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Subject	Final Exam - Chapter Seventeen

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Glycolysis

Introduction:

- The process of breaking down glucose to extract energy is a very important pathway in the human body.
- This pathway consists of 3 main processes, **glycolysis**, which will be discussed in this chapter, **the citric acid cycle** and **oxidative phosphorylation** both of them will be discussed in subsequent chapters.
- The complete aerobic oxidation of glucose to CO_2 and water through the 3 previous processes yields energy equivalents to 32 ATP (Maximum).
- In glycolysis, each glucose molecule is converted to 2 pyruvate molecules in a series of 10 reactions, only one of which is an oxidation. Then pyruvate can be further used in 3 different pathways as we shall see.
- Glycolysis process occurs in the **cytosol**,

- When glucose is converted to pyruvate (glycolysis), an amount of energy is released that is sufficient to be used to produce 2 ATP molecules.

☒ Glycolysis:

Glycolysis (also called **Embden-Meyerhoff** pathway) is divided into 2 main phases:

1. Phase 1 involves the conversion of 1 **Glucose** to 2 **Glyceraldehyde-3-Phosphate**. This process requires energy (endergonic reactions); in which 2 ATP molecules are used.

2. Phase 2 involves the conversion of two **Glyceraldehyde-3-Phosphates** to two **Pyruvate** molecules. This process releases energy (exergonic reaction); in which 4 ATP molecules are produced. In addition, this process is an overall oxidation reaction and requires the conversion of an oxidizing agent (NAD^+) to its reduced form (NADH).

- So, the overall process of glycolysis releases energy that is sufficient to produce 2 ATP molecules (in phase 1; 2 ATP molecules were used, in phase 2; 4 ATP molecules were released, the net ATP change would be 4 – 2 = 2 ATP molecules produced). This is summarized by the following equations:

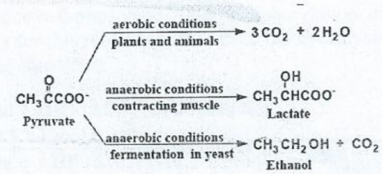
$$\textbf{Glucose (6 C atoms)} \rightarrow \textbf{2 Pyruvate (each 3 C atoms)}$$
$$\textbf{2 ATP + 4 ADP + 2 } P_i \rightarrow \textbf{2 ADP + 4 ATP (Phosphorylation)}$$

$$\textbf{Glucose + 2 ADP + 2 } P_i \rightarrow \textbf{2 Pyruvate + 2 ATP (Net reaction)}$$

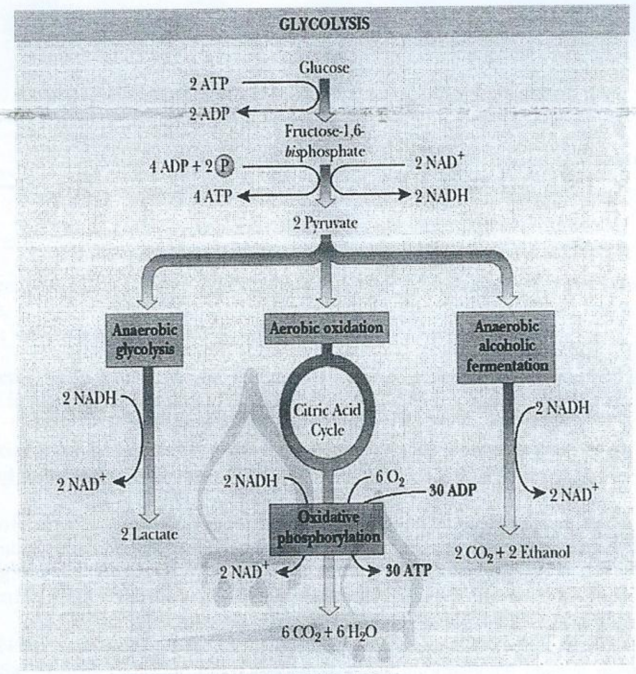
- The produced pyruvate has one of 3 ends:

1. **Aerobic oxidation** in which case pyruvate releases CO_2 and the remainder combines with Coenzyme-A (Co-A) to form acetyl-Co-A, then acetyl-Co-A enters the citric acid cycle and then oxidative phosphorylation to end up as CO_2 and H_2O. Remember that this process occurs in aerobic conditions.

2. **Anaerobic glycolysis** in here pyruvate is converted (reduction) to lactate. This occurs in certain bacteria such as ***Lactobacillus*** and ***Clostridium botulinum***, and in muscles under heavy exercise conditions. Also, it represents the only source of energy in RBCs.



3. **Anaerobic alcoholic fermentation** in organism capable of that, here pyruvate looses CO_2 and is converted to acetaldehyde which is then converted to ethanol.



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☒ Phase 1 of the Glycolytic Pathway:

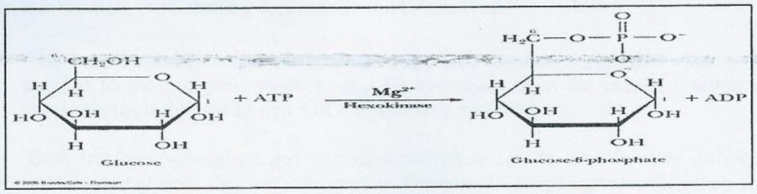
- In this phase which is also called the ***preparation phase*** of glycolysis (the preparation for phosphorylation of ADP). The 6-carbon glucose molecule is converted to the 3 carbon Glyceraldehyde-3- phosphate, this phase consists of 5 steps:

1. **Phosphorylation** of glucose to give glucose-6-phosphate. The phosphorylation of glucose is endergonic reaction, the energy for this reaction comes from the hydrolysis of ATP, this is illustrated by the following equations:

$$\textbf{Glucose} + P_i \rightarrow \textbf{Glucose-6-phosphate} + H_2O \ (\Delta G^{o\prime} = 13.8 \text{ kJ/mole})$$

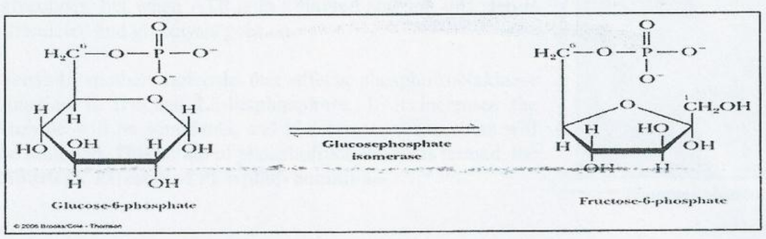
$$ATP + H_2O \rightarrow ADP + P_i \ (\Delta G^{o\prime} = -30.5 \text{ kJ/mole})$$

$$\textbf{Glucose} + ATP \rightarrow \textbf{Glucose-6-phosphate} + ADP \ (\Delta G^{o\prime} = -16.7 \text{ kJ/mole})$$



- The enzyme that catalyzes this reaction is called ***Hexokinase*** (Hexo = 6-carbon atoms, Kinase = transfers phosphate from ATP to other molecules), so hexokinase means the enzyme that transfers a phosphate group to a 6-carbon sugar.
- This enzyme is a non-specific enzyme (it can act on any 6-carbon sugar), and is found in many types of tissues.
- Hexokinase can be inhibited by the accumulation of glucose-6-phosphate which is a **control point** in the pathway.
- One isoform of the enzyme ***hexokinase*** is ***Glucokinase*** which is found in the liver and cannot be inhibited by glucose-6-phosphate; in this way when glucose levels are high, glucose-6-phospahte will accumulate, and the glycolytic pathway will be inhibited in most of the tissues **except in the liver.**
- In hexokinase the binding site for glucose forms a **slit** between 2 lobes of the protein, when the glucose molecule binds the enzyme a conformational change occurs in which, the 2 lobes closes over the binding site. This feature is characteristic of all kinases.

2. **Isomerization** of **Glucose-6-Phosphate** to **Fructose-6-Phosphate**, that is, the C-1 aldehyde group is reduced to a hydroxyl group, and the C-2 hydroxyl group is oxidized to a ketone group, this is done by the enzyme ***Glucose Phosphate Isomerase.***

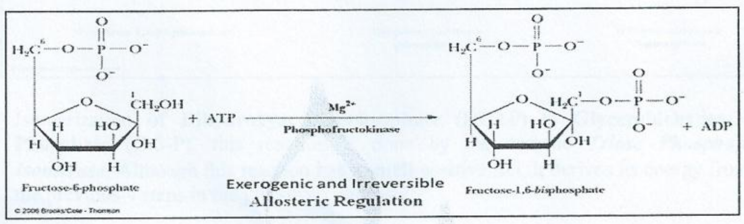


3. **Phosphorylation** of **Fructose-6-Phosphate** to **Fructose-1,6-bisphosphate.** This is done by the enzyme ***Phosphofructokinase***, which is considered as the key regulatory enzyme in glycolysis.

In this reaction, the following concepts should be clarified:

- This reaction itself is an endergonic reaction that requires energy, but since it is coupled to the exergonic reaction of ATP hydrolysis, then the overall reaction is exergonic (as is the case in step 1 of the glycolytic pathway).

- Both fructose-6-phosphate and glucose-6-phosphate can be used in other different pathways, but once they are converted to **fructose-1,6-bisphosphate**, then they are committed to glycolysis; that is this compound cannot be degraded by any other pathway.



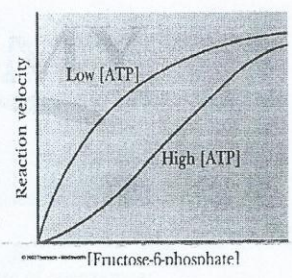
- Phosphofructokinase is a tetramer protein, which means it is composed of 4 subunits, there are 2 types of these subunits, M and L, so there are different possible combinations, M4, M3L1, M2L2, …, L4, these combinations are termed **Isozymes.**

- Different isozymes are found in different body tissues, for example, **M4 is found in muscle tissue** and **L4 is found in the liver**, RBCs, on the other hand, have multiple isozymes.

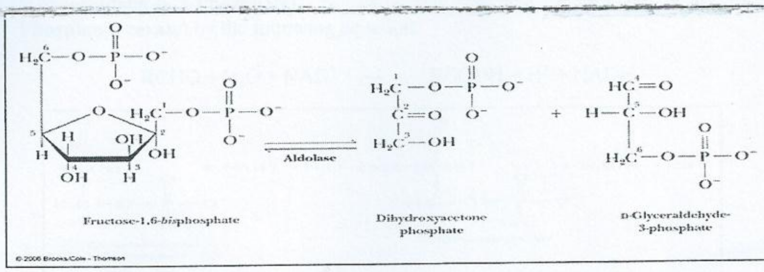
- this step is the **rate limiting step** of glycolysis; because of 2 reasons;

First: ATP acts as a negative feedback inhibitor, so when there is plenty of ATP for energy the cell would shut down glycolysis, but when ATP is in a limited amount, this step is stimulated and glycolysis goes on.

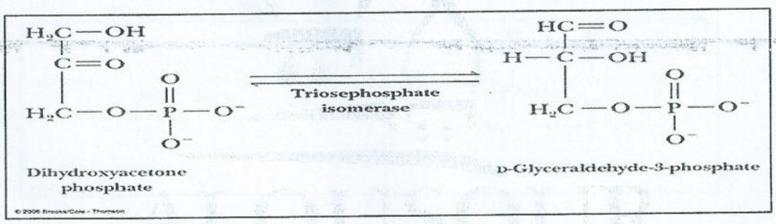
Second: another molecule that affects phosphofructokinase function is **fructose-2,6-bisphosphate**. If it increases the enzyme will be stimulated, and if decreases the enzyme will be inhibited. This action of phosphofructokinase is termed, the **Allosteric Effector of Phosphofructokinase**.



4. Cleavage of **fructose-1,6-bisphosphate** to give **Glyceraldehyde-3-Phosphate** (G-3-P) and **Dihydroxyacetonephosphate** (DHAP), this is done by the enzyme ***Aldolase***.



5. Isomerization of **Dihydroxyacetonephosphate** (DHAP) to **Glyceraldehydes-3-Phosphate** (G-3-P), this reaction is done by the enzyme ***Triose Phosphate Isomerase***. Although this reaction has a small positive ΔG, it derives its energy from the previous 4 steps in the glycolysis pathway.



☒ **Phase 2 of the Glycolytic Pathway:**

- In this phase each Glyceraldehyde-3-phosphate molecule is converted to a molecule of Pyruvate.
- This phase is characterized by 2 facts:

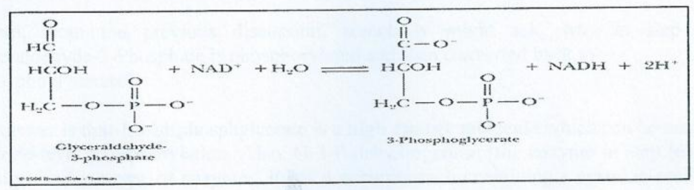
First, since most of the ATP produced by glycolysis is produced in this phase, it is called the **payoff phase** of glycolysis.

Secondly, redox reactions occur in this phase involving the use of **coenzymes**. Since phase 1 consists of 5 steps the first step of phase 2 would be:

6. Oxidation and phosphorylation of **Glyceraldehyde-3-Phosphate** to **1,3-bisphosphoglycerate**.

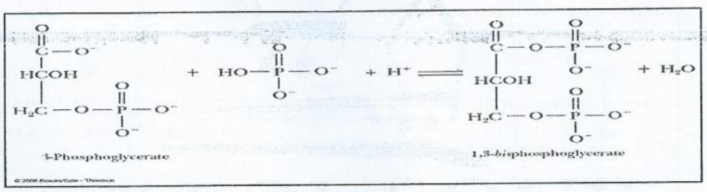
This step consists actually of 2 reactions; the first one is an oxidation reaction in which the coenzyme NAD^+ is used as an oxidizing agent and the aldehyde (**Glyceraldehyde-3-Phosphate**) is converted to the carboxylic acid (**3-Phosphoglycerate**) by the following equation:

$$RCHO + H_2O + NAD^+ \rightarrow RCOOH + H^+ + NADH$$



The second reaction of this step is the phosphorylation of **3-Phosphoglycerate** to 1,3-bisphosphoglycerate. The following equation illustrates this:

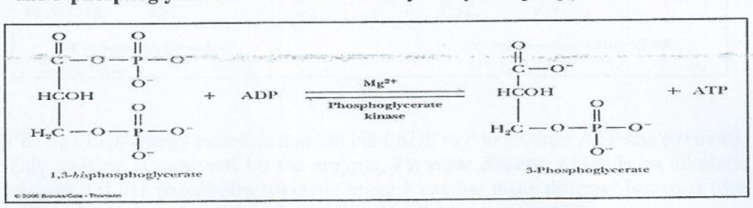
$$\text{3-phosphoglycerate} + P_i \rightarrow \text{1,3-bisphosphoglycerate} + H_2O$$



The overall energy change of this reaction under modified standard states is slightly positive, but when occurs in the cell it becomes slightly negative (higher temperature inside the cell).

The enzyme that catalyzes this reaction is called ***Glyceraldehyde-3-Phosphate Dehydrogenase***.

7. Transfer of a phosphate group from **1,3-bisphosphoglycerate** to ADP to form **ATP** and **3-phosphoglycerate** this reaction is catalyzed by ***Phosphoglycerate Kinase***.



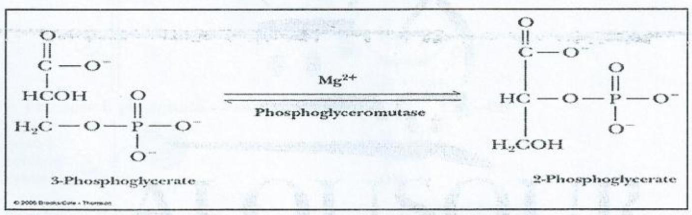
By now we should know 2 things:

First, this process is termed **Substrate-Level Phosphorylation**. In this process energy needed to combine ADP and P to produce ATP comes from the breakdown of a higher energy molecule 1,3-bisphosphoglycerate. Whereas in oxidative phosphorylation, energy comes from the redox reaction involving oxygen as the final electron acceptor.

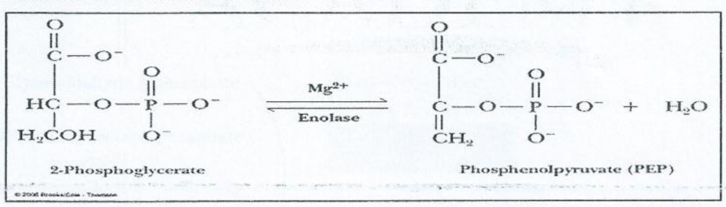
Second, from the previous discussion, somebody might ask, why in step (6) Glyceraldehyde-3-Phosphate is phosphorylated and then converted back to 3-phosphoglycerate?

The answer is that 1,3-bisphosphglcerate is a **high energy molecule** which can be used is substrate-level phosphorylation. Also, G-3-P dehydrogenase [the enzyme in step (6)] is actually a unique type of enzymes, it has 4 subunits each containing a **cysteine residue** that can form a thioester with G-3-P to form a high energy molecule 1,3-bisphosphoglycerate which can then join ADP and P to produce ATP.

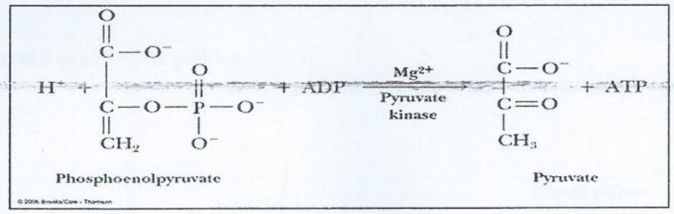
8. Isomerization of **3-Phosphoglycerate** to **2-Phosphoglycerate** by ***Phosphoglyceromutase***.



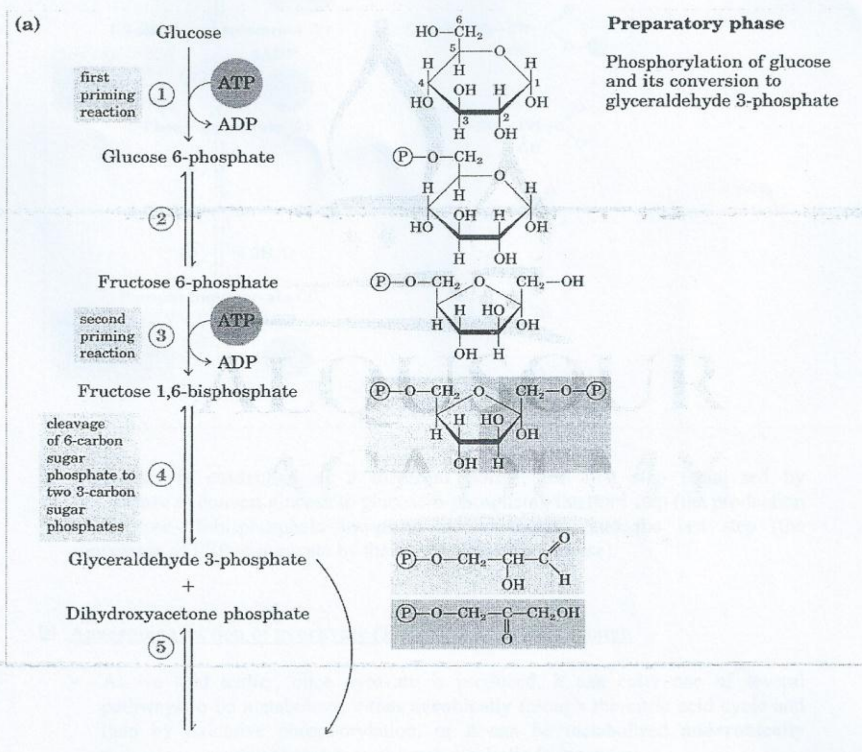
9. Dehydration of **2-Phosphoglycerate** to produce **Phosphoenolpyruvate (PEP)** releasing **water**. This reaction is catalyzed by the enzyme ***Enolase*** which requires **Mg^{+2}** with which the water molecule binds.



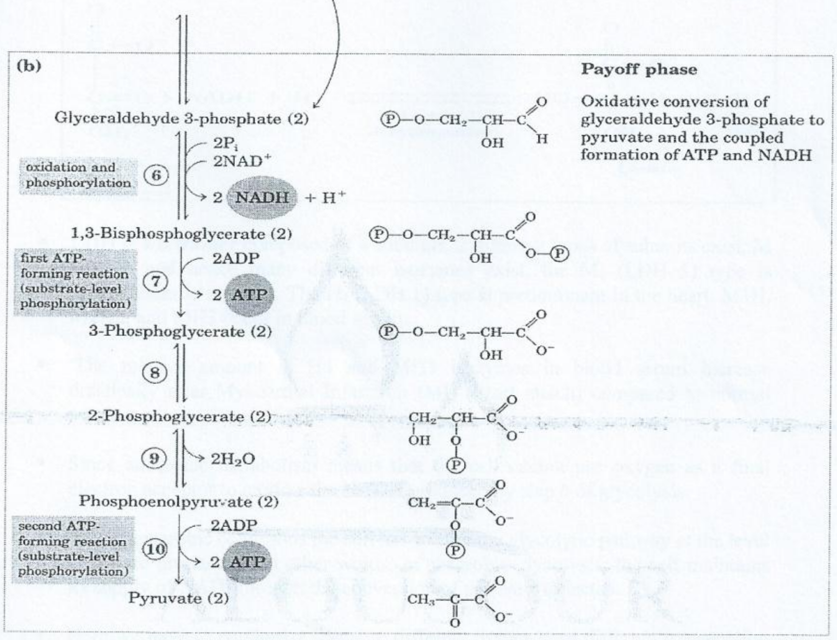
10. **PEP** is a high energy molecule that can bind ADP to P to produce **ATP** and **pyruvate.** This reaction is catalyzed by the enzyme, ***Pyruvate Kinase***, which is an allosteric enzyme just like phosphofructokinase, hence it can has many different isozymes (due to M and L subunits), and it is also inhibited by the increase in ATP.



⊠ **Phase 1 of the glycolytic pathway.**



☒ **Phase 2 of glycolytic pathway**



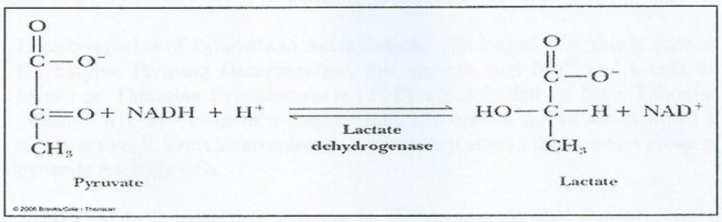
☒ **Glycolysis is controlled at 3 different points**; the first step (catalyzed by ***hexokinase*** to convert glucose to glucose-6-phosphate), the third step (the production of fructose-1,6-bisphosphate by ***phosphofructokinase***), and the last step (the conversion of PEP to pyruvate by the enzyme ***pyruvate kinase***).

☒ <u>**Anaerobic reaction of pyuruvate (The Production of Lactate):**</u>

- As we said earlier, once pyruvate is produced, it can enter one of several pathways to be metabolized either **aerobically** through the citric acid cycle and then by oxidative phosphorylation, or it can be metabolized **anaerobically** through conversion to lactate or through alcoholic fermentation.

- This reaction is also exergonic ($\Delta G°$ is negative).

- **Pyruvate** is converted to **Lactate** by the enzyme ***Lactate Dehydrogenase* (LDH),** this reaction is a **redox** reaction, in which pyruvate is reduced to lactate, with NADH acting as a reducing agent and converted to the oxidized form NAD^+.



- **LDH** is a **tetramer** composed of 4 subunits, 2 different types of subunits exist, M and H, and hence many different isozymes exist, the M_4 (LDH 5) type is predominant in muscles. The H_4 (LDH 1) type is predominant in the heart. M3H, M2H2, and MH3 occur in blood serum.

- The relative amount in H4 and MH3 isozymes in blood serum increase drastically after Myocardial Infarction (MI) (heart attach) compared to normal serum.

- Since anaerobic metabolism means that the cell cannot use oxygen as a final electron acceptor to oxidize the NADH produced by step 6 of glycolysis.

- So, in anaerobic conditions the cell has to stop the glycolytic pathway at the level of lactate production, in other words, in anaerobic glycolysis; the cell maintains its supply of NAD^+ through the conversion of pyruvate to lactate.

- Here we have to emphasize, because different tissues have different oxygenation (for example muscles have less oxygen supply than the heart or the liver), the LDH isozymes have different capabilities (in muscles LDH 5 is allosterically inhibited by the accumulation of pyruvate, whereas in the heart LDH 1 has a higher affinity to lactate as a substrate).

- Muscles cannot metabolize lactate further, so lactate goes through the blood stream to the liver which can then use lactate in a reverse pattern to produce pyruvate or even glucose a process called **Gluconeogenesis**.

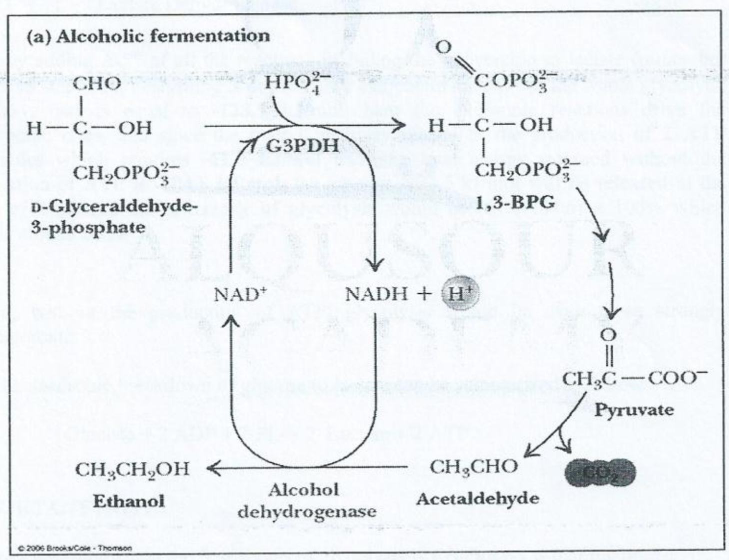
☒ **Alcoholic Fermentation:**

The second way by which organisms can metabolize pyruvate is Alcoholic Fermentation, this occurs in organisms capable of fermentation (ex. yeast), this occurs as 2 reactions:

1. Decarboxylation of **Pyruvate** to **Acetaldehyde** with loss of CO_2, this is done by the enzyme ***Pyruvate Decarboxylase***, this enzyme uses $\mathbf{Mg^{+2}}$ and a cofactor known as **Thiamine Pyrophosphate (TPP)** which is derived from **Thiamine** (Vitamin B_1). TPP contains a thaizole ring, it contain a carbon atoms which is highly active, it forms a carbanion (C^-) which then attacks the carboxyl group of pyruvate releasing CO_2.

2. **Acetaldehyde** is reduced to **Ethanol** by the enzyme ***Alcohol Dehydrogenase***, and again this converts NADH to NAD^+, to maintain the NAD^+ pool in the cell.

Glucose + 2 ADP + 2 Pi + 2H$^+$ → 2 Ethanol + 2 ATP + 2 CO_2 + 2 H2O



☒ **Energy Produced by Glycolysis:**

Before discussing how much energy can be produced by glycolysis, take a good look at table (1):

Step	Enzyme	$\Delta G^{o'}$ (at 25° C and 37° C) (kJ/mol)	ΔG (at 37° C) (kJ/mol)
1	Hexokinase/Glucokinase	-16.7	-33.9
2	Glucose Phosphate Isomerase	+1.67	-2.92
3	Phosphofructokinase	-14.2	-18.8
4	Aldolase	+23.9	-0.23
5	Triose Phosphate Isomerase	+7.56	+2.41
6	G-3-P Dehydrogenase	2(+6.20)	2(-1.29)
7	Phosphoglycerate Kinase	2(-18.8)	2(+0.1)
8	Phosphoglyceromutase	2(+4.4)	2(+0.83)
9	Enolase	2(+1.8)	2(+1.1)
10	Pyruvate Kinase	2(-31.4)	2(-23.0)
11	Lactate Dehydrogenase	-73.3	-98.0

Now by adding $\Delta G^{o'}$ of all the reactions including the conversion to lactate (remember that from step 6 on, everything is doubled) we can obtain the $\Delta G^{o'}$ of the whole glycolytic pathway, that is equal to **-123.5 kJ/mol**, here the exergonic reactions drive the endergonic ones, and since the overall pathway results in the production of **2 ATP** molecules which requires **-61.0 kJ/mol** then the total energy released without the production of ATP is **-184.6 kJ/mol**, the amount -123.5 kJ/mol will be released in the form of heat, and the **efficiency of glycolysis** would be (61.0/184.6) x 100% which equals around **33%**.

❖ So, without the production of ATP, glycolysis would be even more strongly exergonic.

- The anaerobic breakdown of glucose to lactate can be summarized as follows:

Glucose + 2 ADP + 2 Pi → 2 Lactate + 2 ATP

IMPORTANT NOTE:

The key intermediate in the first stages of glycolysis is **Fructose-1,6-bisphosphate.**

QUESTIONS:

1-All considered high energy compound except:
a- ADP b-Phosphoenol pyruvate
c- Glu 1 phosphate d-AMP

2-In anaerobic glcolysis pyruvate is converted to:
a- lactate b- glucose
c- propanol d- none of the above

3-Anerobic glycolysis occurs in all of the following except:
a- lacto bacillus b- clostridium botulinum
c- muscle d- none of the above

4- The isomeration of DHAP to G-3-P done by:
a-trios phosphate isomerase
b-aldolas
c-glucose phosphate isomerase
d-phosphfructokinase
e-all of the above

5-LDH 5 (M4) type is predominant in:
a- muscle b- liver
c- heart d- RBC

6-the conversion of pyruvate to acetaldehyde done by:
a- lactate dehydrogenase b- pyruvate decarboxylase
c- aldolase d- none of the above

7-The intermediate in glycolysis:
a. 1,3- bisphosphoglycerate
b. Fructose 1,6-bisphosphate
c. Glyceraldehyde-3-phosphate
d. Pyruvate

8-Step/s in glycolysis that will be bypassed in gluconeogenesis is/are:
a. Hexokinase –phosphofructokinase - pyruvate kinase
b. Hexokinase – enolase – aldolase
c. Phosphoglycerate kinase – enolase – aldolase
d. Glucokinase – pyruvate kinase – aldolase.

9-Glycolysis is an anaerobic process.
a. True
b. False

Solution

Question number	The answer
1.	d- AMP
2.	a- lactate
3.	d- none of the above
4.	a- trios phosphate isomerase
5.	a- muscle
6.	b- pyruvate decarboxylase
7.	b. Fructose 1,6-bisphosphate
8.	a. Hexokinase –phosphofructokinase - pyruvate kinase
9.	a. True