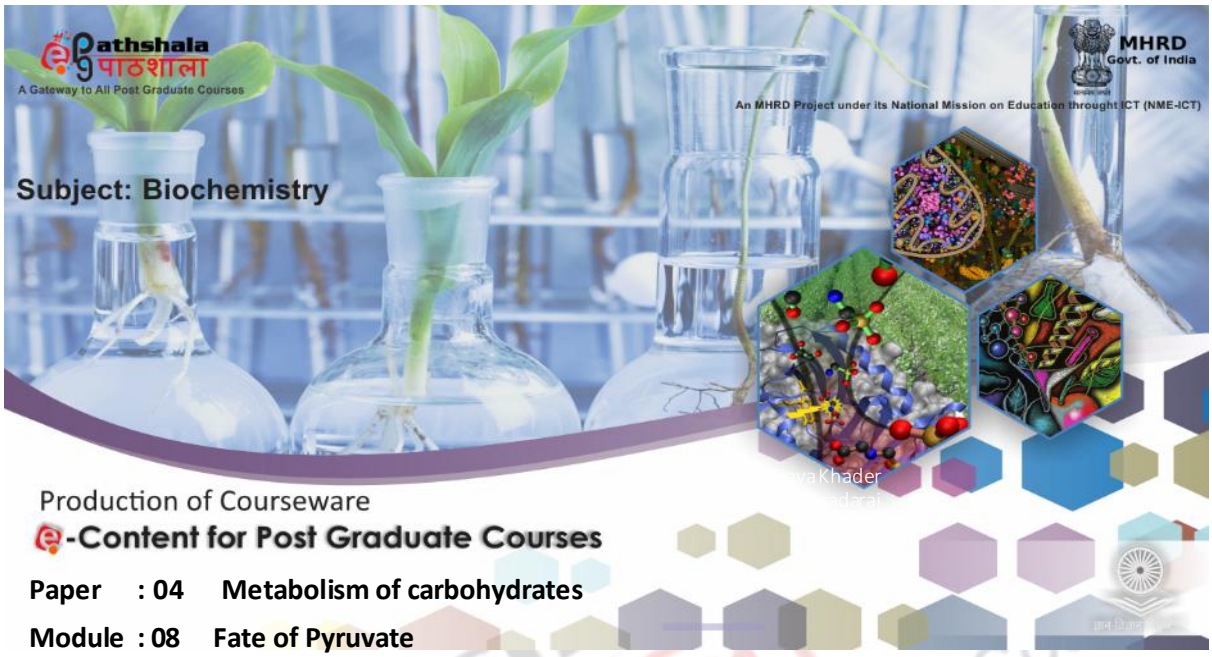

Production of Courseware

e-Content for Post Graduate Courses

Paper : 04 Metabolism of carbohydrates

Module : 08 Fate of Pyruvate

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Description of Module	
Subject Name	Biochemistry
Paper Name	04 Metabolism of carbohydrates
Module Name/Title	Fate of pyruvate

Fate of Pyruvate

Objectives

- To understand fate of pyruvate under different conditions.
- Pyruvate has 3 fates- depending on availability of oxygen.
- In the presence of oxygen (aerobic conditions): enter into the tricarboxylic acid (TCA) cycle- PDH.
- Under the anaerobic conditions: results in formation of lactic acid with help of lactate dehydrogenase or ethanol fermentation- pyruvate decarboxylase, alcohol dehydrogenase.

Introduction

- Pyruvate, a key molecule in metabolism of eukaryotic and human and its fate differs depending upon presence and absence of oxygen.
- It is the end-product of glycolysis and is eventually transported into mitochondria as a major energy and participates in the TCA cycle.
- In the glycolysis, glucose is converted into two molecules of pyruvate with the generation of ATP. However, if reactions stops at pyruvate, due to imbalance redox, it would not proceed for long.
- The enzymatic activity of glyceraldehyde 3-phosphate dehydrogenase produces a molecule containing high phosphoryl-transfer potential and reduces NAD^+ to NADH. However, NAD^+ molecule is present in very limited amount in the cell and it must be regenerated for glycolysis to proceed. This is achieved by the metabolism of pyruvate.
- Pyruvate are mainly converted into ethanol, lactic acid, or carbon dioxide (Figure 1).

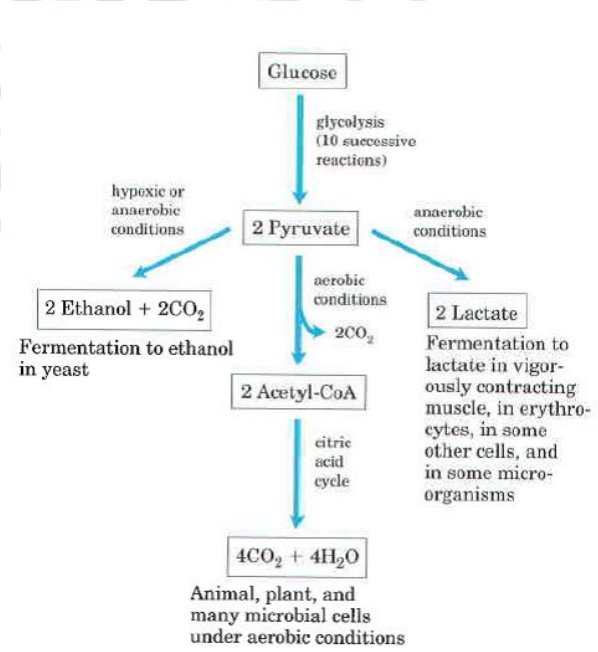

Figure 1. Overview of fate of Pyruvate. (Adapted from lizpaulredd.wordpress.com)

Fate of pyruvate in the presence of aerobic condition

- In the presence of oxygen, molecules like glucose and other sugars, fatty acids, and most amino acids are eventually oxidized to CO_2 and H_2O via the TCA cycle and the respiratory chain.
- The carbon skeletons of sugars and fatty acids are converted into the acetyl group of acetyl-CoA and enters into the TCA cycle, the form in which the cycle accepts most of its fuel input.
- In the matrix of the mitochondria, first pyruvate is converted to Acetyl-CoA by the enzyme pyruvate dehydrogenase complex (PDC) because former cannot enter the TCA cycle (Figure-2).

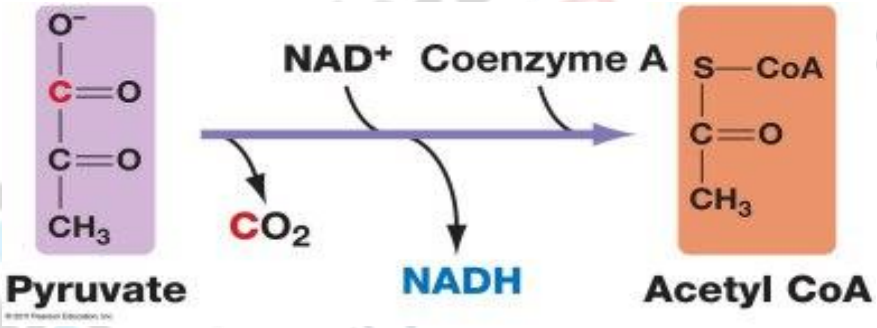

Figure -2 the diagram above illustrates the conversion of pyruvate to Acetyl CoA. (Adapted from – Sachabiochem0001.files.wordpress.com)

- PDC holds a key position in connecting the glycolytic and oxidative pathway of the TCA cycle.
- This catalysis is sequential process which involves the oxidative decarboxylation of pyruvate and the formation of acetyl- CoA, CO_2 and NADH (H^+). This reaction needs five co-factors namely Co-A, TPP, lipoate, FAD and NAD^+.
- PDC are made up of several copies of three catalytic enzymes namely pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3) (Figure 3). They are found in prokaryotes as well as eukaryotes.

E1: Thiamine pyrophosphate (TPP) serves as prosthetic group for Pyruvate dehydrogenase

E2: Lipoamide and coenzyme A (also known as coASH) serves as prosthetic group for enzyme Dihydrolipoyl transacetylase

E3: Dihydrolipoyl dehydrogenase which uses flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD+) as its cofactors.

- A thiamine diphosphate (ThDP) serves as prosthetic group in two step reactions catalysed by E1 and catalyses:
  (i) The decarboxylation of pyruvate to CO_2 with the formation of C2α-hydroxyethylidene- ThDP (enamine) intermediate and
  (ii) The reductive acetylation of the lipoyl groups covalently attached to the E2
- The formation of acetyl-CoA is transfer reaction catalysed by the enzyme E2. The component E3 catalyses the transfer of electrons from the Dihydrolipoyl moieties of E2 to FAD and then to NAD.
- Additional PDCs component are also present in higher eukaryotic cells like dihydrolipoamide dehydrogenase-binding protein (E3BP), and two regulatory enzymes, pyruvate dehydrogenase kinase (PDK, four human isoforms) and pyruvate dehydrogenase phosphatase (PDP, two human isoforms) totalling 11 proteins in PDCh with all isoforms included.
- Moreover, there are two isoforms of the subunit of E1h that are encoded by separate genes in most mammals.
- The X-linked gene (PDHA1 in human) encodes E1 subunit (PDHA1) present in all somatic tissues, whereas an autosomal, intron less gene (PDHA2 in human) is expressed only in the testis.
- In mammals, PDC is serve as a gatekeeper of the metabolism of pyruvate which assist to maintain glucose homeostasis during the fed and fasting states.

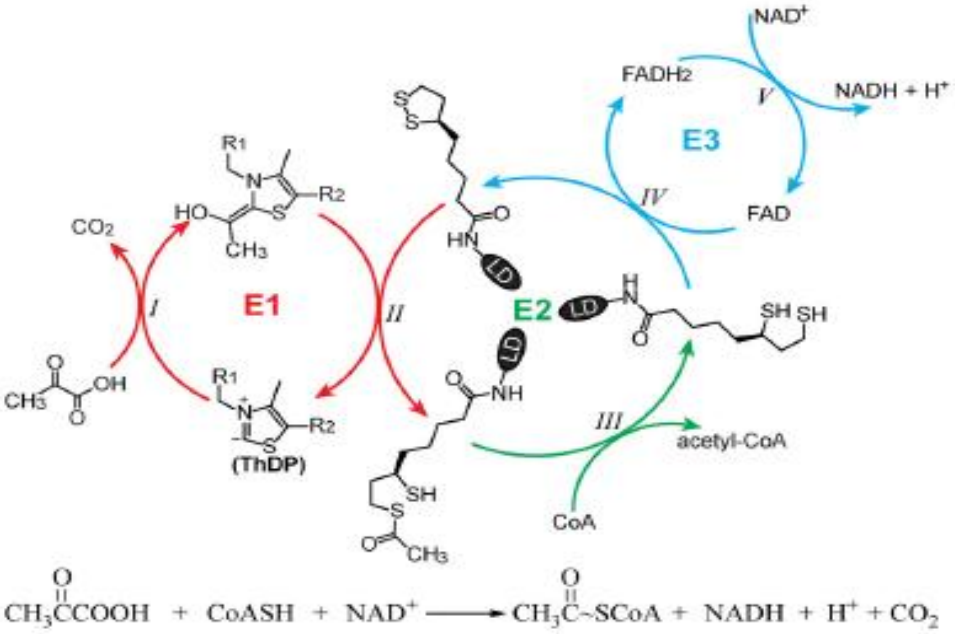

Figure 3. Mechanism of the pyruvate dehydrogenase complex catalysis. There are three catalytic components (E1 is in red; E2 is in green; and E3 is in blue) work sequentially catalyses the oxidative decarboxylation of pyruvate with the formation of acetyl-CoA, CO2, and NADH (H^+) **(Adapted from Patel *et al.*, 2014, J Biol Chem. 289:16615-23.)**

- PDC is very important for health point of view as well. It is involved in degenerative neurological diseases, obesity, type 2 diabetes, and other diseases. More recently, PDC gained attention in cancer biology which is mainly attributed to prominent role played by aerobic glycolysis in some cancers.
- Pyruvate can be converted to oxaloacetate, in a reaction catalysed by the biotin-dependent enzyme pyruvate carboxylas and later molecule enter into TCA cycle to generate energy (Figure 4). It is an important step to replace the intermediates of the TCA cycle and make available as substrates for gluconeogenesis. It also involved in formation of aspartate via transamination reaction.

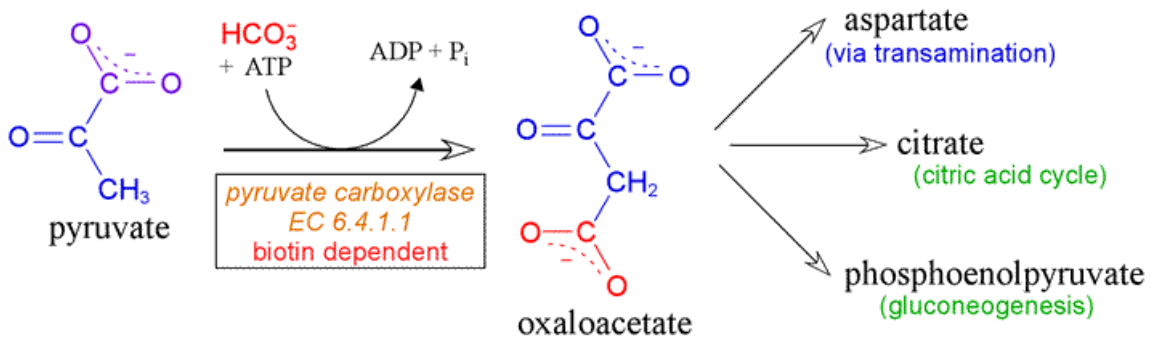

Figure-4. Pyruvate converted to oxaloacetate (https://biochemistryisagoodthing.wordpress.com/2013/03/28/fates-of-pyruvate/)

Fate of Pyruvate under anaerobic condition

- In the absence of oxygen, (anaerobic conditions) pyruvate undergoes fermentation leading to formation of lactic acid or alcohol. In this fermentation reaction reduced NAD^+ is generated which is indirectly help in synthesis ATP in the glycolysis process.
- During the process of evolution, the earliest cells lived in the strict anaerobic condition and had used glycolysis as one of the approaches of metabolism. Most modern organisms have still retained this classical way of metabolism and to produce NAD^+ and as lactate or ethanol as end products.

Lactate fermentation:

- Lactic acid fermentation occurs in many microbes leading to from lactate from pyruvate.
- This conversion is also found in cells of higher animals under certain conditions. For instance extensive exercise would create oxygen limiting condition for muscles cells (state is known as anoxia) and would undergo anaerobic respiration.
- In this process, lactate is formed from pyruvate in the reaction catalysed by Lactate dehydrogenase. It is an important step to restore the supply of NAD^+ in order to ensure that glycolysis long lasts. However, lactic acid is toxic to cells since it causes a change in the pH and leads to acidosis.

- The pyruvate produced in red blood cells (RBC) is converted to lactate by the enzyme lactate dehydrogenase and is a slightly reversible reaction. In this process, NADH is oxidized to NAD^+, which assists the reduction of pyruvate to lactate (Figure 5)

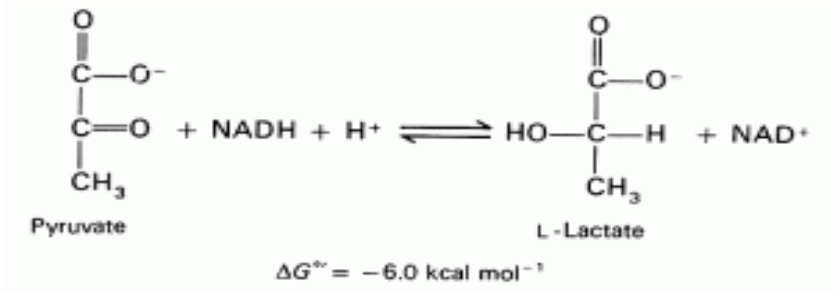

Figure 5. Conversion of Pyruvate to Lactate. (Adapted from biochemistryisagoodthing.wordpress.com)

- Due to lack of mitochondria in RBC, site for the TCA cycle, pyruvate is converted Lactate. This step also helps to regenerate NAD^+ and will further enter into the glycolytic pathway, the site of ATP synthesis for RBC.
- Lactate is also produced in muscles under vigorous muscle contraction due to exercise activities. This leads to building up of lactic acid in the muscles causing cramps and pain.

ETHANOL FERMENTATION:

- In another process, under anaerobic condition pyruvate is further metabolised to ethanol with regeneration of NAD^+ and formation of carbon dioxide.
- In a simple eukaryotic cell like yeast, pyruvate is converted to ethanol with a liberation of carbon dioxide in a two-step fermentation process. These steps are irreversible reactions were catalysed by pyruvate decarboxylase and alcohol dehydrogenase. TPP is a co-factor for both of these enzymes (Figure 6)
- In a first step is catalysed *Pyruvate decarboxylase* in which pyruvate is decarboxylated and converted to acetaldehyde. The product acetaldehyde formed in this reaction serves as the substrate for the next enzyme in the pathway.

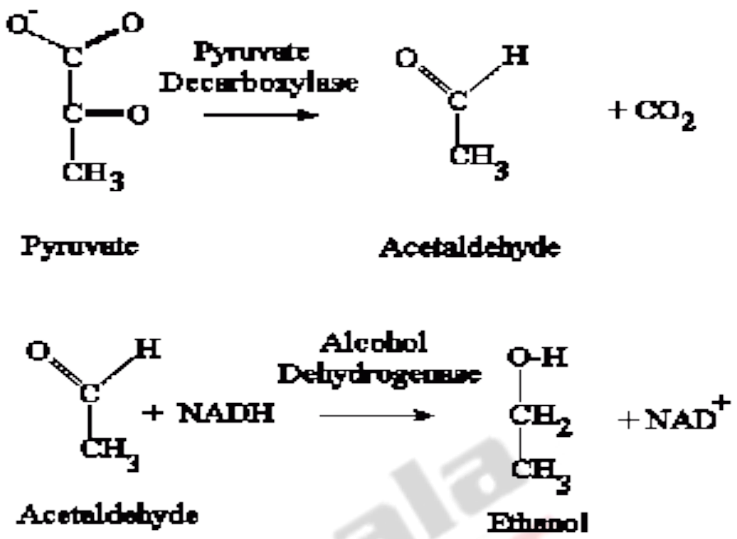

Figure 6. Conversion of Pyruvate to Ethanol. (Adapted from biochemistryisagoodthing.wordpress.com)

- Pyruvate decarboxylase requires Thiamine Pyro Phosphate (TPP) and Mg^{2+} as coenzyme and a cofactor respectively. Thiamine (vitamin B1) contains a thiazolium ring and serve as sources of TPP.

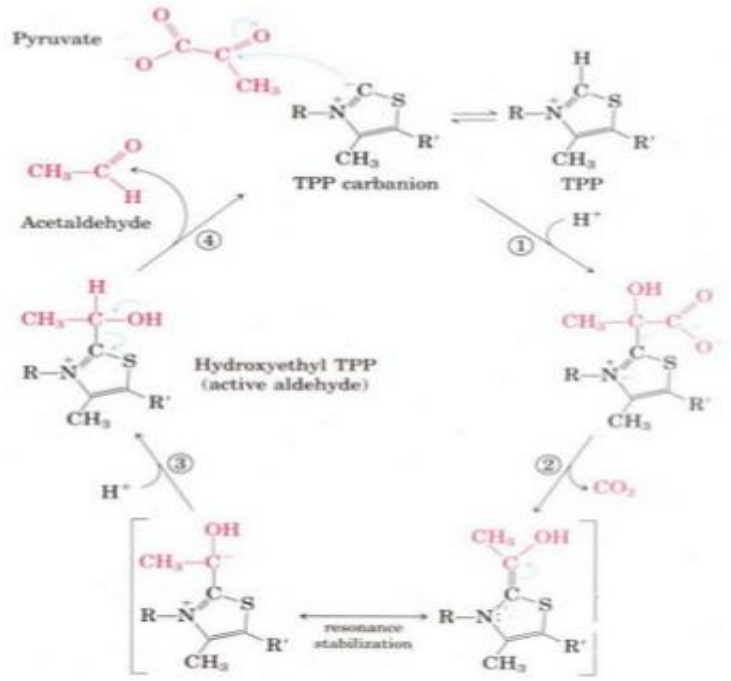

Figure 7. Mechanism of Pyruvate decarboxylase. (Adapted from guweb2.gonzaga.edu)

- Alcohol dehydrogenase catalyses the conversion of acetaldehyde in the presence of NADH to NAD^+, ethanol, and carbon dioxide. It is an important enzyme found in many organisms that including in humans. In the later's liver it carry out the oxidation of ethanol which is either ingested or produced by intestinal microorganisms, with the concomitant reduction of NAD^+ to NADH.
- Lactate and ethanol produced by microbial fermentation are commercially exploited for human use.

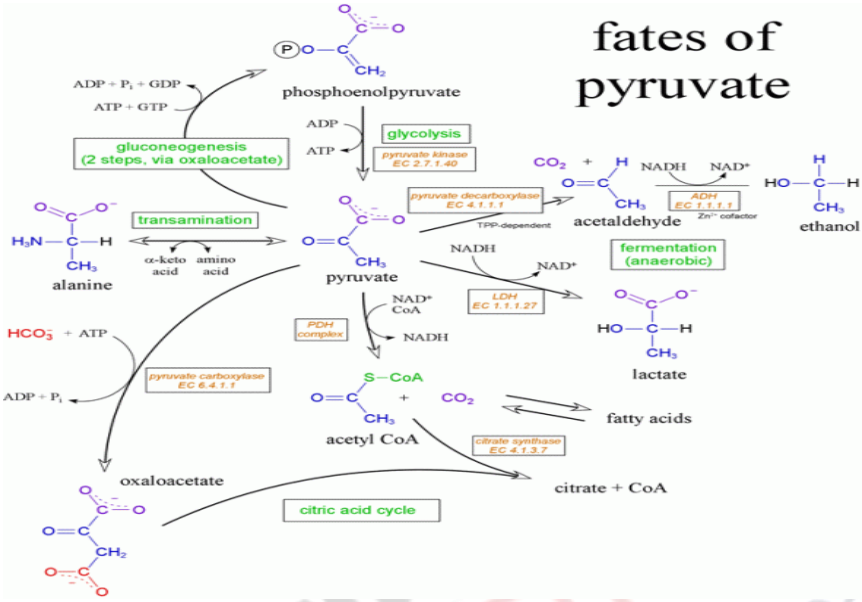

Figure 8. Fate of Pyruvate in aerobic and anaerobic condition. (Adapted from biochemistryisagoodthing.wordpress)

SUMMARY

- Pyruvate, the end product of glycolysis, must be further metabolised to maintain proper redox balance.
- Under aerobic conditions, acetyl-CoA is produced which the starting material for the TCA cycle and the pyruvate dehydrogenase complex plays an important role in this catalysis step (Figure 9).
- Under anaerobic condition, pyruvate is reduced to lactic acid in a reaction catalysed by the lactate dehydrogenase enzyme. During this reduction step, NAD^+ is formed from NADH. Such reactions are observed in the muscle cells are devoid of oxygen and microbes like lactic acid bacteria (Figure 9).
- Microorganisms including yeast opts for fermentation of sugars to ethanol via glycolysis in a two-step process. (i) Pyruvate is converted to acetaldehyde in the presence of Thiamine pyrophosphate and Mg^{2+} and a reaction catalysed by enzyme pyruvate decarboxylase. (ii) Acetaldehyde is further reduced to ethanol by NADH and this reaction catalysed by enzyme alcohol dehydrogenase (Figure 9).

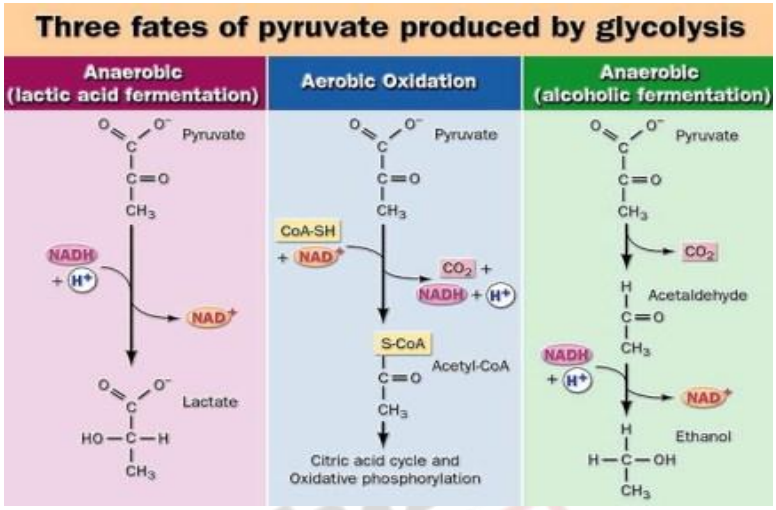

Figure 9. The diagram above illustrates the fate of pyruvate in aerobic and anaerobic conditions. (Adapted from Sachabiochem0001.files.wordpress.com)