CHAPTER III: CARBOHYDRATE CATABOLISM

1. Introduction

Carbohydrate metabolism is the set of various biochemical processes responsible for the formation, degradation, and interconversion of carbohydrates in living organisms. The ***catabolism of carbohydrates*** corresponds to the chemical reactions that lead to the degradation of carbohydrate molecules (catabolism) to produce energy.

Carbohydrates that can be degraded by microorganisms are numerous and varied. Polysaccharides such as starch, cellulose, inulin, and sometimes smaller molecules like sucrose are unable to penetrate the cell. They must first be broken down into low molecular weight fragments by hydrolytic enzymes excreted by the microorganism into the environment. The products formed then enter the cell. In most cases, the transformation of carbohydrate macromolecules, as well as various other organic substances, leads to the formation of **hexoses** (mainly glucose) or **pentoses**. Glucose is the starting point for the main pathways of cellular catabolism.

2. Glucose catabolism

The oldest known hexose degradation pathway is ***Glycolysis***, which leads to the transient formation of pyruvic acid.

There are alternatives to glycolysis in a wide variety of aerobic or anaerobic microorganisms. These pathways are either used exclusively or concurrently with glycolysis.

2.1. Glycolysis or the Embden-Meyerhof-Parnas (EMP) Pathway

This **hexose diphosphate pathway** is a series of reactions allowing the transformation of glucose into two pyruvate molecules, during which two NADH molecules and two ATP molecules are produced (4 ATP formed by substrate-level phosphorylation and 2 ATP consumed).

Glycolysis is widespread among microorganisms: yeasts, molds, aerobic-anaerobic bacteria (*Enterobacteria*, etc.). For some, glucose is almost exclusively degraded by this pathway (*Streptomyces griseus* 97%, *Trypanosoma* 100%).

The key points of the glycolysis pathway are:

- Activation of glucose in the form of glucose-6P using ATP, isomerization, and a second phosphorylation to form fructose-1,6-diphosphate and two ADP molecules.

- Cleavage of fructose-1,6-diphosphate into two triose-phosphate molecules by the action of aldolase (an enzyme characteristic of this metabolic pathway).
- Isomerization of 3-phosphoglyceraldehyde/dihydroxyacetone-phosphate and dehydrogenation with reduction of NAD^+. This reaction is accompanied by substrate-level phosphorylation and leads to the formation of 1,3-diphosphoglycerate (which has an energy-rich bond).
- Transfer of a phosphate ester bond from 1,3-diphosphoglycerate to ADP.
- Transfer of a phosphate ester bond from phosphoenolpyruvate to ADP, forming pyruvate and ATP.

The overall process is:

$$\text{Glucose} + 2NAD^+ + 2\ ADP + 2Pi \rightarrow 2\ \text{pyruvate} + 2NADH + 2H^+ + 2ATP + H_2O$$

2.2. Alternatives to Glycolysis

2.2.1. Pentose Phosphate Pathway or Hexose Monophosphate Pathway (Otto Warburg, Frank Dickens, and Bernard Horecker pathway).

This aerobic pathway is very important because it provides **pentoses**, which are required for the synthesis of nucleic acids and prosthetic groups containing nucleotides. It also provides the elements necessary for the synthesis of aromatic amino acids and vitamins. This pathway is present alongside glycolysis in varying proportions in many microorganisms. It is used, at least partially, by yeasts, molds, and many aerobic-anaerobic bacteria like *Escherichia coli*. It plays a fundamental role in aerobic bacteria that lack glycolysis (*Pseudomonas, Xanthomonas, Acetobacter xylinum*, etc.).

The first steps leading to the formation of **gluconate-6P** are shared with other respiratory and fermentative pathways. From gluconate-6P, ***ribulose-5P*** is formed, which is the starting point of the oxidative **pentose-P cycle**.

The hexose monophosphate pathway does not directly produce energy, but the $NADPH_2$ formed is a source of ATP when electrons are transported to oxygen via the respiratory chain; $NADPH_2$ can also be used by lipid metabolism. The global equation is:

$$3\ \text{glucose-6P} + 6\ NADP^+ + 3\ H_2O \rightarrow 2\ \text{fructose-6P} + \text{glyceraldehyde-3P} + 3\ CO_2 + 6\ \text{NADPH}, H^+ + 6\ H^+$$

After complete degradation of glucose and intermediates:

$$\text{Glucose-6P} + 12\ NADP^+ + 7\ H_2O \rightarrow 6\ CO_2 + 12\ \text{NADPH}, H^+ + 12\ H^+ + Pi$$

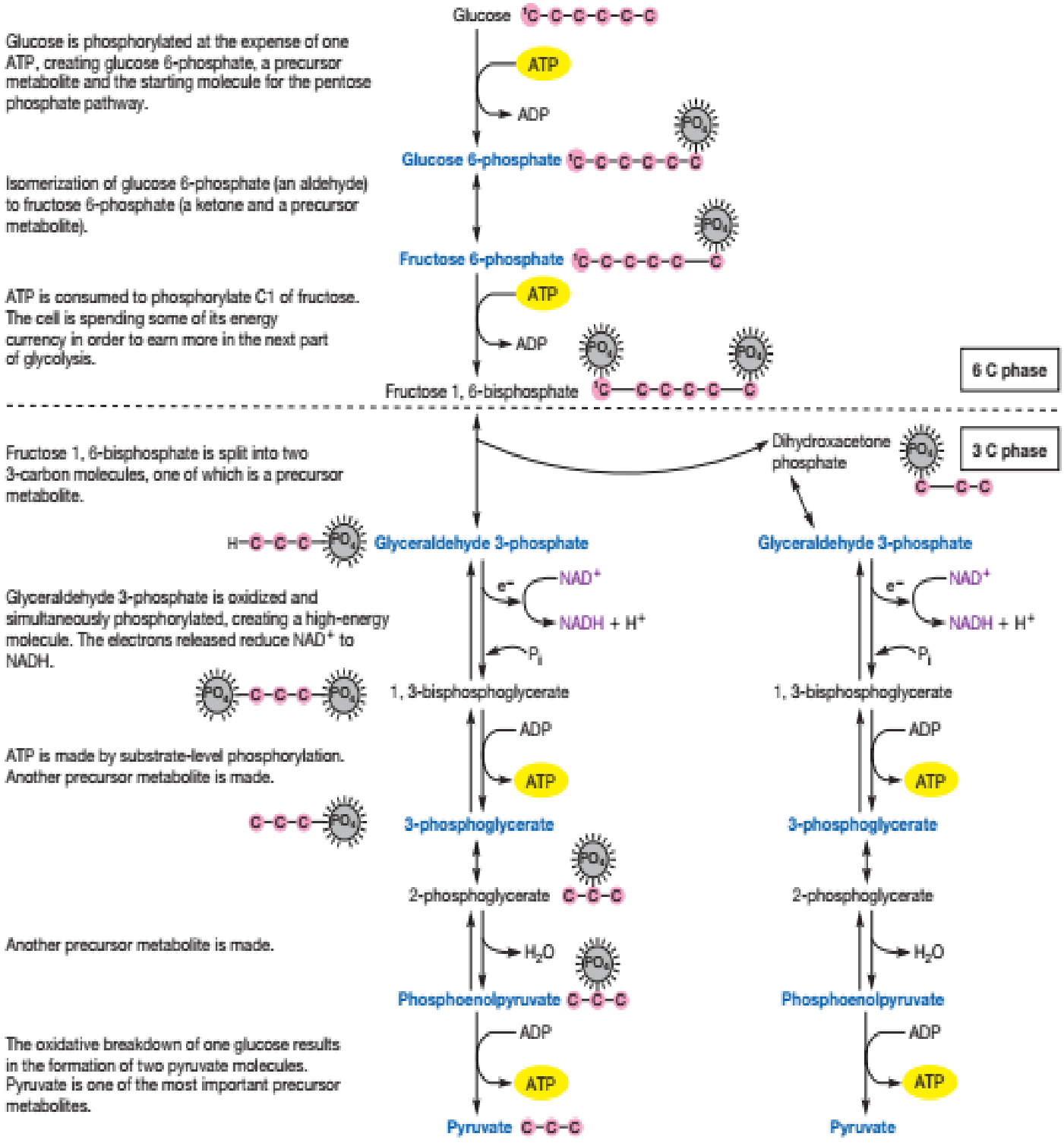

Figure 10: Embden-Meyerhof Pathway.

[This is one of three glycolytic pathways used to catabolize glucose to pyruvate, and it can function during aerobic respiration, anaerobic respiration, and fermentation. When used during a respiratory process, the electrons accepted by NAD^+ are transferred to an electron transport chain and are ultimately accepted by an exogenous electron acceptor. When used during fermentation, the electrons accepted by NAD^+ are donated to an endogenous electron acceptor (e.g., pyruvate). The Embden-Meyerhof pathway is also an important amphibolic pathway, as it generates several precursor metabolites (shown in blue)].

The connections between the glycolysis pathway and the hexose monophosphate pathway are numerous. Glyceraldehyde-3P can be transformed into pyruvate (aerobic-anaerobic bacteria, yeasts, molds). The balance is then:

$$\text{Glucose} + 6\ NADP^+ \rightarrow \text{pyruvate} + 3\ CO_2 + 6\ NADPH, H^+$$

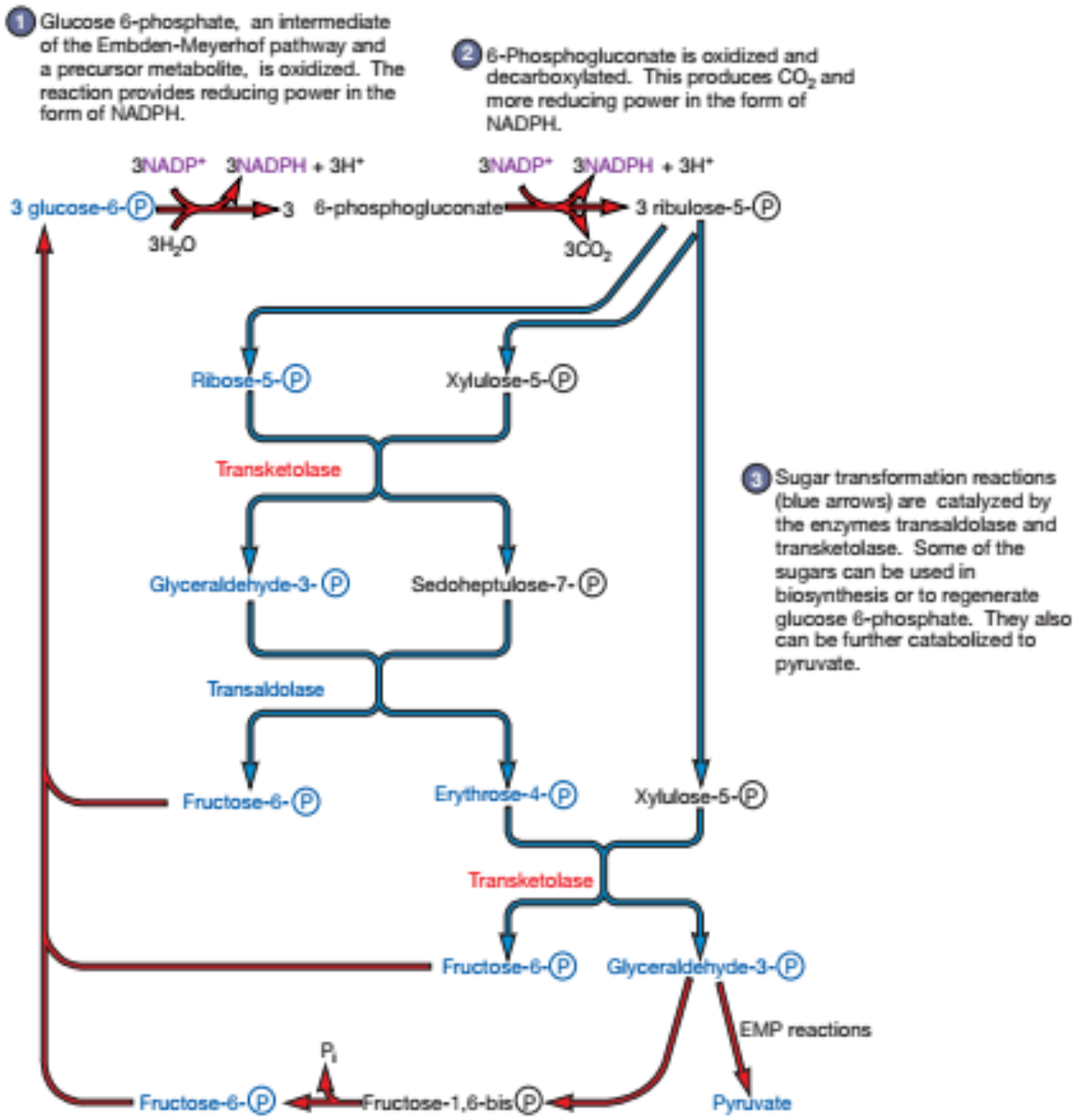

Figure 11: Pentose Phosphate Pathway.

[The catabolism of three glucose 6-phosphate molecules to two fructose 6-phosphates, a glyceraldehyde 3-phosphate, and three CO_2 molecules is traced. Note that the pentose phosphate pathway generates several intermediates that are also intermediates of the Embden-Meyerhof pathway (EMP). These intermediates can be fed into the EMP with two results: (1) continued degradation to pyruvate or (2) regeneration of glucose 6-phosphate. The pentose phosphate pathway also plays a major role in producing reducing power (NADPH) and several precursor metabolites (shown in blue). The sugar transformations are indicated with blue arrows. These reactions are catalyzed by the enzymes transketolase and transaldolase and are shown in more detail in figure 9.7].

- Pyruvate is used by the pathways we will examine later (pyruvate metabolism).
- Glyceraldehyde-3P can also be condensed into fructose-6P by glyceraldehyde-P-aldolase (aerobic bacteria):

$$2 \text{ glyceraldehyde-3P} \rightarrow \text{fructose-6P} + \text{Pi}$$

The balance is then:

$$\text{Glucose} + \text{ATP} + \text{NADP}^+ \rightarrow 6\ \text{CO}_2 + \text{ADP} + \text{Pi} + 12\ \text{NADPH, H}^+$$

The Pentose Phosphate Pathway can be broken down into 3 parts:

- **An oxidative part:** a series of reactions that oxidize glucose-6P, reduce $NADP^+$ to NADPH, and lead to the formation of ribulose-5-phosphate.
- **1st non-oxidative part:** reversible isomerization and epimerization reactions.
- **2nd non-oxidative part:** transketolization and transaldolization reactions (transfer of groups containing several carbons).

a. Oxidative step

Glucose-6-phosphate dehydrogenase catalyzes the oxidation of the aldehyde (hemiketal) group on carbon C1 of glucose-6P to form a carboxylic acid in an ester bond, a ***lactone***. $NADP^+$ acts as the electron acceptor. This reaction is irreversible and controls the flow of the pentose phosphate pathway.

6-Phosphogluconolactonase catalyzes the hydrolysis of the lactone, opening the cycle to form 6-phosphogluconate. While the opening of the cycle can occur without the enzyme, 6-phosphogluconolactonase speeds up the reaction by reducing the lifespan of the highly reactive and potentially toxic 6-phosphogluconolactone.

Phosphogluconate dehydrogenase catalyzes the oxidative decarboxylation of 6-phosphogluconate to form ribulose-5-phosphate. The hydroxyl group at C3 of 6-phosphogluconate is oxidized to a ketone, promoting the loss of the carboxyl group at C1 in the form of CO_2. $NADP^+$ serves as the electron acceptor.

Ribulose-5-phosphate is also a key intermediate in the Calvin cycle (photosynthesis).

b. 1st Non-Oxidative step

- Epimerase interconverts ribulose-5-phosphate and xylulose-5-phosphate.
- Isomerase converts ribulose-5-phosphate (ketose) into ribose-5-phosphate (aldose).

These two reactions are reversible and involve deprotonation to form an enediol intermediate.

c. 2nd Non-Oxidative step

- First Transketolization: involves transferring a ketol group (CH_2OH-CO) from xylulose-5-phosphate to ribose-5-phosphate or erythrose-4-phosphate. The enzyme that catalyzes this reaction is ***transketolase***, which functions in the presence of thiamine pyrophosphate (a derivative of vitamin B1). This produces sedoheptulose-7-phosphate and 3-phospho-D-glyceraldehyde.

- Second Transketolization: transfers the ketol group from xylulose-5-phosphate to erythrose-4-phosphate, producing fructose-6-phosphate and 3-phospho-D-glyceraldehyde.

- Transaldolization: involves transferring a dihydroxyacetone group (CH_2OH-CO-CH_2OH) from sedoheptulose-7-phosphate to 3-phospho-D-glyceraldehyde. The enzyme that catalyzes this reaction is ***transaldolase***, which functions without a coenzyme. This produces erythrose-4-phosphate and fructose-6-phosphate.

This pathway:

- Serves as an alternative to glycolysis with a more anabolic (biosynthesis) than catabolic (degradation) outcome.
- Exists in all eukaryotes and almost all bacteria.
- Is independent of oxygen (it occurs in both aerobic and anaerobic conditions).
- Produces reducing power in the form of NADPH, which is then used for the biosynthesis of fatty acids, cholesterol, and the reduction of glutathione (fighting oxidative stress through reactive oxygen species).
- Produces pentoses, especially ribose-5-phosphate, used for the biosynthesis of pyridine coenzymes (NAD^+ and $NADP^+$), flavin coenzymes (FMN and FAD), coenzyme A, and the biosynthesis of nucleotides.
- Produces erythrose-4-phosphate, a precursor to aromatic amino acids.

2.2.2. 2-Keto-3-Deoxygluconate Pathway or the Entner-Doudoroff Pathway

This pathway shares steps with both the hexose monophosphate pathway and glycolysis. It was discovered by Entner and Doudoroff while studying the oxidation of glucose by species of *Pseudomonas* (aerobic microorganisms). It is also found in *Azotobacter* and some molds. Some references have mentioned that only the bacterium *Zymomonas mobilis* uses this pathway for anaerobic glucose fermentation.

The essential steps of this pathway are:

- Activation of glucose by ATP.
- Oxidation of the aldehyde group of glucose-6P to form 6-phosphogluconate with concurrent reduction of $NADP^+$.
- Dehydration of 6-phosphogluconate and formation of CDPG or KDPG (2-keto-3-deoxy-6-phosphogluconate).
- Cleavage by CDPG-aldolase to produce both glyceraldehyde-3P and pyruvate.
- Conversion of glyceraldehyde-3P to pyruvate via glycolysis, yielding 2 moles of ATP and 1 mole of $NADH_2$ per mole of triose phosphate.

For **one molecule of glucose**, **1 ATP, 1 NADPH2, and 1 NADH2 are formed**.

In *Pseudomonas*, this pathway is used alongside the hexose monophosphate pathway.

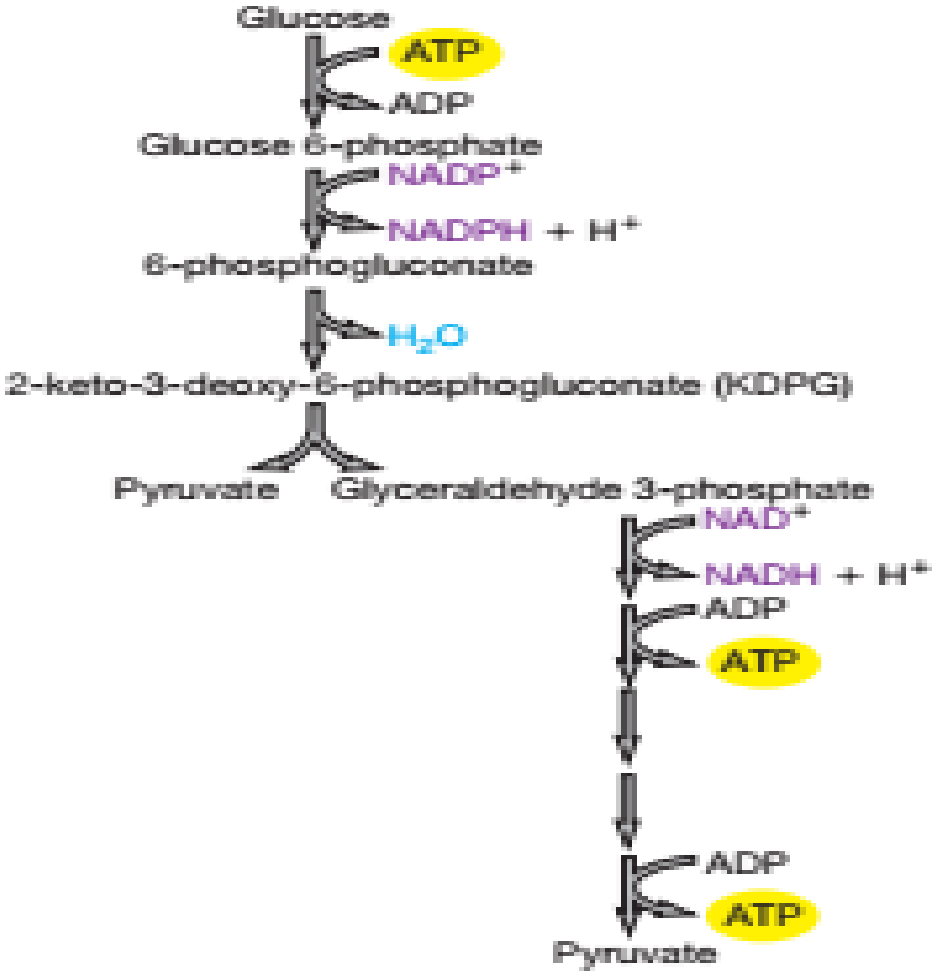

Figure 14: Entner-Doudoroff Pathway.

[The sequence leading from glyceraldehyde 3-phosphate to pyruvate is catalyzed by enzymes common to the Embden-Meyerhof pathway].