الحياتية (3)
المرحلة الرابعة /الفصل الدراسي الأول
Carbohydrate Metabolism (Lec.1)
by Nada Abotimen

Bioenergetics and Metabolism

Living organisms can be divided into two large groups according to the chemical form in which they obtain carbon from the environment:

Autotrophs (such as photosynthetic bacteria and vascular plants) can use carbon dioxide from the atmosphere as their sole source of carbon, from which they construct all their carbon containing biomolecules. Some autotrophic organisms, such as cyanobacteria, can also use atmospheric nitrogen to generate all their nitrogenous components.

Heterotrophs cannot use atmospheric carbon dioxide and must obtain carbon from their environment in the form of relatively complex organic molecules such as glucose.
Multicellular animals and most microorganisms are heterotrophic.

Autotrophic cells and organisms are relatively **self-sufficient**, whereas **heterotrophic** cells and organisms, with their requirements for carbon in more complex forms, must **subsist** on the products of other organisms.
Many **autotrophic** organisms are photosynthetic and obtain their energy from sunlight, whereas **heterotrophic** organisms obtain their energy from the degradation of organic nutrients produced by autotrophs.
In our biosphere, autotrophs and heterotrophs live together in a vast, interdependent cycle in which autotrophic organisms use atmospheric carbon dioxide to build their organic biomolecules, some of them generating oxygen from water in the process. Heterotrophs in turn use the organic products of autotrophs as nutrients and return carbon dioxide to the atmosphere.
Some of the oxidation reactions that produce carbon dioxide also consume oxygen, converting it to water. Thus carbon, oxygen, and water are constantly cycled between the heterotrophic and autotrophic worlds, with solar energy as the driving force for this global process (Fig. 1).

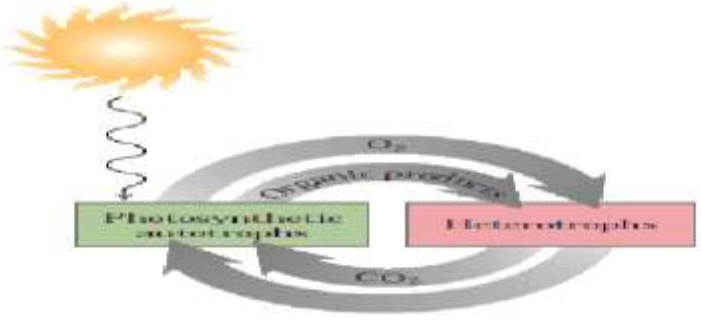


Figure (1) : Cycling of carbon dioxide and oxygen between the autotrophic (photosynthetic) and heterotrophic domains in the biosphere.

All living organisms also require a source of nitrogen, which is necessary for the synthesis of **amino acids, nucleotides,** and other compounds. **Plants** can generally use either **ammonia** or **nitrate** as their sole source of nitrogen, but vertebrates must obtain nitrogen in the form of **amino acids** or other organic compounds. Only a few organisms—the **cyanobacteria** and many species of soil bacteria that live symbiotically on the roots of some plants—are capable of converting **("fixing")** atmospheric nitrogen (N2) into ammonia. Other bacteria (the nitrifying bacteria) oxidize ammonia to nitrites and nitrates; yet others convert nitrate to N2.

Thus, in addition to the global carbon and oxygen cycle, a nitrogen cycle operates in the biosphere, turning over huge amounts of nitrogen (Fig. 2).

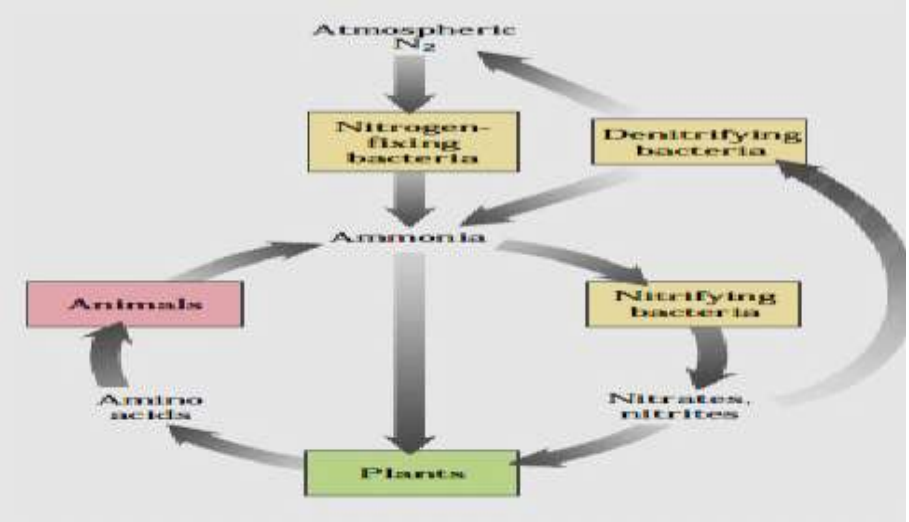


Figure (2): Cycling of nitrogen in the biosphere.

Bioenergetics and Oxidative Phosphorylation

Bioenergetics describes the transfer and utilization of energy in biologic systems. It makes use of a few basic ideas from the field of thermodynamics, particularly the concept of free energy. Changes in free energy (ΔG) provide a measure of the energetic feasibility of a chemical reaction and can, therefore, allow prediction of whether a reaction or process can take place. Bioenergetics concerns only the initial and final energy states of reaction components, not the mechanism or how much time is needed for the chemical change to take place. In short, bioenergetics predicts if a process is possible, whereas kinetics measures how fast the reaction occurs.

Free Energy Change

The change in free energy comes in two forms, ΔG and ΔG^o. The first, ΔG (without the superscript "o"), is the more general because it predicts the change in free energy and, thus, the direction of a reaction at any specified concentration of products and reactants. This contrasts with the change in standard free energy, ΔG^o (with the

superscript “o”), which is the energy change when reactants and products are at a concentration of 1 mol/L.

Sign of ΔG predicts the direction of a reaction

- **ΔG is zero:** If ΔG = 0, the reactants are in equilibrium.

ATP (Adenosine tri phosphate) as An Energy Carrier

Reactions or processes that have a large positive ΔG, such as moving ions against a concentration gradient across a cell membrane, are made possible by coupling the endergonic movement of ions with a second, spontaneous process with a large negative ΔG, such as the hydrolysis of adenosine triphosphate (ATP).

Glucose + ATP → Glucose-6- phosphate + ADP ΔG^{o} **= -4,000 cal/mole**

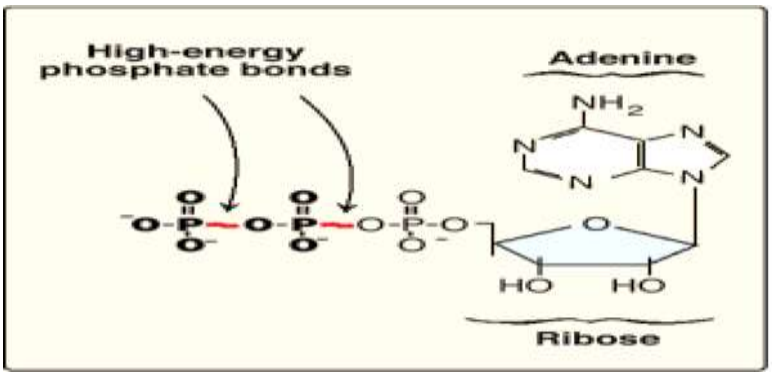
Glucose-6- phosphate → Fructose-6-phosphate ΔG^{o} **= +400 cal/mole**

Glucose + ATP → Fructose-6-phosphate + ADP ΔG^{o} **= -3,600 cal/mole**

Reactions are coupled through common intermediates

Two chemical reactions have a common intermediate when they occur sequentially so that the product of the first reaction is a substrate for the second.

Many coupled reactions use ATP to generate a common intermediate. These reactions may involve ATP cleavage—that is, the transfer of a phosphate group from ATP to another molecule. Other reactions lead to ATP synthesis by transfer of phosphate from an energy-rich intermediate to adenosine diphosphate (ADP), forming ATP.



Figure(3) : Adenosine triphosphate

There are many chemical reactions in a cell that have $\Delta G^{o}(+)$ and will not proceed in a left to right direction without assistance . In general , **an endergonic reaction maybe coupled with an exergonic reaction so that energy is delivered to the endergonic reaction.**

Glucose + pi → Glucose -6-p **ΔG= +3.3 Kcal**
ATP → ADP + pi **ΔG = - 7.3 Kcal**

Glucose + ATP → Glucose -6-p + ADP **ΔG = - 4 Kcal**

Energy carried by ATP

ATP consists of a molecule of adenosine (adenine + ribose) to which three phosphate groups are attached (Fig.3) . If one phosphate is removed, ADP is produced; if two phosphates are removed, adenosine monophosphate (AMP) results. The standard free energy of hydrolysis of ATP, ΔG^{o}, is approximately -7.3 kcal/mol for each of the two terminal phosphate groups. Because of this large, negative ΔG^{o}, ATP is called a **high-energy phosphate compound**.

High energy compounds

High energy compounds are often complex phosphate esters that yield large amount of free energy on hydrolysis. High energy compounds used in synthesis cellular process and in driving other reactions.

ATP $\xrightarrow{\text{hydrolysis}}$ **ADP + pi** $\Delta G^{o} = -7.3$ **Kcal**

ADP $\xrightarrow{\text{hydrolysis}}$ **AMP + pi** $\Delta G^{o} = -6.6$ **Kcal**

AMP $\xrightarrow{\text{hydrolysis}}$ **adenosine + pi** $\Delta G^{o} = -2.2$ **Kcal**

The major reason for the release of energy involves the type of bond structure in these compounds. The phosphate ester bonds in ATP are anhydride linkage that have closely spaced negative charges , which repel each other strongly .Some of this electrical stress is relieved on hydrolysis.
In the intracellular fluid, which contains high concentration of Mg^{+2}, ATP and ADP exist as :

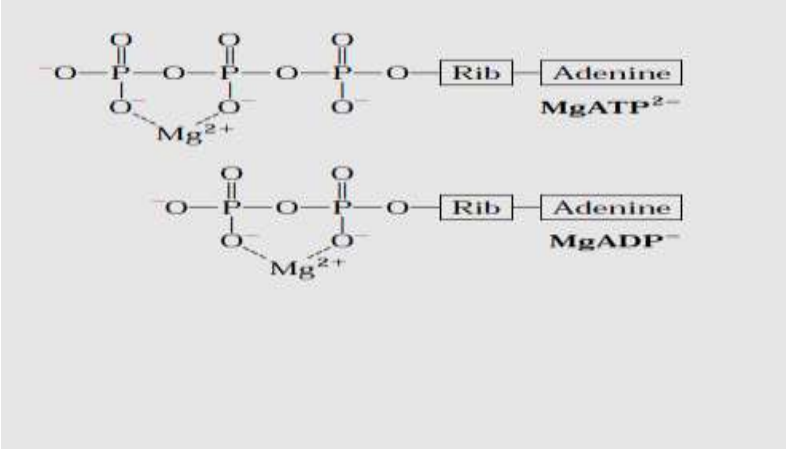


Figure (4) : Formation of Mg^{+2}_ complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP.

The value for the hydrolysis of the terminal phosphate of ATP divides the list into two groups .**Low-energy phosphates,** exemplified by the ester phosphates found in the intermediates of glycolysis, have $\Delta G^{0\prime}$ values smaller than that of ATP, while in **high-energy phosphates** the value is higher than that of ATP. The components of this latter group, including ATP, are usually anhydrides (eg, the 1-phosphate of 1,3-bisphosphoglycerate), enolphosphates (eg, phosphoenolpyruvate), and phosphoguanidines (eg, creatine phosphate, arginine phosphate).

The intermediate position of ATP allows it to play an important role in energy transfer. The high free energy change on hydrolysis of ATP **is due to relief of charge repulsion of adjacent negatively charged oxygen atoms and to stabilization of the reaction products, especially phosphate, as resonance hybrids.** Other "high-energy compounds" are thiol esters involving coenzyme A (eg, acetyl-CoA), acyl carrier protein, amino acid esters involved in protein synthesis, *S*-adenosylmethionine (active methionine), UDPGlc (uridine diphosphate glucose), and PRPP (5-phosphoribosyl-1-pyrophosphate).

Table 10–1. Standard free energy of hydrolysis of some organophosphates of biochemical importance.[1,2]

Compound	$\Delta G^{0\prime}$ kJ/mol	$\Delta G^{0\prime}$ kcal/mol
Phosphoenolpyruvate	−61.9	−14.8
Carbamoyl phosphate	−51.4	−12.3
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	−49.3	−11.8
Creatine phosphate	−43.1	−10.3
ATP → ADP + P_i	−30.5	−7.3
ADP → AMP + P_i	−27.6	−6.6
Pyrophosphate	−27.6	−6.6
Glucose 1-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
AMP	−14.2	−3.4
Glucose 6-phosphate	−13.8	−3.3
Glycerol 3-phosphate	−9.2	−2.2

High-energy phosphates act as the "Energy currency" of the cell

ATP is able to act as a donor of high-energy phosphate to form those compounds below it in Table 10–1. Likewise, with the necessary enzymes, ADP can accept high-energy phosphate to form ATP from those compounds above ATP in the table. In effect, an **ATP/ ADP cycle** connects those processes that generate ~_P to those processes that utilize ~_P **(Figure 10–6)**, continuously consuming and regenerating ATP. This occurs at a very rapid rate, since the total ATP/ADP pool is extremely small and sufficient to maintain an active tissue for only a few seconds.

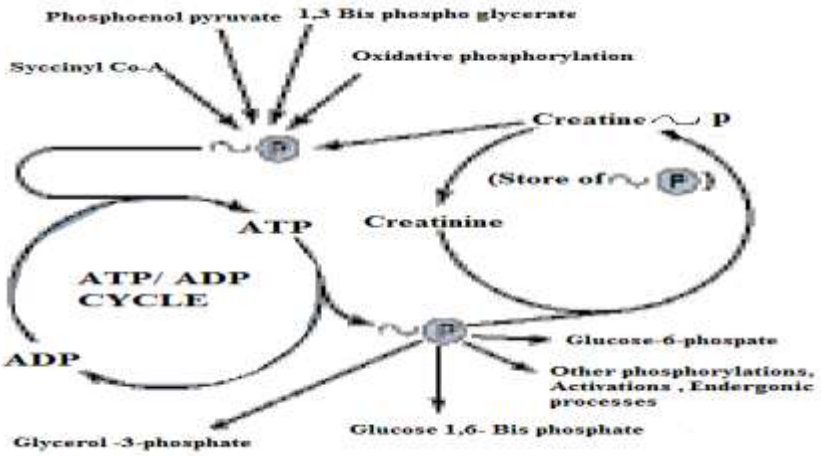


Figure (5): Role of ATP/ADP cycle in transfer of high-energy phosphate.

The formation of ATP

1- Substrate level phosphorylation

A-Glycolysis: A net formation of **two ~_P** results from the formation of lactate from one molecule of glucose, generated in two reactions. These reactions can take place in the absence of O_2 and in the cytoplasm.

1,3 –Bis phosphoglycerate + ADP $\xrightarrow{\text{Phosphoglycero kinase}}$ **3- Phospho glycerate + ATP**

Phosphoenol pyruvate + ADP $\xrightarrow{\text{Pyruvate kinase}}$ **pyruvate + ATP**

B- The citric acid cycle: One ~_P is generated directly in the cycle at the succinyl thiokinase step in mitochondria.

Succinyl- CoA + GDP + Pi $\xrightarrow{\text{Succinyl thiokinase}}$ **Succinate + GTP**

GTP + ADP → GDP +ATP

2- Oxidative phosphorylation: The greatest quantitative source of **~_P** in aerobic organisms. Free energy comes from respiratory chain oxidation using molecular O_2 within mitochondria.
In electron transport system in the mitochondria of the cell actively transports electrons from a reduced metabolite to oxygen with the assistance of enzymes and coenzymes (NAD, FMN and FAD)

NAD + Metabolite.H_2 → Metabolite + NADH + H^+

NADH + 3 ADP + 3 Pi + 1/2 O_2 → NAD + 3 ATP + H_2O

Three moles of ATP may be generated by the passage of electrons from a mole of substrate through **NADH** to molecular oxygen, but only **two moles of ATP** may be generated from the substrate through **$FADH_2$** to molecular oxygen.

Phosphagens act as storage forms of high-energy phosphate and include creatine phosphate, occurring in vertebrate skeletal muscle, heart, spermatozoa, and brain; and arginine phosphate, occurring in invertebrate muscle. When ATP is rapidly being utilized as a source of energy for muscular contraction, phosphagens permit its concentrations to be maintained, but when the ATP/ADP ratio is high, their concentration can increase to act as a **store of high-energy phosphate.**

Other Nucleoside Triphosphates Participate in the Transfer of High-Energy Phosphate

By means of the enzyme **nucleoside diphosphate kinase,** UTP, GTP, and CTP can be synthesized from their diphosphates, eg,

Nucleoside di phosphate kinase

ATP + UDP → ADP + UTP

Uridine triphosphate

kinases catalyze the formation of nucleoside diphosphates from the corresponding monophosphates.

Specific nucleoside mono phosphate kinase

ATP + Nucleoside - P → ADP + Nucleoside - P - P

Thus, adenylyl kinase is a specialized monophosphate kinase.

Metabolism

Metabolism, the sum of all the chemical transformations taking place in a cell or organism, occurs through a series of enzyme-catalyzed reactions that constitute **metabolic pathways.** These reactions include obtaining energy for the organism from its food**.** Each of the consecutive steps in a metabolic pathway brings about a specific, small chemical change, usually the removal, transfer, or addition of a particular atom or functional group. The precursor is converted into a product through a series of metabolic intermediates called **metabolites.**

Metabolism is divided into two parts:

1- Catabolism: is the degradative phase of metabolism in which organic nutrient molecules (carbohydrates, fats, and proteins) are converted into smaller, simpler end products (such as lactic acid, CO2, NH3). Catabolic pathways release energy, some of which is conserved in the formation of ATP and reduced electron carriers (NADH, NADPH, and FADH2); the rest is lost as heat. Catabolic pathways are **exergonic**.

2- Anabolism :also called biosynthesis, small, simple precursors are built up into larger and more complex molecules, including lipids, polysaccharides, proteins, and nucleic acids. Anabolic reactions require an input of energy, generally in the form of the phosphoryl group transfer potential of ATP and the reducing power of NADH, NADPH, and $FADH_2$ (Fig. 6).

The energy released in catabolism is stored and then used in anabolism. Anabolic pathways are **endergonic.**
Anabolism and catabolism occur together and at the same time in the cells .

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

Some metabolic pathways are **linear**, and some are **branched**, yielding multiple useful end products from a single precursor or converting several starting materials into a single product. Some pathways are **cyclic:** one starting component of the pathway is regenerated in a series of reactions that converts another starting component into a product.

A knowledge of normal metabolism is essential for an understanding of abnormalities underlying 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**. An important example of a metabolic disease is **diabetes mellitus.**

Pathways that process the major products of digestion

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. In ruminants (and to a lesser extent in other herbivores), dietary cellulose is fermented by symbiotic microorganisms to short-chain fatty acids (acetic, propionic, butyric), and metabolism in these animals is adapted to use these fatty acids as major substrates.

All the products of digestion are metabolized to a **common product, acetyl-CoA,** which is then oxidized by the **citric acid cycle** (Figure 6).

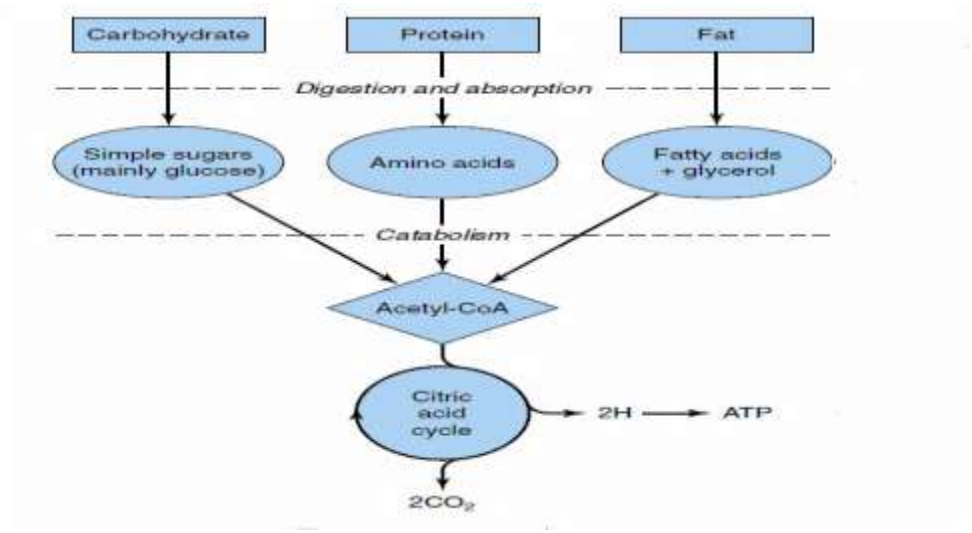


Figure (6): Outline of the pathways for the catabolism of dietary carbohydrate, protein, and fat.

Secondary metabolism

Up to this point we have been speaking largely of the central metabolic pathway in which the bulk nutrients of the cells carbohydrates ,fats and proteins ,are transformed .In these central pathways the flow of metabolic is relatively large for example , several hundred grams of glucose are oxidized to CO_2 and H_2O each day by an adult human being but there are other metabolic pathway in wich the flow is much smaller , involving the formation or degradation of substance in terms of only milligrams per day .These pathways constitute the secondary metabolism of cells ,involving specialized products that are required by cells in only small amounts .Such secondary metabolic pathways are involved in the biosynthesis of coenzymes and hormones , for example ,which are made and used in only trace amounts .The secondary pathways of metabolism in different forms of life lead to hundred of highly specialized biomolecules eg.nucleotides ,pigments , toxins ,antibotic , and alkaloides .While such products are very important to the life of the organism that make them and each has a specialized biological purpose .

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Introduction to Carbohydrates metabolism

Carbohydrates are the most abundant organic molecules in nature. They have a wide range of functions, including providing a significant fraction of the energy in the diet of most organisms, acting as a storage form of energy in the body, and serving as cell membrane components that mediate some forms of intercellular communication. Carbohydrates also serve as a structural component of many organisms, including the cell walls of bacteria, the exoskeleton of many insects, and the fibrous cellulose of plants.

Digestion of Carbohydrates

The principal sites of dietary carbohydrate digestion are the **mouth and intestinal lumen**. This digestion is rapid and is generally completed by the time the stomach contents reach the junction of the duodenum and jejunum. There is little monosaccharide present in diets of mixed animal and plant origin. Therefore, the enzymes needed for degradation of most dietary carbohydrates are primarily **endoglycosidases** that hydrolyze oligosaccharides and polysaccharides, and disaccharidases. Hydrolysis of glycosidic bonds is catalyzed by a family of **glycosidases** that degrade carbohydrates into their reducing sugar components. These enzymes are usually specific for the structure and configuration of the glycosyl residue to be removed, as well as for the type of bond to be broken.

Digestion of carbohydrates begins in the mouth

The major dietary polysaccharides are of **plant** (**starch**, composed of amylose and amylopectin) and **animal** (**glycogen**) origin. During mastication, salivary α-amylase acts briefly on dietary starch and glycogen in a random manner, hydrolyzing some **α (1→4)** bonds. **[Note: There are both α (1→4)- and β (1→4)- endoglucosidases in nature, but humans do not produce and secrete the latter in digestive juices. Therefore, they are unable to digest cellulose—a carbohydrate of plant origin containing β (1→4) glycosidic bonds between glucose residues]**.

Because **branched amylopectin and glycogen** also contain **α (1→6)** bonds, which **α-amylase** cannot hydrolyze, the digest resulting from its action contains a mixture of short, branched oligosaccharides or dextrins **[Note: Disaccharides are also present as they, too, are resistant to the amylase]**.

Carbohydrate digestion halts temporarily in the stomach, because the high acidity inactivates the salivary α-amylase.

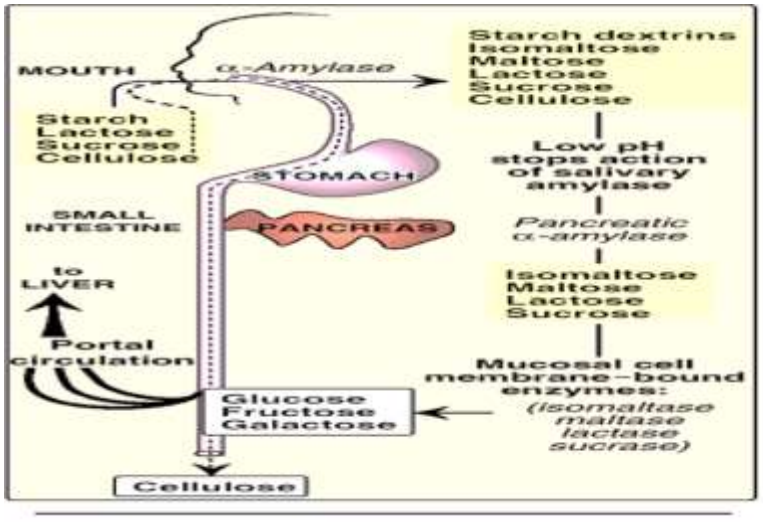


Figure (7): Digestion of carbohydrates.

Further digestion of carbohydrates by pancreatic enzymes occurs in the small intestine

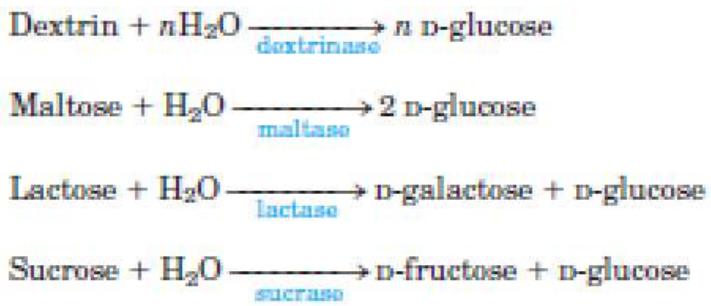
When the acidic stomach contents(chyme) reach the small intestine, they stimulate mucosal cels of the duodenum to release **secretin** and **cholecystokinine(CKK)** two peptide hormones that stimulate the exocrine pencreatic to release pencreatic juice into the intestinal lumen

-**Secretin** stimulate the release of bicarbonate whose function is neutralize the acidic chyme

- **Cholecystokinine**(CKK)stimulate the release of digestive enzymes including pencreatic α-amylase (salivary α-amylase and pancreatic α-amylase are isoenzymes forms).

Final carbohydrate digestion by enzymes synthesized by the intestinal mucosal cells

The final digestive processes occur at the mucosal lining of the upper jejunum, declining as they proceed down the small intestine, and include the action of several disaccharidases and oligosaccharidases. For example, **isomaltase** cleaves the **α (1→6) bond** in isomaltose and **maltase** cleaves **α (1→4)bond** in maltose, both producing glucose, **sucrase** cleaves **α (1→2)** bond in sucrose producing glucose and fructose, and **lactase** (β-galactosidase) cleaves **β(1→4) bond** in lactose producing galactose and glucose. These enzymes are secreted through, and remain associated with, the luminal side of the brush border membranes of the intestinal mucosal cells.



Cellulose has no food value unlike starch because humans lack an enzyme that can hydrolyze the **β (1→4)** glycosidic linkage of polysacchrides, cellulose cannot be digested and absorbed. However, ruminant can utilize cellulose because they have in their digestive tract microorganisms whose enzymes hydrolyse cellulose.

Absorption of monosaccharides by intestinal mucosal cells

Carbohydrates are absorbed as monosaccharides from the intestinal lumen through the mucosal epithelial cells into the blood stream of the portal venous system. Two mechanisms are responsible for the absorbtionn of monosaccharides:

1-Active transport against concentration gradient

The transport of **glucose and galactose** across the brush border membrane of mucosal cells occurs by an active transport, energy requiring process that requires a specific transport protein and the presence of sodium ions.

2- Facilitative transport (with the concentration gradient)

Fructose and mannose are transported across the brush border by Na^+ independent facilitative diffusion process. Movement of sugar in facilitative diffusion is strictly downhill, going from a higher concentration to a lower one until it reaches an equilibrium.

Disorders of digestion and absorption of carbohydrate

Any conditions that results in impaired ability to digest and absorb carbohydrate may result in bacterial fermentation in the large intestine with the production of H_2 and CO_2 gases and low molecular weight acids like acetic acid, propionic acid and butyric acid which are somatically active.

Abdominal cramps and flatulence results from the accumulation of gases and the osmatically active products draw water from the intestinal cells into the lumen resulting in diarrhea and dehydration.

Genetic deficiencies in most of the disaccharides digestion or absorption result in the symptoms described above.

Lactose intolerance

Intolerance to lactose (the sugar of milk) not to milk. This is the most common disorder due to deficiency of enzyme **lactase**. Treatment for this disorder is simply to remove lactose from the diet.

Lactose intolerance: More than three quarters of the world's adults are lactose intolerant (Figure 9). The mechanism by which this age-dependent loss of the enzyme occurs is not clear, but it is determined genetically and represents a **reduction in the amount** of enzyme protein rather than a modified inactive enzyme. Treatment for this disorder is to reduce consumption of milk while eating yogurts and cheeses.

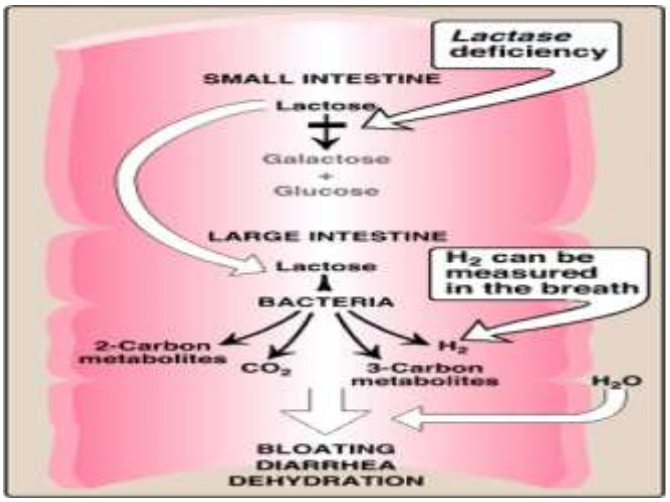


Figure (8): Abnormal lactose metabolism.

Metabolic fate of carbohydrates

After being absorbed from the intestinal tract the monosaccharides are carried by the portal circulation directly to the liver.

• **In the liver most of the entering free D-glucose is phosphorylated to glucose -6-phosphate and sugar is trapped within the cell and it cannot diffuse back out of the cell because its plasma membrane is impermeable to the glucose-6-phosphate.**

•The remainder of the glucose passes into the systematic blood supply

•Other dietary monosaccharides D-fructose and D-galactose are phosphorylated and maybe converted into glucose in the liver.

Glucose -6-phosphate is an intermediate in several metabolites; pathway that uses glucose in the liver depending upon the supply and demand includes:

•Glycolysis

•Pentose phosphate pathway

•Glycogenesis and

•Glycogenolysis

Glycolysis

Definition

Glycolysis is the sequence of reactions that converts glucose into pyruvate in the presence of oxygen **(aerobic)** or lactate in the absence of oxygen **(anaerobic)** with the production of **ATP .** The pathway is also called **Embden Meyerhof pathway**.
It is a unique pathway since it can utilize oxygen if available, or it can function in the total absence of oxygen.

Location

Glycolysis is the major pathway for the utilization of glucose and is found in cytosol of all cells.

Reactions of glycolysis

The breakdown of glucose (6-carbon compound) to two moles of pyruvate (3-carbon compound) is brought about by sequential action of 10 enzymes which can be divided into two phases

1- First phase, energy requiring phase or preparative phase
2- Second phase, energy generating phase

First phase, energy requiring phase or preparative phase

First five reactions of glycolysis correspond to the phase where phosphorylated form of glucose and fructose are synthesized at the expense of two moles of ATP per glucose molecule.

Reactions of first phase

1- Glucose enters into the glycolytic pathway by phosphorylation to glucose -6-phosphate by the enzyme **hexokinase** and ATP is required as a phosphate donor . The reaction is accompanied by a considerable loss of free energy as heat and therefore under physiologic conditions is regarded as irreversible.

Phosphorylated sugar molecules do not readily penetrate cell membranes, because there are no specific transmembrane carriers for these compounds, and because they are too polar to diffuse through the cell membrane. The irreversible phosphorylation of glucose, therefore, effectively traps the sugar as cytosolic glucose 6-phosphate, thus committing it to further metabolism in the cell.

Mammals have several isozymes of the enzyme **hexokinase** that catalyze the phosphorylation of glucose to glucose 6-phosphate. Hexokinase occurs in different isoenzyme forms (I to IV).

In the liver the principle form of hexokinase is type IV , commonly known as **glucokinase** .Hexokinase isoenzymes catalyzed the same type of reaction but they differ with respect to their **kinetic properties** . The different between muscle hexokinase and liver hexokinase is listed in the following table:

Table: Difference between muscle hexokinase and glucokinase (liver hexokinase)

Muscle hexokinase	Glucokinase (liver hexokinase)
Distributed in exterahepatic tissue	Present in liver and β-cell of pancreas
High affinity for its substrate glucose (low K_m)	Low affinity for its substrate glucose (high K_m)
Inhibited by its product glucose -6-phosphate ,in an allosteric manner	No inhibition by its product
Its function is to ensure supply of glucose for the tissues irrespective of blood glucose concentration	Its function to remove glucose from the blood , when the blood glucose level increases (following meal)
Catalyze the phosphorylation of other hexoses like fructose ,galactose	Specific for glucose
Its activity is not affected by insulin	It is an inducible enzyme that increase its synthesis response to insulin

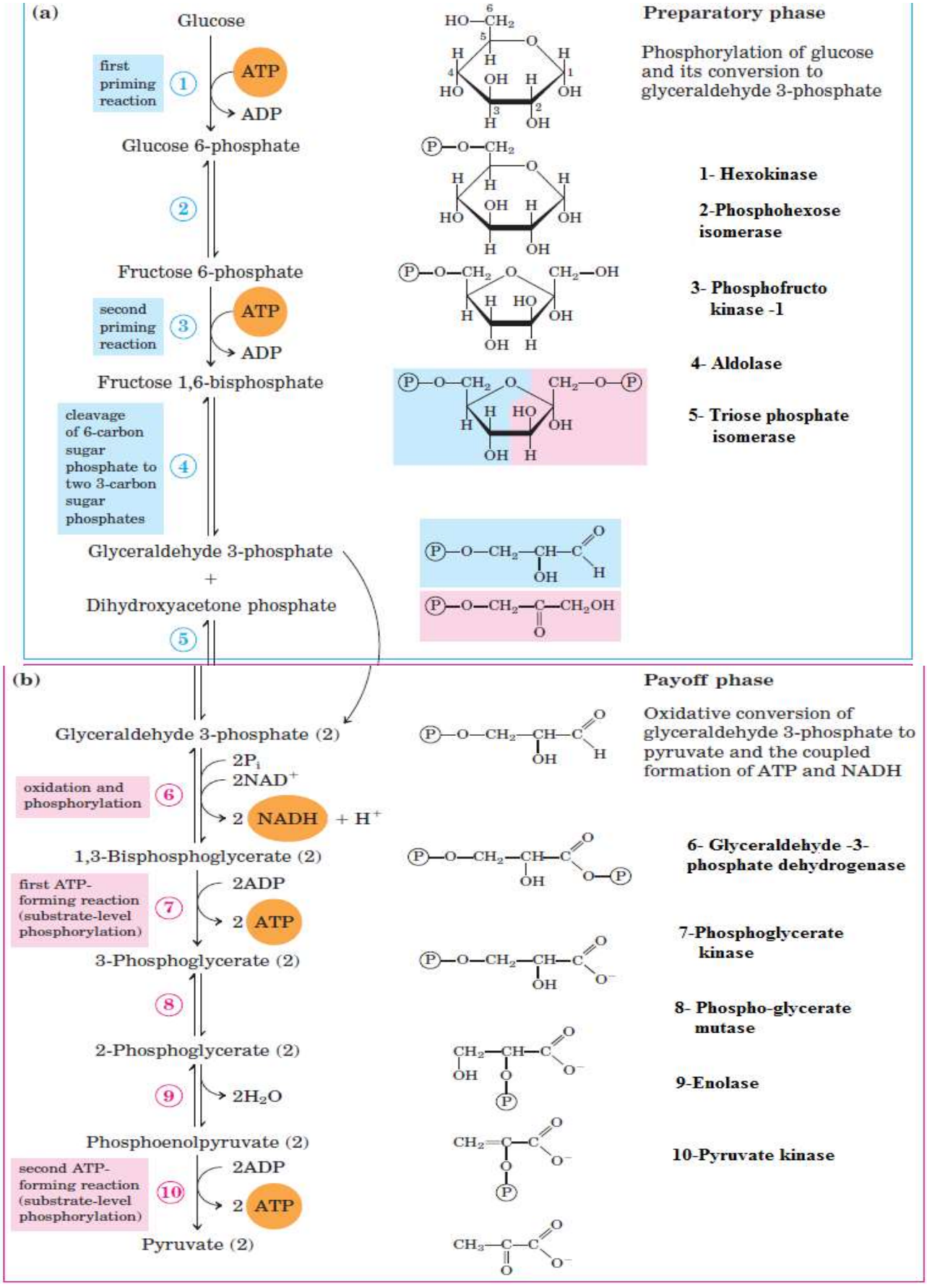


Figure (9) : The two phases of glycolysis

2- Conversion of glucose -6- phosphate to fructose -6- phosphate by an enzyme **phosphohexose isomerase** which involve aldose-ketose isomerization and is freely reversible reaction.

3- Fructose -6-phosphate to fructose 1,6-bisphosphate , second phosphorylation reaction with ATP catalyzed by an enzyme **phosphofructokinase -1** . This step is irreversible under physiological conditions .Phosphofructokinase-1 is both allosteric and an inducible enzyme which is the rate limiting ,regulatory enzyme of glycolysis.

4- Fructose -1,6 bisphosphate is cleaved by **aldolase** to two triose phosphates , glyceraldehyde -3- phosphate and dihydroxy acetone phosphate (DHAP) .

5- DHAP is isomerized to glyceraldehyde -3-phosphate by the enzyme phosphotriose isomerase, so that for every molecule of glucose entering glycolysis **,2 moles of glyceraldehyde-3-phosphate** are formed.

Second phase, energy generating phase (Payoff phase)

The subsequent reactions of glycolysis constitute an energy generation phase where molecules are formed per glucose molecules.

1) Oxidation of glyceraldehyde -3-phosphate to 1,3 – bis phosphoglycerate .The enzyme responsible for the oxidation ,**glyceraldehyde -3-phosphate dehydrogenase**, is a NAD^+ dependent phosphorylation ,occurs at the expense of inorganic phosphate (Pi) and is a reversible reaction. The reducing equivalents $NADH+H^+$ formed ,are reoxidized by electron transport chain , to generate 3ATP molecules per $NADH +H^+$.

2) 1,3- bis phosphoglycerate to 3-phosphoglycerate catalyzed by **phosphoglycerate kinase** .This is the first step in glycolysis that generates ATP , an example of substrate level phosphorylation .Since two molecules of triose phosphate are formed per molecule of glucose undergoing glycolysis two molecules of ATP are generated at this stage per molecule of glucose . **Arsenate** can uncouple oxidation and phosphorylation at this step.

3) 3- phosphoglycerate to 2-phosphoglycerate is a reversible reaction catalyzed by **phosphoglycerate mutase.**

4) 2-phosphoglycerate to phosphoenol pyruvate .This reaction catalyzed by **enolase** and involves dehydration and redistribution of energy within the molecule, raising the phosphate on position 2 to high energy state ,thus forming phosphoenol pyruvate **.Enolase is inhibited by fluoride ,a property that can be made use of when it is required to prevent glycolysis in blood prior to the estimation of glucose.**

5) phosphoenol pyruvate ,this is an irreversible reaction .The high energy phosphate of phosphoenol pyruvate is transferred to ADP by the enzyme **pyruvate kinase** to generate 2 molecules of ATP per molecule of glucose oxidized ,**another example of substrate level phosphorylation.**

Enol pyruvate formed in this reaction is converted spontaneously to the keto form of pyruvate .

All of the intermediate in the glycolysis pathway between glucose and pyruvate are phosphorylated .At the pH of the cell(pH≈7) the phosphate group is fully ionized .As ,a result ,these compounds cannot escape from the cell ,because the cell membrane does not allow highly polar molecules to pass .This prevents the intermediates from leaking out of the cell.

Under **aerobic condition** pyruvate is taken up into mitochondria and after conversion to acetyl-CoA is oxidized to CO_2 by citric acid cycle.

Fructose is more rapidly glycolyzed by the liver than glucose , because it bypasses the step in glucose metabolism catalyzed by phosphofructokinase ,at which point metabolic control is exerted on the rate of catabolism of glucose.

Anaerobic glycolysis

●If **anaerobic** condition prevail ,the reoxidation of NADH (formed in the glyceraldehyde-3-phosphate step) by transfer of reducing equivalents through the respiratory chain to oxygen is prevented and get reoxidized by conversion of pyruvate to :

1- Lactate by lactate dehydrogenase.

Conversion of pyruvate to lactate (lactate fermentation) occurs in higher organisms as well as in microorganism under anaerobic conditions ,the NADH formed in the oxidation of glyceraldehyde -3-phosphate is consumed in the reduction of pyruvate to lactate.

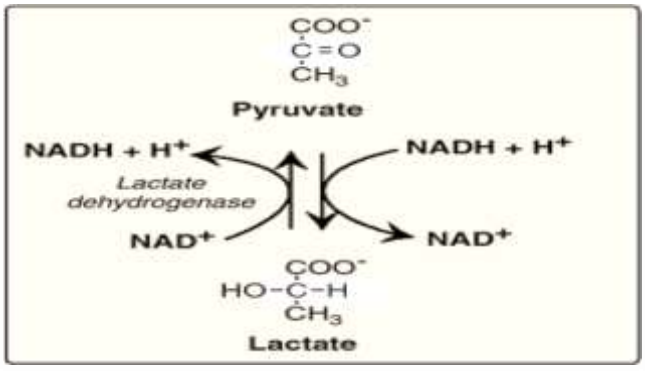


Figure (10): Lactate fermentation

●Tissue that function under **hypoxic conditions** produce lactate ,e.g. skeletal muscle ,smooth muscle and erythrocytes

●**In erythrocytes** even under aerobic conditions , glycolysis terminates in **lactate** because of absence of mitochondria

●**In contracting skeletal muscle** during vigorous exercise ,production of pyruvate exceeds the rate at which the citric acid cycle oxidizes it. Moreover under these conditions the rate

of formation of NADH by glycolysis is greater than the rate of its oxidation by aerobic metabolism.

●Accumulated NADH in muscles oxidized by lactate dehydrogenase to NAD^+ during reduction of pyruvate to lactate .**The only role of the reduction of pyruvate to lactate is to generate NAD^+ ,so that glycolysis can proceed in skeletal muscle and erythrocytes.**

2-Conversion of pyruvate to ethanol

●Conversion of pyruvate to ethanol occurs in yeast and microorganism under **anaerobic conditions.**

●First pyruvate is decarboxylated to acetaldehyde by the enzyme **pyruvate decarboxylase**, which requires the coenzymes thiamine pyrophosphate (TPP)

●Then acetaldehyde is converted to ethanol by **alcohol dehydrogenase** .In this process NADH generated by oxidation of glyceraldehyde -3-phosphate in glycolysis is consumed .Thus there is no net generation of NADH in the conversion of glucose to ethanol

●The conversion of glucose into ethanol is an example of **alcoholic fermentation**

●The ethanol formed in alcohol fermentation is used for brewing and winemaking

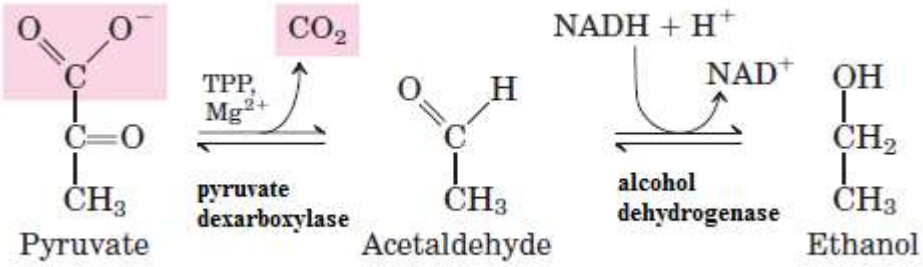


Figure (11): Alcoholic fermentation.

Significance of anaerobic glycolysis

●The reoxidation of NADH via lactate or ethanol formation allows glycolysis to proceed in the absence of oxygen by regeneration sufficient NAD^+ for another cycle of the reaction catalyzed by glyceraldehyde -3- phosphate dehydrogenase

●Anaerobic glycolysis is an emergency source of ATP

Regulation of glycolysis

One of the major mechanism for the control of the rate of the glycolytic pathway is the use of **allosteric enzymes** .In addition to the active site , which binds the substrate ,allosteric

enzymes have an effector binding site , which binds a chemical signal that has the ability to alter the rate at which the enzyme catalyze the reaction. Effector binding may increase (**positive allosterism**) or decrease the rate of reaction (**negative allosterism**).

The chemical signals , or effectors, that indicate the energy needs of the cell include molecules such as ATP .If the ATP concentration is high , the cell must have a sufficient energy reserve . Similarly , ADP and AMP ,which are precursors of ATP , are indicators that the cell is in need of ATP.

Glycolysis is regulated at 3 steps which are irreversible and exergonic

These reactions are catalyzed by :

●Hexokinase and glucokinase

Hexokinase is an allosteric enzyme, that is inhibited by its product glucose -6- phosphate in extrahepatic tissue

Liver glucokinase is an inducible enzyme that increase its synthesis in response to **insulin** and decrease in response to **glucagon**. The activity of glucagon is also influenced by carbohydrate intake. The activity increase with carbohydrate intake and decrease during starvation and diabetes mellitus

●Phosphofructokinase-1

The enzyme that catalyze the third reaction in glycolysis , is a key regulatory enzyme in the pathway .**ATP** is an allosteric inhibitor of phosphofructokinase, whereas **AMP** and **ADP** are allosteric activators .Another allosteric inhibitor of phosphofructokinase is citrate .**Citrate** is the first intermediate in the citric acid cycle .The citric acid cycle is a pathway that results in the complete oxidation of the pyruvate produced by glycolysis .A high concentration of citrate signals that sufficient substrate is entering the citric acid cycle .The inhibition of phosphofructokinase by citrate is an example of **feedback inhibition**: the product ,citrate ,allosterically inhibit the activity of an enzyme early in the pathway.

Phospho fructokinase-1 is an inducible enzyme that increases its synthesis in response to insulin and decrease in response to glucagon.

●Pyruvate kinase

Is also subject to allosteric regulation .In this case . fructose -1,6- bis phosphate is the allosteric activator .It is interesting that fructose-1,6-bisphospate is the product of the reaction catalyzed by phosphofructokinase .Thus , activation of phosphofructokinase results in the activation of pyruvate kinase . This is an example of **feedforward activation** because the product of an earlier reaction causes activation of an enzyme later in the pathway.

In Erythrocytes, the First Site in Glycolysis for ATP Generation May Be Bypassed

Mature erythrocyte contains no mitochondria. So, they are totally dependent upon glycolysis for ATP production. Erythrocyte metabolizes excessive amount of glucose in the glycolytic pathway; which will generate much of ATP, which is not required and cannot be used by the erythrocytes and may inhibit glycolysis by inhibiting the enzyme phosphofructokinase-1.

In the erythrocytes of many mammals, ATP production by substrate phosphorylation from 1,3-bis phospho glycerate is bypassed in the erythrocyte by taking a diversion pathway
It is a side reaction of the glycolytic pathway. occurring in erythrocyte, in which:

●1,3-bis phospho glycerate is converted to 2,3- bis phospho glycerate by an enzyme **bis phospho glycerate mutase**

●Then 2,3-bis phospho glycerate converted to 3- phosphor glycerate by **2,3-bis phospho glycerate phosphatase, which** a loss of high energy, phosphate (energy is dissipated as heat) and there is no net production of ATP when glycolysis takes this rout**.**

This alternative pathway **prevent accumulation of ATP not needed by the erythrocyte** . and provide 2,3-bisphosphoglycerate, which is required for the haemoglubin function. 2,3-bis phospho glycerate regulates the binding and release of oxygen from haemoglubin and so making oxygen more readily available to tissues**.**

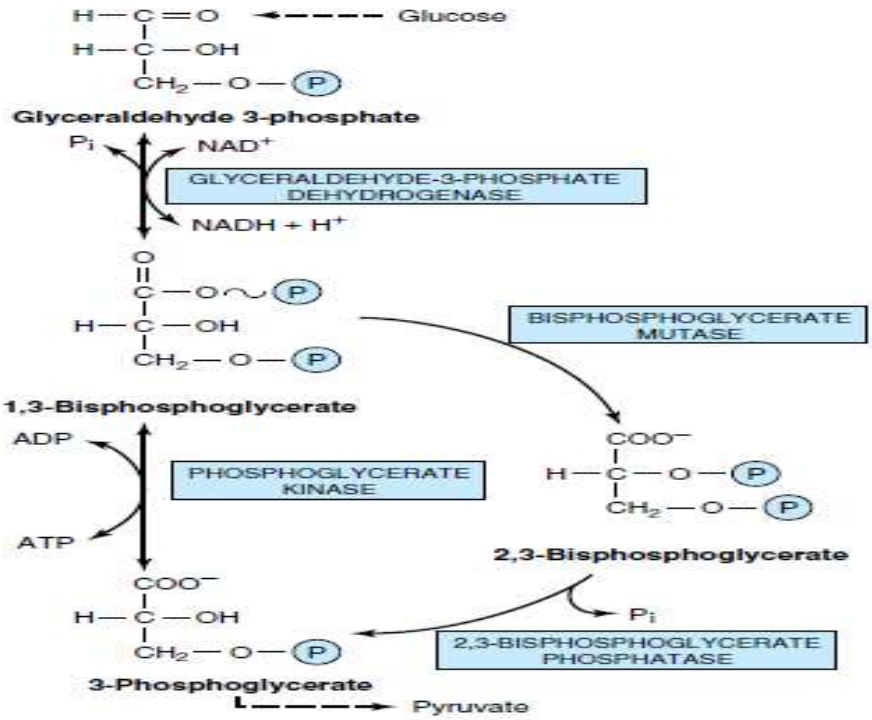


Figure (12): 2,3-Bisphosphoglycerate pathway in erythrocytes.

Significance of glycolysis

●Glycolysis is the principal route for glucose metabolism for the production of ATP molecules

●It is also provide pathway for the metabolism of fructose and galactose derived from diet

●Important biochemical significance is the ability of glycolysis to provide ATP in the absence of oxygen and allow tissues to survive

●It generate precursors for biosynthetic pathway ,e.g.

-Pyruvate may be transaminated to aminoacid alanine .In the liver pyruvate provides substrate ,acetyl -CoA for fatty acid biosynthesis.

- Glycerol -3- phosphate ,which forms the backbone of triacylglycerol is derived from glycolytic pathway

●In erythrocytes glycolysis supplies 2,3-bis phospho glycerate which is required for the haemoglubin function in the transport of oxygen

Disorder of glycolysis

Pyruvate kinase deficiency

●Pyruvate kinase is a key enzyme in glycolysis , catalyzes the final step with formation of ATP

●Genetic deficiency of pyruvate kinase in the erythrocyte lead to haemolytic anaemia due to excessive erythrocyte destruction

●In erythrocyte deficiency of pyruvate kinase leads to reduced rate of glycolysis and the rate of ATP being inadequate to meet the energy needs of the cells and maintain the structural integrity of the erythrocyte membrane

Hexokinase deficiency

●Genetic defect in the hexokinase of erythrocyte reduces the amount of oxygen that is available for the tissues. Because hexokinase is the first enzyme in glycolysis ,the red blood cells of these patients contain low concentration of 2,3-bis phospho glycerate which normally allows haemoglubin to release oxygen in tissue

●Consequently due to low level of 2,3-bis phosphor glycerate less oxygen is available for the tissue

●This defect will also result in anaemia

Lactic acidosis

●Lactic acidosis is the accumulation of lactic acid in the blood to level that significantly affects the blood pH

●The high concentration of lactate result in lowered blood pH and can result from increased formation or decreased utilization of lactate

●This pathological state (increased lactate level and decrease of blood pH) results from

-Inability to reoxidize NADH in the electron transport chain

-Excessive NADH production e.g. ethanol intoxication

-Impaired pyruvate dehydrogenase activity ,e.g. severe thiamine deficiency

-Inhibition of lactate utilization for gluconeogenesis

Glycolysis in cancer cell

●It has been known that hyper metabolism of glucose occur in cancer cells .Cancer cell stimulate glucose uptake and glycolysis

●As cancer cells grow more rapidly ,blood vessels cannot supply oxygen efficiently to fulfill the required demand of oxygen by the rapidly grown tumor cells

●For the survival of tumor cells ,some metabolic adaptations occur

●They begin to grow in hypoxic (absence of oxygen) conditions

●Under these conditions glucose is oxidized anaerobically to lactic acid and this pathway becomes primary source of ATP for tumor cells.

Energy yield from glycolysis

Despite the production of some ATP during glycolysis, the end products, pyruvate or lactate, still contain most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely .

• **Anaerobic glycolysis**: **Two molecules of ATP** are generated for each molecule of glucose converted to two molecules of lactate (Figure 13). **There is no net production or consumption of NADH.**

•**Aerobic glycolysis:** The direct consumption and formation of ATP is the same as in anaerobic glycolysis—that is, a net gain of **two ATP** per molecule of glucose. **Two molecules** of NADH are also produced per molecule of glucose. Ongoing aerobic glycolysis requires the oxidation of most of this NADH by the electron transport chain, producing approximately **three ATP for each NADH molecule entering the chain** . [Note: NADH cannot cross the inner mitochondrial membrane, and shuttle mechanisms are required]

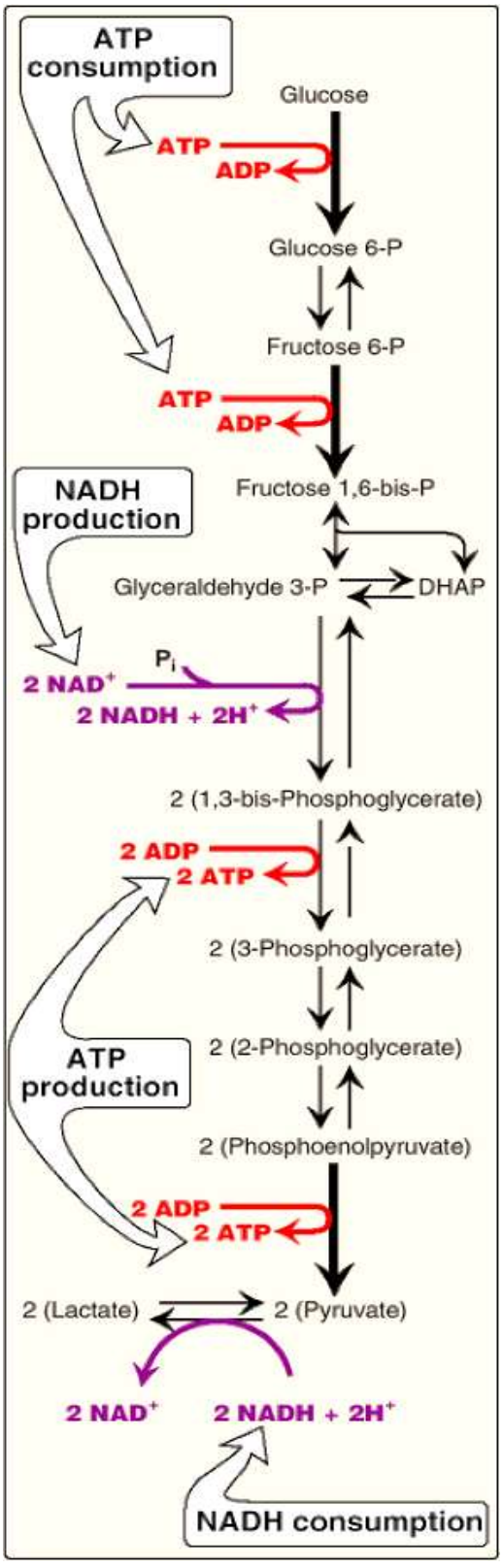


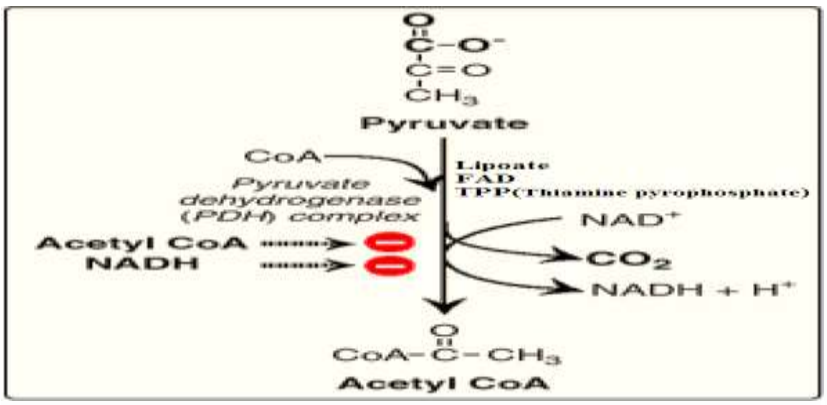
Figure (13): Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or NADH are indicated. The three irreversible reactions of glycolysis are shown with thick arrows. DHAP = dihydroxyacetone phosphate.

الحياتية (3)
المرحلة الرابعة /الفصل الدراسي الأول
Carbohydrate Metabolism (Lec.3)
by Nada Abotimen

Conversion of pyruvate to acetyl-CoA

For the further metabolism of pyruvate ,oxidative decarboxylation to acetyl-CoA is primary .This step occurs only in mitochondria ,therefore pyruvate must be transported into the mitochondria via special pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane.

The first loss of carbon in the metabolism of glucose takes place as CO_2 in the formation of acetyl –CoA from pyruvate.



Figure(14): Oxidative decarboxylation of pyruvate. Product inhibition is shown, but covalent modification is the key method of regulation for PDH. PDH is active when dephosphorylated.

Within the mitochondria , pyruvate is oxidatively decarboxylated to acetyl-CoA catalyzed by a multienzyme complex called **pyruvate dehydrogenase (PDH)** that is associated with the inner mitochondrial membrane and work sequentially.

Coenzymes required for conversion of pyruvate to acetyl – CoA

●TPP(thiamine pyrophosphate)

●Lipoate

●CoA-SH

●FAD

●NAD

Significance

●The conversion of pyruvate to acetyl-CoA is a central step ,linking the glycolytic pathway with citric acid cycle

●Acetyl-CoA is also an important intermediate in lipid metabolism , cholesterol biosynthesis and acetylation reactions.

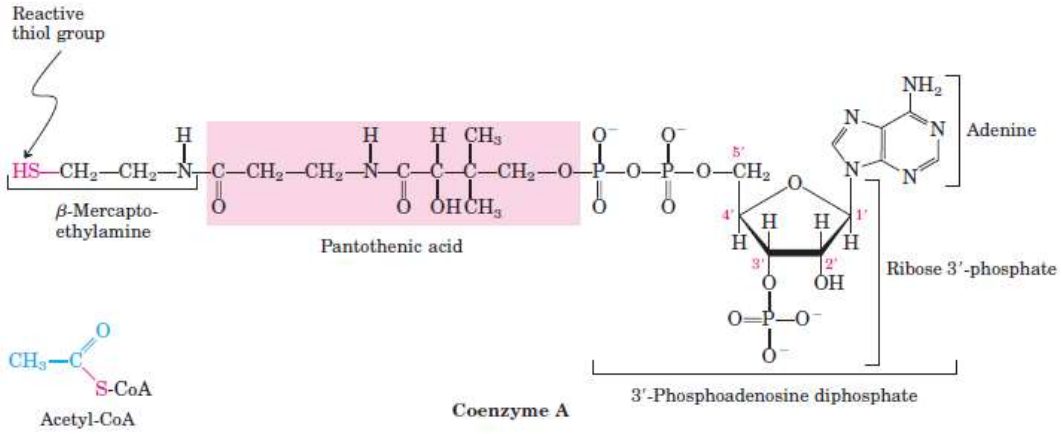


Figure (15): Structure of Coenzyme A

Inhibition of PDH (pyruvate dehydrogenase) by alcohol

●Many alcoholics develop thiamine deficiency because alcohol inhibits the transport of thiamine through the intestinal mucosal cells. Thiamine is converted to thiamine pyrophosphate (TPP) and acts as a coenzyme of PDH.

●lack of TPP inhibits PDH enzyme as a result pyruvate is converted to lactate leading to lactic acidosis and neurological disorders.

Metabolic fates of acetyl-CoA

Acetylcholine **Acetylation of amino sugar** **Ketones bodies**

Glycoprotein synthesis

acetyl-CoA

catabolism

TCA cycle

ATP

Cholesterol **CO_2 + H_2O** **Fatty acids synthesis**

Figure (16) : Metabolic fates of acetyl -CoA

Citric acid cycle

For every glucose molecule two pyruvate molecules are produced by glycolysis. These pyruvates are transferred into mitochondria, where decarboxylation leads to formation of two acetyl-CoA and two CO_2 molecules ,then acetyl-CoA are further degraded to CO_2 by citric acid cycle.Once acetyl-CoA is produced ,from whatever precursor ,it is degraded via the **citric acid cycle** .Citric acid cycle is also called **Krebs cycle** or **tricarboxylic acid (TCA) cycle**.

●It is called **citric acid cycle** because citrate was one of the first compounds known to participate

●It is called **Krebs cycle** , because its reaction were formulated into a cycle by Sir Hans Krebs

●The most common name for the this pathway is ,the **tricarboxylic acid or TCA cycle** ,due to involvement of the tricarboxylates citrate and isocitrate

Definition

The citric acid cycle is a series of reaction in mitochondria that bring about the catabolism of acetyl –CoA ,liberating reducing equivalents ,which ,upon oxidation through respiratory chain of mitochondria ,generate ATP.

Amphibolic nature of citric acid cycle

Citric acid cycle has a dual function , it functions in both catabolism(of carbohydrates ,fatty acids and amino acids) and anabolism .It provides intermediates for the synthesis of many compound required for the body and hence the cycle is said to be an amphibolic .

Location of TCA cycle

The enzymes of citric acid cycle are located in the mitochondria matrix ,either free or attached to the inner surface of the inner mitochondrial membrane ,which facilitates the transfer of reducing equivalents to the adjacent enzymes of the respiratory chain ,situated in the inner mitochondrial membrane.

Reactions of citric acid cycle

1- Entrance of 2-carbon acetyl –CoA into the citric acid cycle occurs by condensation of acetyl-CoA with oxaloacetate to yield citrate ,catalyzed by **citrate synthase**

2- Citrate is converted to isocitrate by an enzyme **aconitase .**

This conversion take place in two steps: 1-dehydration to cis-aconitate ,and

2 -rehydration to isocitrate

The reaction is inhibited by fluoroacetate

3-Isocitrate undergoes dehydrogenation in the presence of **isocitrate dehydrogenase** to form oxalosuccinate . There follows a decarboxylation to α-ketoglutarate also catalyzed by **isocitrate dehydrogenase** .

The formation of NADH and liberation of CO_2 occurs at this stage.

4- Next **α-ketoglutarate** undergoes oxidative decarboxylation ,catalyzed by a multienzyme complex **, α-ketoglutarate dehydrogenase , which requires cofactors: thiamine pyrophosphate(TPP), Lipoate, NAD, FAD, and coenzyme A and results in the formation of succinyl-CoA , a high energy thioester .The reaction is physiological irreversible .At this stage second NADH is produced with liberation of second CO_2 molecule.**

5- Succinyl –CoA is converted to succinate by the enzyme **succinyl-CoA synthetase .**The energy conserved from the previous step in the succinyl-CoA as the thioester bond is now liberated in the form of ATP .**This is the only example in citric acid cycle of generation of ATP at the substrate level phosphorylation.**

6- Succinate is metabolized **by succinate dehydrogenase ,**catalyze a reversible dehydrogenation of succinate to fumarate . Succinate dehydrogenase contains FAD ,the reaction results in the production of $FADH_2$.

7- Next **fumarase** catalyzes the addition of water to fumarate to give malate .Malate is freely permeable to the mitochondrial membrane.

8- Malate is converted to oxaloacetate by **malate dehydrogenase** and reguires NAD^+ .**The third synthesis of NADH occurs at this stage .**The oxaloacetate which can combination with another molecule of acetyl –CoA and continue the cycle.

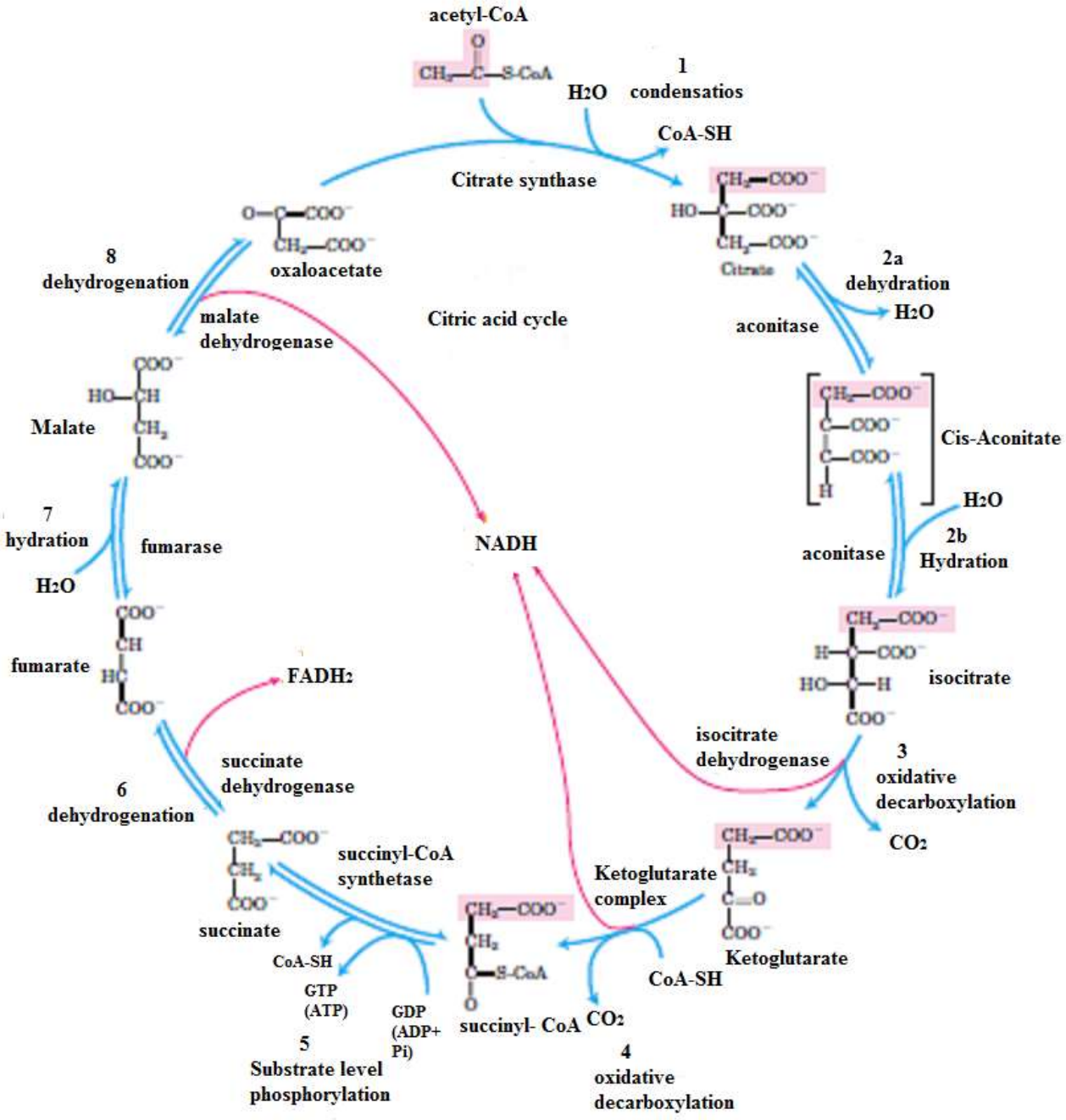


Figure (17) : Citric acid cycle(TCA)

Generation of ATP in citric acid cycle

As a result of oxidation of acetyl-CoA to H_2O and CO_2 catalyzed by dehydrogenase enzymes of citric acid cycle **three molecules of NADH and one $FADH_2$** are produced for each molecule of acetyl-CoA catabolized in one turn of the cycle .These reducing equivalents are transferred to respiratory chain in the inner mitochondrial membrane :

●During passage along the chain reducing equivalents from NADH generate 3 ATP

●However $FADH_2$ generate 2ATP molecules

●A further ATP is generated at substrate level phosphorylation during the conversion of succinyl-CoA to succinate

Thus 12 ATP molecules are generated for each turn of the cycle

Table: Generation of high-energy phosphate in the catabolism of glucose.

Pathway	Reaction Catalyzed by	Method of ~Ⓟ Production	Number of ~Ⓟ Formed per Mole of Glucose
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	6*
	Phosphoglycerate kinase	Phosphorylation at substrate level	2
	Pyruvate kinase	Phosphorylation at substrate level	2
			10
	Allow for consumption of ATP by reactions catalyzed by hexokinase and phosphofructokinase		−2
			Net 8
Citric acid cycle	Pyruvate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	Isocitrate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	α-Ketoglutarate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	Succinate thiokinase	Phosphorylation at substrate level	2
	Succinate dehydrogenase	Respiratory chain oxidation of 2 $FADH_2$	4
	Malate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
			Net 30
	Total per mole of glucose under aerobic conditions		38
	Total per mole of glucose under anaerobic conditions		2

Role of vitamins in citric acid cycle

Four water soluble vitamins of B-complex have a precise role in the function of the citric acid cycle

Vitamin	Coenzyme form
Thiamine(B_1)	**TPP (Thiamine pyrophosphate)**
Riboflavin(B_2)	**FAD (Flavin adenine dinucleotide)**
Niacin (B_3)	**NAD^+ (Nicotin amide adenine dinucleotide)**
Pantothenic acid (B_5)	**Coenzyme -A**

Significance of citric acid cycle

●The primary function of the citric acid cycle is to provide energy in the form of ATP.

●Citric acid cycle provide substrate for the respiratory chain. During the course of oxidation of acetyl-CoA in the cycle, reducing equivalents are formed ,these then enter the respiratory chain where ATPs are generated in the process of oxidative phosphorylation.

●Citric acid cycle is the final common pathway for the oxidation of carbohydrates ,lipids ,and protein .As glucose ,fatty acids and many amino acids are all metabolized to acetyl-CoA or intermediates of the cycle

●Citric acid cycle is an amphibolic process , it plays roles in both oxidative (catabolic) and synthetic (anabolic) process .Some metabolic pathways end in the constituent of the citric acid cycle while other pathways originate from the cycle

-gluconeogenesis

-transamination

-fatty acid synthesis

-porphyrin synthesis

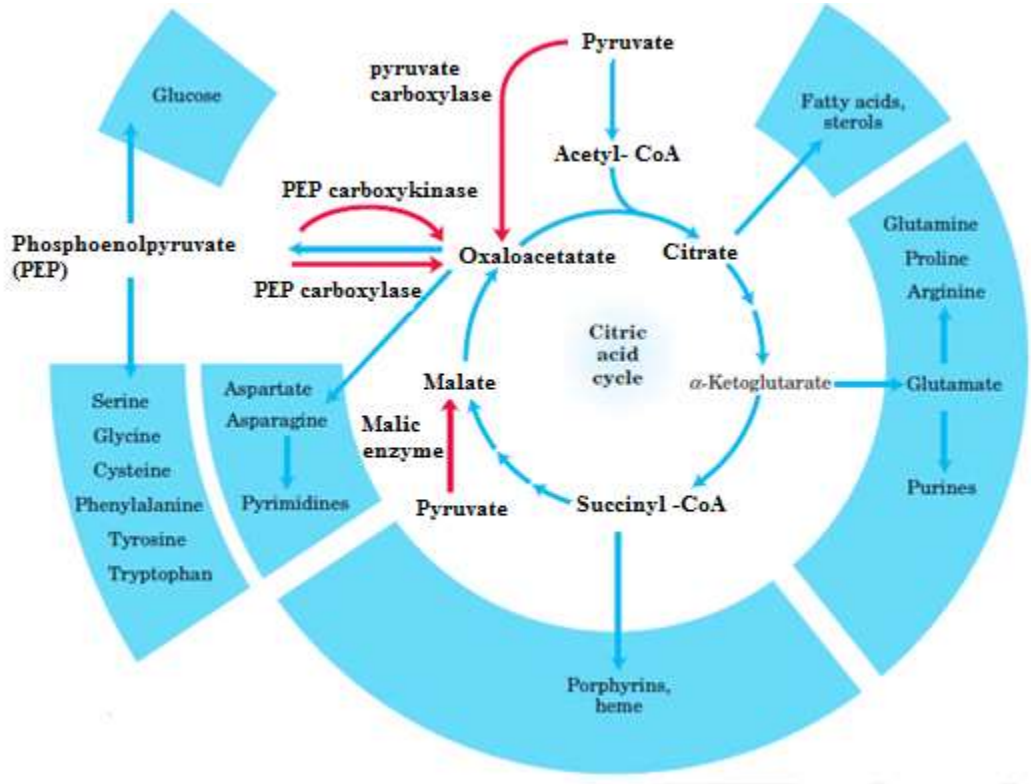


Figure (18): Role of the citric acid cycle in anabolism.

Regulation of citric acid cycle

●Citric acid cycle is regulated at four steps which are non –equilibrium reactions. These are catalyzed by:

-pyruvate dehydrogenase

-citrate synthase

-isocitrate dehydrogenase

- α- ketoglutarate dehydrogenase

●The activity of citric acid cycle is dependent on the rate of utilization of ATP:

- High ratios of **ATP/ ADP** , acetyl- CoA/CoA and NADH/NAD^+ will serve as signals to inhibit the operation of the cycle.

-An excess of ATP ,NADH, and acetyl –CoA occurs when energy supply is sufficient for the cell.

-As energy is used ,the ratio of **ATP/ ADP** declines and the inhibition of the cycle is relieved.

Glyoxylate cycle

●Glyoxylate cycle is a modification of the citric acid cycle , which occurs in **plants and some microorganisms but not in animals** due to lack of enzymes **isocitrate lyase and malate synthase** ,key enzymes involved in the glyoxylate cycle.

●In plants glyoxylate cycle occurs in cytoplasmic organelles called **glyoxysomes.**

●In each turn of glyoxylate cycle **two molecules of acetyl -CoA** enter and **one molecule of succinate** is formed, which is used for biosynthesis of glucose.

●The glyoxylate cycle allows cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available

Reactions of glyoxylate cycle

●Acetyl –CoA condenses with oxaloacetate to form citrate ,which is isomerized to isocitrate like in the citric acid cycle

●Instead of being decarboxylated , isocitrate is cleaved by **isocitrate lyase** into succinate and glyoxylate

●The glyoxylate then condenses with second molecule of acetyl –CoA to yield malate by **malate synthase**.

●Finally malate is oxidized to oxaloacetate , as in the citric acid cycle.

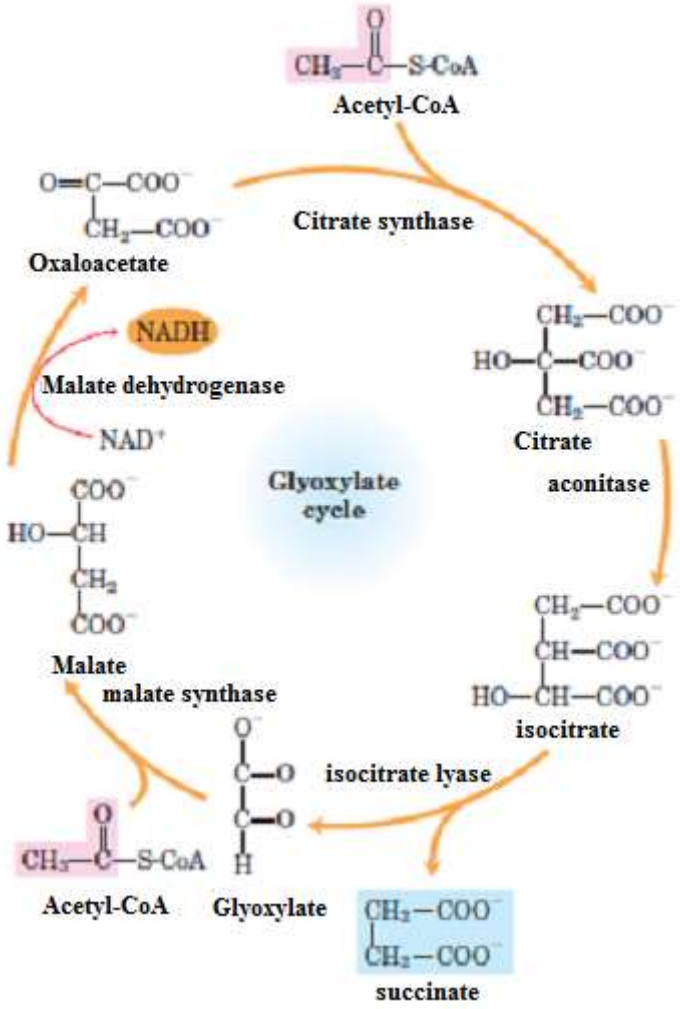


Figure (19) : Glyoxylate cycle.

Role of glyoxylate cycle in germinating plant seeds

Glyoxylate cycle is very active in germinating plant seeds, which it converts acetate derived from the fatty acids of storage triacylglycerol into glucose.

Characteristic differences between citric acid cycle and glyoxylate cycle

●Glyoxylate cycle consists of (5) reactions while citric acid cycle consists of (8) reactions

●Glyoxylate cycle bypasses the two decarboxylation reactions of the citric acid cycle

●Two molecules of acetyl –CoA enter per turn of the glyoxylate cycle , compared with one in the citric acid cycle.

الحياتية (3)
المرحلة الرابعة /الفصل الدراسي الأول
Carbohydrate Metabolism (Lec.4)
by Nada Abotimen

Gluconeogenesis

Definition The formation of glucose or glycogen from non- carbohydrate precursors is called gluconeogenesis (synthesis of new glucose) .The major non-carbohydrate substrates for gluconeogenesis are the :

●Lactate

●Glycerol

●Glucogenic amino acids

●Propionate

●Intermediate of the citric acid cycle

Location of gluconeogenesis

Liver is the major tissue involved. During starvation ,the kidney is capable of making glucose by gluconeogenesis .Certain enzymes required in gluconeogenesis are present only in these organs.

Characteristic of gluconeogenesis

●Gluconeogenesis involves glycolysis ,the citric acid cycle plus some special reactions

●Glycolysis and gluconeogenesis share the same pathway but in opposite direction

●Seven of the reactions of glycolysis are reversible and are used in the synthesis of glucose by gluconeogenesis

●However ,three of the reactions of glycolysis are irreversible and must be circumvented by four special reactions which are unique to gluconeogenesis and catalyzed by:

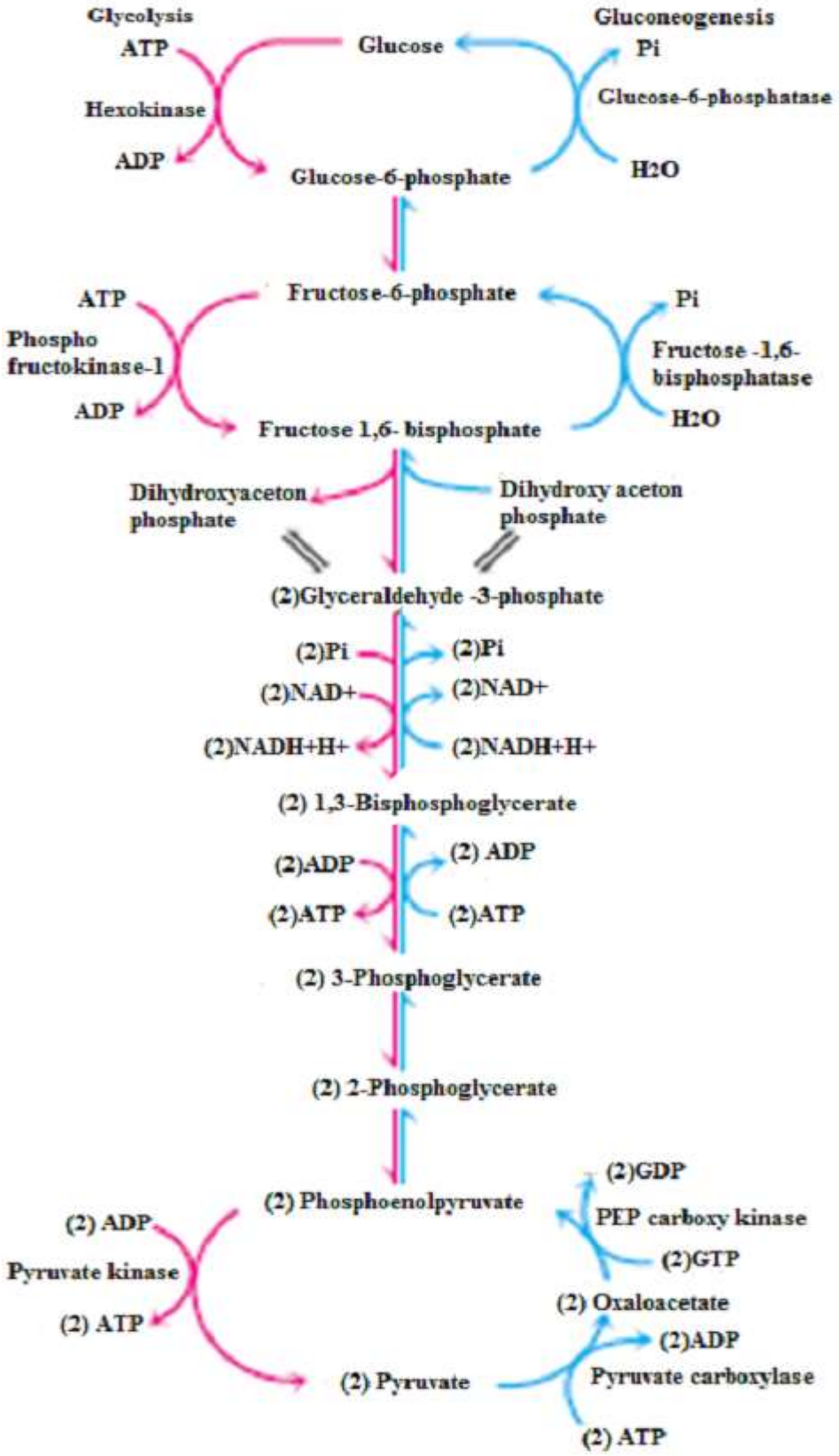
-Pyruvate carboxylase

-Phosphoenol pyruvate carboxy kinase

-Fructo-1,6-bisphosphotase

-Glucose -6-phosphotase

Figure (20): Opposing pathways of glycolysis and gluconeogenesis in the liver.



Reactions of gluconeogenesis

1- Carboxylation of pyruvate to oxaloacetate: In gluconeogenesis pyruvate is first carboxylated to oxaloacetate .**Pyruvate carboxylase** which in presence of **ATP ,vitamin biotin and CO_2 converts pyruvate to oxaloacetate in mitochondria.**

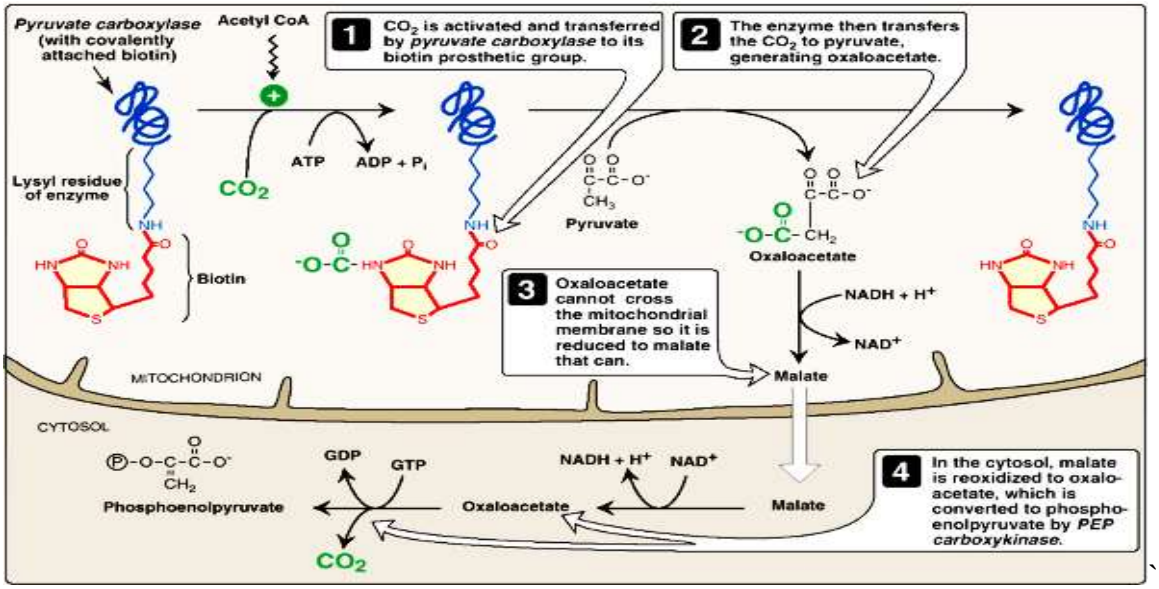
2-Transport of oxaloacetate to cytosol : Oxaloacetate formed in mitochondria , must enter the cytosol, where the other enzymes of gluconeogenesis are located .

However ,as oxaloacetate is unable to cross the inner mitochondrial membrane directly ,it must be reduced to malate which can be transported from the mitochondria to the cytosol , malate is deoxidized to oxaloacetate .

3-Decarboxylation of cytosolic oxaloacetate to phosphoenol pyruvate(PEP): Oxaloacetate is decarboxylated and phosphorylated in the cytosol by phosphoenol pyruvate carboxykinase .High energy phosphate in the form of GTP is required in this reaction .PEP then enters the reversed reaction of glycolysis until it reaches fructose-1,6-bisphosphate.

4-Dephosphorylation of fructose -1,6-bisphosphate to fructose -6-phosphate: Hydrolysis of fructose-1,6-bisphosphate to fructose -6- phosphate by fructose -1,6-bisphosphotase by passes the irreversible phosphofructokinase -1 reaction in glycolysis **.Fructose-1,6-bisphosphatase is an allosteric enzyme which is the major regulatory enzyme in gluconeogenesis .**

5-Dephosphorylation of glucose-6-phosphate to glucose : Hydrolysis of glucose-6-phosphate to glucose by **glucose-6-phosphatase** bypasses the irreversible glucokinase and hexokinase reaction of glycolysis .**Glucose -6-phosphatase is only present in liver and kidney but not in muscle .Thus muscle cannot provide blood glucose by gluconeogenesis**.



Figure(21):Activation and transfer of CO_2 to pyruvate ,followed by transport of oxaloacetate to the cytosol and subsequent decarboxylation.

Substrate for gluconeogenesis

1-Lactate

Lactate formed by the oxidation of glucose in skeletal muscle and by erythrocytes, is transported to the liver and kidney where it reform glucose ,which again become available via circulation for the oxidation in the tissue .This cycling of lactate between muscle and liver is known as **the Cori cycle or Lactic acid cycle.**

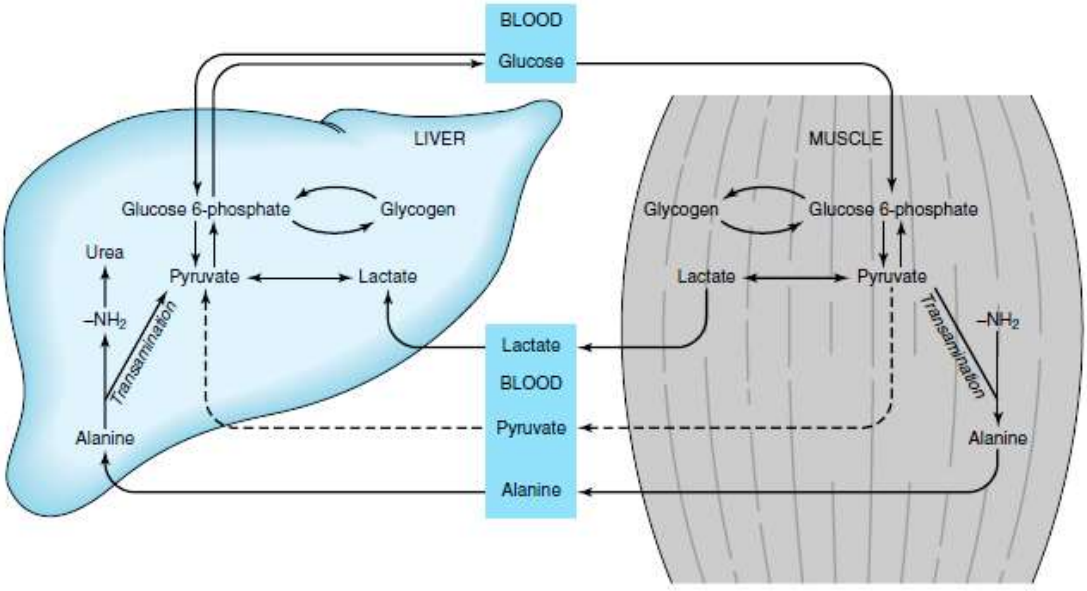


Figure (22): The lactic acid (Cori) cycle and glucose-alanine cycle.

2-Glycerol

Glycerol is released during hydrolysis of triacylglycerol in adipose tissue ,which cannot be utilized by adipose tissue due to poor content of enzyme **glycerol kinase .**Therefore , it diffuses out into the blood and is delivered to the liver and kidney ,where it is converted back to glucose by gluconeogenesis .

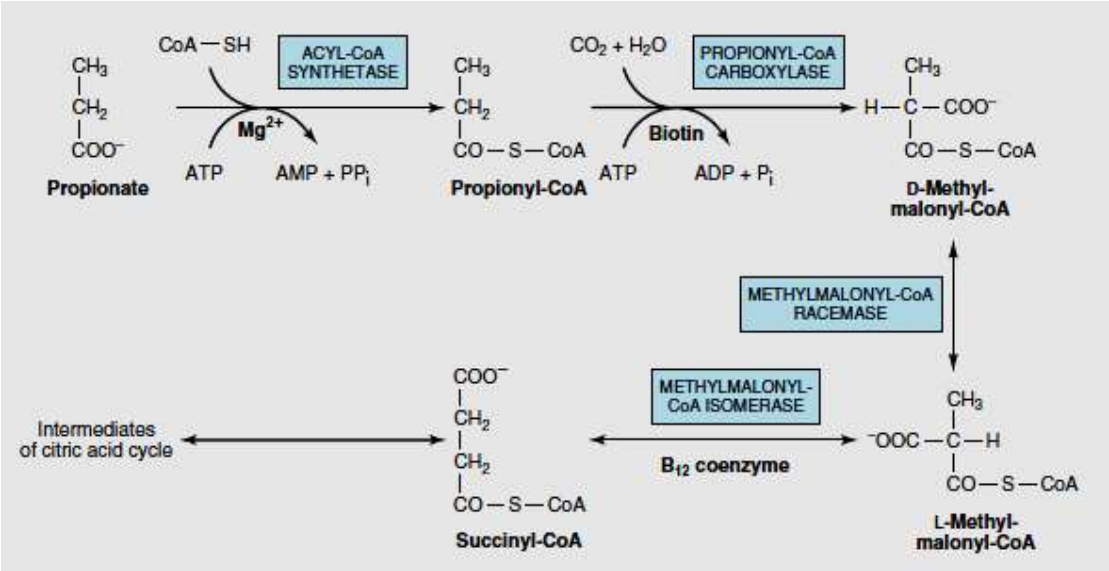
3-Amino acids and intermediates of citric acid cycle

Alanine is the predominant amino acid released from muscle to liver during fasting by **glucose – alanine cycle .**Some of the pyruvate resulting from glycolysis in skeletal muscle ,is transaminated to alanine ,and transported from skeletal muscle to liver , where it is transaminated back to pyruvate ,the pyruvate in liver is used to synthesize glucose , which can be returned to skeletal muscle ,completing the cycle. This process also helps to maintain nitrogen balance.

●In addition to alanine ,many other amino acids are converted to pyruvate or citric acid cycle intermediates ,which are metabolized to oxaloacetate to an intermediate of gluconeogenesis.

4-Propionate

Fatty acids with an odd number of carbons and carbon skeleton of some amino acids produce propionyl-CoA .These are minor precursor of gluconeogenesis in human .Propionate enters the main gluconeogenic pathway via citric acid cycle after conversion of succinyl-CoA .



Figure(23): Conversion of propionate to succinyl –CoA

<u>Regulation of gluconeogenesis</u>

Since glycolysis and gluconeogenesis share the same pathway but in opposite direction ,they may be regulated reciprocally .The rate of gluconeogenesis depends on the:

1-**Availability of substrate**

Gluconeogenesis is stimulated by the flow of its major substrate from peripheral tissues to the liver

Thus gluconeogenesis is stimulated during

●Fasting

●Prolonged exercise

●By a high protein diet

●Under conditions of stress

2-Enzyme regulation

Gluconeogenesis is regulated by four key enzymes

●Pyruvate carboxylase

●Phosphoenol pyruvate carboxykinase

●Fructose-1,6-bisphosphatase

●Glucose -6-phosphatase

Significance of gluconeogenesis

●Gluconeogenesis meets the needs of the body glucose and maintains blood glucose homeostasis;when carbohydrate is not available in sufficient amounts from the diet.

●Some tissues such as the brain ,erythrocytes ,lens ,cornea of the eye and kidney medulla require a continuous supply of the glucose as a source of energy .Liver glycogen stores can meet these needs for only 12-18 hours in the absence of dietary intake of carbohydrate .As the glycogen store starts depleting ,gluconeogenesis takes place ,which ensure a continuous supply of glucose to the brain and other tissues.

●Gluconeogenesis mechanism are used to clear the product of the metabolism of other tissues from the blood ,e.g.,

-Lactate ,produced by muscle and erythrocytes

-Glycerol ,produced by adipose tissues

- Propionyl –CoA produced by oxidation of odd carbon number fatty acids and carbon skeleton of some amino acids.

Glycogen Metabolism

●Glycogen is the major storage form of glucose mainly in the liver and muscle ,although most of the cells may store minute amounts.

●Glycogen composed of glycosyl units linked by α-1,4 glycosidic bonds with α-1,6 branches occurring about every 8-10 glycosyl units.

Glycogenesis

Definition

Glycogenesis is the pathway for the formation of glycogen from glucose .Glycogen is synthesized from α-D-glucose ,the process requires energy , supplied by ATP (for phosphorylation of glucose) and uridine triphosphate (UTP).

Location

It occurs in muscle and in liver in the fed state, when insulin/glucagon ratio is high.

Reactions of glycogenesis

1- Glucose is phosphorylated to glucose -6-phosphate catalyzed by **hexokinase** in muscle and **glucokinase** in liver.

2-Glucose-6-phosphate is converted to glucose-1-phosphate by the enzyme **phosphoglucomutase.**

3- Glucose- 1- phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide , uridine di phosphate glucose(UDP- glucose) .The reaction is catalyzed by the enzyme **UDP-glucose pyrophosphorylase** .

4- By the action of the enzyme glycogen synthase the C_1 of the activated glucose of UDP-glucose forms a glycosidic bond with C_4 of terminal glucose residue of pre-existing glycogen molecule **(glycogen primer) ,** liberating uridine di phosphate(UDP) **[.Glycogen primer:preformed α-(1,4) polyglucose chain having at least eight glucose residues].**

5- In the above reaction , a new α-1,4 linkage is established between carbon atom one of the incoming glucose and carbon 4 of the terminal glucose of a glycogen primer.

6- When the chain has been lengthened to a minimum of 11 residues , a second enzyme , **the branching enzyme** (amylo-1,4 to 1,6 –transglucosidase) transfer a part of the 1,4 –chain minimum length of 6- glucose residues to a neighboring chain to form a α-1,6- linkage thus establishing a branching point in the molecule. The branches grow by further additions of 1,4-glycosyl units and further branching.

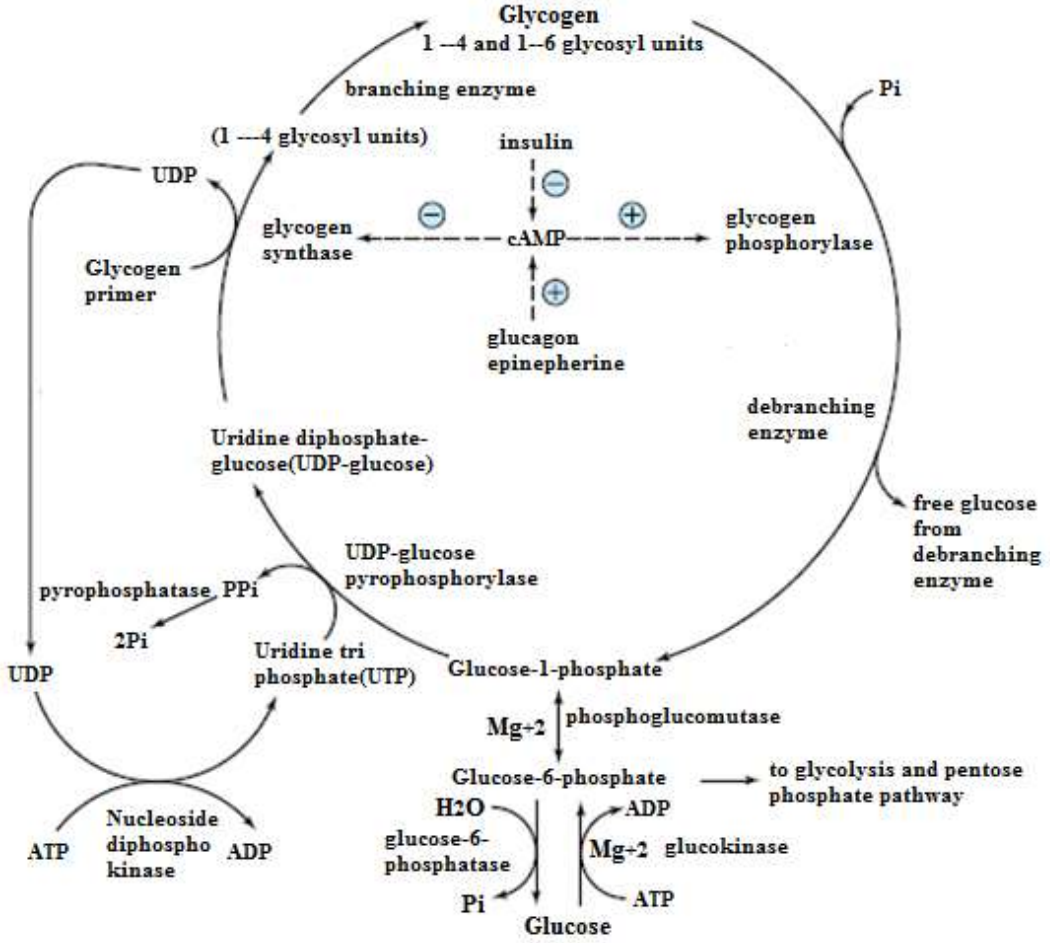


Figure (24): Pathway of glycogenesis and of glycogenolysis in the liver.

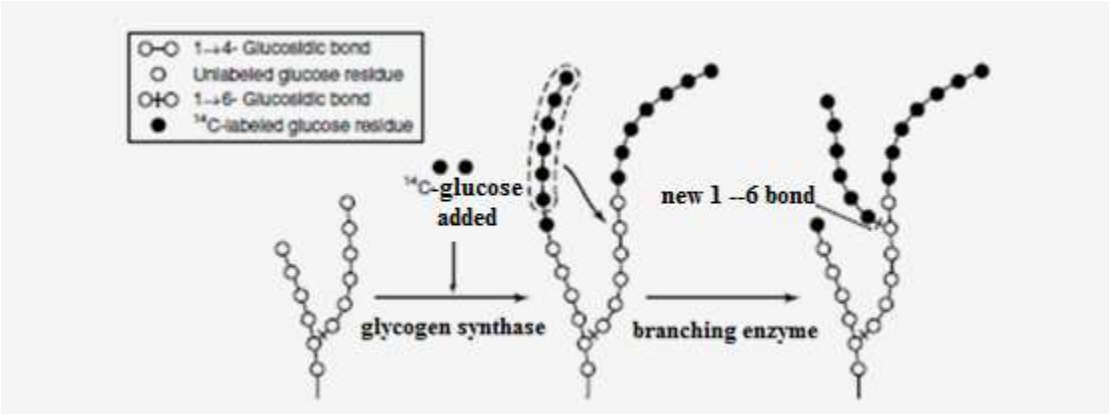


Figure (25): The biosynthesis of glycogen.

Glycogenolysis

Definition and location

Glycogenolysis is the degradation of glycogen to glucose-6-phosphate and glucose in muscle and liver respectively .Glycogenolysis is not the reverse of the glycogenesis but is a separate pathway.

Reactions of glycogenolysis

1- Glycogenolysis occurs primarily by the phosphorolytic breaking (phosphorolysis)of α-1,4- glycosidic bonds of glycogen to yield glucose-1- phosphate and residual glycogen molecule .This process is catalyzed by the enzyme **glycogen phosphorylase** .

The terminal glycosyl residues from outermost chain of the glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a branch point (α-1,6 linkage).

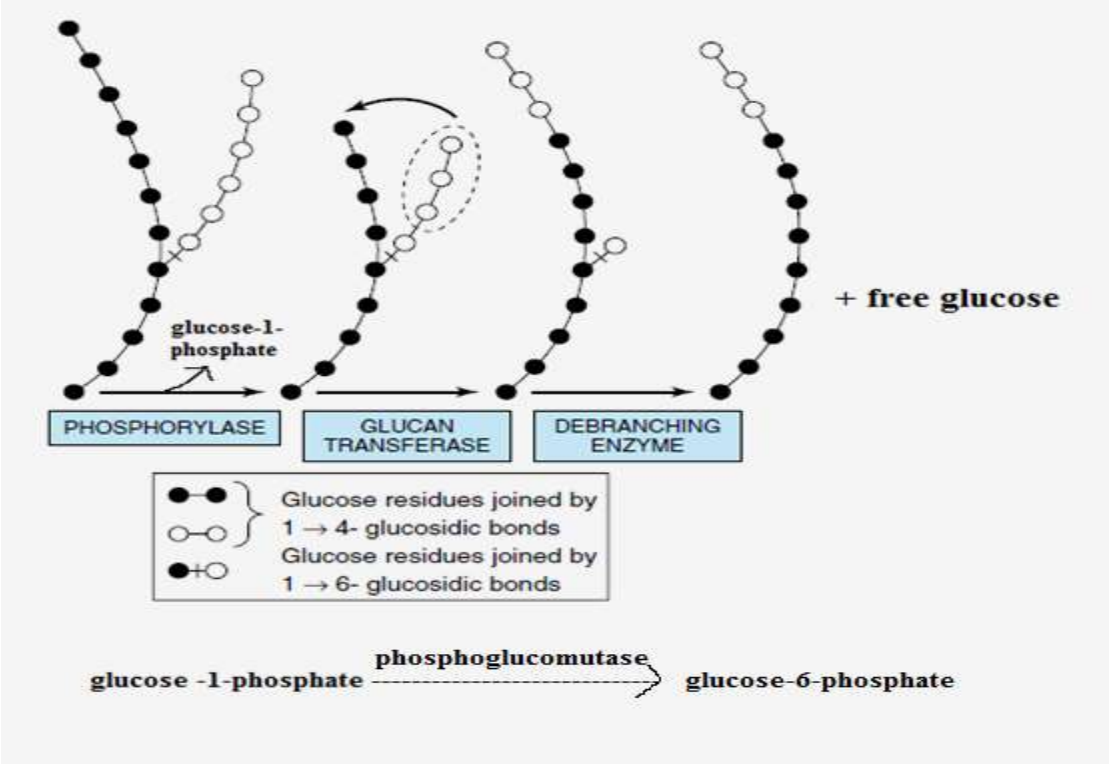
2- Phosphorolysis cannot continue until the branch is removed . This is accomplished by **debranching enzyme**, which acts in two distinct steps:

- First ,it acts as an α-1,4 to α-1,4 **glucan transferase** and transfers three of the remaining residues as a trisaccharide unit from one branch to the other.This exposes the α-1,6 branch point.

-In the second step , the hydrolytic splitting of the α-1,6 linkages occurs by the action of a specific debranching enzyme **amylo-1,6 –glucosidase** .This step release **free glucose** .Further splitting of the glycogen can then proceed by the actions of phosphorylase until the neighbourhood of another branch point is reached .The action of transferase and debranching enzymes are repeated.
The combined action of **phosphorylase ,glucan transferase and debranching enzyme** leads to the complete breakdown of glycogen with the formation of glucose-1-phosphate and free glucose (from hydrolytic cleavage of the 1,6-glycosidic bond).

3- Next ,glucose -1- phosphate is converted to glucose-6-phospate by **phosphoglucomutase** . This is a reversible reaction.

4- In the liver but not in the muscle ,there is a specific enzyme, glucose -6-phosphatase , that removes phosphate from glucose-6-phosphate ,enabling glucose to be formed and diffuse from the cell into the blood.



Figure(26): Schematic representation of glycogenolysis (mechanism of debranching enzyme)

Significance of glycogenolysis and glycogenesis

In liver

Following a meal ,excess glucose is removed from the portal circulation and stored as glycogen by glycogenesis .Conversely ,between meals ,blood glucose levels are maintained within the normal range by release of glucose from liver glycogen by glycogenolysis.

In muscle

The function of muscle glycogen is to act as a readily available source of glucose for glycolysis within the muscle itself during muscle contraction .The muscle cannot release glucose into the blood ,because of the absence of glucose -6- phosphatase .Therefore ,muscle glycogen stores are used exclusively by muscle.

Regulation of glycogenesis and glycogenolysis

As glycogenesis and glycogenolysis are involved in maintaining blood glucose levels ,these are tightly regulated .Inhibition of glycogenolysis enhances net glycogenesis and inhibition of glycogenesis enhances net glycogenolysis

●In the liver ,glycogen synthesis accelerates during well-fed state ,whereas glycogen degradation accelerates during fasting conditions

●In a skeletal muscle degradation occurs during contraction and synthesis occurs during relaxation (rest) of the muscle.

The principle enzymes controlling glycogen metabolism are **glycogen phosphorylase and glycogen synthase** which are regulated reciprocally.

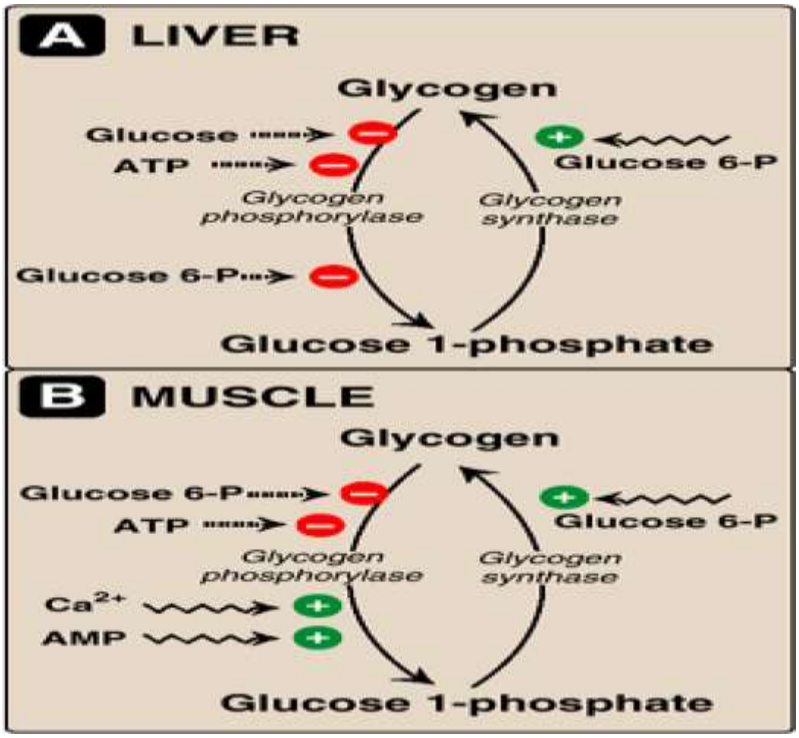


Figure (27): Allosteric regulation of glycogen synthesis and degradation

Glycogen storage diseases

This is a group genetic diseases, that result from a defect in an enzyme required for either glycogen synthase or degradation and characterized by deposition of either normal or abnormal glycogen in the specific tissues.

One example is an inherited defect of glycogen metabolism known as **Von Gierke ، s disease .**This disease results from a defective gene for **glucose-6-phosphatase ,**which catalyze the final step of gluconeogenesis and glycogenolysis .People who lack glucose-6-phosphatase cannot convert glucose-6-phosphate to glucose .As we have seen ,the liver is the primary sources of blood glucose ,and much of this glucose is produced by gluconeogenesis.Glucose-6-phosphate ,unlike glucose ,cannot cross the cell membrane ,and the liver of a person suffering from **Von Gierke ، s disease cannot** provide the organism with glucose .The blood sugar level **falls** precipitously low between meals .In addition ,the lack of glucose -6-phosphatase also affects glycogen metabolism .Because **glucose-6-phosphatase** is absent ,the supply of glucose-6-phosphate in the liver is large .This glucose-6-phosphate can also be converted to glycogen. Person suffering from **Von Gierke ، s disease** has a massively **enlarged liver** as a result of enormously increased stores of glycogen.

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المرحلة الرابعة /الفصل الدراسي الأول
Carbohydrate Metabolism (Lec.5)
by Nada Abotimen

Pentose Phosphate Pathway and NADPH

●The pentose phosphate pathway **(also called the hexose monophosphate shunt, or 6-phosphogluconate pathway)** occurs in the cytosol of the cell.
● It includes two, irreversible oxidative reactions, followed by a series of reversible sugar–phosphate interconversions
●No ATP is directly consumed or produced in the cycle.
● Carbon one of glucose 6-phosphate is released as CO_2, and two NADPH are produced for each glucose 6-phosphate molecule entering the oxidative part of the pathway.
●The rate and direction of the reversible reactions of the pentose phosphate pathway are determined by the supply of and demand for intermediates of the cycle.
●The pathway provides a major portion of the body's **NADPH**, which functions as a biochemical reductant. It also produces **ribose 5-phosphate**, required for the biosynthesis of nucleotides and
● provides a mechanism for the metabolic use of five-carbon sugars obtained from the diet or the degradation of structural carbohydrates in the body.

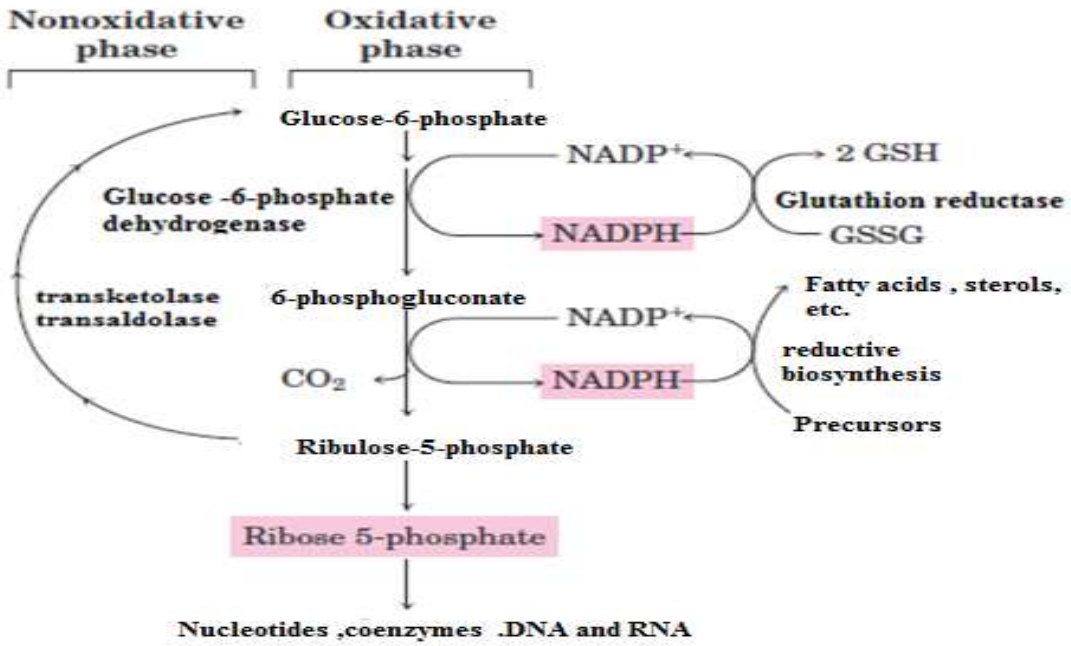


Figure (28): General scheme of the pentose phosphate pathway.

The different between glycolysis and pentose phosphate pathway

Glycolysis	Pentose phosphate pathway
Oxidation occur utilizing NAD^+ as a H-acceptor	Oxidation occurs utilizing NADP as A H-acceptor
Aerobic as well as anaerobic process	Anaerobic process
CO_2 is not produced at all	CO_2 is a characteristic product
ATP is generated ,where it is a major function	ATP is not generated
Ribose phosphate are not generated	Ribose phosphate are generated
80-90% of glucose oxidized by glycolysis	10-20% glucose oxidized by pentose phosphate pathway

Role of NADPH in red blood cells

●Red blood cells need NADPH to maintain glutathione ,a tri-peptide ,in reduced form to protect themselves from oxidizing agents

●The red blood cells is exposed to large amounts of molecular oxygen .Some of the molecular oxygen is converted to superoxide and hydrogen peroxide that can cause irreversible damage to the cell

●Glutathione , a strong reducing agent, protect against oxidative damage by reducing H_2O_2 to water.

●The role of NADPH is to maintain the concentration of reduced glutathione (GSH) at a concentration of about 5mM in the RBC membrane.

●In RBC ,pentose phosphate pathway is the only means of generating NADPH ,other tissues have alternative sources for NADPH production .

●It also keeps iron of the hemoglobin in reduced ferrous state

1- Red blood cell + Oxidants → H_2O_2

Glutathion peroxidase

2- H_2O_2 + 2 GSH → $2H_2O$ + GSSG

Reduced glutathione Se oxidized glutathione

Glutathione reductase

3- GSSG + NADPH →2 GSH + $NADP^+$

from (PPP)

Figure (29):Role of NADPH in disposal of H_2O_2

Disorder of pentose phosphate pathway

Deficiency of glucose -6-phosphate dehydrogenase :Enzyme deficient cells have a lower rate of NADPH production , resulting in a deficiency of reduced glutathione (GSH) ,which is essential to maintain the integrity of the erythrocyte membrane and for keeping Hb in the ferrous state.Cells with a lowered levels of reduced glutathione are more susceptible to **haemolysis.**

Metabolism of other important sugars

1- Fructose metabolism

fructokinase

a) Fructose → Fructose -1-phosphate

ATP ADP

Fructose-1-phosphate

aldolase

Fructose -1-phosphate → Dihydroxy aceton phosphate +Glyceraldehyde

ATP

Triosphosphate isomerase **Glyceraldehyde kinase**

↓ ↓ **ADP**

Glyceraldehyde-3-phosphate

↓

Glycolysis

Hexokinase

b) Fructose → Fructose-6-phosphate

ATP ADP

2- Galactose metabolism

Galactokinase

Galactose → Galactose-1-phosphate

ATP ADP

Galactose-1- phosphate uridyltransferase

Galactose-1-phosphate → UDP-galactose

UDP-glucose glucose-1-phospate

UDP-gluco-4-epimerase

UDP-galactose → UDP- glucose

3- Mannose metabolism

Hexokinase

Mannose → Mannose -6-phosphate

ATP ADP

Phosphomannose isomerase

Mannose -6-phosphate → Fructose-6-phosphate

Biological oxidative and oxidative phosphorylation

●Oxidation . which occurs in living systems, is called oxidation .Biological oxidations are exergonig .The energy released as heat is converted to chemical energy by formation of energy rich compound ATP.

●The formation of ATP from ADP and Pi is termed phosphorylation, as phosphorylation is coupled with biological oxidation ,the process is called **biological oxidative phosphorylation.**

●During biological oxidations ,the reacting chemical system move from a higher energy level to a lower one and therefore there is liberation of energy.

Electron carriers

●**Cytochromes** are iron containing electron transferring proteins ,in which the iron atom is present either Fe^{+3} (ox) or Fe^{+2} (red) form during oxidation reduction .Iron is present as an iron –porphyrin (haem) prosthetic group.

●There are three classes of cytochromes **a, b ,c .**

●Except cytochrome oxidase (cyt aa_3) , the cytochromes are also classified as dehydrogenase.

●The cytochromes in the respiratory chain are arranged in the sequence **b→c_1→ c→aa_3**.

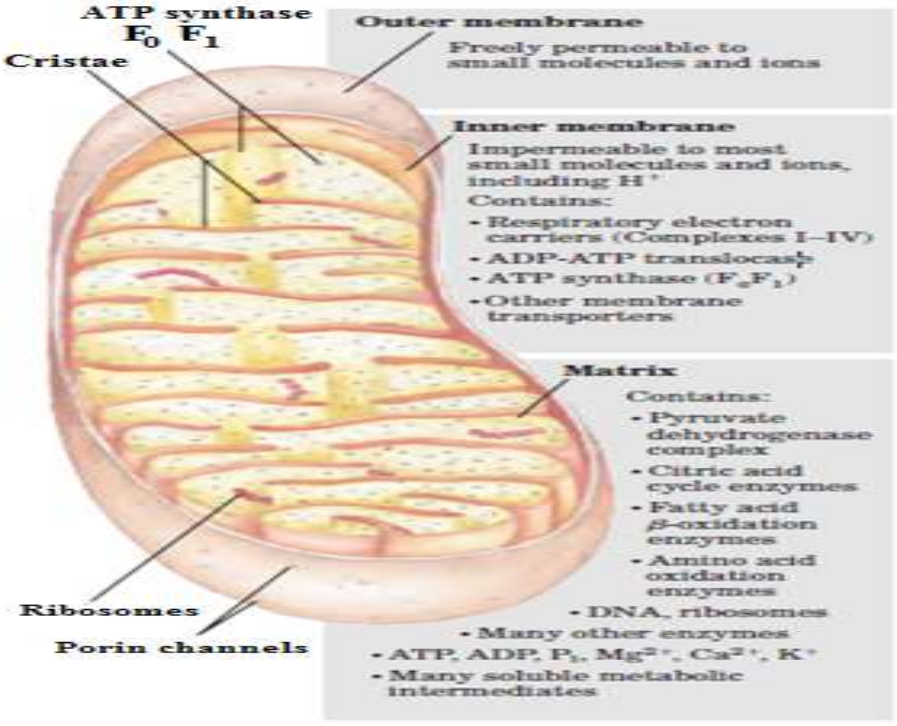
●Besides the respiratory chain , cytochromes are also found in endoplasmic reticulum (cyt p $_{450}$ and cyt b_5)

Mitochondrial electron transport chain and oxidative phosphorylation

The electron transport chain or respiratory chain is the final common pathway in aerobic cells by which electrons (reducing equivalent)derived from various substrates are transferred to oxygen .Electron transport chain is a series of highly organized oxidation reduction enzymes ,co-enzymes and electron carrier cytochromes.

Localization of the electron transport chain

The electron transport chain is present in the **inner mitochondrial membrane .**The enzymes of the electron transport chain are embedded in the inner membrane in association with the enzymes of oxidative phosphorylation .



Figure(30): Biochemical anatomy of a mitochondrion

Function of respiratory chain

●Respiratory chain collects and oxidizes reducing equivalents with liberation of energy in the form of ATP .

●The energy liberated during oxidation of carbohydrates ,fatty acids and amino acids is made available within the mitochondria as reducing equivalents (H or e^-).

●The mitochondria which contains respiratory chain that collects and transport reducing equivalents and hands them over to their final acceptor ,oxygen to form water.

●Liberated free energy in the mitochondria by oxidative phosphorylation.

Components of the respiratory chain or electron transport chain

The major components of the respiratory chain include

●Nicotinamide adenine dinucleotide **(NAD^+)** of various dehydrogenase.

●Flavin mononucleotide **(FMN)** of NADH-dehydrogenase and flavin adenine dinucleotide **(FAD).**

●Ubiquinone or **coenzyme Q** . Except coenzyme Q ,all members of this chain are proteins . Coenzyme Q (CoQ) is a fat soluble quinone (ubiquinone)and is a constituent of mitochondrial ,lipids.

Two different types of iron containing proteins, the **iron-sulfur (Fe-S) protein** associated with FMN and cytochrome b. The iron take part in the oxidation –reduction mechanism ,which accepts only single electron.

●Cytochromes (haem –protein), **b ,c_1 ,c , aa_3** . Cytochrome aa_3 ,are also called cytochrome oxidase and are copper containing haemproteins.

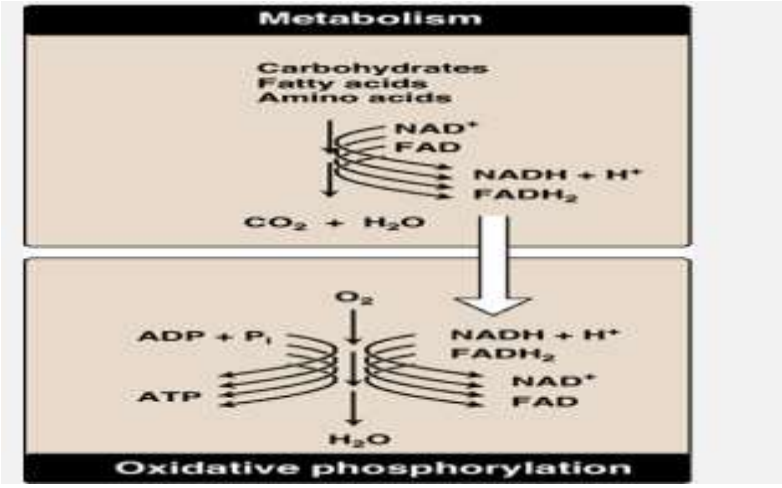


Figure (31): The metabolic breakdown of energyyielding molecules.

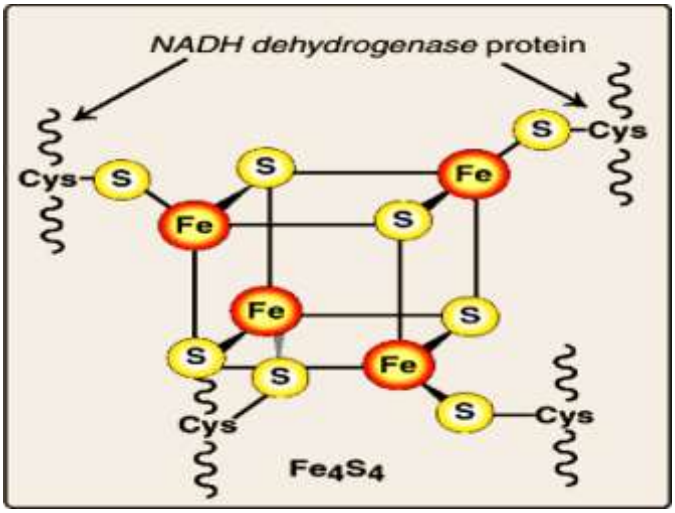


Figure (32): Iron-sulfur center of NADH dehydrogenase.

Substrate (e.i. succinate)

↓

FAD

↓

Malate, Isocitrate **FeS**

↓ ↓

Substrate → NAD^+ → FMN→(FeS) →Coenzyme Q →Cyt b → (FeS)→Cyt c_1 →Cyt c →Cyt aa_3 → O_2

(i.e. pyruvate,

α-ketoglutarate)

Figure (33): Components of the respiratory chain in mitochondria

Structural organization of components of respiratory chain

Components of the respiratory chain are arranged in order of increasing redox potential and reducing equivalents flow through the chain from the components of more negative redox to the components of more positive redox potential .The components of the respiratory chain are present in the inner mitochondrial membrane as four complexes:

1. **Complex –I NADH: Ubiquinone oxidoreductase.**
2. **Complex –II Succinate :Ubiquinone oxidoreductase**
3. **Complex –III Ubiquinole : Cytochrome c oxidoreductase**
4. **Complex –IV cytochrome c : Oxygen oxidoreductase.**

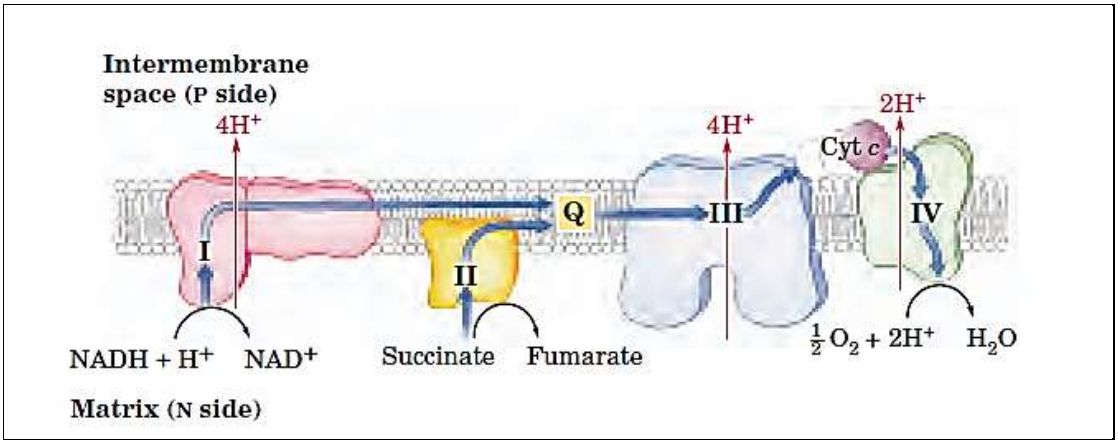


Figure (33): Summary of the flow of electrons and protons through the four complexes of the respiratory chain.

Complex –I	**NADH**				
NADH:Ubiquinone	↓				
Oxidoreductase	**FMN**				
	↓				
	Fe-S			**Succinate**	
	↓			↓	
	Ubiquinone (CoQ)	←	**Fe-S**	←	**FAD**
			Complex –II		
	↓		**Succinate – ubiquinone**		
	Cyt b		**oxidoreductase**		
Complex –III	↓				
Ubiquinol :Cytochrome c	**Fe-S**				
Oxidoreductase	↓				
	Cyt c_1				
	↓				
	Cyt c				
	↓				
Complex – IV	**Cyt aa_3**				
Cytochrome c : Oxygen	↓				
Oxidoreductase	**O_2**				

Figure(34): The electron transport complexes.

Reaction of electron transport or respiratory chain

●NAD^+ molecule accepts and transfers one hydride ion (H^- i.e. one H^+ and $2e^-$).
●FMN or FAD or coenzyme Q accepts and donates two hydrogen (H_2 i.e. $2H^+$ and $2e^-$) at a time.

●While each cytochrome or iron –sulfur protein molecule accepts and transfers only one electron but not hydrogen ion (H^+).

The following sequence of reactions occurs in the transfer of electrons to the ultimate acceptor oxygen

1- Formation of NADH:

●Most of the reducing equivalent entering the respiratory chain arise from the action of dehydrogenases that use the coenzyme **NAD^+**
●Thus **NAD^+** is reduced to **NADH** by various dehydrogenases which remove two hydrogen atoms **($2H^+$+$2e^-$)** from their metabolite **(MH_2)** , which get oxidized to **M.**
●In the oxidation reduction reaction , two electrons and a proton **($2e^- + H^+ = H^-$, hydride ion)** Are actually accepted by **NAD^+** to form **NADH** ,while the second proton **(H^+)** is released into the aqueous medium.

2- Formation of $FMNH_2$

●The free proton and the hydride ion carried by **NADH** are next transferred to **FMN** coenzyme of **NADH-dehydrogenase** and get reduced to **$FMNH_2$** with oxidation of **NADH** to **NAD^+.**

3- Collection of electrons from $FMNH_2$ by Fe-S protein

In addition to **FMN** ,**NADH dehydrogenase** also consists of **Fe-S** protein , which accepts only electron from **$FMNH_2$** .Thus **two Fe-S** protein molecules accept **two electrons** from **one $FMNH_2$** molecule with release of two protons **($2H^+$)** into the medium.

4- Collection of reducing equivalents from **Fe-S protein** of **$FMNH_2$ and FAD- linked dehydrogenase** by ubiquinone **(CoQ).**

●**CoQ** accepts two electrons from **two Fe-S protein** molecules and **two protons($2H^+$)** from the medium to get reduced to **ubiqunol ($CoQH_2$).**

●**Ubiquinone (CoQ)** Collects reducing equivalents from **NADH –dehydrogenase ,i.e. from $FMNH_2$** as well as from other substrates that are linked directly to the respiratory chain through **FAD –linked dehydrogenases** of mitochondria .The major substrates includes succinate , glycerol-3-phosphate and fatty acyl- CoA .

5- Removal of electrons by cytochroms

●Beyond ubiquinone **(CoQ),** oxidation reduction process occurs by removal of electrons with the help of **cytochromes**.
●Each **cytochrome** is a protein that contains iron atom bound to porphyrin nucleus.
●The iron atoms in the cytochromes are in the **ferric (Fe^{+3})** state.
●As they accept an electron , they are reduced to ferrous **Fe^{+2}** .
●As the electrons pass to the next component of the electron transport chain ;they are reoxidized to **Fe^{+3}**.
●**Coenzyme QH_2** get oxidized by donating its electrons to next members of electron transport chain in a sequence of:

Cyt b → Fe-S → Cyt c → Cyt c_1 → Cyt aa_3 → O_2

●**Cytochromes** accept only electrons from **coenzyme QH_2** With the release **of $2H^+$** in the medium.

●As a **cytochrome** can accept only one electron , **$CoQH_2$** transfers its two electrons to two molecules of cytochrome **b,c_1, c, and aa_3** sequnentially.

6- Reduction of oxygen to water

●The last cytochrome complex **is cytochrome oxidase (cyt aa_3)** which passes electrons **from cytochrome c to molecular oxygen.**

●A whole **O_2** molecule must accept **4 electrons** to be reduced **to $2H_2O$.**
Since there are **only two electrons** ,they are removed per turn of cycle , the electron transport chain must cycle **twice** to pass along **4 electrons** to the **O_2** molecule .
Each **oxygen atom** with two electrons accept two protons from the medium and a molecule of water results.
The reduction of O_2 by cytochrome oxidase reaction accounts for the production of about **300 ml of water / day .This water is called metabolic water.**

Formation of ATP in oxidative phosphorylation

During the transfer of electrons through the electron transport chain energy is produced .This energy is coupled to the formation of ATP molecules by phosphorylation of ADP by an enzyme **F_0F_1 ATPase.**

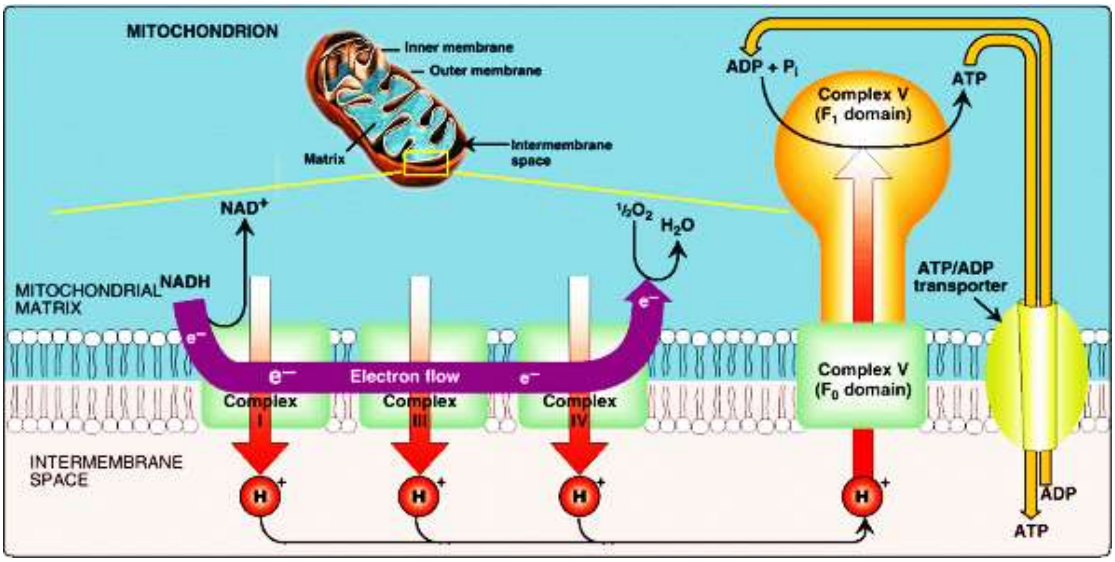


Figure (35): Electron transport chain shown coupled to the transport of protons.

Coupling sites for ATP synthesis

●There are three energy –conserving or coupling sites of the electron transport chain these are **complex-I ,complex-III , and complex –IV** that provides the energy required to make ATP from ADP and inorganic phosphate by an enzyme **F_0 F_1 ATPase** in the process of oxidation phosphorylation .

●Electron that enter the chain through NADH pass through all three energy –conserving sites and thus yield three ATPs.

●However electrons that enter the chain through $FADH_2$ pass through only two energy conserving sites ,as they bypass site 1 , they yield two ATPs.

F_0 F_1 ATPase

●The enzyme complex **F_0 F_1 ATPase** that synthesize ATP is also called **ATP synthase**

● **F_0 F_1 ATPase** is composed of two protein subunits , **F_0 and F_1** .

● **F_0 and F_1** which are embedded in the inner membrane and extends across it.

F_0

The **F_0** unit forms a channel or path , through which hydrogen ions may pass across the membrane.

F_1

It is tightly bound to **F_0** and protruding into the matrix from the inner mitochondrial membrane .It contains the catalytic site for ATP synthesis.

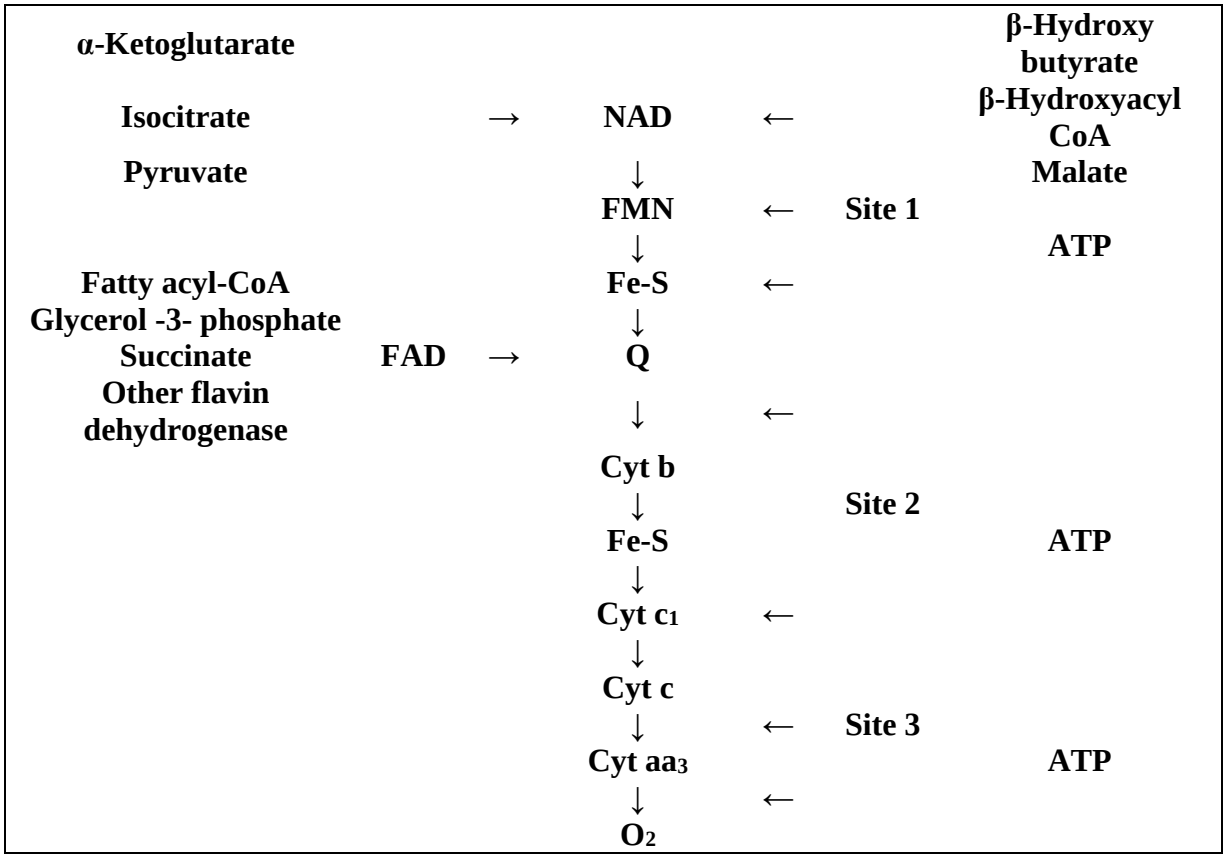


Figure (36): Coupling sites of oxidative phosphorylation for the formation of ATP.

Inhibitors of electron transport chain and oxidative phosphorylation

In the cell , electron flow in the electron transport chain must be sequential from NADH or a flavoprotein all the way to oxygen to generate ATP. The three complexes **I ,III ,IV** of a electron transport chain is associated with generation of ATP .Blocking the chain at any point prevents the formation of any ATP ,because pumping of protons is associated with the movement of the electrons from one carrier to the next.
If the movement is blocked anywhere in the chain ,the carriers on the oxidized site of the block have no source of electrons and the carrers on the reduced side of the block have nowhere to donate their electrons.

Inhibitors that arrest respiration by interrupting the flow of electrons through the respiratory chain and thus blocking the respiratory chain at three saites:

1- Complex-I
2- Complex-III
3-Complex-IV

This results in a block in

1- Proton pumping 2-ATP synthesis 3- Oxygen uptake

Inhibitors of first site or complex-I

These inhibitors prevents the oxidation of substrates that communicate directly with the respiratory chain via NAD-linked dehydrogenase by blocking the transfer of reducing equivalents from **Fe-S protein to ubiquinone(Q)** , e.g. **the insecticide rotenone**.

Inhibitors of second site or complex-III

These inhibitors prevent transfer of electrons from **cytochrome b to cytochrome c_1 ,** e.g. **antimycin A**

Inhibitors of three site or complex-IV

These inhibitors prevent transfer of electrons from **cytochrome aa_3 to molecular oxygen** by inhibiting cytochrome oxidase and can therefore totally arrest respiration.

Inhibitors of oxidative phosphorylation (F_0 F_1 ATPase)

Another set of inhibitors do not inhibit special complexes .Instead these compounds block phosphorylation directly by inhibiting **F_0 F_1 ATPase** enzyme .In a tightly coupled system ,inhibition of phosphorylation can lead to inhibition of oxidation .For example **antibiotic oligomycin.**

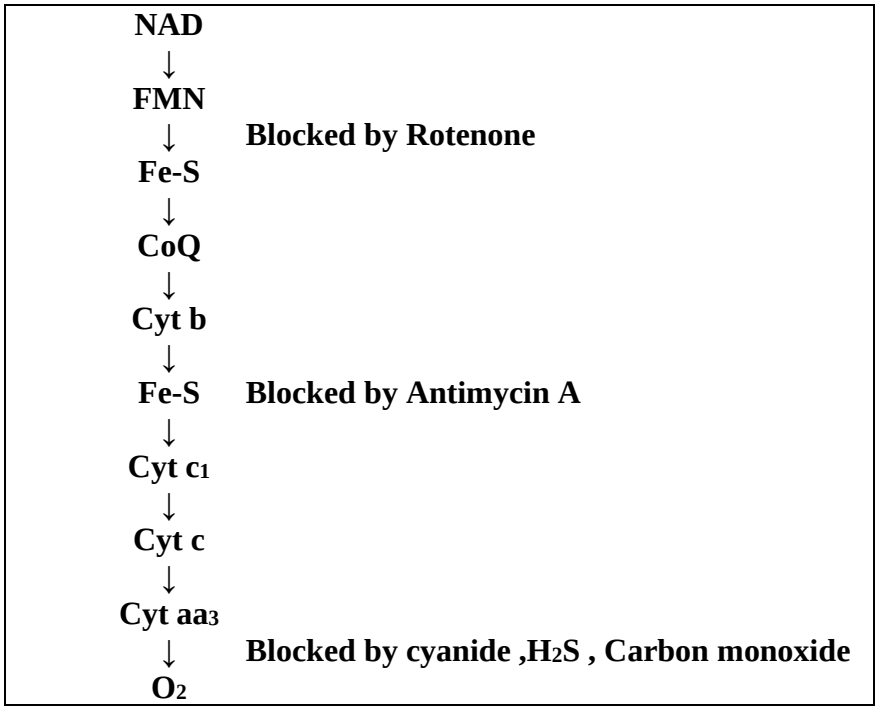


Figure (37): Sites of action of various inhibitors of electron transport chain

Uncoupled Mitochondria in Brown Fat Produce Heat

There is a remarkable and instructive exception to the general rule that respiration slows when the ATP supply is adequate. Most newborn mammals, including humans,have a type of adipose tissue called **brown fat** in which fuel oxidation serves not to produce ATP but to generate heat to keep the newborn warm. This specialized adipose tissue is brown because of the presence of large numbers of mitochondria and thus large amounts of cytochromes, whose heme groups are strong absorbers of visible light.

The mitochondria of brown fat are like those of other mammalian cells in all respects, except that they have a unique protein in their inner membrane. **Thermogenin,** also called the **uncoupling protein** provides a path for protons to return to the matrix without passing through the **F_0 F_1 complex** .

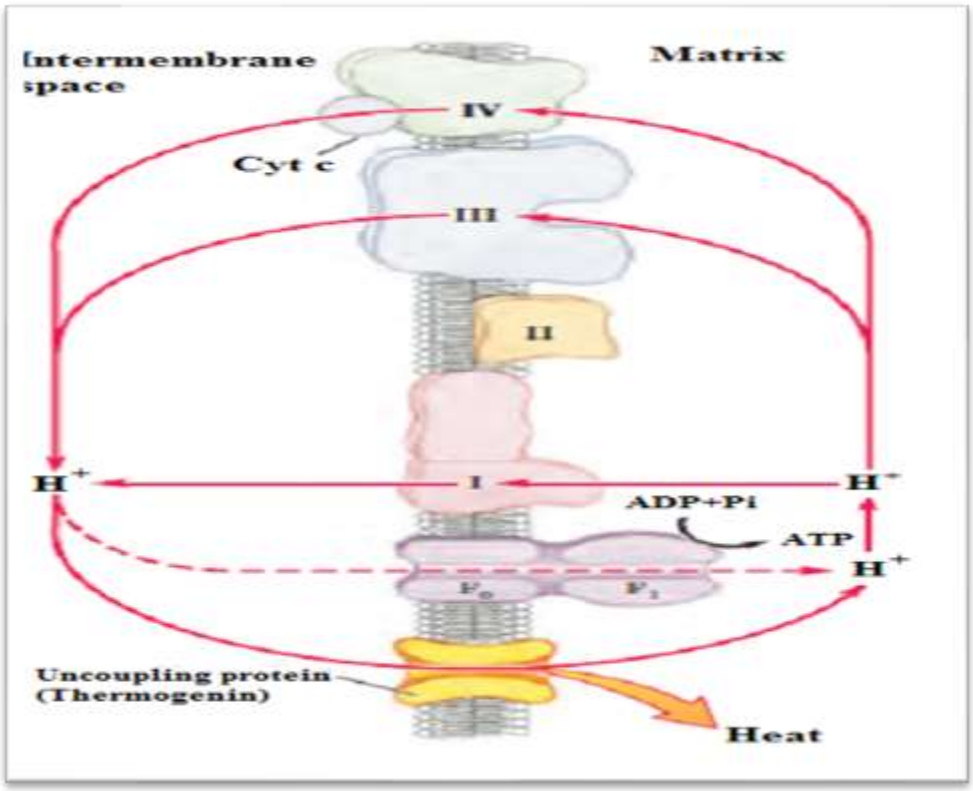


Figure (38): Heat generation by uncoupled mitochondria

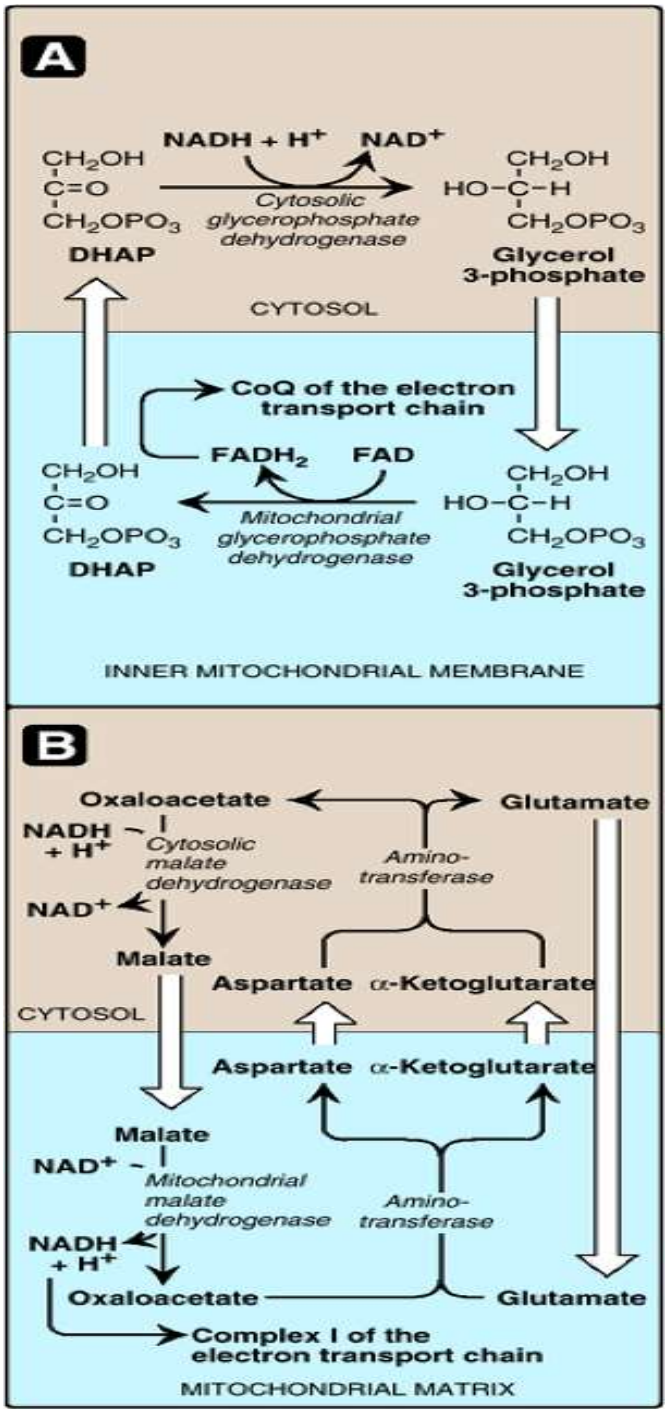
Shuttle system for oxidation of extramitochondrial NADH

●Most of the **NADH and $FADH_2$** entering the mitochondrial electron transport chain arises from Kreb's cycle and β- oxidation of fatty acids , located in the mitochondria itself.

●Since the inner mitochondrial membrane is not permeable to cytoplasmic NADH , **how can the NADH generate by glycolysis ,which take place outside of the mitochondria ,be oxidized to NADH by respiratory chain located in mitochondria.**

●Special shuttle systems carry reducing equivalents from cytosolic NADH into the mitochondria by an indirect route. This transport of reducing equivalent as NADH regardless of sources by enzymatic processes **called Shuttle mechanism.**

●Two such mechanisms that can lead to the transport of reducing equivalent from the cytoplasm into mitochondria are, (1)The malate-aspartate shuttle,
(2)The glycerol phosphate shuttle



Figure(39): Shuttle pathways for the transport of electrons across the inner mitochondrial membrane. A. Glycerolphosphate shuttle. B. Malateaspartate shuttle.