluconeogenesis and Glucocorticoids

Glucocorticoids are synthesized in the kidney under stressful condition like starvation and intense exercise. Thus cortisol is stimulated by low blood sugar and increased demand. They increase Gluconeogenesis by increasing the breakdown of Glucogenic amino acid. They increase Gluconeogenesis in kidney more than liver through a series of complicated biochemical steps.

## Gluconeogenesis and Insulin:

It is the anti diabetic hormone synthesized by beta cells of pancreas and its main function is to lower the blood glucose level. Increased glucose level leads to increased production of ATP. This ATP acts on potassium and calcium channel of the beta cells of pancreas and leads to release of insulin. Insulin inhibits Gluconeogenesis and causes glucose uptake by cells. It reduces both hepatic and renal Gluconeogenesis to equal extent.

# Insulin and counter regulatory hormones

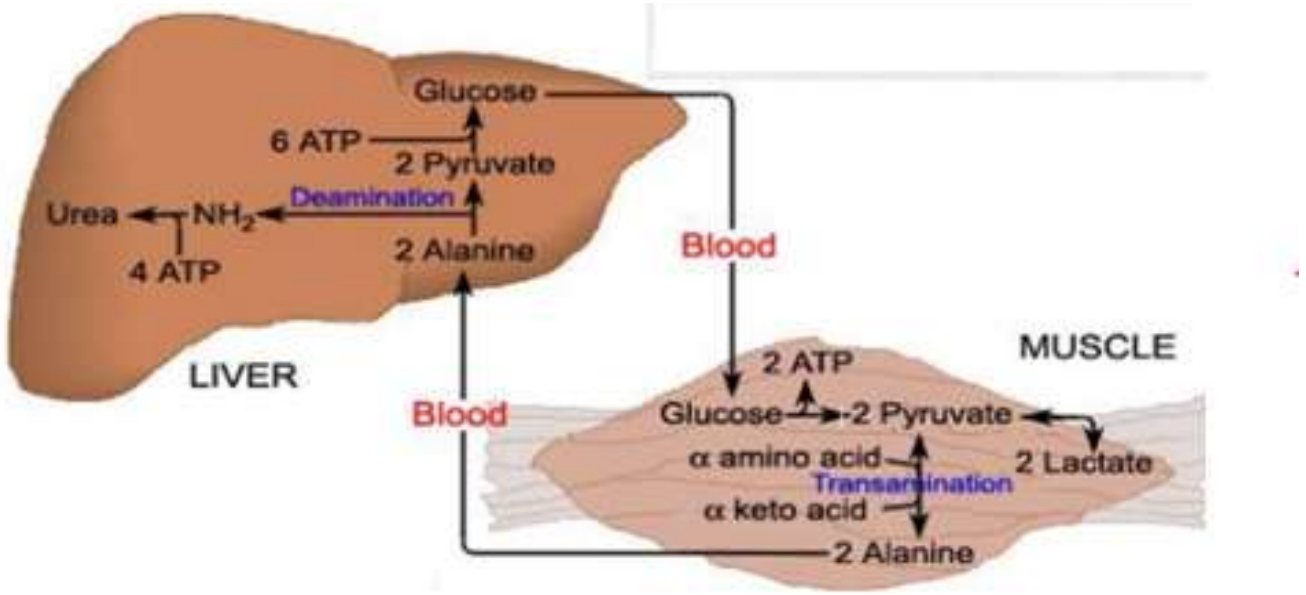
| Hormone | functions | major metabolic paths |
|---|---|---|
| Insulin | promotes storage | stimulate glucose storage in muscle, liver |
| | promotes growth | stimulates protein synthesis, fatty acid synthesis |
| Glucagon | mobilizes fuels | activates gluconeogenesis and glycogenolysis |
| | maintains blood glucose in fasting | activates fatty acid release |
| Epinphrine | mobilizes fuels in acute stress | stimulate glycogenolysis<br>stimulate fatty acid release |
| Cortisol | changing long term | amino acid mobilization<br>gluconeogenesis |

# Organs of Gluconeogenesis

- The major tissues capable of synthesizing glucose are liver and kidney. Only they have the sufficient gluconeogenic enzyme activity and glucose 6 phosphatase activity to release glucose into circulation. It occurs in small intestine to a small extent in fasting state.
- **Hepatic Gluconeogenesis – Gluconeogenesis in liver**
- Liver is the major site of Gluconeogenesis. During first 12 hrs of fasting, the glycogen reserve gets depleted dramatically and Gluconeogenesis increases by its regulatory mechanism. Liver primarily uses lactate, alanine and glycerol. Lactate gets converted to pyruvate by Cori cycle and then undergoes Gluconeogenesis in liver. Alanine gets converted to glucose by Glucose – Alanine cycle. A cetyl-CoA is also formed in the liver and also participate in gluconeogenesis.

## The glucose/alanine cycle:

- During extended periods of fasting (eg baby not feeding well, Ramadan fasting), skeletal muscle is degraded as an alternative source of energy.
- Alanine is the major amino acid present when muscle (protein) is degraded.
- When muscle amino acids for energy needs, the resulting nitrogen is transaminated to pyruvate to form alanine. This alanine is shuttled to the liver where the nitrogen enters the urea cycle and the pyruvate is used to make glucose.



Renal Gluconeogenesis – Gluconeogenesis in kidney

liver is the major organ of Gluconeogenesis but also the kidney is important as the liver. Gluconeogenesis occurs in the outer tissue of kidney which is the cortex.

It mainly uses lactate, glutamine and glycerol. Lactate dehydrogenase, glucose 6 phosphatase and fructose 1, 6 bisphosphatase makes it possible for Gluconeogenesis to occur in kidney. Renal Gluconeogenesis is greatly stimulated by glucocorticoids. This helps to maintain the normal blood sugar in people with liver disease and contributes to excess glucose in **diabetes** type 1 and 2. It is increased by acidosis in contrast to hepatic Gluconeogenesis. It is suppressed by insulin as much as in the liver.

# Alcohol – Gluconeogenesis

Alcoholics have impaired Gluconeogenesis and are more prone to low blood sugar because the metabolism of alcohol by alcohol dehydrogenase and aldehyde dehydrogenase, forms chemical molecules which results in the deviation of the substrates of Gluconeogenesis to other pathways to lipid accumulation. This results in the fatty liver in the alcoholics and more chances for hypoglycemic suddenly. Thus any disease condition damaging the liver will affect Gluconeogenesis.

## Pyruvate carboxylase deficiency

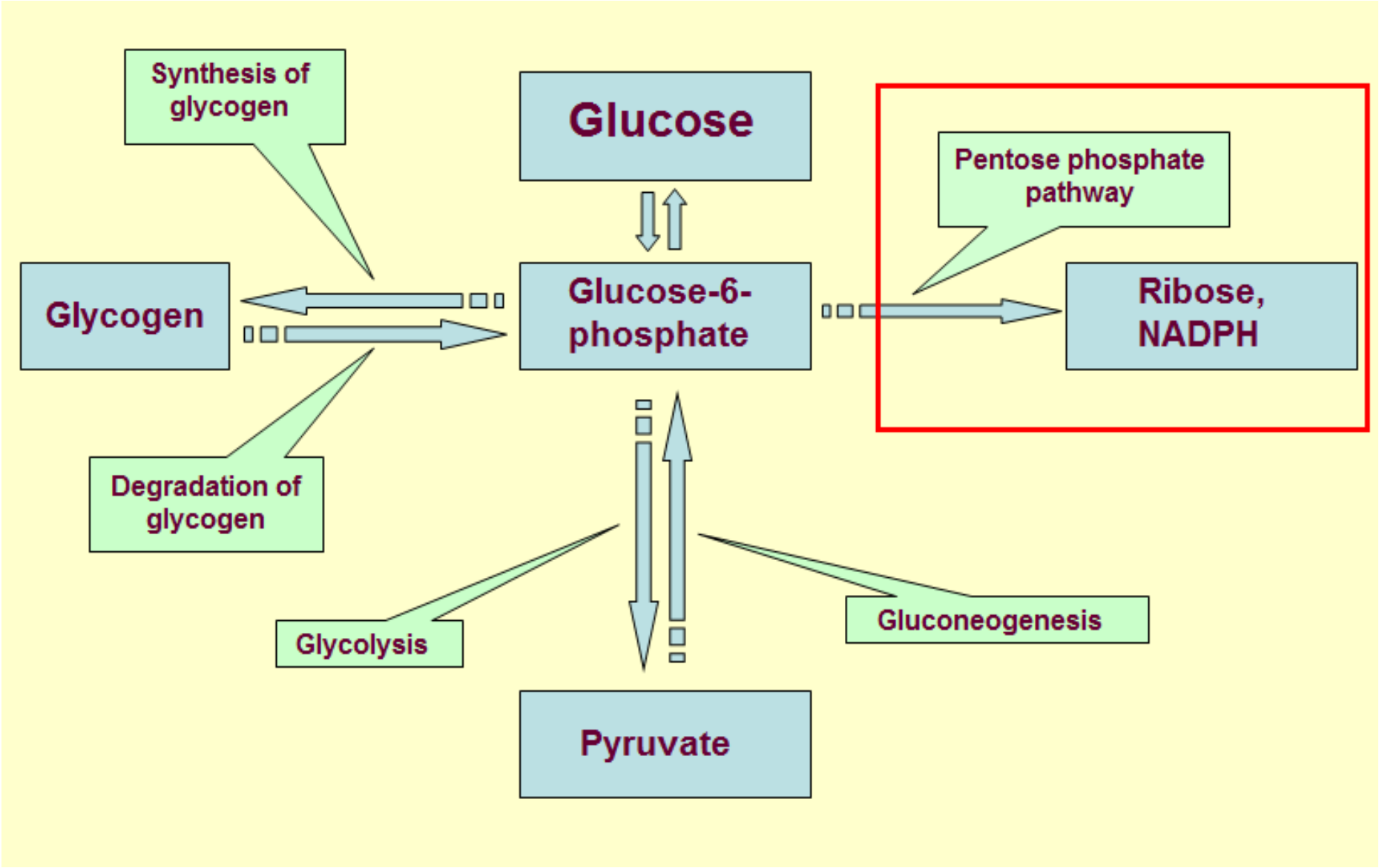
- Pyruvate carboxylase (PC) deficiency is a rare disorder that can cause developmental delay and failure to the neonatal or early infantile period. All patients who develop symptoms in the first weeks and months of life have lactic acidosis.
- PC is a biotin-dependent mitochondrial enzyme that plays an important role in energy production. PC catalyzes the conversion of pyruvate to oxaloacetate. Oxaloacetate is 1 of 2 essential substrates needed to produce citrate, the first substrate in gluconeogenesis.
- PC deficiency results in malfunction of the citric acid cycle and of gluconeogenesis, thereby depriving the body of energy.
- Phosphoenolpyruvate carboxykinase deficiency

impairs gluconeogenesis and results in symptoms and signs similar to the hepatic forms of glycogen storage disease but without hepatic glycogen accumulation.

# The Pentose Phosphate Pathway

By: Inaam A. Ameen

# The fate of glucose molecule in the cell



# The Role of Pentose Phosphate Pathway (phosphogluconate pathway)

- (1) Synthesis of NADPH (for reductive reactions in biosynthesis of fatty acids, neurotransmitter and steroids)
- (2) Synthesis of Ribose 5-phosphate (for the biosynthesis of ribonucleotides (RNA, DNA) and several cofactors)
- (3) Pentose phosphate pathway also provides a means for the metabolism of “unusual sugars”, 4, 5 and 7 carbons.
- Pentose phosphate pathway does not function in the production of high energy compounds like ATP.

# Occurrence of the pentose phosphate pathway

- Liver, mammary and adrenal glands, and adipose tissue
- Red blood cells (NADPH maintains reduced iron)
- NOT present in skeletal muscles.
- All enzymes in the cycle occur in the cytosol

**Tissues with active pentose phosphate pathways**

| Tissue | Function |
|---|---|
| Adrenal gland | Steroid synthesis |
| Liver | Fatty acid and cholesterol synthesis |
| Testes | Steroid synthesis |
| Adipose tissue | Fatty acid synthesis |
| Ovary | Steroid synthesis |
| Mammary gland | Fatty acid synthesis |
| Red blood cells | Maintenance of reduced glutathione |

# The pentose pathway is a shunt

- It is called the pentose phosphate shunt because the pathway allows for carbon atoms from glucose 6-phosphate to take a brief deviation(a shunt) before they continue the glycolytic pathway.

- It is a pathway begins with hexose oxidation in which glucose-6-phosphate (glycolytic intermediate)undergoes two successive oxidations by NADP, the final  forming a pentose phosphate.

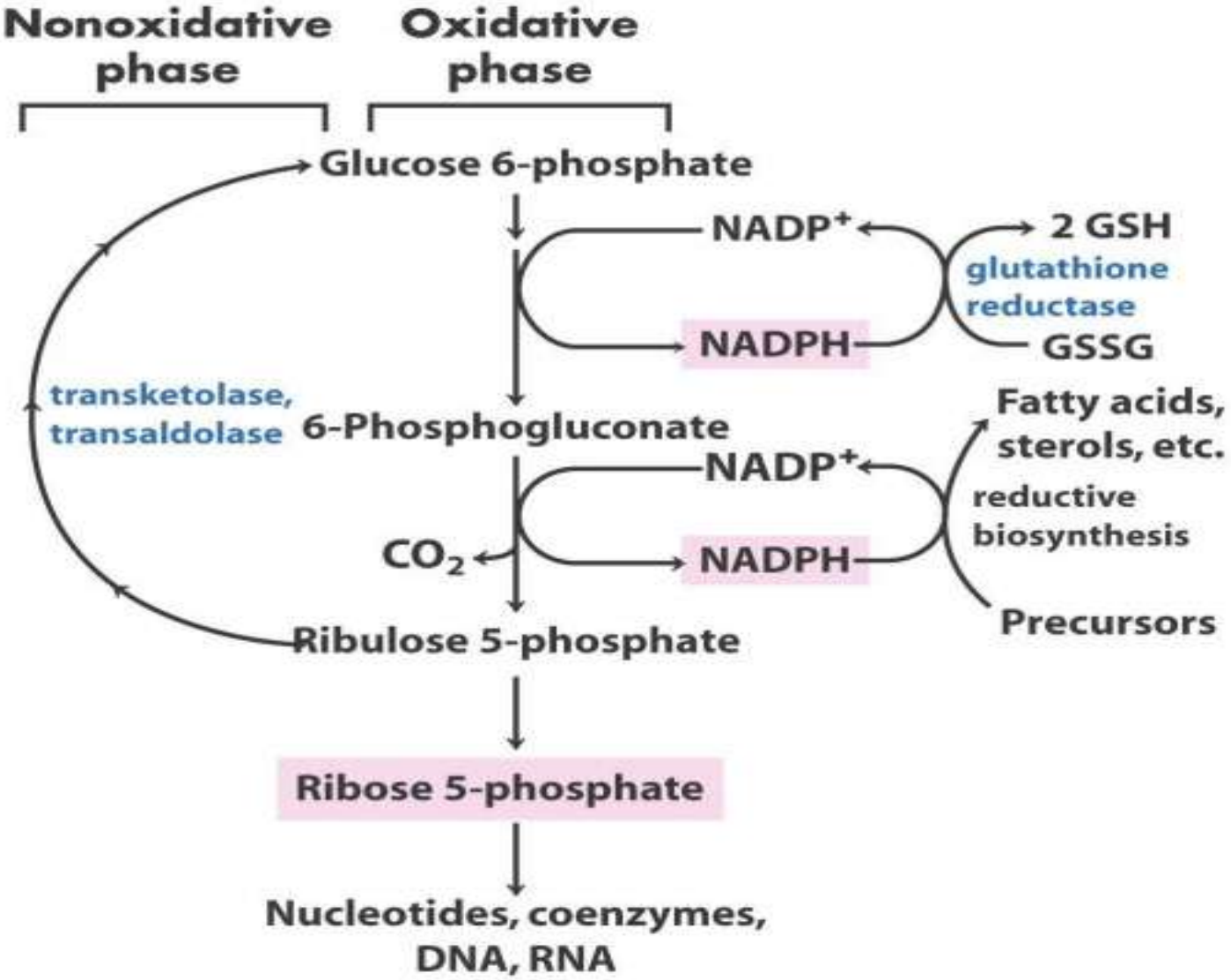
- It reconnects with glycolysis because two of the end products of the pentose pathway are glyceraldehyde 3-P and fructose 6-P; two intermediates further down in the glycolytic pathway.

## The carry out of pentose phosphate pathway for the cell

- The oxidative phase generates NADPH which is required for many biosynthetic pathways and for detoxification of reactive oxygen species.

- The nonoxidative phase interconverts $C_3$, $C_4$, $C_5$, $C_6$ and $C_7$ monosaccharides to produce ribose-5P for nucleotide synthesis, and also to regenerate glucose-6P to maintain NADPH production by the oxidative phase.

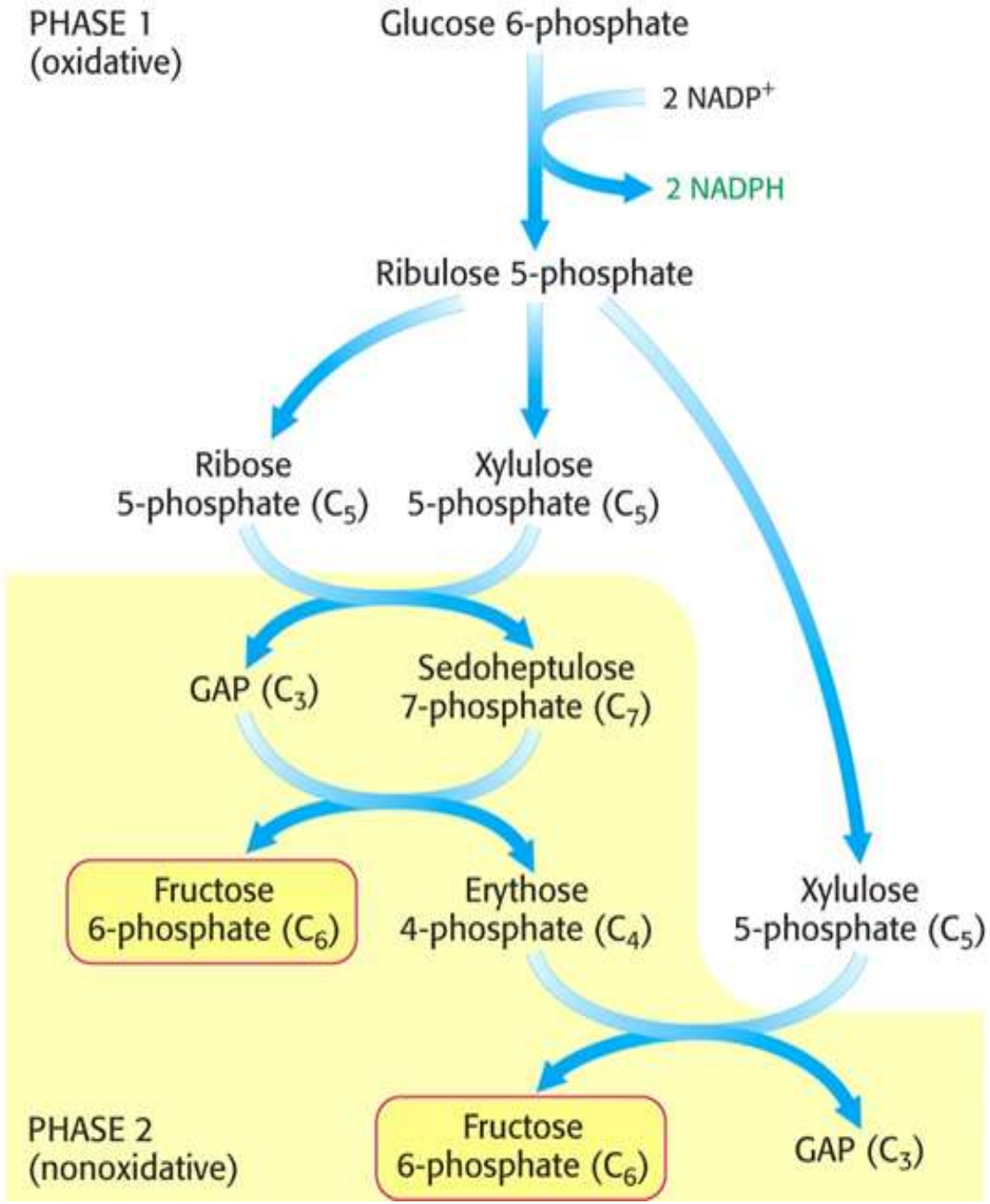
## Two Phases of the Pentose Pathway



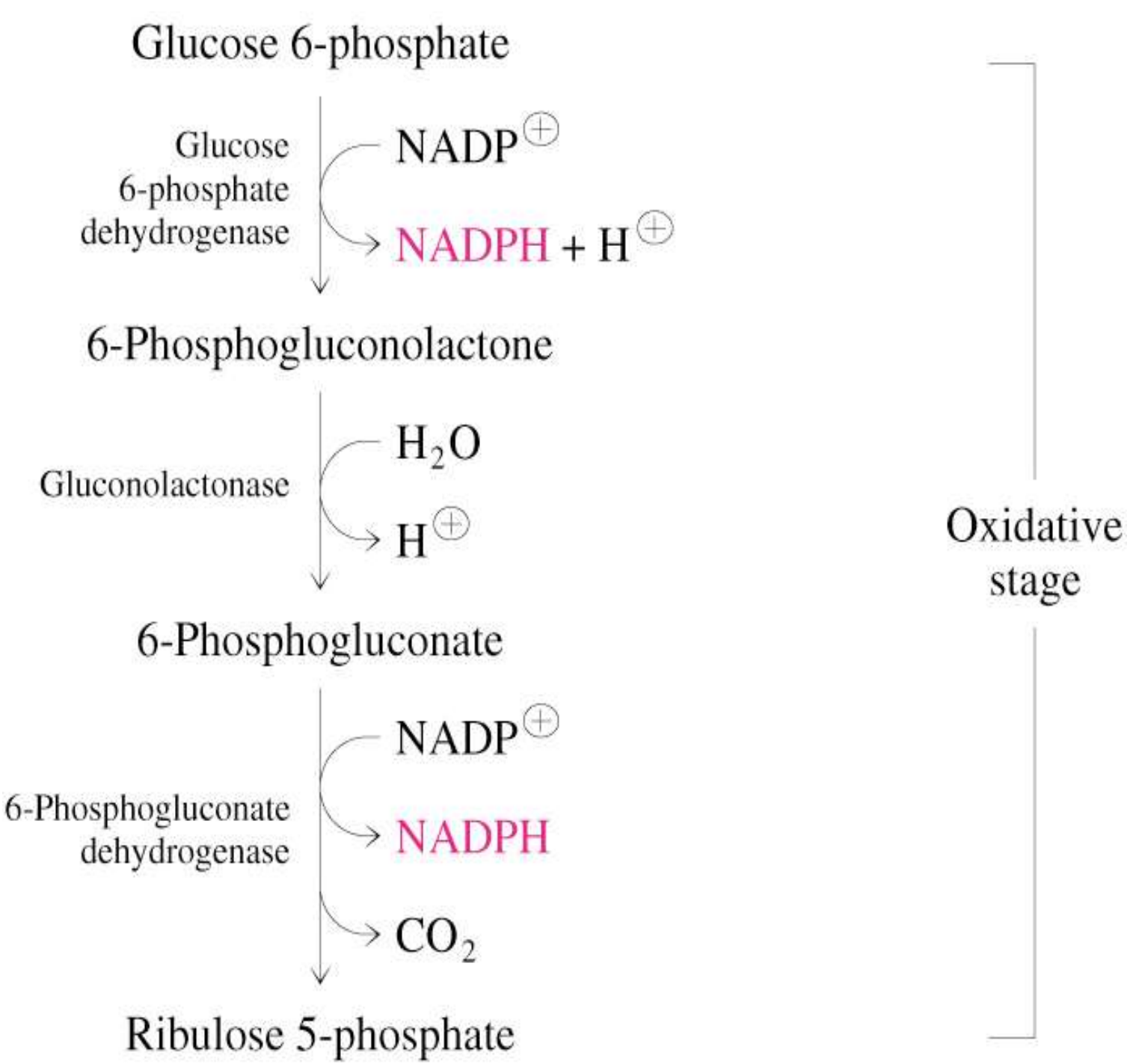
# Two phases:

1) The oxidative phase that generates NADPH

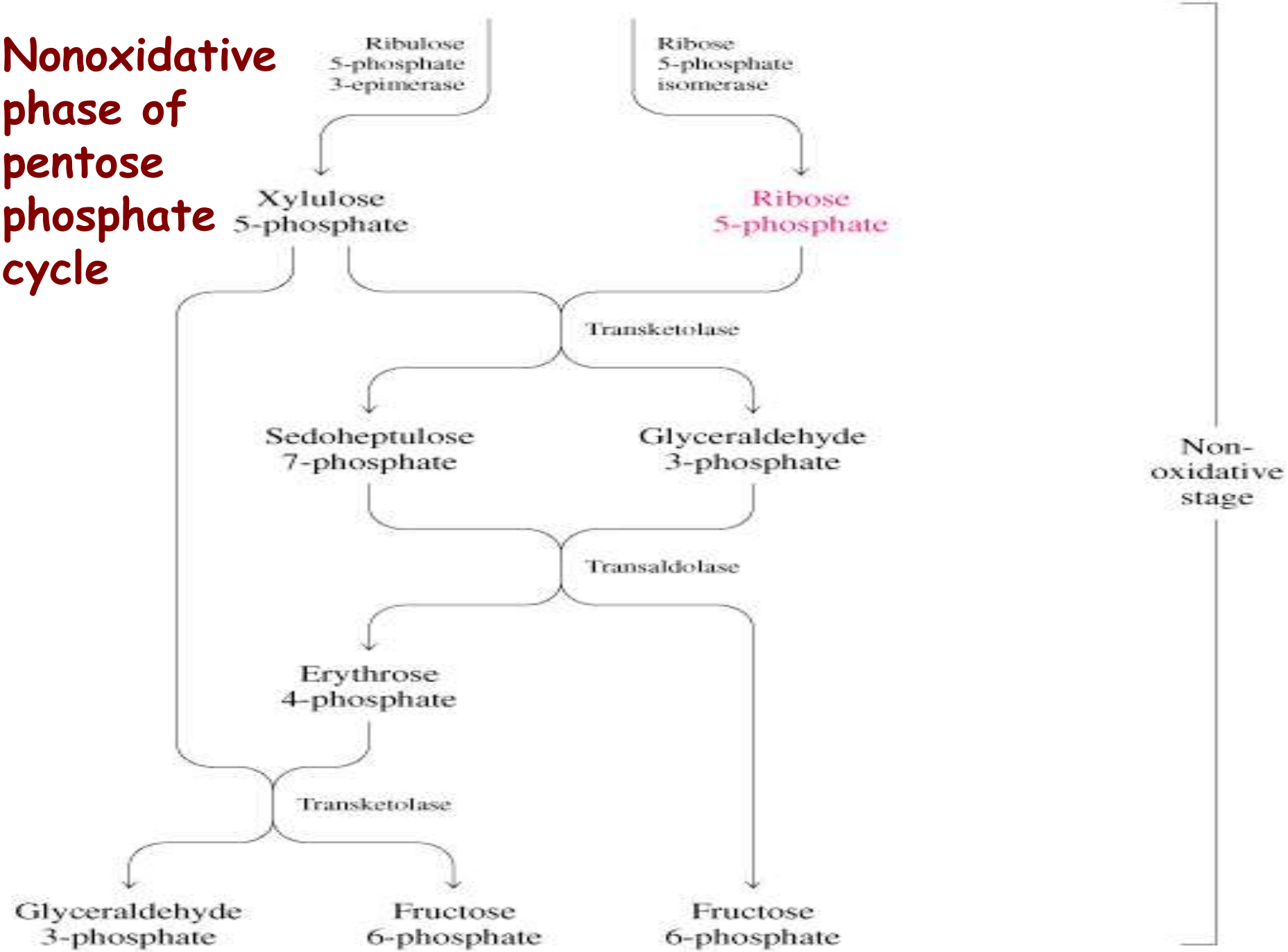
2) The nonoxidative phase (transketolase/ transaldolase system) that interconvert phosphorylated sugars.



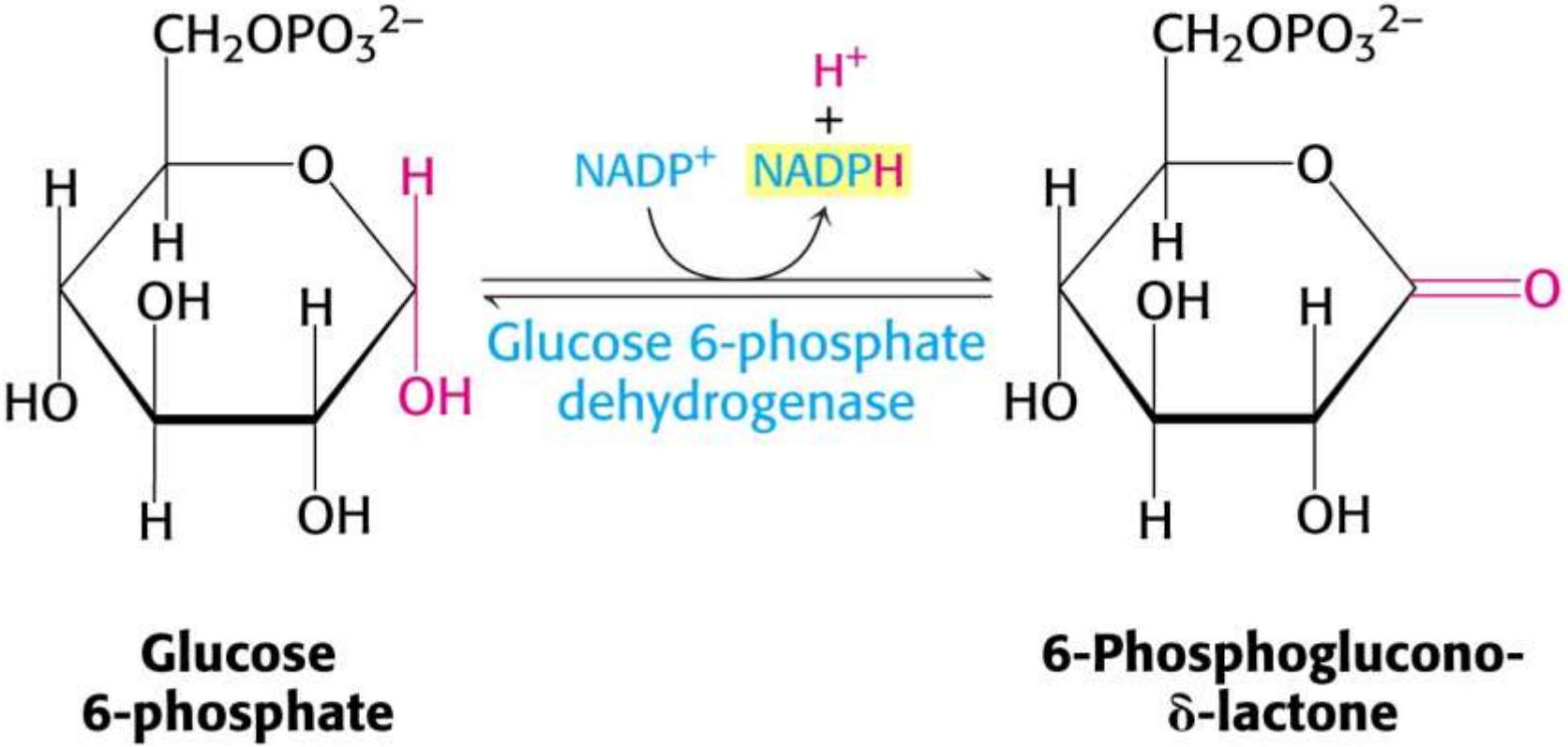
**Oxidative phase of pentose phosphate cycle**



# Nonoxidative phase of pentose phosphate cycle



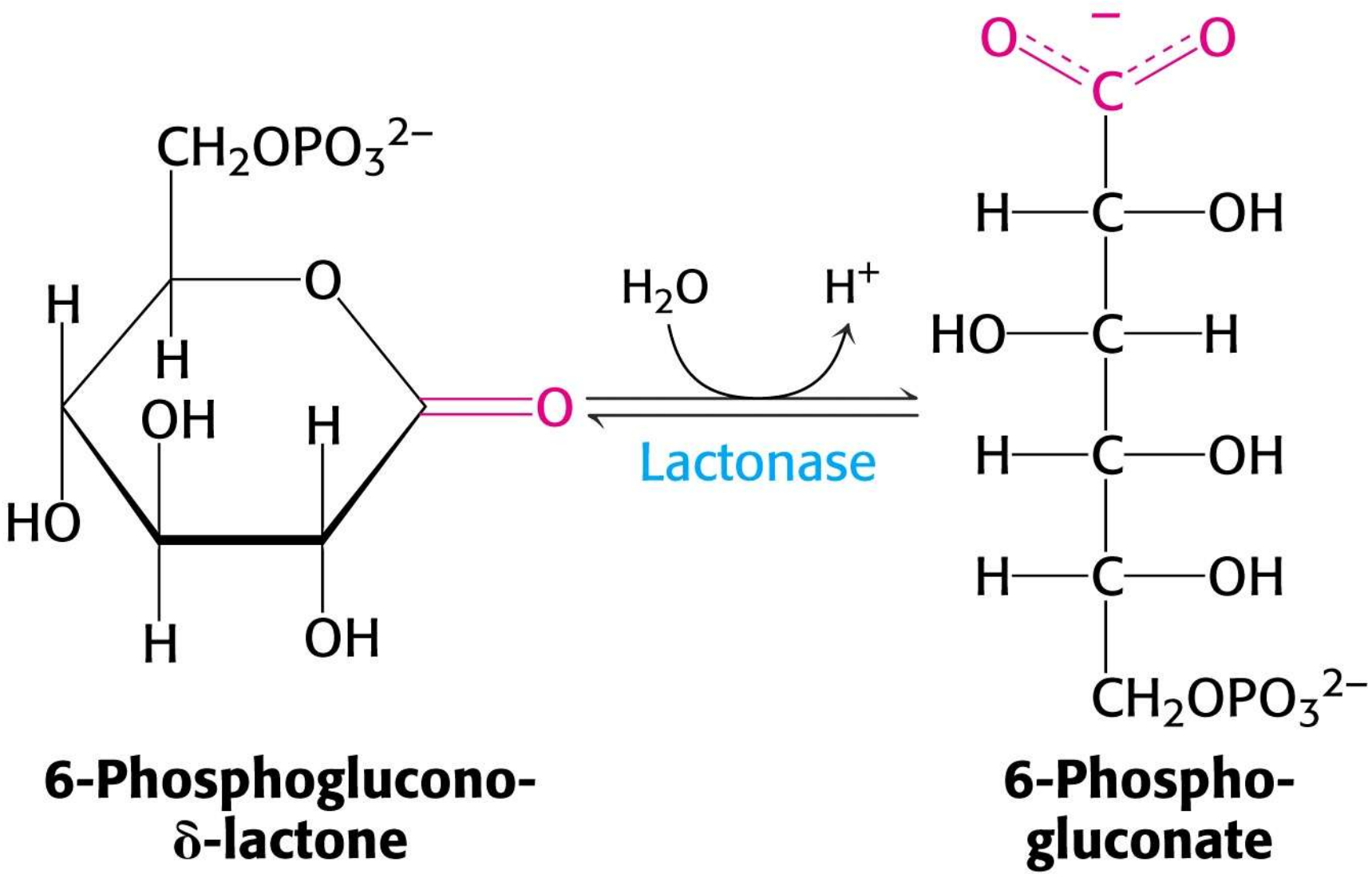
# Conversion of glucose-6-phosphate to 6-phosphogluconolactone



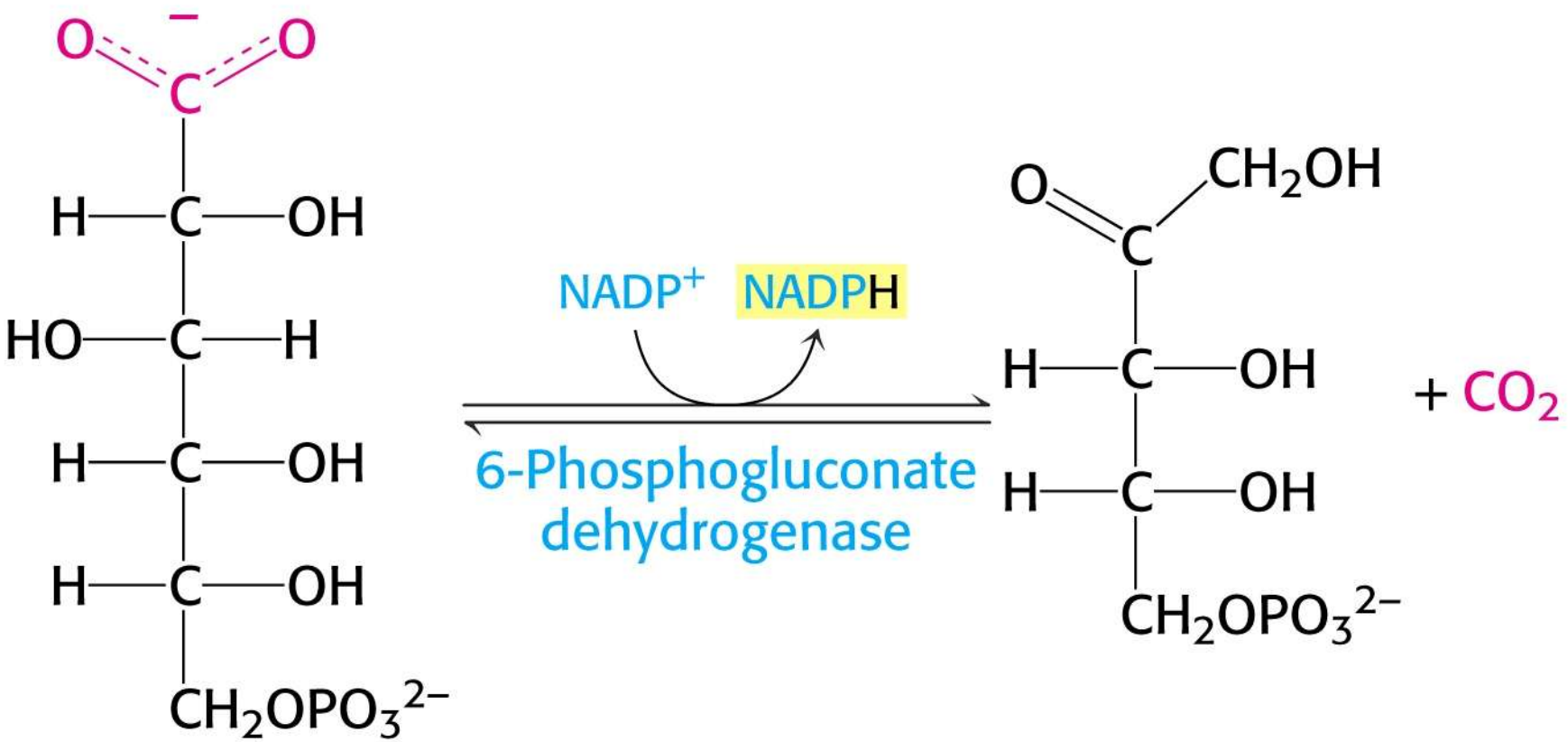

**Glucose 6-phosphate**

**6-Phosphoglucono-δ-lactone**

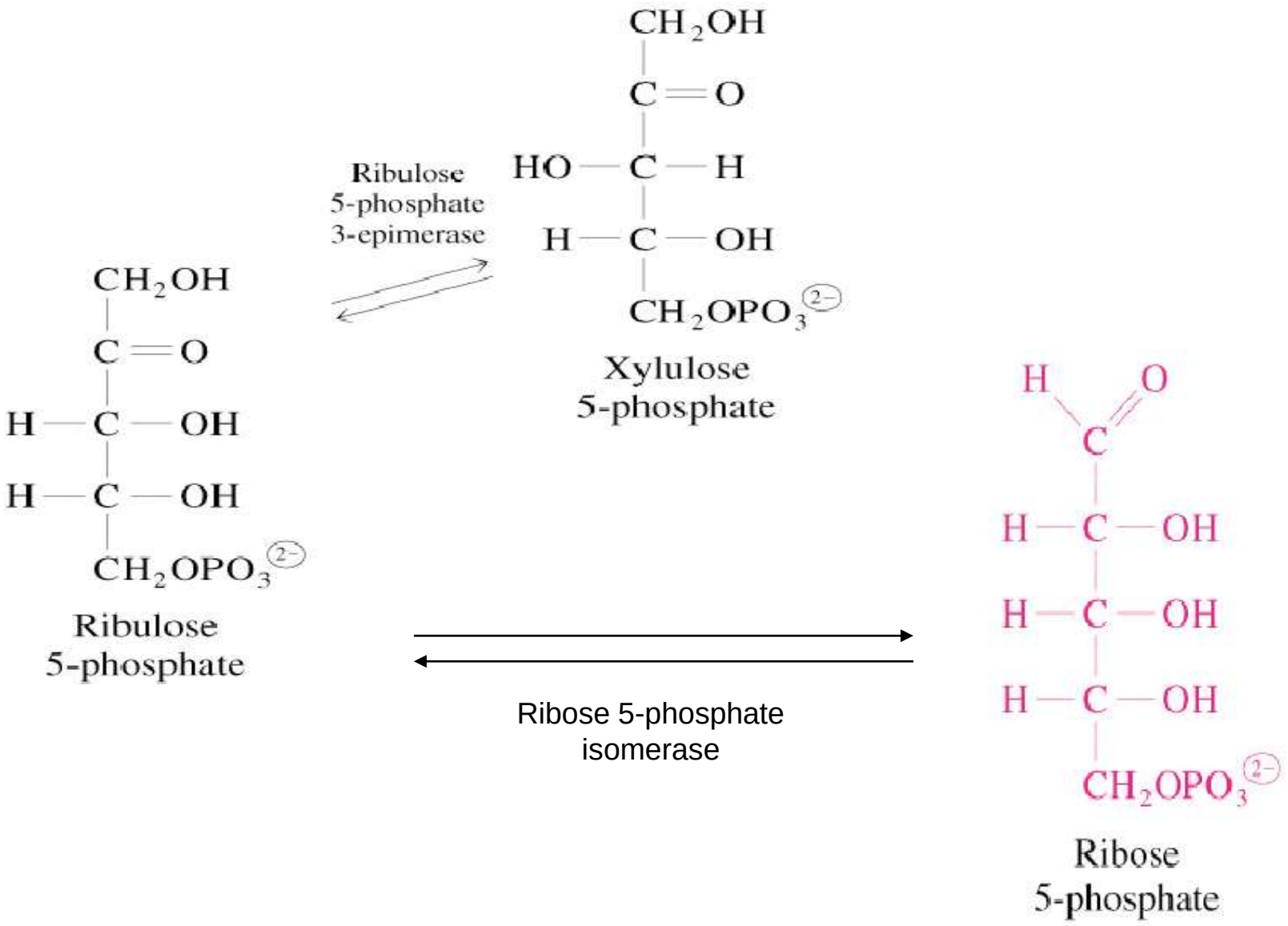
# Conversion of 6-phosphogluconolactone to 6-phosphogluconate



# Conversion of 6-phosphogluconate to ribuloso 5-phosphate



# Conversions of ribulose 5-phosphate



The pentose phosphate pathway ends with these five reactions in some tissue.

In others it continue in nonoxidative mode to make fructose 6-phosphate and glyceraldehyde 3-phosphate. These reactions link pentose phosphate pathway with glycolysis.

## The net reaction for the pentose phosphate pathway

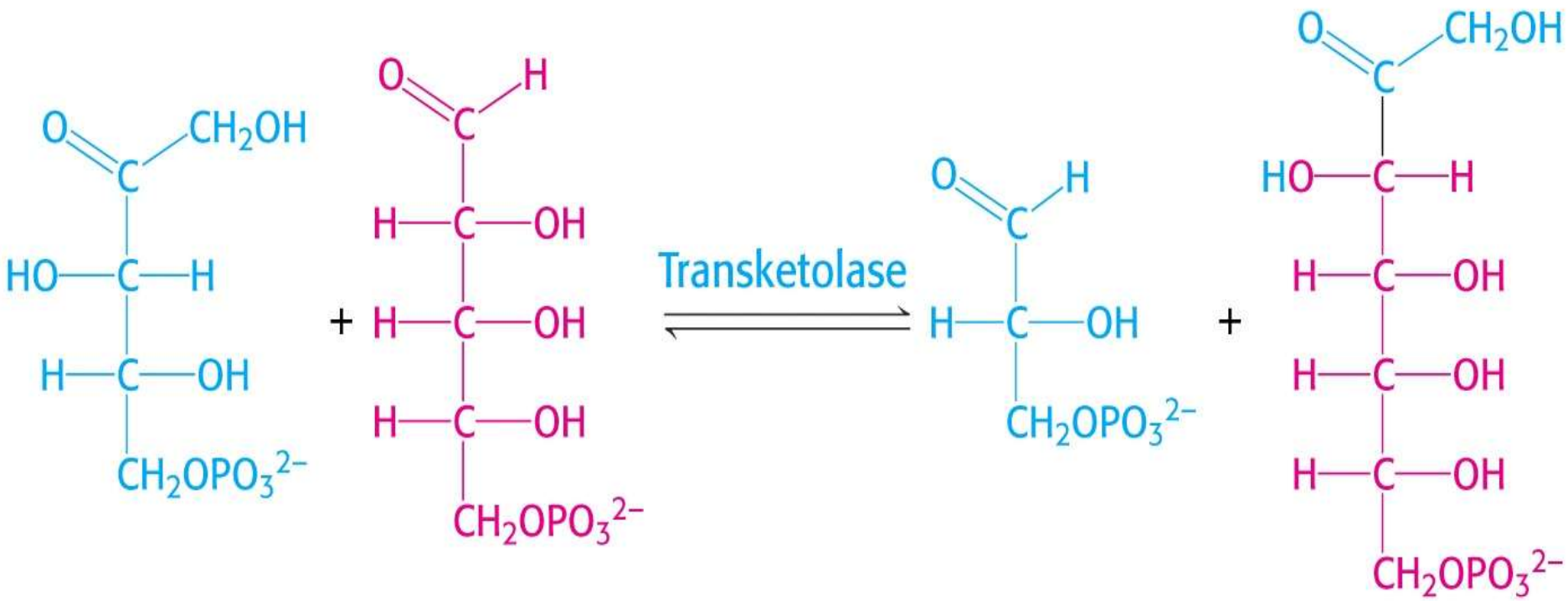
$$\text{Glucose} + \text{ATP} + 2\text{NADP}^+ + \text{H}_2\text{O} \longrightarrow$$

$$\text{ribose 5-phosphate} + \text{CO}_2 + 2\text{NADPH} + 2\text{H}^+ + \text{ADP}$$

## Interconversions Catalyzed by Transketolase and Transaldolase

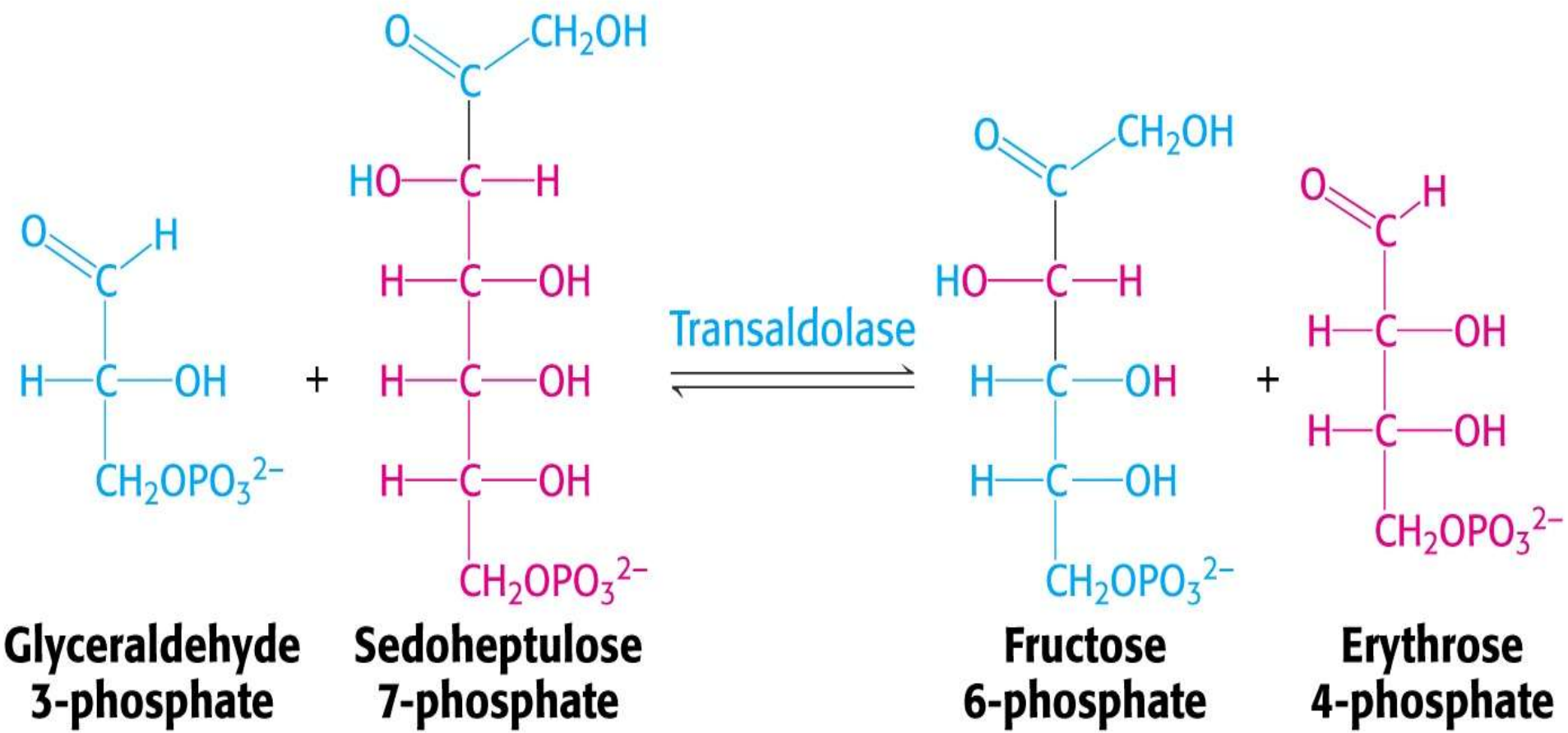
- Transketolase and transaldolase have broad substrate specificities
- They catalyze the exchange of two- and three-carbon fragments between sugar phosphates
- For both enzymes, one substrate is an aldose, one substrate is a ketose

# Reaction catalyzed by transketolase

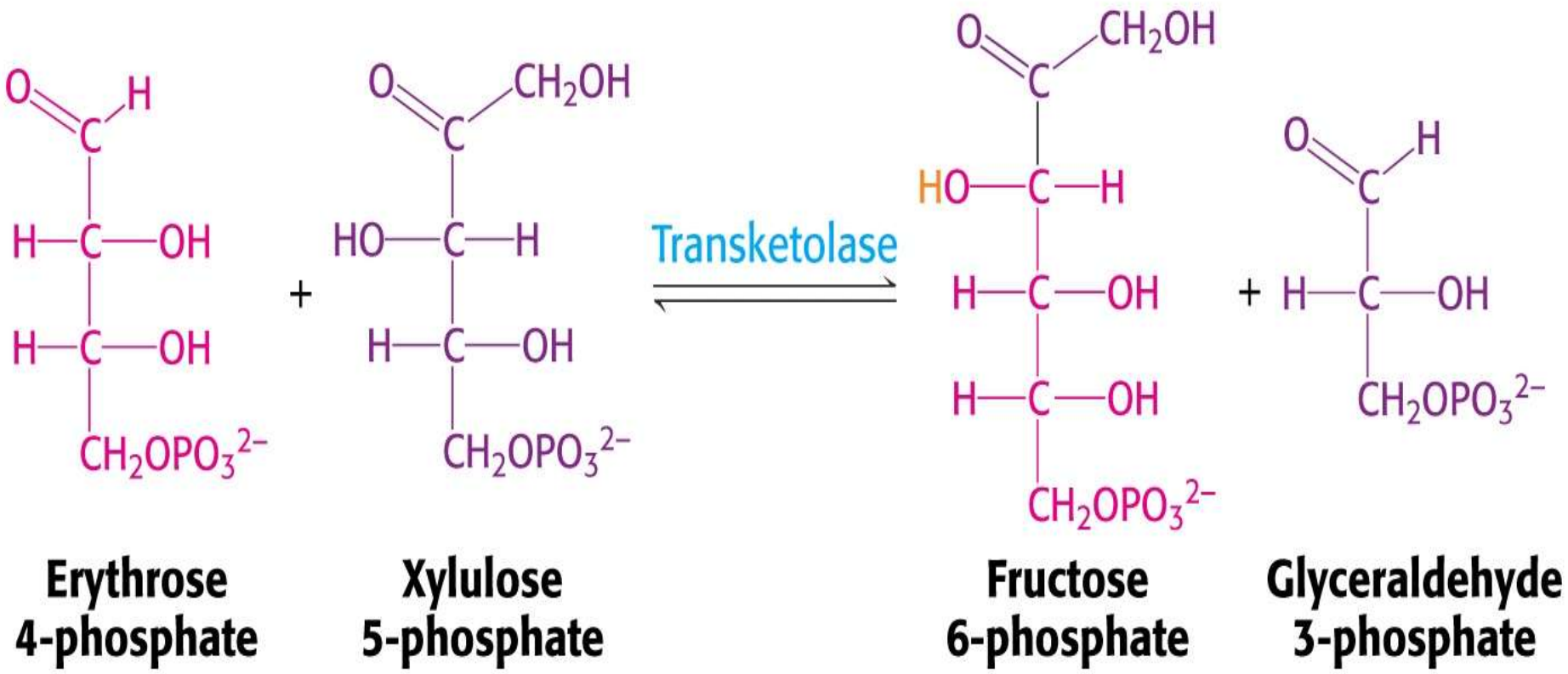



**Xylulose 5-phosphate** + **Ribose 5-phosphate** ⇌ **Glyceraldehyde 3-phosphate** + **Sedoheptulose 7-phosphate**

# Reaction catalized by transaldolase



# Reaction catalyzed by transketolase

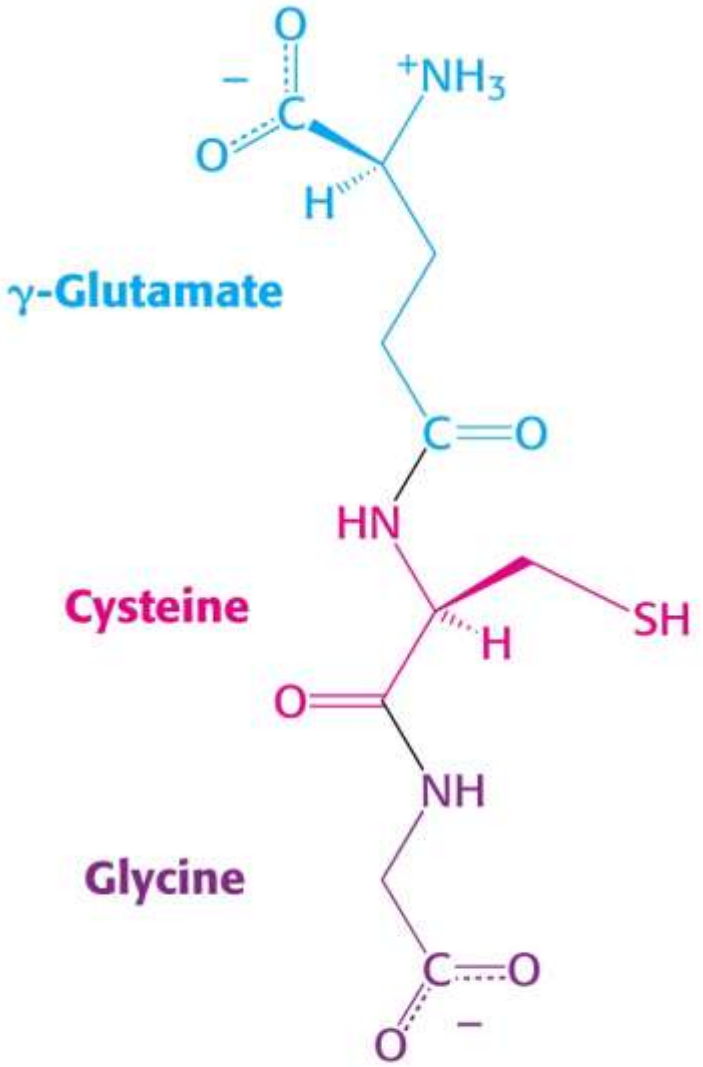


# Glucose-6-phosphate dehydrogenase deficiency

NADPH is required for the proper action of the tripeptide glutathione (GSH) (maintains it in the reduced state).

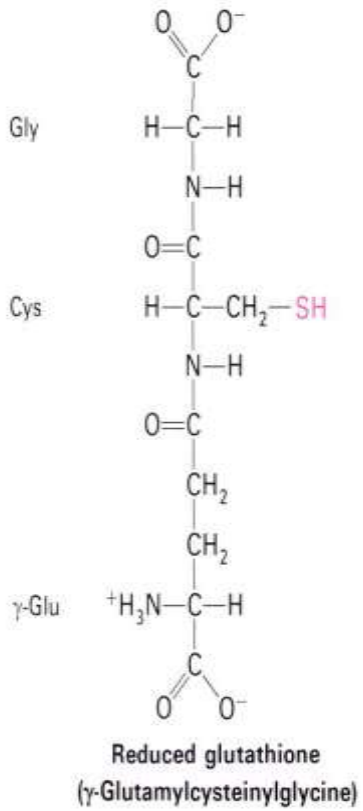
GSH in erythrocytes maintains hemoglobin in the reduced Fe(II) state necessary for oxygen binding.

GSH also functions to eliminate $H_2O_2$ and organic peroxides.
Peroxides can cause irreversible damage to hemoglobin and destroy cell membranes.




**Glutathione (reduced)**
**(γ-Glutamylcysteinylglycine)**

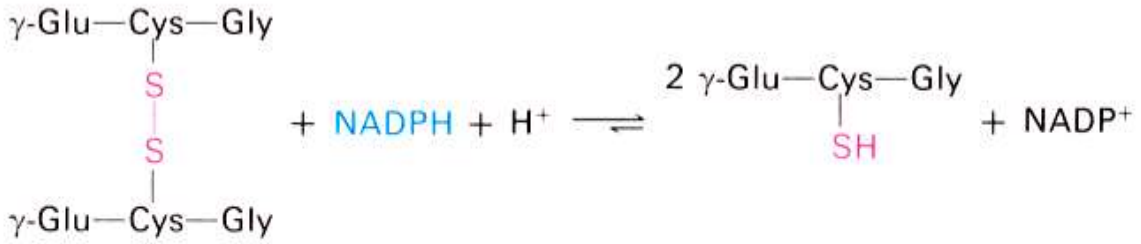
# Role of Glucose 6-phosphate Dehydrogenase in the Red Blood Cell



Reduced glutathione
(γ-Glutamylcysteinylglycine)

In the RBC glucose serves as the primary energy source. RBC's lack mitochondria and thus lack the enzymes of the citric acid cycle. Therefore, glucose is metabolized completely by the glycolytic pathway (90%) and the pentose phosphate pathway (10%).

The most important function of the pentose phosphate pathway in the RBC is to maintain the tripeptide glutathione in a reduced state. Oxidized glutathione is reduced by the enzyme glutathione reductase in a reaction which utilizes NADPH:

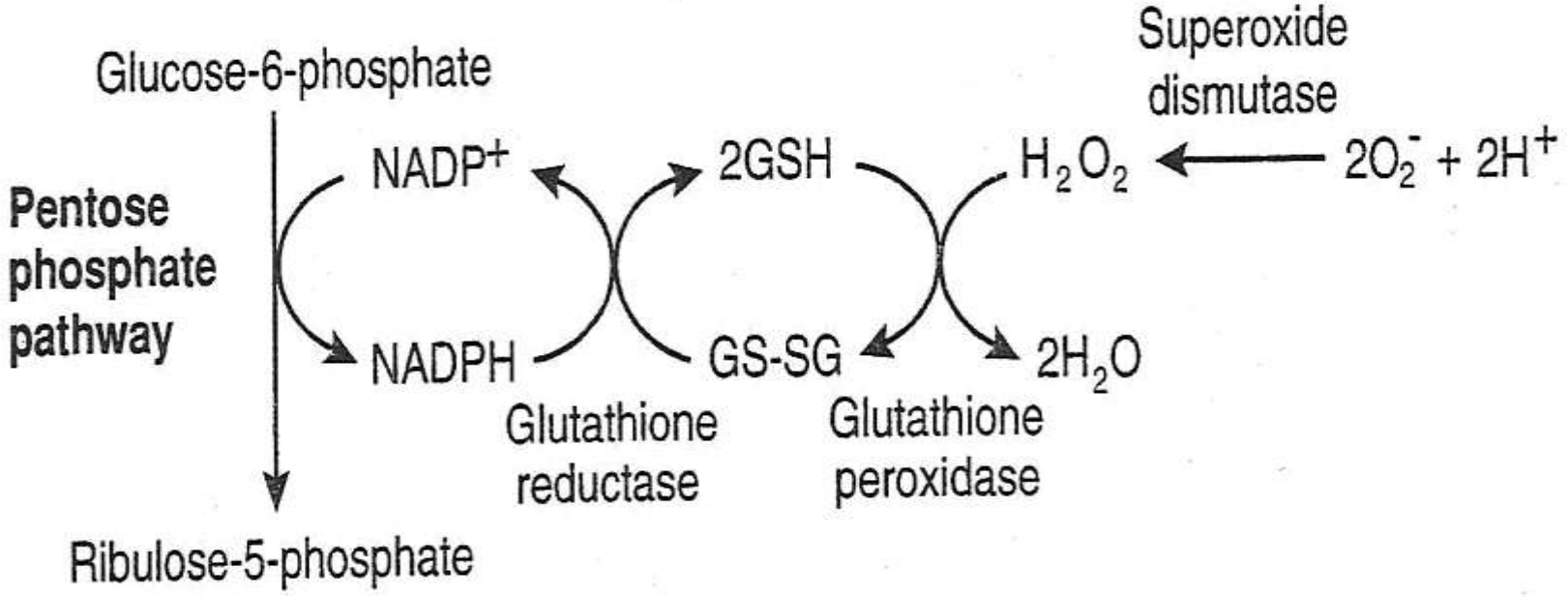


Oxidized glutathione (GSSG)

Reduced glutathione (GSH)

# Detoxification of Superoxide Anion and Hydrogen Peroxide

- Antioxidant enzymes
  - Superoxide dismutase
  - Glutathione peroxidase
  - Glutathione reductase



**Glucose-6-phosphate dehydrogenase deficiency** – **the most common enzymopathy** affecting hundreds of millions of people.

- • Mutations present in some populations causes a deficiency in glucose 6-phosphate dehydrogenase, with consequent impairment of NADPH production.
- • Detoxification of H2O2 is inhibited, and cellular damage results - lipid peroxidation leads to erythrocyte membrane breakdown and hemolytic anemia.
- Exposure to anti-malarial drugs (Primaquine) results in increased cellular production of superoxide and hydrogen peroxide (Primaquine sensitivity)
- In severe cases, the massive destruction of red blood cells causes death.
- Other chemicals known to increase oxidant stress
  - Sulfonamides (antibiotic)
  - Asprin and NSAIDs
  - Quinadine and quinine
  - Napthlane (mothballs)
  - Fava beans (vicine & isouramil)

# Regulation of pentose phosphate pathway

• The entry of glucose 6-phosphate into the pentose phosphate pathway is controlled by the cellular concentration of NADPH

• NADPH is a strong inhibitor of glucose 6- phosphate dehydrogenase

• As NADPH is used in various pathways, inhibition is reduced, and the enzyme is accelerated to produce more NADPH

• The synthesis of glucose 6-phosphate dehydrogenase is induced by the increased insulin/glucagon ratio after a high carbohydrate

- Glucose-6-P dehydrogenase
  - First step
  - Rate limiting
- Allosteric Regulation
  - Feedback inhibited by NADPH
- Inducible enzyme
  - Induced by insulin

# SUMMARY

- The pentose phosphate pathway, present in the cytosol, can account for the complete oxidation of glucose, producing NADPH and $CO_2$ but not ATP.

- The pathway has an oxidative phase, which is irreversible and generates NADPH; and a nonoxidative phase, which is reversible and provides ribose precursors for nucleotide synthesis.

The complete pathway is present only in those tissues having a requirement for NADPH like, lipogenesis or steroidogenesis, whereas the nonoxidative phase is present in all cells requiring ribose.

- In erythrocytes, the pathway has a major function in preventing hemolysis by providing NADPH to maintain glutathione in the reduced state as the substrate for glutathione peroxidase.